

Article

Design, Synthesis and Antitumor Evaluation of Novel Pyrazolopyrimidines and Pyrazoloquinazolines

Mohamed El-Naggar ¹, Ashraf S. Hassan ^{2,*} , Hanem M. Awad ³ and Mohamed F. Mady ^{4,5,*} 

¹ Chemistry Department, Faculty of Sciences, University of Sharjah, Sharjah 27272, UAE; M5elnaggar@yahoo.com

² Organometallic and Organometalloid Chemistry Department, National Research Centre, Dokki, Cairo 12622, Egypt

³ Department of Tanning Materials and Leather Technology, National Research Centre, Dokki, Cairo 12622, Egypt; hanem_awad@yahoo.com

⁴ Department of Green Chemistry, National Research Centre, Dokki, Cairo 12622, Egypt

⁵ Department of Chemistry, Bio Science and Environmental Technology, Faculty of Science and Technology, University of Stavanger, N-4036 Stavanger, Norway

* Correspondence: ashraf_salmoon@yahoo.com (A.S.H.); mohamed.mady@uis.no (M.F.M.); Tel.: +20-100-664-5444 (A.S.H.); +47-912-569-33 (M.F.M.)

Received: 2 May 2018; Accepted: 22 May 2018; Published: 23 May 2018



Abstract: A series of *N*-aryl-7-aryl-pyrazolo[1,5-*a*]pyrimidines **18a–u** and *N*-aryl-pyrazolo[1,5-*a*]quinazolines **25a–c** were designed and synthesized via the reaction of 5-aminopyrazoles **11a–c** with enaminones **12a–g** or **19**, respectively. The new compounds were screened for their *in vitro* antitumor activity toward liver (HepG-2) and breast (MCF-7) human cancer cells using 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2*H*-tetrazolium bromide MTT assay. From the results, it was found that all compounds showed dose-dependent cytotoxic activities against both HepG-2 and MCF-7 cells. Two compounds **18o** and **18a** were selected for further investigations. Cell cycle analysis of liver (HepG-2) cells treated with **18o** and breast (MCF-7) cells treated with **18a** showed cell cycle arrest at G2/M phase and pro-apoptotic activity as indicated by annexin V-FITC staining.

Keywords: pyrazolopyrimidines; pyrazoloquinazolines; synthesis; antitumor activity; cell cycle analysis

1. Introduction

Pyrazolo[1,5-*a*]pyrimidine ring **1** and its derivatives occupy a unique place in medicinal chemistry due to its various pharmacological activities [1–6] especially antitumor properties [7–9]. In 2006, Li et al. synthesized compound **2** which exhibited significant *in vitro* antitumor activity against Bel-7402 (liver) and HT-1080 (fibrosarcoma) cell lines [10]. In 2009, Ahmed et al., prepared compound **3** which was more effective and exhibited cytotoxicity against HCT116 (colon) and HeLa (cervix) cell lines [11]. In 2010, Abdel-Aziz and co-workers have described a facile synthesis of compound **4** which exhibited promising *in vitro* antitumor activity against CaCo-2 (colon) and BHK (normal fibroblast) cell lines [12]. Furthermore, we have reported the synthesis of compounds **5** and **6** in high yield by treating 5-aminopyrazole with 2-(2-chlorobenzylidene)malononitrile and ethyl acetoacetate, respectively, these compounds show good antitumor activities against HCT-116 and HepG2 cells [13,14] (Figure 1).

In addition, pyrazolo[1,5-*a*]pyrimidine derivatives have been reported as potent enzymes inhibitors [15–17]. Mukaiyama et al., have prepared compound **7** which exhibited potent inhibitory activity against c-Src Kinase and good CNS penetration [18]. Very recently, Kumar et al., have prepared pyrazolo[1,5-*a*]pyrimidine carboxamide **8** which showed good aurora kinase A and B activity [19] (Figure 1).

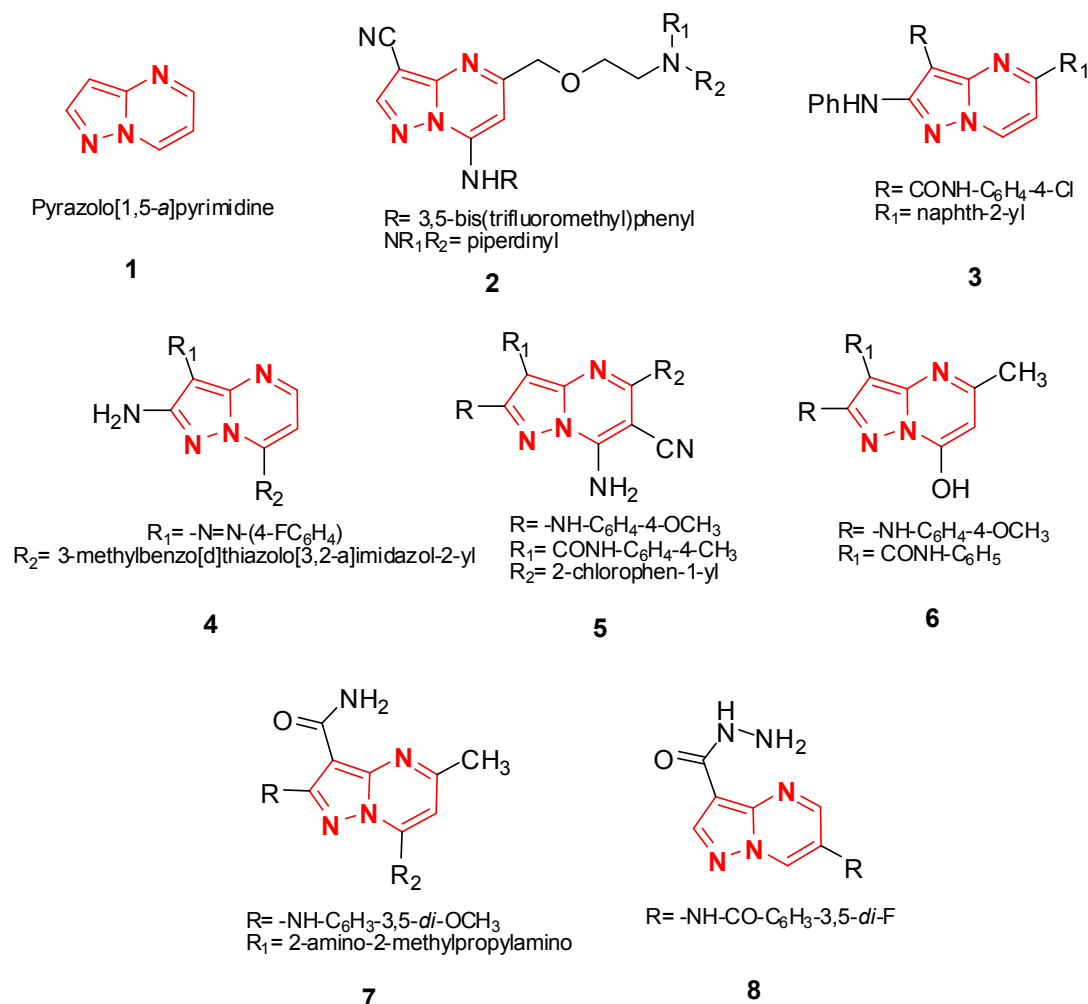


Figure 1. Structures of the antitumor activity compounds 1–6 and enzymes inhibitors 7–8.

In continuation of our research program [20–27] and following the potent biological activity results against MCF-7 and HepG2 carcinoma cells which were obtained from our synthesized compounds such as 7-(4-chlorophenyl)-2-(phenylamino)pyrazolo[1,5-*a*]pyrimidine (**9**, IC₅₀ = 63.2 ± 5.9 μM) and 2-(phenylamino)-pyrazolo[1,5-*a*]quinazoline (**10**, IC₅₀ = 77.6 ± 4.3 μM) compared to doxorubicin [28]. In this work, we have planned to modify the pyrazolo[1,5-*a*]pyrimidine **9** and pyrazolo[1,5-*a*]quinazoline **10** to obtain *N*-aryl-7-aryl-pyrazolo[1,5-*a*]pyrimidines **18a–u** and *N*-aryl-pyrazolo[1,5-*a*]quinazolines **25a–c**, respectively, incorporating different aryl groups (blue and green) into the structures to evaluate their in vitro antitumor activities against HepG-2 and MCF-7 human cells to find novel and potent antitumor compounds (Figure 2).

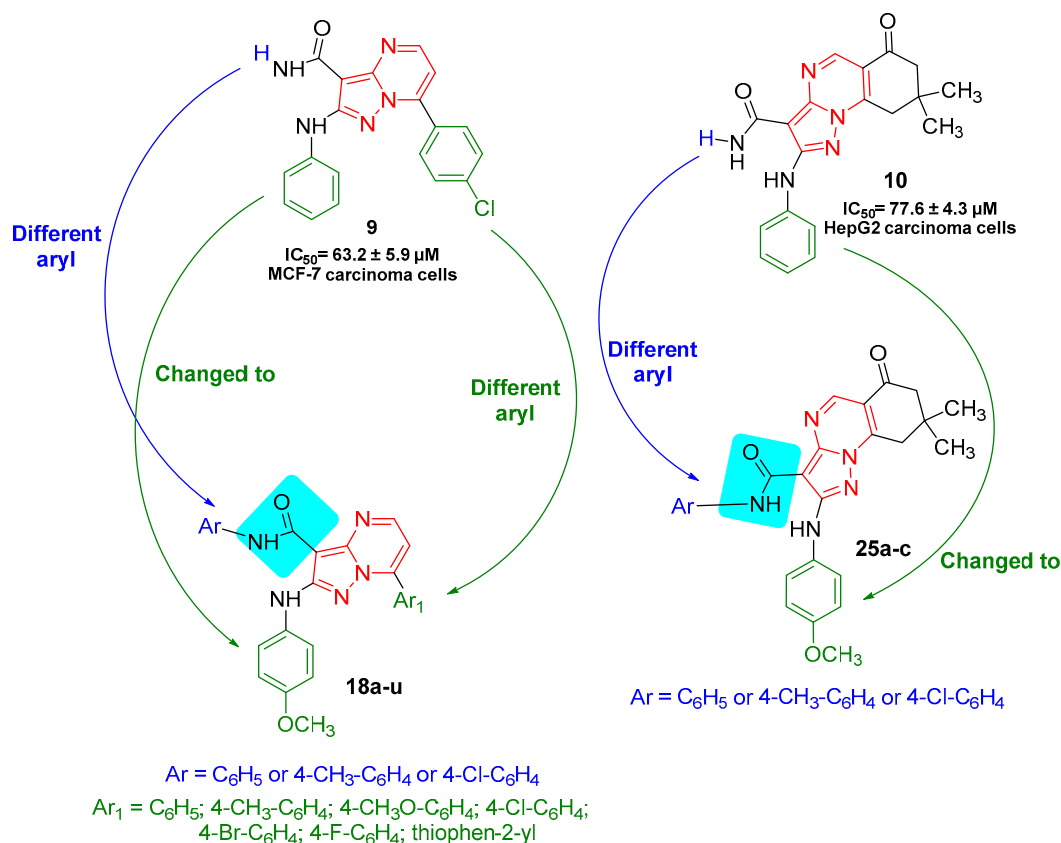


Figure 2. Design of novel *N*-aryl-pyrazolo[1,5-*a*]pyrimidines **18a–u** and *N*-aryl-pyrazolo[1,5-*a*]quinazolines **25a–c**-based amide linkages.

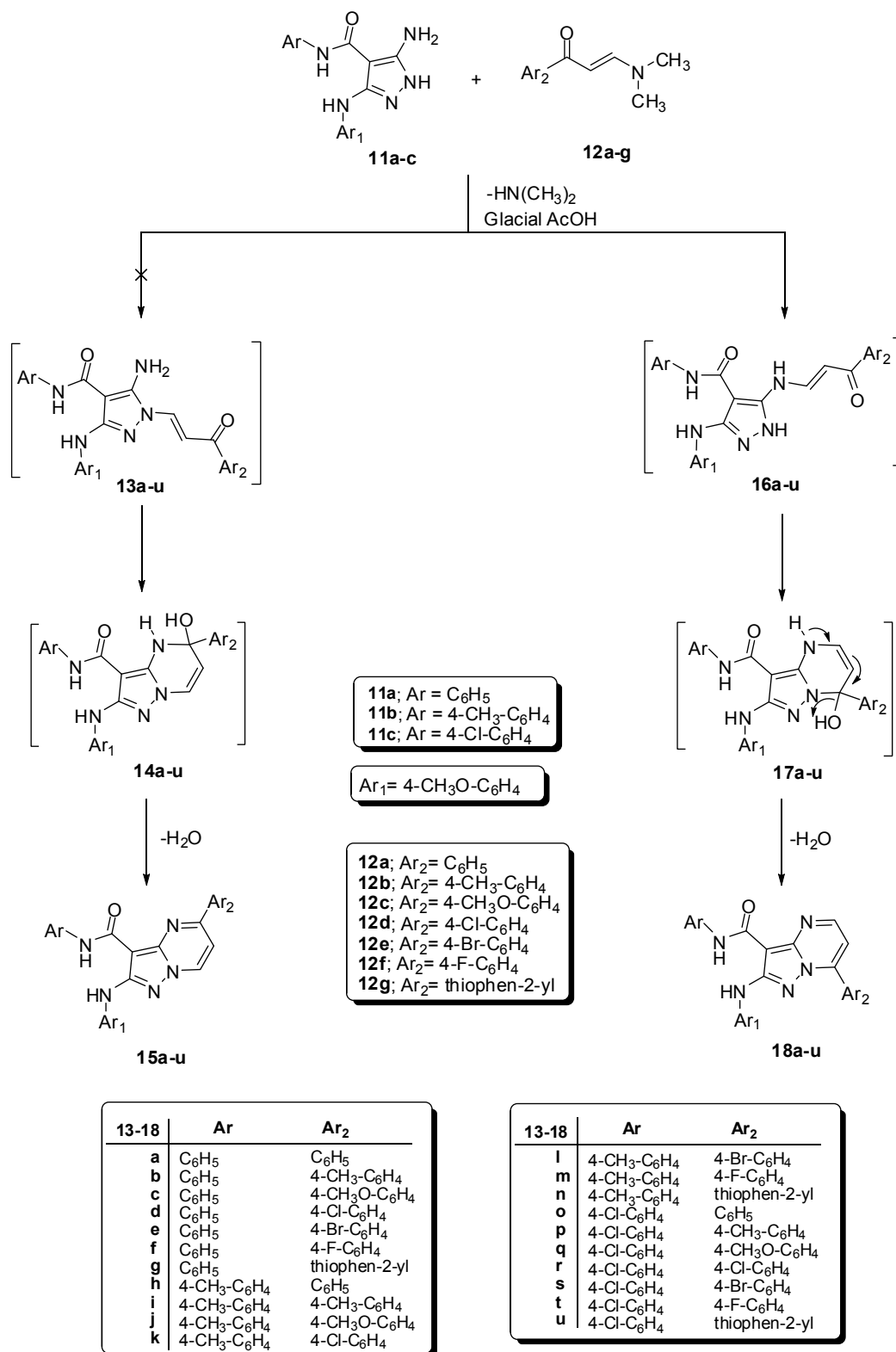
2. Results and Discussion

2.1. Chemistry

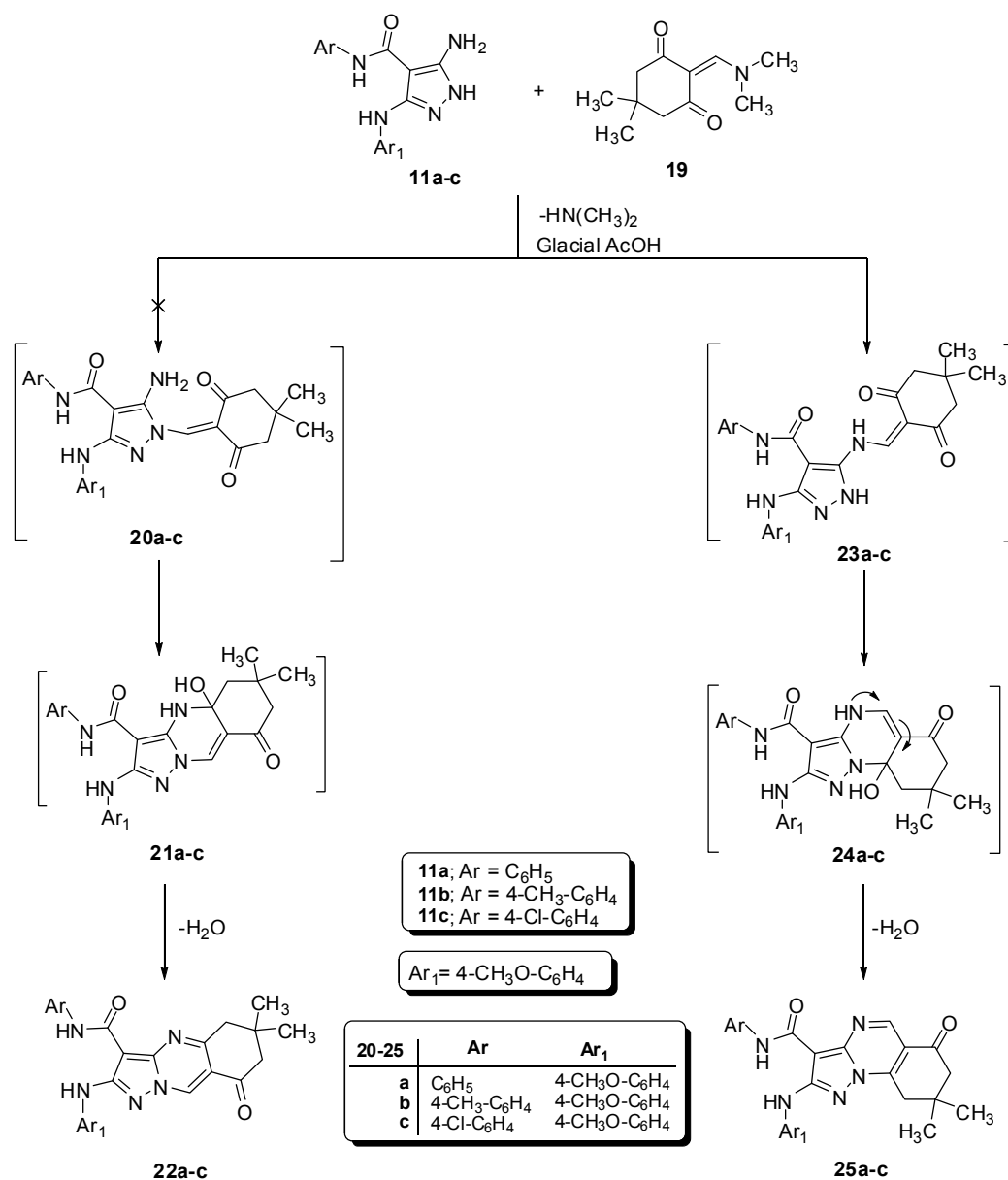
The syntheses of target compounds **18a–u** and **25a–c** are illustrated in Schemes 1 and 2. The starting materials, 5-amino-*N*-aryl-1*H*-pyrazole-4-carboxamides **11a–c** were synthesized according to our previous work [29]. Reaction of compounds **11a–c** with 1-(aryl)-3-(dimethylamino)prop-2-en-1-ones **12a–g** in glacial acetic acid furnished one isolable product 5-aryl-pyrazolo[1,5-*a*]pyrimidines **15a–u** or 7-aryl-pyrazolo[1,5-*a*]pyrimidines **18a–u**. As depicted in Scheme 1, the final products were confirmed by the spectral analysis.

The 1H -NMR spectrum ($CDCl_3$, δ ppm) exhibited, in each case **18c** or **15c**, characteristic two doublets of the pyrimidine protons at 6.87 (1H, H-6) and at 8.40 (1H, H-5) (each with $J = 8.4$ Hz) and four signals at 3.80, 3.91, 9.36 and 10.02 due to $2OCH_3$ and $2NH$, respectively. The ^{13}C -NMR spectrum ($CDCl_3$, δ ppm), in each case **18c** or **15c**, also characterized by signals of $-OCH_3$, $-OCH_3$, C_3 -pyrazolopyrimidine, C_6 -pyrazolopyrimidine, C_5 -pyrazolopyrimidine and $C=O$ at 55.53, 55.60, 87.45, 106.43, 157.52 and 163.21, respectively.

Although, 1H - and ^{13}C -NMR spectra cannot differentiate between **15a–u** and **18a–u**, 1H - ^{15}N HMBC spectrum used for differentiating between the two isomers. The 1H - ^{15}N HMBC spectrum of the final product shows the most important correlated coupling between the proton H-5 of pyrazolopyrimidine (1H , at 8.40 ppm) with N-4 of pyrimidine (^{15}N , at 255 ppm) 2J (H-5, N-4) gave absolute confirmation for the structure of **18a–u** and conclude **15a–u** (cf. Supporting Information). Also, the structure of **18a–u** was supported by X-ray crystallography of similar analogs and products [12].

Scheme 1. Synthesis of 7-aryl-pyrazolo[1,5-*a*]pyrimidines 18a-u.

In addition, *N*-aryl-2-(arylamino)-pyrazolo[1,5-*a*]quinazolines **25a-c** were formed by the condensation of **11a-c** with 2-((dimethylamino)methylene)-5,5-dimethylcyclohexane-1,3-dione (**19**) in a glacial AcOH (Scheme 2) while pyrazolo[1,5-*a*]quinazolines **22a-c** were not formed. The spectral analysis of the products supported the structures of **25a-c**.



Scheme 2. Synthesis of *N*-aryl-2-(arylamino)-pyrazolo[1,5-*a*]quinazolines **25a-c**.

The mass spectrum of **25b** confirmed the molecular formula C₂₇H₂₇N₅O₃ (469.53) {MS (*m/z*, %): 469 (M⁺, 93.88)}. The ¹H-NMR (CDCl₃, 400 MHz, δ ppm) spectrum of **25b** was characterized by sharp signals of 2CH₃, CH₂ and CH₂ groups of the dimedone at 1.19, 2.52 and 3.22, respectively. The OCH₃ group, H-5 proton of quinazoline and 2NH protons appeared as singlet signals at 3.81, 8.90, 9.41 and 9.72, respectively. The aromatic protons of 4-methoxyphenylamino ring appeared as two doublets at 6.91 (2*H*) and 7.58 (2*H*) with the coupling constant *J* = 9.0 Hz and the four aromatic protons of *N*-(4-methylphenyl) ring appeared as two doublets at 7.16 (*J* = 8.2 Hz) and 7.54 (*J* = 8.3 Hz). Also, the ¹³C-NMR (CDCl₃, 100 MHz, δ ppm) spectrum showed characteristic signals at 28.70 corresponding to 2CH₃, at 32.65 for a C₈ (quinazoline), two signals at 37.52 and 50.99 corresponding to 2CH₂ and signal at 194.01 due to C=O (quinazoline). The ¹H-¹⁵N HMBC spectrum showed that the two most important correlated coupling which gave absolute and unique confirmation for the structure of **25a-c**, the first was between the proton H-5 of quinazoline (¹H, at 8.90 ppm) with N-4 of quinazoline (¹⁵N, at 260 ppm) ²*J* (H-5, N-4) and the second was between the CH₂ of quinazoline

(^1H , at 3.22 ppm) with N-10 of quinazoline (^{15}N , at 216 ppm) 3J (H-9, N-10) (cf. Supporting Information) (Figure 3).

If the compound **22b** was obtained, its ^1H - ^{15}N HMBC spectrum would have exhibited that correlated coupling between the proton H-9 of quinazoline with N-10 of quinazoline 2J (H-9, N-10) and correlated coupling between the CH_2 of quinazoline with N-4 of quinazoline 3J (H-5, N-4), but, these two correlated coupling were not detected in ^1H - ^{15}N HMBC spectrum (Figure 3). Furthermore, X-ray diffraction of similar analogs added unequivocal evidence for the structures of **25a–c** and confirmed the reaction mechanism [30].

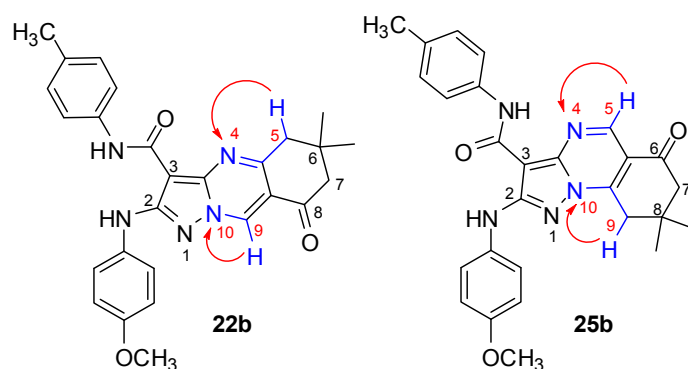


Figure 3. Diagnostic correlations in the ^1H - ^{15}N HMBC spectrum for the two isomers **22b** and **25b**.

2.2. In Vitro Cytotoxic Activity

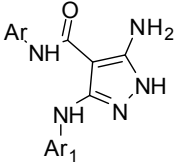
For evaluation of in vitro cytotoxic activity of compounds {5-aminopyrazoles **11a–c**, pyrazolo[1,5-*a*]pyrimidines **18a–u** and pyrazolo[1,5-*a*]quinazolines **25a–c**} against liver (HepG-2) and breast (MCF-7) human carcinoma cell lines, MTT assay was used [31–33]. Doxorubicin[®] was used as a reference cytotoxic compound. The results were expressed as growth inhibitory concentration (IC_{50}) values (Table 1).

From the results of in vitro cytotoxic activity, it was found that most of the prepared compounds displayed comparable IC_{50} values against liver (HepG-2) and breast (MCF-7) cancer cell lines compared to positive control.

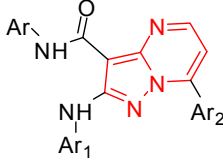
For HepG-2 cancer cells, most of the tested compounds did not show any significant difference compared to the positive control. Only four compounds (**11c**, **18b**, **18f** and **18g**) showed significant difference in their activities. Compounds **18c** ($\text{IC}_{50} = 75.9 \pm 5.3 \mu\text{M}$), **18d** ($\text{IC}_{50} = 77.1 \pm 4.2 \mu\text{M}$), **18h** ($\text{IC}_{50} = 73.2 \pm 3.2 \mu\text{M}$), **18j** ($\text{IC}_{50} = 77.4 \pm 2.9 \mu\text{M}$), **18k** ($\text{IC}_{50} = 74.0 \pm 3.1 \mu\text{M}$), **18l** ($\text{IC}_{50} = 78.7 \pm 5.1 \mu\text{M}$), **18o** ($\text{IC}_{50} = 72.2 \pm 3.8 \mu\text{M}$), **18q** ($\text{IC}_{50} = 72.8 \pm 3.9 \mu\text{M}$), **18r** ($\text{IC}_{50} = 73.0 \pm 1.9 \mu\text{M}$), **18s** ($\text{IC}_{50} = 78.2 \pm 3.2 \mu\text{M}$), **18t** ($\text{IC}_{50} = 78.7 \pm 4.7 \mu\text{M}$) and **25c** ($\text{IC}_{50} = 79.5 \pm 4.8 \mu\text{M}$) showed slightly higher activities than doxorubicin ($\text{IC}_{50} = 80.9 \pm 2.1 \mu\text{M}$). In addition, compound **18m** ($\text{IC}_{50} = 80.3 \pm 3.9 \mu\text{M}$) was almost equipotent as doxorubicin ($\text{IC}_{50} = 80.9 \pm 2.1 \mu\text{M}$), while, compounds **11a** ($\text{IC}_{50} = 81.3 \pm 4.1 \mu\text{M}$), **18e** ($\text{IC}_{50} = 81.2 \pm 5.5 \mu\text{M}$), **18n** ($\text{IC}_{50} = 82.5 \pm 5.7 \mu\text{M}$) and **25b** ($\text{IC}_{50} = 81.9 \pm 5.9 \mu\text{M}$) displayed slightly less activities compared to doxorubicin ($\text{IC}_{50} = 80.9 \pm 2.1 \mu\text{M}$).

In case of MCF-7 cell lines, none of the compounds showed any significant differences compared to the positive control. Compounds **18a** ($\text{IC}_{50} = 63.1 \pm 3.1 \mu\text{M}$), **18b** ($\text{IC}_{50} = 64.9 \pm 3.1 \mu\text{M}$), **18c** ($\text{IC}_{50} = 64.3 \pm 4.2 \mu\text{M}$), **18j** ($\text{IC}_{50} = 64.3 \pm 3.1 \mu\text{M}$), **11o** ($\text{IC}_{50} = 64.7 \pm 1.9 \mu\text{M}$) and **18u** ($\text{IC}_{50} = 64.5 \pm 2.9 \mu\text{M}$) showed slightly higher activities than doxorubicin ($\text{IC}_{50} = 65.6 \pm 4.2 \mu\text{M}$). Whilst, compounds **11a** ($\text{IC}_{50} = 65.5 \pm 4.3 \mu\text{M}$), **18d** ($\text{IC}_{50} = 65.1 \pm 2.8 \mu\text{M}$), **18n** ($\text{IC}_{50} = 65.9 \pm 3.1 \mu\text{M}$), **18q** ($\text{IC}_{50} = 65.5 \pm 2.1 \mu\text{M}$) and **18r** ($\text{IC}_{50} = 65.9 \pm 2.6 \mu\text{M}$) displayed equipotent as doxorubicin ($\text{IC}_{50} = 65.6 \pm 4.2 \mu\text{M}$). Whereas, compounds **18f** ($\text{IC}_{50} = 66.1 \pm 2.9 \mu\text{M}$), **18h** ($\text{IC}_{50} = 66.8 \pm 2.6 \mu\text{M}$), **18k** ($\text{IC}_{50} = 66.8 \pm 3.9 \mu\text{M}$), **18l** ($\text{IC}_{50} = 66.7 \pm 3.2 \mu\text{M}$), **18m** ($\text{IC}_{50} = 66.2 \pm 3.8 \mu\text{M}$), **18s** ($\text{IC}_{50} = 66.8 \pm 5.0 \mu\text{M}$), **25b** ($\text{IC}_{50} = 66.2 \pm 2.9 \mu\text{M}$) and **25c** ($\text{IC}_{50} = 66.5 \pm 3.1 \mu\text{M}$) displayed slightly less activities.

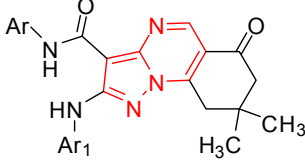
Table 1. The IC₅₀ (μM) values of compounds **11a–c**, **18a–u** and **25a–c** using MTT assay against two human carcinoma cell lines (HepG-2 and MCF-7).



11a-c



18a-u



25a-c

Compounds	Ar	Ar ₁	Ar ₁	IC ₅₀ (μM)	
				HepG-2	MCF-7
11a	C ₆ H ₅	4-CH ₃ O-C ₆ H ₄	-	81.3 ± 4.1	65.5 ± 4.3
11b	4-CH ₃ -C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	-	86.2 ± 4.5	69.2 ± 3.9
11c	4-Cl-C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	-	94.8 ± 6.5	69.1 ± 3.7
18a	C ₆ H ₅	4-CH ₃ O-C ₆ H ₄	C ₆ H ₅	85.4 ± 5.1	63.1 ± 3.1 *
18b	C ₆ H ₅	4-CH ₃ O-C ₆ H ₄	4-CH ₃ -C ₆ H ₄	90.9 ± 6.5	64.9 ± 3.1
18c	C ₆ H ₅	4-CH ₃ O-C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	75.9 ± 5.3	64.3 ± 4.2
18d	C ₆ H ₅	4-CH ₃ O-C ₆ H ₄	4-Cl-C ₆ H ₄	77.1 ± 4.2	65.1 ± 2.8
18e	C ₆ H ₅	4-CH ₃ O-C ₆ H ₄	4-Br-C ₆ H ₄	81.2 ± 5.5	68.1 ± 4.0
18f	C ₆ H ₅	4-CH ₃ O-C ₆ H ₄	4-F-C ₆ H ₄	92.8 ± 6.7	66.1 ± 2.9
18g	C ₆ H ₅	4-CH ₃ O-C ₆ H ₄	thiophen-2-yl	91.1 ± 6.4	69.2 ± 3.2
18h	4-CH ₃ -C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	C ₆ H ₅	73.2 ± 3.2	66.8 ± 2.6
18i	4-CH ₃ -C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	4-CH ₃ -C ₆ H ₄	83.3 ± 4.3	67.7 ± 2.7
18j	4-CH ₃ -C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	77.4 ± 2.9	64.3 ± 3.1
18k	4-CH ₃ -C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	4-Cl-C ₆ H ₄	74.0 ± 3.1	66.8 ± 3.9
18l	4-CH ₃ -C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	4-Br-C ₆ H ₄	78.7 ± 5.1	66.7 ± 3.2
18m	4-CH ₃ -C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	4-F-C ₆ H ₄	80.3 ± 3.9	66.2 ± 3.8
18n	4-CH ₃ -C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	thiophen-2-yl	82.5 ± 5.7	65.9 ± 3.1
18o	4-Cl-C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	C ₆ H ₅	72.2 ± 3.8 *	64.7 ± 1.9
18p	4-Cl-C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	4-CH ₃ -C ₆ H ₄	87.8 ± 5.4	67.1 ± 2.1
18q	4-Cl-C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	72.8 ± 3.9	65.5 ± 2.1
18r	4-Cl-C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	4-Cl-C ₆ H ₄	73.0 ± 1.9	65.9 ± 2.6
18s	4-Cl-C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	4-Br-C ₆ H ₄	78.2 ± 3.2	66.8 ± 5.0
18t	4-Cl-C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	4-F-C ₆ H ₄	78.7 ± 4.7	67.0 ± 1.8
18u	4-Cl-C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	thiophen-2-yl	83.1 ± 5.1	64.5 ± 2.9
25a	C ₆ H ₅	4-CH ₃ O-C ₆ H ₄	-	87.9 ± 6.0	68.9 ± 4.2
25b	4-CH ₃ -C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	-	81.9 ± 5.9	66.2 ± 2.9
25c	4-Cl-C ₆ H ₄	4-CH ₃ O-C ₆ H ₄	-	79.5 ± 4.8	66.5 ± 3.1
Doxorubicin	-	-	-	80.9 ± 2.1	65.6 ± 4.2

* The most potent compound and selected for further experiments.

2.3. Structure Activity Relationship (SAR)

From the results of in vitro cytotoxic activity of the synthesized compounds against liver (HepG2) cell lines, we found that, **25c** (IC₅₀ = 79.5 ± 4.8 μM) > **25b** (IC₅₀ = 81.9 ± 5.9 μM) > **25a** (IC₅₀ = 87.9 ± 6.0 μM) in the series of pyrazolo[1,5-*a*]quinazolines **25a–c**, in addition, **18o** (IC₅₀ = 72.2 ± 3.8 μM) > **18h** (IC₅₀ = 73.2 ± 3.2 μM) > **18a** (IC₅₀ = 85.4 ± 5.1 μM); **18r** (IC₅₀ = 73.0 ± 1.9 μM) > **18k** (IC₅₀ = 74.0 ± 3.1 μM) > **18d** (IC₅₀ = 77.1 ± 4.2 μM); **18s** (IC₅₀ = 78.2 ± 3.2 μM) > **18l** (IC₅₀ = 78.7 ± 5.1 μM) > **18c** (IC₅₀ = 75.9 ± 5.3 μM) and **18t** (IC₅₀ = 78.7 ± 4.7 μM) > **18m** (IC₅₀ = 80.3 ± 3.92 μM) > **18f** (IC₅₀ = 92.8 ± 6.7 μM) in the series of pyrazolo[1,5-*a*]pyrimidines **18a–u**. This was concerning the effect of 4-Cl-C₆H₄ group (chloride atom as electron withdrawing group) and 4-CH₃-C₆H₄ group (methyl as electron releasing group) in the two series. Whence, the derivatives bearing Ar = 4-Cl-C₆H₄ group (at position 3 in the two series) were slightly more active than those bearing Ar = 4-CH₃-C₆H₄ group than those bearing Ar = C₆H₅ group.

In addition, we observed that, there was a ranking in the order of rings bearing halogen atoms (Cl, Br and F) in the series of **18a–u**, where, **18d** (IC₅₀ = 77.1 ± 4.2 μM) > **18e** (IC₅₀ = 81.2 ± 5.5 μM)

>**18f** ($IC_{50} = 92.8 \pm 6.7 \mu M$); **18k** ($IC_{50} = 74.0 \pm 3.1 \mu M$) >**18l** ($IC_{50} = 78.7 \pm 5.1 \mu M$) >**18m** ($IC_{50} = 80.3 \pm 3.9 \mu M$) and **18r** ($IC_{50} = 73.0 \pm 1.9 \mu M$) >**18s** ($IC_{50} = 78.2 \pm 3.2 \mu M$) >**18t** ($IC_{50} = 78.7 \pm 4.7 \mu M$). Therefore, the derivatives bearing $Ar_2 = 4\text{-Cl-C}_6\text{H}_4$ group (at position 7) > $Ar_2 = 4\text{-Br-C}_6\text{H}_4$ group > $Ar_2 = 4\text{-F-C}_6\text{H}_4$ group.

Moreover, the derivatives bearing $Ar_2 = 4\text{-CH}_3\text{O-C}_6\text{H}_4$ group (at position 7) > $Ar_2 = 4\text{-CH}_3\text{-C}_6\text{H}_4$ group, where, **18c** ($IC_{50} = 75.9 \pm 5.3 \mu M$) >**18b** ($IC_{50} = 90.9 \pm 6.5 \mu M$); **18j** ($IC_{50} = 77.4 \pm 2.9 \mu M$) >**18i** ($IC_{50} = 83.3 \pm 4.3 \mu M$) and **18q** ($IC_{50} = 72.8 \pm 3.9 \mu M$) >**18p** ($IC_{50} = 87.8 \pm 5.4 \mu M$). Therefore, the replacement of the $4\text{-CH}_3\text{-C}_6\text{H}_4$ group by $4\text{-CH}_3\text{O-C}_6\text{H}_4$ group was impacted and increased the activity against liver cancer.

Furthermore, we observed that, the derivatives bearing phenyl group (at position 7) more active than those bearing thiophen-2-yl group, where, **18a** ($IC_{50} = 85.4 \pm 5.1 \mu M$) >**18g** ($IC_{50} = 91.1 \pm 6.4 \mu M$); **18h** ($IC_{50} = 73.2 \pm 3.2 \mu M$) >**18n** ($IC_{50} = 82.5 \pm 5.7 \mu M$) and **18o** ($IC_{50} = 72.2 \pm 3.8 \mu M$) >**18u** ($IC_{50} = 83.1 \pm 5.1 \mu M$). Therefore, the introduction of thiophen-2-yl group in the series decreased the activity. A brief Structure-activity relationship (SAR) study has been presented in Figure 4.

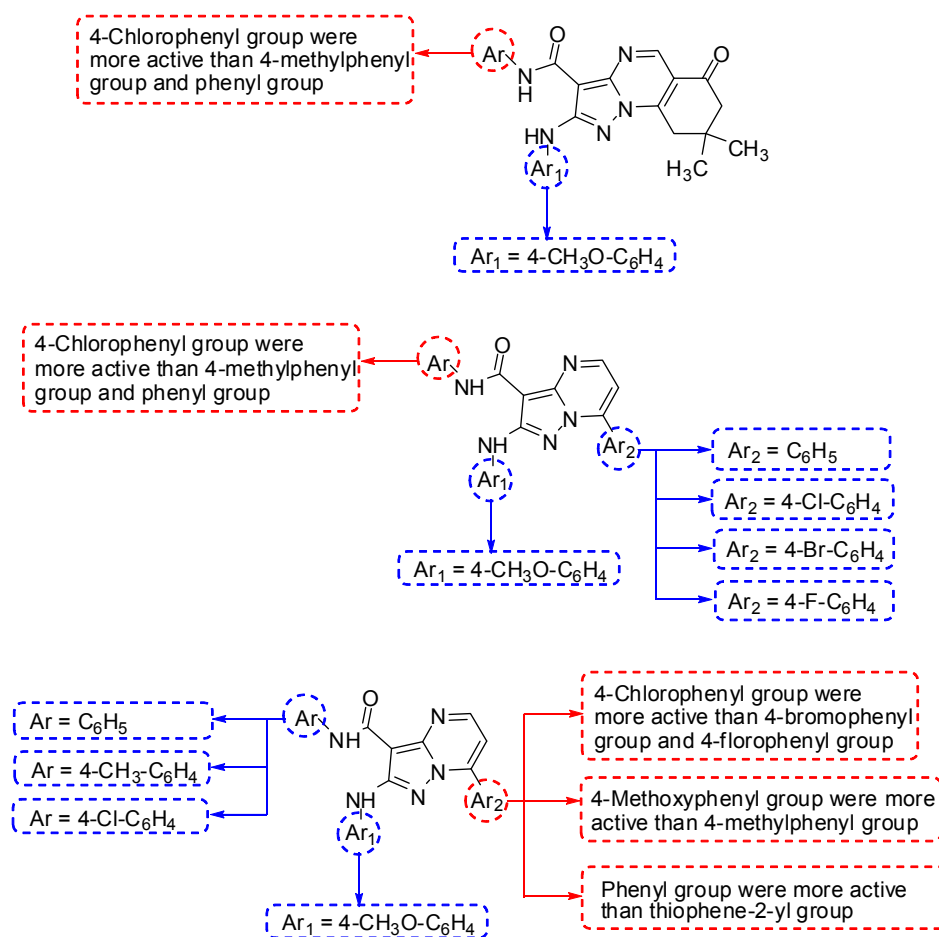


Figure 4. A brief Structure-activity relationship (SAR) study of **18a–u** and **25a–c** against liver (HepG2) cell lines.

2.4. Cell-Cycle Analysis and Apoptotic Changes

Cell cycle can be defined as cell reproduction via replication of the DNA followed by division of the nucleus and partitioning of the cytoplasm to yield two daughter cells. This cell cycle comprises four different phases. G1 phase occurs between nuclear division (M phase) and DNA synthesis (S phase); G2 phase occurs between S phase and M phase. These gaps allow for the repair of DNA damage

and replication errors [34]. According to the cytotoxicity screening in Table 1, and because most of the compounds did not show statistical significant differences compared to the positive control, two compounds (**18o** and **18a**) have been selected for further experiments. The effect of compounds **18o** and **18a** after 24 h of treatment by propidium iodide on cell cycle progression, using the flow cytometry (Figure 5a), was investigated against HepG-2 and MCF-7, respectively.

Compound **18o** induced significant alterations in the cell-cycle phases of HepG2 cells when compared to control. Interestingly, exposure of HepG2 cells to **18o** induced a significant increase in the percentage of cells at pre-G1 and G2/M phases by 6.6 folds and 1.7 folds, with a concurrent significant reduction in the percentage of cells at G0/G1 by 1.2 folds without any significant changes in S phase compared to control, respectively.

Moreover, treatment of MCF-7 cancer cells with compound **18a** caused a significant increase in pre-G1 and G2/M phases percent by 7.9 folds and 3.5 folds with a significant reduction in the percentage of cells at G0/G1 by 1.4 folds and slightly increase in S phases by 0.9 folds compared to control, respectively (Figure 5b). However, the positive control showed better results. In case of HepG-2 cancer cells, Doxorubicin-induced a significant increase in the percentage of cells at pre-G1 and G2/M phases by 2.2 folds and 1.6 folds, with a significant reduction in the percentage of cells at G0/G1 and S phases by 1.14 and 1.46 folds compared to compound **18o**. In addition, in case of MCF-7 cancer cells, Doxorubicin-induced a significant increase in the percentage of cells at pre-G1 and G2/M phases by 1.7-folds and 1.4 folds, with a significant reduction in the percentage of cells at G0/G1 and did not show any significant increase in S phases compared to compound **18o**. From these results, it can be concluded that compounds **18o** and **18a** inhibit the cell growth through cell cycle arrest at G2/M phase, which in turn induces cell death by apoptosis. These results are in agreement with the cytotoxicity screening results.

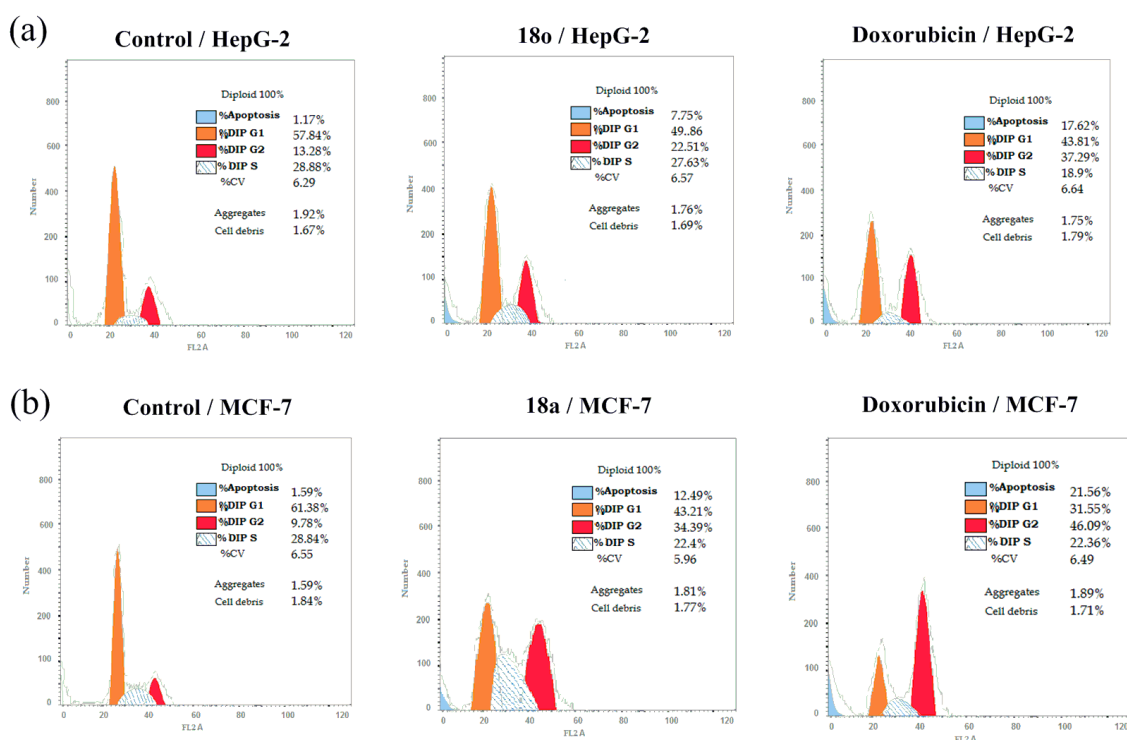


Figure 5. (a) Effect of compound **18o** on DNA-ploidy flow cytometric analysis of HepG-2 cancer cells, the cells were treated with DMSO as control and with doxorubicin as a positive control, for 24 h. (b) Effect of compound **18a** on DNA-ploidy flow cytometric analysis of MCF-7 cancer cells, the cells were treated with DMSO as control and with doxorubicin as a positive control, for 24 h.

2.5. Annexin V-FITC Apoptosis Assay

The apoptotic effect of compounds **18o** and **18a** was carried out using Annexin V-FITC/PI (AV/PI) dual staining assay (Figure 6). The results revealed that HepG2 and MCF-7 cells, treated with compounds **18o** and **18a**, respectively, showed a significant increase in the percent of annexin V-FITC positive apoptotic cells (UR & LR) by 11.6 folds and 9.8 folds compared to control, respectively. However, doxorubicin showed 2.2 folds and 1.7 folds increases in apoptotic cells % compared to compounds **18o** and **18a**, respectively. These results reveal that the cytotoxicity activities of compounds **18o** and **18a** are due to their potent pro-apoptotic activity.

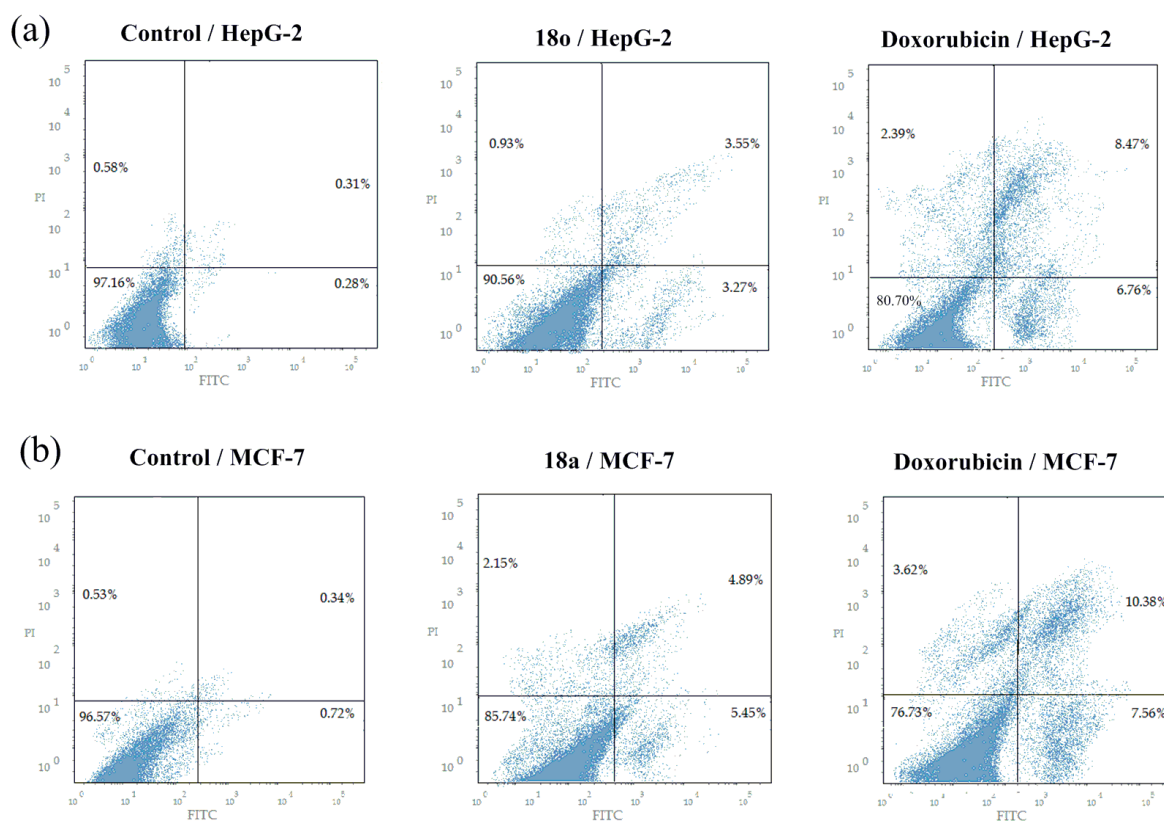


Figure 6. (a) Effect of compound **18o** on the percentage of annexin V-FITC positive staining in HepG-2 cancer cells, the cells were treated with DMSO as control and with doxorubicin as a positive control, for 24 h. (b) Effect of compound **18a** on the percentage of annexin V-FITC positive staining in MCF-7 cancer cells, the cells were treated with DMSO as control and with doxorubicin as a positive control, for 24 h.

3. Materials and Methods

3.1. Chemistry

All melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. The IR spectra were recorded (KBr disk) on a 1650 FT-IR instrument (Perkin Elmer, Waltham, MA, USA). ¹H-NMR (400 MHz) and ¹³C-NMR (100 MHz) spectra were recorded on a Varian spectrometer (Varian, Inc., Palo Alto, CA, USA) using DMSO-*d*₆ or CDCl₃ as solvent and TMS as an internal standard. Chemical shifts are reported in ppm. Coupling constants (*J*) are expressed in Hz. Mass spectra were recorded on a Varian MAT 112 spectrometer at 70 eV. Elemental analyses were performed at the Microanalytical Center, Cairo University, Egypt. The progress of the reactions was monitored by thin-layer chromatography (TLC) using aluminum sheets coated with silica gel F₂₅₄ (Merck, Darmstadt,

Germany), viewing under a short-wavelength UV lamp effected detection. All evaporations were carried out under reduced pressure at 40 °C.

Synthesis of 5-amino-3-(arylamino)-1H-pyrazole-4-carboxamides 11a–c. Compounds of this series were prepared according to the literature procedure.

5-Amino-3-(4-methoxyphenylamino)-N-phenyl-1H-pyrazole-4-carboxamide (11a). White crystals; m.p. 175–177 °C [29].

5-Amino-3-(4-methoxyphenylamino)-N-(4-methylphenyl)-1H-pyrazole-4-carboxamide (11b). White crystals; m.p. 198–200 °C [29].

5-Amino-3-(4-methoxyphenylamino)-N-(4-chlorophenyl)-1H-pyrazole-4-carboxamide (11c). White crystals; m.p. 190–192 °C [29].

General Procedure for Synthesis of 7-aryl-2-(arylamino)pyrazolo[1,5-a]pyrimidine-3-carboxamides 18a–u. A mixture of compounds **11a–c** (0.01 mol) with enaminones **12a–g** {e.g., 3-(dimethylamino)-1-phenylprop-2-en-1-one (**12a**), 3-(dimethylamino)-1-(4-methylphenyl)prop-2-en-1-one (**12b**), 3-(dimethylamino)-1-(4-methoxyphenyl)prop-2-en-1-one (**12c**), 1-(4-chlorophenyl)-3-(dimethylamino)prop-2-en-1-one (**12d**), 1-(4-bromophenyl)-3-(dimethylamino)prop-2-en-1-one (**12e**), 3-(dimethylamino)-1-(4-fluorophenyl)prop-2-en-1-one (**12f**) or 3-(dimethylamino)-1-(thiophen-2-yl)prop-2-en-1-one (**12g**)} (0.01 mol) in glacial acetic acid (25 mL), the reaction mixture was refluxed for 1 h and then left to cool. The solid product was filtered off, washed with ethanol, dried and finally recrystallized from DMF/H₂O to afford the corresponding pyrazolo[1,5-a]pyrimidine derivatives **18a–u**.

2-(4-Methoxyphenylamino)-N,7-diphenylpyrazolo[1,5-a]pyrimidine-3-carboxamide (18a). Yellow crystals, m.p. 218–220 °C, yield (72%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3346 (NH), 1658 (C=O). ¹H-NMR (CDCl₃, 400 MHz, δ ppm): 3.80 (s, 3H, OCH₃), 6.88 (d, 2H, *J* = 9.0 Hz, ArH), 6.96 (d, 1H, *J* = 4.8 Hz, pyrimidine), 7.12 (t, 1H, ArH), 7.36–7.42 (m, 5H, ArH), 7.62 (d, 2H, *J* = 9.0 Hz, ArH), 7.74 (d, 2H, *J* = 8.4 Hz, ArH), 8.11 (d, 2H, *J* = 8.3 Hz, ArH), 8.49 (d, 1H, *J* = 4.8 Hz, pyrimidine), 9.40 (s, 1H, NH), 10.05 (s, 1H, NH). ¹³C-NMR (CDCl₃, 100 MHz, δ ppm): 55.7 (C, OCH₃), 87.8 (C, C₃-pyrazolopyrimidine), 107.0 (C, C₆-pyrazolopyrimidine), 114.4, 119.2, 120.2, 123.7, 127.7, 129.1, 129.5, 129.6 (14C, Ar), 134.1 (C, C_{3a}-pyrazolopyrimidine), 138.8, 142.4, 146.7 (3C, Ar), 147.9 (C, C₇-pyrazolopyrimidine), 149.6 (C, Ar), 154.5 (C, C₂-pyrazolopyrimidine), 157.8 (C, C₅-pyrazolopyrimidine), 163.3 (C=O). MS (*m/z*, %): 435 (M⁺, 73.86). Anal. Calcd. (%) for C₂₆H₂₁N₅O₂ (435.48): C, 71.71; H, 4.86; N, 16.08. Found: C, 71.80; H, 4.81; N, 16.00%.

2-(4-Methoxyphenylamino)-N-phenyl-7-(4-methylphenyl)-pyrazolo[1,5-a]pyrimidine-3-carboxamide (18b). Yellow crystals, m.p. 219–221 °C, yield (77%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3337 (NH), 1658 (C=O). ¹H-NMR (CDCl₃, 400 MHz, δ ppm): 2.49 (s, 3H, CH₃), 3.80 (s, 3H, OCH₃), 6.87 (d, 2H, *J* = 8.9 Hz, ArH), 6.91 (d, 1H, *J* = 4.7 Hz, pyrimidine), 7.12 (t, 1H, ArH), 7.36 (d, 2H, *J* = 8.3 Hz, ArH), 7.38 (t, 2H, ArH), 7.60 (d, 2H, *J* = 8.9 Hz, ArH), 7.73 (d, 2H, *J* = 7.6 Hz, ArH), 8.08 (d, 2H, *J* = 8.1 Hz, ArH), 8.43 (d, 1H, *J* = 4.7 Hz, pyrimidine), 9.38 (s, 1H, NH), 10.01 (s, 1H, NH). ¹³C-NMR (CDCl₃, 100 MHz, δ ppm): 21.8 (C, CH₃), 55.7 (C, OCH₃), 87.7 (C, C₃-pyrazolopyrimidine), 107.0 (C, C₆-pyrazolopyrimidine), 114.4, 119.1, 120.1, 123.7, 127.6, 129.1, 129.4, 129.6 (14C, Ar), 134.1 (C, C_{3a}-pyrazolopyrimidine), 138.8, 142.3, 146.6 (3C, Ar), 147.8 (C, C₇-pyrazolopyrimidine), 149.6 (C, Ar), 154.4 (C, C₂-pyrazolopyrimidine), 157.7 (C, C₅-pyrazolopyrimidine), 163.3 (C=O). MS (*m/z*, %): 449 (M⁺, 67.43). Anal. Calcd. (%) for C₂₇H₂₃N₅O₂ (449.50): C, 72.14; H, 5.16; N, 15.58. Found: C, 72.10; H, 5.20; N, 15.60%.

7-(4-Methoxyphenyl)-2-(4-methoxyphenylamino)-N-phenylpyrazolo[1,5-a]pyrimidine-3-carboxamide (18c). Yellow crystals, m.p. 206–208 °C, yield (76%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3340 (NH), 1646 (C=O). ¹H-NMR (CDCl₃, 400 MHz, δ ppm): 3.80 (s, 3H, OCH₃), 3.91 (s, 3H, OCH₃), 6.87 (d, 2H, *J* = 8.9 Hz, ArH), 6.89 (d, 1H, *J* = 4.8 Hz, pyrimidine), 7.05 (d, 2H, *J* = 8.8 Hz, ArH), 7.11 (t, 1H, ArH), 7.37 (t, 2H, ArH), 7.60 (d, 2H, *J* = 8.9 Hz, ArH), 7.72 (d, 2H, *J* = 7.6 Hz, ArH), 8.18 (d, 2H, *J* = 8.8 Hz, ArH), 8.40 (d, 1H, *J* = 4.8 Hz, pyrimidine), 9.36 (s, 1H, NH), 10.02 (s, 1H, NH). ¹³C-NMR (CDCl₃, 100 MHz, δ ppm): 55.5 (C, OCH₃), 55.6 (C, OCH₃), 87.4 (C, C₃-pyrazolopyrimidine), 106.4 (C, C₆-pyrazolopyrimidine), 113.9,

114.2, 119.0, 120.0, 122.4, 123.5, 128.9, 131.3 (14C, Ar), 134.0 (C, C_{3a}-pyrazolopyrimidine), 138.6, 146.0 (2C, Ar), 147.7 (C, C₇-pyrazolopyrimidine), 149.3 (C, Ar), 154.3 (C, C₂-pyrazolopyrimidine), 157.5 (C, C₅-pyrazolopyrimidine), 162.2 (C, Ar), 163.2 (C=O). MS (*m/z*, %): 465 (M⁺, 69.48). Anal. Calcd. (%) for C₂₇H₂₃N₅O₃ (465.50): C, 69.66; H, 4.98; N, 15.04. Found: C, 69.70; H, 4.95; N, 15.00%.

7-(4-Chlorophenyl)-2-(4-methoxyphenylamino)-N-phenylpyrazolo[1,5-a]pyrimidine-3-carboxamide (**18d**). Yellow crystals, m.p. 252–253 °C, yield (72%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3343 (NH), 1648 (C=O). ¹H-NMR (CDCl₃, 400 MHz, δ ppm): 3.81 (s, 3H, OCH₃), 6.88 (d, 2H, *J* = 9.0 Hz, ArH), 6.94 (d, 1H, *J* = 4.7 Hz, pyrimidine), 7.13 (t, 1H, ArH), 7.39 (t, 2H, ArH), 7.58 (d, 4H, *J* = 8.8 Hz, ArH), 7.74 (d, 2H, *J* = 8.6 Hz, ArH), 8.15 (d, 2H, *J* = 8.7 Hz, ArH), 8.52 (d, 1H, *J* = 4.7 Hz, pyrimidine), 9.42 (s, 1H, NH), 9.99 (s, 1H, NH). ¹³C-NMR (CDCl₃, 100 MHz, δ ppm): 55.7 (C, OCH₃), 88.0 (C, C₃-pyrazolopyrimidine), 107.0 (C, C₆-pyrazolopyrimidine), 114.4, 119.2, 120.2, 123.8, 129.1, 129.1, 130.9, 131.8 (14C, Ar), 133.9 (C, C_{3a}-pyrazolopyrimidine), 134.6, 138.0, 138.7 (3C, Ar), 145.3 (C, C₇-pyrazolopyrimidine), 149.7 (C, Ar), 154.6 (C, C₂-pyrazolopyrimidine), 157.9 (C, C₅-pyrazolopyrimidine), 163.2 (C=O). MS (*m/z*, %): 469 (M⁺, 78.23). Anal. Calcd. (%) for C₂₆H₂₀ClN₅O₂ (469.92): C, 66.45; H, 4.29; N, 14.90. Found: C, 66.40; H, 4.30; N, 14.95%.

7-(4-Bromophenyl)-2-(4-methoxyphenylamino)-N-phenylpyrazolo[1,5-a]pyrimidine-3-carboxamide (**18e**). Yellow crystals, m.p. 278–280 °C, yield (69%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3365 (NH), 1650 (C=O). ¹H-NMR (DMSO-*d*₆, 400 MHz, δ ppm): 3.73 (s, 3H, OCH₃), 6.93 (d, 2H, *J* = 9.0 Hz, ArH), 7.12 (t, 1H, ArH), 7.39 (t, 2H, ArH), 7.41 (d, 1H, *J* = 4.8 Hz, pyrimidine), 7.59 (d, 2H, *J* = 9.0 Hz, ArH), 7.73 (d, 2H, *J* = 7.6 Hz, ArH), 7.90 (d, 2H, *J* = 8.7 Hz, ArH), 8.20 (d, 2H, *J* = 8.7 Hz, ArH), 8.74 (d, 1H, *J* = 4.8 Hz, pyrimidine), 9.26 (s, 1H, NH), 10.03 (s, 1H, NH). ¹³C-NMR (DMSO-*d*₆, 100 MHz, δ ppm): 55.7 (C, OCH₃), 87.6 (C, C₃-pyrazolopyrimidine), 106.9 (C, C₆-pyrazolopyrimidine), 114.4, 119.1, 120.5, 123.3, 129.4, 129.8, 131.0, 131.6 (14C, Ar), 133.7 (C, C_{3a}-pyrazolopyrimidine), 133.4, 136.1, 138.7 (3C, Ar), 145.2 (C, C₇-pyrazolopyrimidine), 149.5 (C, Ar), 154.8 (C, C₂-pyrazolopyrimidine), 157.1 (C, C₅-pyrazolopyrimidine), 163.7 (C=O). MS (*m/z*, %): 514 (M⁺, 81.26). Anal. Calcd. (%) for C₂₆H₂₀BrN₅O₂ (514.37): C, 60.71; H, 3.92; N, 13.62. Found: C, 60.65; H, 3.97; N, 13.65%.

7-(4-Fluorophenyl)-2-(4-methoxyphenylamino)-N-phenylpyrazolo[1,5-a]pyrimidine-3-carboxamide (**18f**). Yellow crystals, m.p. 237–239 °C, yield (70%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3343 (NH), 1647 (C=O). ¹H-NMR (DMSO-*d*₆, 400 MHz, δ ppm): 3.73 (s, 3H, OCH₃), 6.91 (d, 2H, *J* = 9.0 Hz, ArH), 7.11 (t, 1H, ArH), 7.37 (d, 1H, *J* = 4.9 Hz, pyrimidine), 7.39 (d, 2H, *J* = 7.6 Hz, ArH), 7.52 (t, 2H, ArH), 7.58 (d, 2H, *J* = 9.0 Hz, ArH), 7.71 (d, 2H, *J* = 8.6 Hz, ArH), 8.31 (d, 2H, *J* = 8.9 Hz, ArH), 8.71 (d, 1H, *J* = 4.8 Hz, pyrimidine), 9.23 (s, 1H, NH), 10.01 (s, 1H, NH). ¹³C-NMR (DMSO-*d*₆, 100 MHz, δ ppm): 55.2 (C, OCH₃), 86.7 (C, C₃-pyrazolopyrimidine), 108.3 (C, C₆-pyrazolopyrimidine), 114.3, 115.7, 115.9, 118.8, 119.4, 123.5, 126.4, 129.1 (14C, Ar), 132.4 (C, C_{3a}-pyrazolopyrimidine), 133.3, 138.4 (2C, Ar), 145.0 (C, C₇-pyrazolopyrimidine), 147.1 (C, Ar), 151.1 (C, C₂-pyrazolopyrimidine), 154.1 (C, C₅-pyrazolopyrimidine), 156.6 (C, Ar), 162.2 (C=O). MS (*m/z*, %): 453 (M⁺, 87.33). Anal. Calcd. (%) for C₂₆H₂₀FN₅O₂ (453.47): C, 68.86; H, 4.45; N, 15.44. Found: C, 68.95; H, 4.40; N, 15.50%.

2-(4-Methoxyphenylamino)-N-phenyl-7-(thiophen-2-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (**18g**). Yellow crystals, m.p. 233–235 °C, yield (71%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3356 (NH), 1652 (C=O). ¹H-NMR (DMSO-*d*₆, 400 MHz, δ ppm): 3.78 (s, 3H, OCH₃), 7.03 (d, 2H, *J* = 8.4 Hz, ArH), 7.12 (t, 1H, ArH), 7.40 (t, 2H, ArH), 7.47 (t, 1H, *J* = 4.9 Hz, thiophene), 7.74 (d, 2H, *J* = 7.8 Hz, ArH), 7.84 (d, 2H, *J* = 8.5 Hz, ArH), 7.90 (d, 1H, *J* = 4.6 Hz, pyrimidine), 8.28 (d, 1H, *J* = 4.4 Hz, thiophene), 8.58 (d, 1H, *J* = 2.8 Hz, thiophene), 8.71 (d, 1H, *J* = 4.4 Hz, pyrimidine), 9.44 (s, 1H, NH), 10.07 (s, 1H, NH). ¹³C-NMR (DMSO-*d*₆, 100 MHz, δ ppm): 55.7 (C, OCH₃), 86.8 (C, C₃-pyrazolopyrimidine), 107.3 (C, C₆-pyrazolopyrimidine), 114.8, 119.2, 120.8, 126.3 (7C, Ar), 128.1, 129.8 (2C, thiophene), 130.1 (2C, Ar), 133.2 (C, thiophene), 133.9 (C, C_{3a}-pyrazolopyrimidine), 134.5, 137.1 (2C, Ar), 139.4 (C, thiophene), 147.3 (C, Ar), 150.8 (C, C₂-pyrazolopyrimidine), 154.4 (C, C₅-pyrazolopyrimidine),

156.3 (C, C₇-pyrazolopyrimidine), 162.9 (C=O). MS (*m/z*, %): 441 (M⁺, 100). Anal. Calcd. (%) for C₂₄H₁₉N₅O₂S (441.50): C, 65.29; H, 4.34; N, 15.86. Found: C, 65.35; H, 4.30; N, 15.90%.

2-(4-Methoxyphenylamino)-7-phenyl-N-(4-methylphenyl)-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**18h**). Yellow crystals, m.p. 251–253 °C, yield (76%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3374 (NH), 1660 (C=O). ¹H-NMR (CDCl₃, 400 MHz, δ ppm): 2.35 (s, 3H, CH₃), 3.80 (s, 3H, OCH₃), 6.88 (d, 2H, *J* = 9.0 Hz, ArH), 6.95 (d, 1H, *J* = 4.8 Hz, pyrimidine), 7.18 (d, 2H, *J* = 8.2 Hz, ArH), 7.40 (d, 2H, *J* = 8.2 Hz, ArH), 7.60–7.64 (m, 5H, ArH), 8.11 (d, 2H, *J* = 8.2 Hz, ArH), 8.48 (d, 1H, *J* = 4.8 Hz, pyrimidine), 9.42 (s, 1H, NH), 9.97 (s, 1H, NH). ¹³C-NMR (CDCl₃, 100 MHz, δ ppm): 21.0 (C, CH₃), 55.7 (C, OCH₃), 87.8 (C, C₃-pyrazolopyrimidine), 106.9 (C, C₆-pyrazolopyrimidine), 114.4, 119.1, 120.2, 127.7, 129.4, 129.6, 133.3 (14C, Ar), 134.2 (C, C_{3a}-pyrazolopyrimidine), 136.2, 142.4, 146.6 (3C, Ar), 147.8 (C, C₇-pyrazolopyrimidine), 149.6 (C, Ar), 154.4 (C, C₂-pyrazolopyrimidine), 157.8 (C, C₅-pyrazolo-pyrimidine), 163.2 (C=O). MS (*m/z*, %): 449 (M⁺, 92.11). Anal. Calcd. (%) for C₂₇H₂₃N₅O₂ (449.50): C, 72.14; H, 5.16; N, 15.58. Found: C, 72.20; H, 5.11; N, 15.50%.

2-(4-Methoxyphenylamino)-N,7-di-(4-methylphenyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**18i**). Yellow crystals, m.p. 261 °C, yield (74%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3293 (NH), 1642 (C=O). ¹H-NMR (CDCl₃, 400 MHz, δ ppm): 2.35 (s, 3H, CH₃), 2.49 (s, 3H, CH₃), 3.80 (s, 3H, OCH₃), 6.87 (d, 2H, *J* = 9.0 Hz, ArH), 6.91 (d, 1H, *J* = 4.8 Hz, pyrimidine), 7.18 (d, 2H, *J* = 8.2 Hz, ArH), 7.37 (d, 2H, *J* = 8.0 Hz, ArH), 7.60 (d, 2H, *J* = 9.0 Hz, ArH), 7.61 (d, 2H, *J* = 8.5 Hz, ArH), 8.08 (d, 2H, *J* = 8.3 Hz, ArH), 8.43 (d, 1H, *J* = 4.8 Hz, pyrimidine), 9.40 (s, 1H, NH), 9.94 (s, 1H, NH). ¹³C-NMR (CDCl₃, 100 MHz, δ ppm): 21.0 (C, CH₃), 21.8 (C, CH₃), 55.7 (C, OCH₃), 87.7 (C, C₃-pyrazolopyrimidine), 106.9 (C, C₆-pyrazolopyrimidine), 114.4, 119.1, 120.1, 127.6, 129.4, 129.5, 129.6, 133.2 (14C, Ar), 134.1 (C, C_{3a}-pyrazolopyrimidine), 136.2, 142.3, 146.5 (3C, Ar), 147.7 (C, C₇-pyrazolopyrimidine), 149.5 (C, Ar), 154.4 (C, C₂-pyrazolopyrimidine), 157.7 (C, C₅-pyrazolopyrimidine), 163.2 (C=O). MS (*m/z*, %): 463 (M⁺, 100). Anal. Calcd. (%) for C₂₈H₂₅N₅O₂ (463.53): C, 72.55; H, 5.44; N, 15.11. Found: C, 72.55; H, 5.44; N, 15.11%.

7-(4-Methoxyphenyl)-2-(4-methoxyphenylamino)-N-(4-methylphenyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**18j**). Yellow crystals, m.p. 244–245 °C, yield (75%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3368 (NH), 1649 (C=O). ¹H-NMR (CDCl₃, 400 MHz, δ ppm): 2.35 (s, 3H, CH₃), 3.80 (s, 3H, OCH₃), 3.94 (s, 3H, OCH₃), 6.88 (d, 2H, *J* = 9.0 Hz, ArH), 6.93 (d, 1H, *J* = 4.8 Hz, pyrimidine), 7.10 (d, 2H, *J* = 9.0 Hz, ArH), 7.18 (d, 2H, *J* = 8.2 Hz, ArH), 7.61–7.64 (m, 4H, ArH), 8.22 (d, 2H, *J* = 8.9 Hz, ArH), 8.45 (d, 1H, *J* = 4.8 Hz, pyrimidine), 9.42 (s, 1H, NH), 9.98 (s, 1H, NH). ¹³C-NMR (CDCl₃, 100 MHz, δ ppm): 21.0 (C, CH₃), 55.7 (C, OCH₃), 55.7 (C, OCH₃), 87.7 (C, C₃-pyrazolopyrimidine), 106.5 (C, C₆-pyrazolopyrimidine), 114.1, 114.4, 119.1, 120.2, 122.7, 129.6, 131.5, 133.2 (14C, Ar), 134.2 (C, C_{3a}-pyrazolopyrimidine), 136.2, 146.2 (2C, Ar), 147.9 (C, C₇-pyrazolopyrimidine), 149.5 (C, Ar), 154.4 (C, C₂-pyrazolopyrimidine), 157.8 (C, C₅-pyrazolopyrimidine), 162.3 (C, Ar), 163.3 (C=O). MS (*m/z*, %): 479 (M⁺, 92.77). Anal. Calcd. (%) for C₂₈H₂₅N₅O₃ (479.53): C, 70.13; H, 5.25; N, 14.60. Found: C, 70.05; H, 5.30; N, 14.55%.

7-(4-Chlorophenyl)-2-(4-methoxyphenylamino)-N-(4-methylphenyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**18k**). Yellow crystals, m.p. 267–269 °C, yield (71%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3315 (NH), 1662 (C=O). ¹H-NMR (CDCl₃, 400 MHz, δ ppm): 2.35 (s, 3H, CH₃), 3.81 (s, 3H, OCH₃), 6.88 (d, 2H, *J* = 9.0 Hz, ArH), 6.93 (d, 1H, *J* = 4.7 Hz, pyrimidine), 7.19 (d, 2H, *J* = 8.2 Hz, ArH), 7.57–7.62 (m, 6H, ArH), 8.14 (d, 2H, *J* = 8.7 Hz, ArH), 8.50 (d, 1H, *J* = 4.7 Hz, pyrimidine), 9.43 (s, 1H, NH), 9.91 (s, 1H, NH). ¹³C-NMR (CDCl₃, 100 MHz, δ ppm): 21.0 (C, CH₃), 55.7 (C, OCH₃), 88.0 (C, C₃-pyrazolopyrimidine), 107.0 (C, C₆-pyrazolopyrimidine), 114.4, 119.2, 120.2, 129.1, 129.6, 130.9, 131.7, 133.4 (14C, Ar), 134.1 (C, C_{3a}-pyrazolopyrimidine), 134.4, 136.0, 137.9 (3C, Ar), 146.1 (C, C₇-pyrazolopyrimidine), 149.7 (C, Ar), 154.6 (C, C₂-pyrazolopyrimidine), 159.4 (C, C₅-pyrazolopyrimidine), 163.1 (C=O). MS (*m/z*, %): 483 (M⁺, 87.08). Anal. Calcd. (%) for C₂₇H₂₂ClN₅O₂ (483.95): C, 67.01; H, 4.58; N, 14.47. Found: C, 67.10; H, 4.50; N, 14.50%.

7-(4-Bromophenyl)-2-(4-methoxyphenylamino)-N-(4-methylphenyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**18l**). Yellow crystals, m.p. 278–279 °C, yield (68%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3325 (NH), 1649 (C=O).

¹H-NMR (DMSO-*d*₆, 400 MHz, δ ppm): 2.30 (s, 3H, CH₃), 3.74 (s, 3H, OCH₃), 6.95 (d, 2H, *J* = 9.3 Hz, ArH), 7.20 (d, 2H, *J* = 8.6 Hz, ArH), 7.44 (d, 1H, *J* = 4.1 Hz, pyrimidine), 7.62 (d, 2H, *J* = 8.4 Hz, ArH), 7.63 (d, 2H, *J* = 7.8 Hz, ArH), 7.93 (d, 2H, *J* = 8.3 Hz, ArH), 8.22 (d, 2H, *J* = 8.3 Hz, ArH), 8.77 (d, 1H, *J* = 4.3 Hz, pyrimidine), 9.31 (s, 1H, NH), 9.98 (s, 1H, NH). ¹³C-NMR (DMSO-*d*₆, 100 MHz, δ ppm): 21.0 (C, CH₃), 55.7 (C, OCH₃), 88.0 (C, C₃-pyrazolopyrimidine), 107.0 (C, C₆-pyrazolopyrimidine), 114.9, 119.2, 120.5, 125.2, 129.7, 129.4, 131.0, 133.5 (14C, Ar), 134.2 (C, C_{3a}-pyrazolopyrimidine), 134.4, 135.9, 137.9 (3C, Ar), 146.0 (C, C₇-pyrazolopyrimidine), 149.7 (C, Ar), 154.5 (C, C₂-pyrazolopyrimidine), 159.3 (C, C₅-pyrazolopyrimidine), 163.1 (C=O). MS (*m/z*, %): 528 (M⁺, 26.25). Anal. Calcd. (%) for C₂₇H₂₂BrN₅O₂ (528.40): C, 61.37; H, 4.20; N, 13.25. Found: C, 61.45; H, 4.16; N, 13.30%.

7-(4-Fluorophenyl)-2-(4-methoxyphenylamino)-N-(4-methylphenyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**18m**). Yellow crystals, m.p. 255–257 °C, yield (69%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3329 (NH), 1652 (C=O). ¹H-NMR (DMSO-*d*₆, 400 MHz, δ ppm): 2.30 (s, 3H, CH₃), 3.73 (s, 3H, OCH₃), 6.93 (d, 2H, *J* = 9.0 Hz, ArH), 7.19 (d, 2H, *J* = 8.3 Hz, ArH), 7.39 (d, 1H, *J* = 4.8 Hz, pyrimidine), 7.54 (d, 2H, *J* = 8.9 Hz, ArH), 7.60 (d, 2H, *J* = 9.0 Hz, ArH), 7.62 (d, 2H, *J* = 8.4 Hz, ArH), 8.32 (d, 2H, *J* = 9.0 Hz, ArH), 8.74 (d, 1H, *J* = 4.8 Hz, pyrimidine), 9.27 (s, 1H, NH), 9.97 (s, 1H, NH). ¹³C-NMR (DMSO-*d*₆, 100 MHz, δ ppm): 21.0 (C, CH₃), 55.8 (C, OCH₃), 87.9 (C, C₃-pyrazolopyrimidine), 107.0 (C, C₆-pyrazolopyrimidine), 114.6, 116.1, 119.5, 120.5, 129.5, 130.9, 131.7 (13C, Ar), 134.2 (C, C_{3a}-pyrazolopyrimidine), 134.2, 136.1, 138.0 (3C, Ar), 146.2 (C, C₇-pyrazolopyrimidine), 149.6 (C, Ar), 154.3 (C, C₂-pyrazolopyrimidine), 159.0 (C, C₅-pyrazolopyrimidine), 160.1 (C, Ar), 162.9 (C=O). MS (*m/z*, %): 467 (M⁺, 45.13). Anal. Calcd. (%) for C₂₇H₂₂FN₅O₂ (467.49): C, 69.37; H, 4.74; N, 14.98. Found: C, 69.30; H, 4.80; N, 15.05%.

2-(4-Methoxyphenylamino)-7-(thiophen-2-yl)-N-(4-methylphenyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**18n**). Yellow crystals, m.p. 278–279 °C, yield (70%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3345 (NH), 1652 (C=O). ¹H-NMR (DMSO-*d*₆, 400 MHz, δ ppm): 2.30 (s, 3H, CH₃), 3.78 (s, 3H, OCH₃), 7.03 (d, 2H, *J* = 8.9 Hz, ArH), 7.20 (d, 2H, *J* = 8.1 Hz, ArH), 7.47 (t, 1H, thiophene), 7.62 (d, 2H, *J* = 8.0 Hz, ArH), 7.83 (d, 2H, *J* = 8.6 Hz, ArH), 7.90 (d, 1H, *J* = 3.6 Hz, pyrimidine), 8.28 (d, 1H, *J* = 4.7 Hz, thiophene), 8.59 (d, 1H, *J* = 2.3 Hz, thiophene), 8.70 (d, 1H, *J* = 4.8 Hz, pyrimidine), 9.46 (s, 1H, NH), 10.00 (s, 1H, NH). ¹³C-NMR (DMSO-*d*₆, 100 MHz, δ ppm): 21.0 (C, CH₃), 55.7 (C, OCH₃), 87.5 (C, C₃-pyrazolopyrimidine), 107.1 (C, C₆-pyrazolopyrimidine), 114.1, 119.5, 120.3 (6C, Ar), 127.6, 128.1, 129.8 (3C, thiophene), 130.0 (2C, Ar), 133.6 (C, C_{3a}-pyrazolopyrimidine), 133.5, 134.5, 137.1 (3C, Ar), 139.8 (C, thiophene), 147.8 (C, Ar), 151.5 (C, C₂-pyrazolopyrimidine), 154.1 (C, C₅-pyrazolopyrimidine), 157.2 (C, C₇-pyrazolopyrimidine), 163.0 (C=O). MS (*m/z*, %): 455 (M⁺, 65.71). Anal. Calcd. (%) for C₂₅H₂₁N₅O₂S (455.53): C, 65.92; H, 4.65; N, 15.37. Found: C, 66.00; H, 4.60; N, 15.31%.

N-(4-Chlorophenyl)-2-(4-methoxyphenylamino)-7-phenylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**18o**). Yellow crystals, m.p. 252–254 °C, yield (73%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3336 (NH), 1650 (C=O). ¹H-NMR (DMSO-*d*₆, 400 MHz, δ ppm): 3.72 (s, 3H, OCH₃), 6.90 (d, 2H, *J* = 9.0 Hz, ArH), 7.40 (d, 1H, *J* = 4.8 Hz, pyrimidine), 7.44 (d, 2H, *J* = 8.8 Hz, ArH), 7.61 (d, 2H, *J* = 9.0 Hz, ArH), 7.68–7.70 (m, 3H, ArH), 7.78 (d, 2H, *J* = 8.9 Hz, ArH), 8.23 (d, 2H, *J* = 7.2 Hz, ArH), 8.75 (d, 1H, *J* = 4.8 Hz, pyrimidine), 9.20 (s, 1H, NH), 10.11 (s, 1H, NH). ¹³C-NMR (DMSO-*d*₆, 100 MHz, δ ppm): 55.7 (C, OCH₃), 87.9 (C, C₃-pyrazolopyrimidine), 107.0 (C, C₆-pyrazolopyrimidine), 114.5, 119.1, 120.2, 124.0, 128.4, 129.1, 130.9, 131.8 (13C, Ar), 134.0 (C, C_{3a}-pyrazolopyrimidine), 134.6, 135.9, 138.0, 138.7 (4C, Ar), 145.6 (C, C₇-pyrazolopyrimidine), 149.7 (C, Ar), 154.8 (C, C₂-pyrazolopyrimidine), 158.0 (C, C₅-pyrazolopyrimidine), 163.8 (C=O). MS (*m/z*, %): 469 (M⁺, 29.83). Anal. Calcd. (%) for C₂₆H₂₀ClN₅O₂ (469.92): C, 66.45; H, 4.29; N, 14.90. Found: C, 66.40; H, 4.35; N, 14.85%.

N-(4-Chlorophenyl)-2-(4-methoxyphenylamino)-7-(4-methylphenyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**18p**). Yellow crystals, m.p. 261 °C, yield (75%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3322 (NH), 1658 (C=O). ¹H-NMR (DMSO-*d*₆, 400 MHz, δ ppm): 2.32 (s, 3H, CH₃), 3.74 (s, 3H, OCH₃), 6.93 (d, 2H, *J* = 7.6 Hz, ArH), 7.42 (d, 1H, *J* = 4.8 Hz, pyrimidine), 7.45 (d, 2H, *J* = 7.7 Hz, ArH), 7.51 (d, 2H, *J* = 8.6 Hz, ArH), 7.64 (d, 2H, *J* = 7.8 Hz, ArH), 7.79 (d, 2H, *J* = 8.4 Hz, ArH), 8.19 (d, 2H, *J* = 7.5 Hz, ArH), 8.74 (d, 1H,

$J = 4.8$ Hz, pyrimidine), 9.23 (s, 1H, NH), 10.14 (s, 1H, NH). ^{13}C -NMR (DMSO- d_6 , 100 MHz, δ ppm): 21.0 (C, CH_3), 55.7 (C, OCH_3), 87.5 (C, C_3 -pyrazolopyrimidine), 107.0 (C, C_6 -pyrazolopyrimidine), 114.4, 119.3, 120.9, 129.0, 129.6, 131.0, 131.7, 133.4 (14C, Ar), 134.0 (C, C_{3a} -pyrazolopyrimidine), 134.3, 136.0, 137.9 (3C, Ar), 146.1 (C, C_7 -pyrazolopyrimidine), 149.7 (C, Ar), 154.5 (C, C_2 -pyrazolopyrimidine), 159.4 (C, C_5 -pyrazolopyrimidine), 163.1 (C=O). MS (m/z , %): 483 (M^+ , 22.71). Anal. Calcd. (%) for $\text{C}_{27}\text{H}_{22}\text{ClN}_5\text{O}_2$ (483.95): C, 67.01; H, 4.58; N, 14.47. Found: C, 67.10; H, 4.50; N, 14.55%.

N-(4-Chlorophenyl)-7-(4-methoxyphenyl)-2-(4-methoxyphenylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**18q**). Yellow crystals, m.p. 266–267 °C, yield (74%). IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 3365 (NH), 1661 (C=O). ^1H -NMR (DMSO- d_6 , 400 MHz, δ ppm): 3.74 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 6.95 (d, 2H, $J = 8.8$ Hz, ArH), 7.24 (d, 2H, $J = 8.7$ Hz, ArH), 7.40 (d, 1H, $J = 4.7$ Hz, pyrimidine), 7.44 (d, 2H, $J = 8.7$ Hz, ArH), 7.65 (d, 2H, $J = 8.8$ Hz, ArH), 7.78 (d, 2H, $J = 8.6$ Hz, ArH), 8.31 (d, 2H, $J = 8.6$ Hz, ArH), 8.70 (d, 1H, $J = 4.7$ Hz, pyrimidine), 9.22 (s, 1H, NH), 10.15 (s, 1H, NH). ^{13}C -NMR (DMSO- d_6 , 100 MHz, δ ppm): 55.6 (C, OCH_3), 55.7 (C, OCH_3), 87.8 (C, C_3 -pyrazolopyrimidine), 106.4 (C, C_6 -pyrazolopyrimidine), 114.2, 114.6, 119.0, 120.5, 122.8, 129.5, 131.6 (13C, Ar), 133.1 (C, C_{3a} -pyrazolopyrimidine), 134.8, 135.0, 136.1 (3C, Ar), 147.9 (C, C_7 -pyrazolopyrimidine), 149.4 (C, Ar), 154.5 (C, C_2 -pyrazolopyrimidine), 157.8 (C, C_5 -pyrazolopyrimidine), 161.5 (C, Ar), 163.2 (C=O). MS (m/z , %): 499 (M^+ , 18.46). Anal. Calcd. (%) for $\text{C}_{27}\text{H}_{22}\text{ClN}_5\text{O}_3$ (499.95): C, 64.86; H, 4.44; N, 14.01. Found: C, 64.95; H, 4.40; N, 14.05%.

N,7-bis(4-Chlorophenyl)-2-(4-methoxyphenylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**18r**). Yellow crystals, m.p. 282–284 °C, yield (70%). IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 3317 (NH), 1653 (C=O). ^1H -NMR (DMSO- d_6 , 400 MHz, δ ppm): 3.74 (s, 3H, OCH_3), 6.94 (d, 2H, $J = 8.8$ Hz, ArH), 7.44 (d, 2H, $J = 8.6$ Hz, ArH), 7.45 (d, 1H, $J = 3.8$ Hz, pyrimidine), 7.61 (d, 2H, $J = 8.8$ Hz, ArH), 7.78 (d, 4H, $J = 8.4$ Hz, ArH), 8.29 (d, 2H, $J = 8.6$ Hz, ArH), 8.76 (d, 1H, $J = 4.7$ Hz, pyrimidine), 9.23 (s, 1H, NH), 10.10 (s, 1H, NH). ^{13}C -NMR (DMSO- d_6 , 100 MHz, δ ppm): 55.8 (C, OCH_3), 87.4 (C, C_3 -pyrazolopyrimidine), 106.9 (C, C_6 -pyrazolopyrimidine), 115.0, 119.3, 120.4, 129.1, 129.7, 129.9, 131.6 (13C, Ar), 133.1 (C, C_{3a} -pyrazolopyrimidine), 133.8, 134.3, 136.0, 137.9 (4C, Ar), 146.0 (C, C_7 -pyrazolopyrimidine), 149.8 (C, Ar), 154.5 (C, C_2 -pyrazolopyrimidine), 159.4 (C, C_5 -pyrazolopyrimidine), 162.9 (C=O). MS (m/z , %): 504 (M^+ , 22.87). Anal. Calcd. (%) for $\text{C}_{26}\text{H}_{19}\text{Cl}_2\text{N}_5\text{O}_2$ (504.37): C, 61.91; H, 3.80; N, 13.89. Found: C, 62.00; H, 3.75; N, 13.80%.

7-(4-Bromophenyl)-*N*-(4-chlorophenyl)-2-(4-methoxyphenylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**18s**). Yellow crystals, m.p. 275–277 °C, yield (67%). IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 3327 (NH), 1648 (C=O). ^1H -NMR (DMSO- d_6 , 400 MHz, δ ppm): 3.74 (s, 3H, OCH_3), 6.94 (d, 2H, $J = 7.3$ Hz, ArH), 7.44 (d, 2H, $J = 7.2$ Hz, ArH), 7.45 (d, 1H, $J = 4.4$ Hz, pyrimidine), 7.61 (d, 2H, $J = 7.1$ Hz, ArH), 7.79 (d, 2H, $J = 7.9$ Hz, ArH), 7.92 (d, 2H, $J = 7.3$ Hz, ArH), 8.21 (d, 2H, $J = 7.8$ Hz, ArH), 8.76 (d, 1H, $J = 4.3$ Hz, pyrimidine), 9.23 (s, 1H, NH), 10.11 (s, 1H, NH). ^{13}C -NMR (DMSO- d_6 , 100 MHz, δ ppm): 55.7 (C, OCH_3), 87.6 (C, C_3 -pyrazolopyrimidine), 106.9 (C, C_6 -pyrazolopyrimidine), 114.4, 119.2, 120.2, 123.2, 129.1, 129.6, 130.7, 131.4 (14C, Ar), 132.7 (C, C_{3a} -pyrazolopyrimidine), 134.2, 136.0, 137.5 (3C, Ar), 146.0 (C, C_7 -pyrazolopyrimidine), 149.8 (C, Ar), 154.5 (C, C_2 -pyrazolopyrimidine), 159.5 (C, C_5 -pyrazolopyrimidine), 163.2 (C=O). MS (m/z , %): 548 (M^+ , 20.55). Anal. Calcd. (%) for $\text{C}_{26}\text{H}_{19}\text{BrClN}_5\text{O}_2$ (548.82): C, 56.90; H, 3.49; N, 12.76. Found: C, 57.00; H, 3.40; N, 12.80%.

N-(4-Chlorophenyl)-7-(4-fluorophenyl)-2-(4-methoxyphenylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**18t**). Yellow crystals, m.p. 251–252 °C, yield (67%). IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 3339 (NH), 1651 (C=O). ^1H -NMR (DMSO- d_6 , 400 MHz, δ ppm): 3.74 (s, 3H, OCH_3), 6.93 (d, 2H, $J = 7.8$ Hz, ArH), 7.44 (d, 2H, $J = 8.7$ Hz, ArH), 7.45 (d, 1H, $J = 4.2$ Hz, pyrimidine), 7.56 (d, 2H, $J = 8.0$ Hz, ArH), 7.62 (d, 2H, $J = 8.0$ Hz, ArH), 7.78 (d, 2H, $J = 7.4$ Hz, ArH), 8.33 (d, 2H, $J = 8.1$ Hz, ArH), 8.75 (d, 1H, $J = 4.5$ Hz, pyrimidine), 9.21 (s, 1H, NH), 10.10 (s, 1H, NH). ^{13}C -NMR (DMSO- d_6 , 100 MHz, δ ppm): 55.8 (C, OCH_3), 86.7 (C, C_3 -pyrazolopyrimidine), 108.3 (C, C_6 -pyrazolopyrimidine), 114.3, 115.9, 119.0, 119.4, 124.6, 128.1, 129.1 (13C, Ar), 132.3 (C, C_{3a} -pyrazolopyrimidine), 133.2, 134.3, 138.4 (3C, Ar), 145.0 (C, C_7 -pyrazolopyrimidine), 147.3 (C, Ar), 151.1 (C, C_2 -pyrazolopyrimidine), 154.0

(C, C₅-pyrazolopyrimidine), 156.6 (C, Ar), 162.3 (C=O). MS (*m/z*, %): 487 (M⁺, 21.30). Anal. Calcd. (%) for C₂₆H₁₉ClFN₅O₂ (487.91): C, 64.00; H, 3.93; N, 14.35. Found: C, 64.10; H, 4.00; N, 14.30%.

N-(4-Chlorophenyl)-2-(4-methoxyphenylamino)-7-(thiophen-2-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**18u**). Yellow crystals, m.p. 289–291 °C, yield (71%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3293 (NH), 1644 (C=O). ¹H-NMR (DMSO-*d*₆, 400 MHz, δ ppm): 3.78 (s, 3H, OCH₃), 7.04 (d, 2H, *J* = 9.0 Hz, ArH), 7.33 (d, 2H, *J* = 9.2 Hz, ArH), 7.47 (t, 1H, thiophene), 7.79 (d, 2H, *J* = 9.1 Hz, ArH), 7.84 (d, 2H, *J* = 8.9 Hz, ArH), 7.91 (d, 1H, *J* = 4.8 Hz, pyrimidine), 8.29 (d, 1H, *J* = 5.1 Hz, thiophene), 8.60 (d, 1H, *J* = 2.9 Hz, thiophene), 8.71 (d, 1H, *J* = 5.7 Hz, pyrimidine), 9.39 (s, 1H, NH), 10.14 (s, 1H, NH). ¹³C-NMR (DMSO-*d*₆, 100 MHz, δ ppm): 55.7 (C, OCH₃), 87.8 (C, C₃-pyrazolopyrimidine), 107.1 (C, C₆-pyrazolopyrimidine), 114.0, 119.5, 120.4, (6C, Ar), 127.6, 128.2, 129.7 (3C, thiophene), 130.0 (2C, Ar), 133.1 (C, C_{3a}-pyrazolopyrimidine), 133.6, 134.5, 136.9 (3C, Ar), 139.7 (C, thiophene), 148.0 (C, Ar), 151.4 (C, C₂-pyrazolopyrimidine), 154.2 (C, C₅-pyrazolopyrimidine), 157.2 (C, C₇-pyrazolopyrimidine), 162.9 (C=O). MS (*m/z*, %): 475 (M⁺, 74.59). Anal. Calcd. (%) for C₂₄H₁₈ClN₅O₂S (475.95): C, 60.56; H, 3.81; N, 14.71. Found: C, 60.50; H, 3.90; N, 14.80%.

*General Procedure for Synthesis of N-aryl-2-(arylamino)-8,8-dimethyl-6-oxo-6,7,8,9-tetrahydropyrazolo[1,5-*a*]quinazoline-3-carboxamides 25a–c*. A mixture of compounds **11a–c** (0.01 mol) with 2-((dimethyl-amino)methylene)-5,5-dimethylcyclohexane-1,3-dione (**19**, 0.01 mol, 1.95 g) in glacial acetic acid (25 mL), the reaction mixture was refluxed for 1 h and then left to cool. The solid product was filtered off, washed with ethanol, dried and finally recrystallized from DMF/H₂O to afford the corresponding pyrazolo[1,5-*a*]quinazolines **25a–c**.

2-(4-Methoxyphenylamino)-8,8-dimethyl-6-oxo-*N*-phenyl-6,7,8,9-tetrahydropyrazolo[1,5-*a*]quinazoline-3-carboxamide (**25a**). Yellow crystals, m.p. 270–272 °C, yield (73%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3302 (NH), 1655 (C=O). ¹H-NMR (CDCl₃, 400 MHz, δ ppm): 1.24 (s, 6H, 2CH₃), 2.58 (s, 2H, CH₂), 3.31 (s, 2H, CH₂), 3.82 (s, 3H, OCH₃), 6.95 (d, 2H, *J* = 9.0 Hz, ArH), 7.14 (t, 1H, ArH), 7.39 (t, 2H, ArH), 7.64 (d, 2H, *J* = 9.0 Hz, ArH), 7.71 (d, 2H, *J* = 7.5 Hz, ArH), 8.99 (s, 1H, quinazoline), 9.48 (s, 1H, NH), 9.88 (s, 1H, NH). ¹³C-NMR (CDCl₃, 100 MHz, δ ppm): 28.7 (2C, 2CH₃), 32.7 (C, C₈-quinazoline), 37.7 (C, CH₂), 51.1 (C, CH₂), 55.7 (C, OCH₃), 90.9 (C, C₃-quinazoline), 113.8 (C, C_{5a}-quinazoline), 114.5, 119.6, 120.3, 124.2, 129.2 (9C, Ar), 133.4 (C, C_{3a}-quinazoline), 138.2, 147.1, 148.6 (3C, Ar), 151.5 (C, C₂-quinazoline), 155.0 (C, C₅-quinazoline), 159.1 (C=O), 162.6 (C, C_{9a}-quinazoline), 193.9 (C=O). MS (*m/z*, %): 455 (M⁺, 71.06). Anal. Calcd. (%) for C₂₆H₂₅N₅O₃ (455.51): C, 68.56; H, 5.53; N, 15.37. Found: C, 68.50; H, 5.55; N, 15.40%.

2-(4-Methoxyphenylamino)-8,8-dimethyl-6-oxo-*N*-(4-methylphenyl)-6,7,8,9-tetrahydropyrazolo[1,5-*a*]quinazoline-3-carboxamide (**25b**). Yellow crystals, m.p. 266–268 °C, yield (77%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3316 (NH), 1659 (C=O). ¹H-NMR (CDCl₃, 400 MHz, δ ppm): 1.19 (s, 6H, 2CH₃), 2.34 (s, 3H, CH₃), 2.52 (s, 2H, CH₂), 3.22 (s, 2H, CH₂), 3.81 (s, 3H, OCH₃), 6.91 (d, 2H, *J* = 9.0 Hz, ArH), 7.16 (d, 2H, *J* = 8.2 Hz, ArH), 7.54 (d, 2H, *J* = 8.3 Hz, ArH), 7.58 (d, 2H, *J* = 9.0 Hz, ArH), 8.90 (s, 1H, quinazoline), 9.41 (s, 1H, NH), 9.72 (s, 1H, NH). ¹³C-NMR (CDCl₃, 100 MHz, δ ppm): 21.0 (C, CH₃), 28.7 (2C, 2CH₃), 32.6 (C, C₈-quinazoline), 37.5 (C, CH₂), 50.9 (C, CH₂), 55.6 (C, OCH₃), 90.7 (C, C₃-quinazoline), 113.7 (C, C_{5a}-quinazoline), 114.4, 119.4, 120.1, 129.6 (8C, Ar), 133.4 (C, C_{3a}-quinazoline), 133.7, 135.7, 146.8, 148.5 (4C, Ar), 151.5 (C, C₂-quinazoline), 154.8 (C, C₅-quinazoline), 158.8 (C=O), 162.3 (C, C_{9a}-quinazoline), 194.0 (C=O). MS (*m/z*, %): 469 (M⁺, 93.88). Anal. Calcd. (%) for C₂₇H₂₇N₅O₃ (469.53): C, 69.07; H, 5.80; N, 14.92. Found: C, 69.15; H, 5.75; N, 15.00%.

N-(4-Chlorophenyl)-2-(4-methoxyphenylamino)-8,8-dimethyl-6-oxo-6,7,8,9-tetrahydropyrazolo[1,5-*a*]quinazoline-3-carboxamide (**25c**). Yellow crystals, m.p. 291–293 °C, yield (72%). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3299 (NH), 1662 (C=O). ¹H-NMR (DMSO-*d*₆, 400 MHz, δ ppm): 1.16 (s, 6H, 2CH₃), 2.59 (s, 2H, CH₂), 3.36 (s, 2H, CH₂), 3.76 (s, 3H, OCH₃), 6.98 (d, 2H, *J* = 8.8 Hz, ArH), 7.45 (d, 2H, *J* = 8.6 Hz, ArH), 7.72 (d, 2H, *J* = 8.6 Hz, ArH), 7.76 (d, 2H, *J* = 8.8 Hz, ArH), 8.95 (s, 1H, quinazoline), 9.30 (s, 1H, NH), 10.01 (s, 1H, NH). ¹³C-NMR (DMSO-*d*₆, 100 MHz, δ ppm): 28.7 (2C, 2CH₃), 32.6 (C, C₈-quinazoline), 37.6 (C, CH₂), 50.0 (C, CH₂), 55.7

(C, OCH₃), 90.8 (C, C₃-quinazoline), 113.7 (C, C_{5a}-quinazoline), 114.4, 119.3, 120.1, 129.3 (8C, Ar), 133.4 (C, C_{3a}-quinazoline), 133.8, 136.3, 146.9, 148.1 (4C, Ar), 151.5 (C, C₂-quinazoline), 154.8 (C, C₅-quinazoline), 158.9 (C=O), 162.4 (C, C_{9a}-quinazoline), 193.9 (C=O). MS (*m/z*, %): 489 (M⁺, 63.07). Anal. Calcd. (%) for C₂₆H₂₄ClN₅O₃ (489.95): C, 63.74; H, 4.94; N, 14.29. Found: C, 63.80; H, 5.00; N, 14.20%.

3.2. Biological Evaluation

3.2.1. In-Vitro Anticancer Activity

Cell culture of HepG-2 (human liver carcinoma) and MCF-7 (human breast adenocarcinoma) cell lines were purchased from the American Type Culture Collection (Rockville, MD, USA) and maintained in DMEM medium which was supplemented with 10% heat-inactivated FBS (fetal bovine serum), 100 U/mL penicillin and 100 U/mL streptomycin. The cells were grown at 37 °C in a humidified atmosphere of 5% CO₂.

3.2.2. MTT Cytotoxicity Assay

The antitumor activity against HepG-2 and MCF-7 human cancer cell lines was estimated using the 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, which is based on the cleavage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells [31–33]. Cells were dispensed in a 96 well sterile microplate (5 × 10⁴ cells/well), and incubated at 37 °C with series of different concentrations, in DMSO, of each tested compound or Doxorubicin[®] (positive control) for 48 h in a serum free medium prior to the MTT assay. After incubation, media were carefully removed, 40 µL of MTT (2.5 mg/mL) were added to each well and then incubated for an additional 4 h. The purple formazan dye crystals were solubilized by the addition of 200 µL of DMSO. The absorbance was measured at 590 nm using a SpectraMax[®], Paradigm[®] Multi-Mode microplate reader. The relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells.

3.2.3. Statistical Analysis

All experiments were conducted in triplicate and repeated on three different days. All the values were represented as mean ± SD. IC₅₀s were determined by probit analysis using the SPSS software program (SPSS Inc., Chicago, IL, USA).

3.2.4. Cell Cycle Analysis and Apoptosis Detection

Cell cycle analysis and apoptosis detection were carried out by flow cytometry [35]. Both HepG-2 and MCF-7 cells were seeded at 8 × 10⁴ and incubated at 37 °C, 5% CO₂ overnight, after treatment with the tested compounds, for 24 h. Cell pellets were collected and centrifuged (300 g, 5 min). For cell cycle analysis, cell pellets were fixed with 70% ethanol on ice for 15 min and collected again. The collected pellets were incubated with propidium iodide (PI) staining solution (50 µg/mL PI, 0.1 mg/mL RNaseA, 0.05% Triton X-100) at room temperature for 1 h and analyzed by Gallios flow cytometer (Beckman Coulter, Brea, CA, USA). Apoptosis detection was performed by FITC Annexin-V/PI commercial kit (Becton Dickinson, Franklin Lakes, NJ, USA) following the manufacture protocol. The samples were analyzed by fluorescence-activated cell sorting (FACS) with a Gallios flow cytometer (Beckman Coulter) within 1 h after staining. Data were analyzed using Kaluza v. 1.2 (Beckman Coulter).

4. Conclusions

A series of *N*-aryl-7-aryl-pyrazolo[1,5-*a*]pyrimidines **18a–u** and *N*-aryl-pyrazolo[1,5-*a*]quinazolines **25a–c** have been synthesized and investigated for their in vitro antitumor activity. All the investigated compounds showed dose-dependent cytotoxic activities against two cancer types (liver and breast cancer). The IC₅₀ values of these compounds did not reveal statistical significant differences compared to the positive control (doxorubicin). Therefore, two compounds (**18o** and **18a**) have been selected to study their cell cycle and apoptotic effect against HepG2 and

MCF-7 cancer cell lines. Compounds **18o** and **18a** showed slightly higher cytotoxicity compared to doxorubicin against HepG-2 cells ($IC_{50} = 72.2 \pm 3.8$ vs. $80.9 \pm 2.1 \mu M$) and against MCF-7 cells ($IC_{50} = 63.1 \pm 3.1$ vs. $65.6 \pm 4.2 \mu M$), respectively. Cell cycle analysis of HepG-2 cells treated with **18o** and MCF-7 cells treated with **18a** revealed a significant G2/M phase arrest coupled with an increase in the percentage of cells in pre-G phase, which is indicative of apoptosis. The pro-apoptotic activity of **18a** and **18o** was inferred by the significant increase in the percentage of annexin V-FITC-positive apoptotic cells.

Supplementary Materials: Spectra of compounds are available online.

Author Contributions: A.S.H. formulated the research idea; M.E.-N., A.S.H. and M.F.M. carried out the experimental, interpreted the data and prepared the manuscript; H.M.A. performed the biological screening. All authors have read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Abd El Razik, H.A.; Abdel Wahab, A.E. Synthesis and Biological Evaluation of Some Novel Fused Pyrazolopyrimidines as Potential Anticancer and Antimicrobial Agents. *Arch. Pharm. Chem. Life Sci.* **2011**, *11*, 184–196. [[CrossRef](#)] [[PubMed](#)]
2. Aggarwal, R.; Masan, E.; Kaushik, P.; Kaushik, D.; Sharma, C.; Aneja, K.R. Synthesis and biological evaluation of 7-trifluoromethylpyrazolo[1,5-*a*]pyrimidines as anti-inflammatory and antimicrobial agents. *J. Fluor. Chem.* **2014**, *168*, 16–24. [[CrossRef](#)]
3. Kaping, S.; Kalita, U.; Sunn, M.; Singha, L.I.; Vishwakarma, J.N. A facile, regioselective synthesis of pyrazolo[1,5-*a*]pyrimidine analogs in the presence of $KHSO_4$ in aqueous media assisted by ultrasound and their anti-inflammatory and anti-cancer activities. *Monatsh. Chem.* **2016**, *147*, 1257–1276. [[CrossRef](#)]
4. Deshmukh, S.; Dingore, K.; Gaikwad, V.; Jachak, M. An efficient synthesis of pyrazolo[1,5-*a*]pyrimidines and evaluation of their antimicrobial activity. *J. Chem. Sci.* **2016**, *128*, 1459–1468. [[CrossRef](#)]
5. El-Mekabaty, A.; Habib, O.M.O.; Moawad, E.B.; Hasel, A.M. Synthesis and Antioxidant Activity of New Pyrazolo[1,5-*a*]Pyrimidine Derivatives Incorporating a Thiazol-2-ylidiazanyl Moiety. *J. Heterocycl. Chem.* **2016**, *53*, 1820–1826. [[CrossRef](#)]
6. Hassan, A.S.; Masoud, D.M.; Sroor, F.M.; Askar, A.A. Synthesis and biological evaluation of pyrazolo[1,5-*a*]pyrimidine-3-carboxamide as antimicrobial agents. *Med. Chem. Res.* **2017**, *26*, 2909–2919. [[CrossRef](#)]
7. Kumar, A.K.A.; Bodke, Y.D.; Lakra, P.S.; Sambasivam, G.; Bhat, K.G. Design, synthesis and anti-cancer evaluation of a novel series of pyrazolo[1,5-*a*]pyrimidine substituted diamide derivatives. *Med. Chem. Res.* **2017**, *26*, 714–744. [[CrossRef](#)]
8. Rahmouni, A.; Souiei, S.; Belkacem, M.A.; Romdhane, A.; Bouajila, J.; Ben Jannet, H. Synthesis and biological evaluation of novel pyrazolopyrimidines derivatives as anticancer and anti-5-lipoxygenase agents. *Bioorg. Chem.* **2016**, *66*, 160–168. [[CrossRef](#)] [[PubMed](#)]
9. Zhao, M.; Ren, H.; Chang, J.; Zhang, D.; Yang, Y.; He, Y.; Qi, C.; Zhang, H. Design and Synthesis of pyrazolo[1,5-*a*]pyrimidine derivatives bearing nitrogen mustard moiety and evaluation of their antitumor activity in vitro and in vivo. *Eur. J. Med. Chem.* **2016**, *119*, 183–196. [[CrossRef](#)] [[PubMed](#)]
10. Li, J.; Zhao, Y.F.; Zhao, X.L.; Yuan, X.Y.; Gong, P. Synthesis and Anti-tumor Activities of Novel Pyrazolo[1,5-*a*]pyrimidines. *Arch. Pharm. Chem. Life Sci.* **2006**, *339*, 593–597. [[CrossRef](#)] [[PubMed](#)]
11. Ahmed, O.M.; Mohamed, M.A.; Ahmed, R.R.; Ahmed, S.A. Synthesis and anti-tumor activities of some new pyridines and pyrazolo[1,5-*a*]pyrimidines. *Eur. J. Med. Chem.* **2009**, *44*, 3519–3523. [[CrossRef](#)] [[PubMed](#)]
12. Abdel-Aziz, H.A.; Saleh, T.S.; El-Zahabi, H.S.A. Facile Synthesis and In-Vitro Antitumor Activity of Some Pyrazolo[3,4-*b*]pyridines and Pyrazolo[1,5-*a*]pyrimidines Linked to a Thiazolo[3,2-*a*]benzimidazole Moiety. *Arch. Pharm. Chem. Life Sci.* **2010**, *343*, 24–30. [[CrossRef](#)]
13. Hassan, A.S.; Mady, M.F.; Awad, H.M.; Hafez, T.S. Synthesis and antitumor activity of some new pyrazolo[1,5-*a*]pyrimidines. *Chin. Chem. Lett.* **2017**, *28*, 388–393. [[CrossRef](#)]
14. Hassan, A.S.; Hafez, T.S.; Osman, S.A.M.; Ali, M.M. Synthesis and In Vitro cytotoxic activity of novel pyrazolo[1,5-*a*]pyrimidines and related Schiff bases. *Turk. J. Chem.* **2015**, *39*, 1102–1113. [[CrossRef](#)]

15. Gopalsamy, A.; Ciszewski, G.; Hu, Y.; Lee, F.; Feldberg, L.; Frommer, E.; Kimb, S.; Collins, K.; Wojciechowicz, D.; Mallon, R. Identification of pyrazolo[1,5-*a*]pyrimidine-3-carboxylates as B-Raf kinase inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2735–2738. [[CrossRef](#)] [[PubMed](#)]
16. Mukaiyama, H.; Nishimura, T.; Kobayashi, S.; Komatsu, Y.; Kikuchi, S.; Ozawa, T.; Kamada, N.; Ohnota, H. Novel pyrazolo[1,5-*a*]pyrimidines as c-Src kinase inhibitors that reduce I_{Kr} channel blockade. *Bioorg. Med. Chem.* **2008**, *16*, 909–921. [[CrossRef](#)] [[PubMed](#)]
17. Liu, Y.; Laufer, R.; Patel, N.K.; Ng, G.; Sampson, P.B.; Li, S.-W.; Lang, Y.; Feher, M.; Brokx, R.; Beletskaya, I.; et al. Discovery of Pyrazolo[1,5-*a*]pyrimidine TTK Inhibitors: CFI-402257 is a Potent, Selective, Bioavailable Anticancer Agent. *Med. Chem. Lett.* **2016**, *7*, 671–675. [[CrossRef](#)] [[PubMed](#)]
18. Mukaiyama, H.; Nishimura, T.; Shiohara, H.; Kobayashi, S.; Komatsu, Y.; Kikuchi, S.; Tsuji, E.; Kamada, N.; Ohnota, H.; Kusama, H. Discovery of Novel 2-Anilinopyrazolo[1,5-*a*]pyrimidine Derivatives a c-Src Kinase Inhibitors for the Treatment of Acute Ischemic Stroke. *Chem. Pharm. Bull.* **2007**, *55*, 881–889. [[CrossRef](#)] [[PubMed](#)]
19. Kumar, A.K.A.; Bodke, Y.D.; Sambasivam, G.; Lakra, P.S. Design, synthesis, and evaluation of novel hydrazide hydrochlorides of 6-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxamides as potent Aurora kinase inhibitors. *Monatsh. Chem.* **2017**, *148*, 1767–1780. [[CrossRef](#)]
20. Hassan, A.S.; Awad, H.M.; Magd-El-Din, A.A.; Hafez, T.S. Synthesis and in vitro antitumor evaluation of novel Schiff bases. *Med. Chem. Res.* **2018**, *27*, 915–927. [[CrossRef](#)]
21. Hassan, A.S.; Hafez, T.S.; Ali, M.M.; Khatib, T.K. Design, synthesis and cytotoxic activity of some new pyrazolines bearing benzofuran and pyrazole moieties. *Res. J. Pharm. Biol. Chem. Sci.* **2016**, *7*, 417–429.
22. Abd El-All, A.S.; Hassan, A.S.; Osman, S.A.; Yosef, H.A.A.; Abdel-Hady, W.H.; El-Hashash, M.A.; Atta-Allah, S.R.; Ali, M.M.; El Rashedy, A.A. Synthesis, characterization and biological evaluation of new fused triazine derivatives based on 6-methyl-3-thioxo-1,2,4-triazin-5-one. *Acta Poloniae Pharm. Drug Res.* **2016**, *73*, 79–92.
23. Osman, S.A.; Mousa, H.A.; Yosef, H.A.A.; Hafez, T.S.; El-Sawy, A.A.; Abdallah, M.M.; Hassan, A.S. Synthesis, characterization and cytotoxicity of mixed ligand Mn(II), Co(II) and Ni(II) complexes. *J. Serb. Chem. Soc.* **2014**, *79*, 953–964. [[CrossRef](#)]
24. Hafez, T.S.; Osman, S.A.; Yosef, H.A.A.; Abd El-All, A.S.; Hassan, A.S.; El-Sawy, A.A.; Abdallah, M.M.; Youns, M. Synthesis, structural elucidation and in vitro antitumor activities of some pyrazolopyrimidines and Schiff bases derived from 5-amino-3-(arylamino)-1H-pyrazole-4-carboxamides. *Sci. Pharm.* **2013**, *81*, 339–357. [[CrossRef](#)] [[PubMed](#)]
25. Osman, S.A.; Yosef, H.A.A.; Hafez, T.S.; El-Sawy, A.A.; Mousa, H.A.; Hassan, A.S. Synthesis and antibacterial activity of some novel chalcones, pyrazoline and 3-cyanopyridine derivatives based on khellinone as well as Ni(II), Co(II) and Zn(II) complexes. *Aust. J. Basic Appl. Sci.* **2012**, *6*, 852–863.
26. Elgemeie, G.H.; Elsayed, S.H.; Hassan, A.S. Design and synthesis of the first thiophene thioglycosides. *Synth. Commun.* **2009**, *39*, 1781–1792. [[CrossRef](#)]
27. Elgemeie, G.H.; Elsayed, S.H.; Hassan, A.S. Direct route to a new class of acrylamide thioglycosides and their conversions to pyrazole derivatives. *Synth. Commun.* **2008**, *38*, 2700–2706. [[CrossRef](#)]
28. Hassan, A.S.; Moustafa, G.O.; Awad, H.M. Synthesis and in vitro anticancer activity of pyrazolo[1,5-*a*]pyrimidines and pyrazolo[3,4-*d*][1,2,3]triazines. *Synth. Commun.* **2017**, *47*, 1963–1972. [[CrossRef](#)]
29. Hassan, A.S.; Hafez, T.S.; Osman, S.A. Synthesis, characterization, and cytotoxicity of some new 5-aminopyrazole and pyrazolo[1,5-*a*]pyrimidine derivatives. *Sci. Pharm.* **2015**, *83*, 27–39. [[CrossRef](#)] [[PubMed](#)]
30. Sadek, K.U.; Mekheimer, R.A.; Mohamed, T.M.; Moustafa, M.S.; Elnagdi, M.H. Regioselectivity in the multicomponent reaction of 5-aminopyrazoles, cyclic 1,3-diketones and dimethyl formamide dimethylacetal under controlled microwave heating. *Beilstein J. Org. Chem.* **2012**, *8*, 18–24. [[CrossRef](#)] [[PubMed](#)]
31. Hamdy, N.A.; Anwar, M.M.; Abu-Zied, K.M.; Awad, H.M. Synthesis, tumor inhibitory and antioxidant activity of new polyfunctionally 2-substituted 5,6,7,8-tetrahydronaphthalene derivatives containing pyridine, thioxopyridine and pyrazolopyridine moieties. *Acta Poloniae Pharm. Drug Res.* **2013**, *70*, 987–1001.
32. Awad, H.M.; Abd-Alla, H.I.; Mahmoud, K.H.; El-Toumy, S.A. In vitro anti-nitrosative, antioxidant, and cytotoxicity activities of plant flavonoids: A comparative study. *Med. Chem. Res.* **2014**, *23*, 3298–3307. [[CrossRef](#)]

33. Soliman, H.A.; Yousif, M.N.M.; Said, M.M.; Hassan, N.A.; Ali, M.M.; Awad, H.M.; Abdel-Megeid, F.M.E. Synthesis of novel 1,6-naphthyridines, pyrano[3,2-c]pyridines and pyrido[4,3-d]pyrimidines derived from 2,2,6,6-tetramethylpiperidin-4-one for in vitro anticancer and antioxidant evaluation. *Der Pharma Chem.* **2014**, *6*, 394–410.
34. Massagué, J. G1 cell-cycle control and cancer. *Nature* **2004**, *432*, 298–306. [[CrossRef](#)] [[PubMed](#)]
35. Thornton, T.M.; Rincon, M. Non-classical P38 map kinase functions: Cell cycle checkpoints and survival. *Int. J. Biol. Sci.* **2009**, *5*, 44–52. [[CrossRef](#)] [[PubMed](#)]

Sample Availability: Samples of the compounds are all available from the authors.



© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).