Research article

Synthesis, Structural Elucidation, and *In Vitro* Antitumor Activities of Some Pyrazolopyrimidines and Schiff Bases Derived from 5-Amino-3-(arylamino)-1*H*-pyrazole-4-carboxamides

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Abstract

The reaction of 5-amino-3-(arylamino)-1*H*-pyrazole-4-carboxamides **1a,b** with acetylacetone **2** and arylidenemalononitriles **5a–c** yielded the pyrazolo[1,5-*a*]-pyrimidine derivatives **4a,b** and **7a–f** respectively. On the other hand, Schiff bases **9a,b** and **12a–j** were obtained upon treatment of carboxamides **1a,b** with isatin **8** and some selected aldehydes **11a–e**. The newly synthesized compounds were characterized by analytical and spectroscopic data. Representative examples of the synthesized products **4a,b, 7e, 7f, 9b, 12b–f, 12h,** and **12j** were screened for their *in vitro* antitumor activities against different human cancer cell lines and the structure-activity relationship (SAR) was discussed.

Keywords

5-Aminopyrazole-4-carboxamides • Pyrazolo[1,5-*a*]pyrimidines • Schiff bases • Antitumor agents • Ferrocenecarboxaldehyde

Introduction

Of the various human diseases, cancer, human immunodeficiency virus (HIV), and hepatitis C virus (HCV) are the major scourges of humanity. Therefore, the identification of novel, potent, selective, less toxic anticancer and antiviral agents remains one of the most pressing health problems. A literature survey revealed that pyrazolo[1,5-*a*]pyrimidines are purine analogues which are well-known for their importance in biological applications as antimetabolites [1]. They act as potent inhibitors of the enzymes HCV polymerase [2], checkpoint kinase 1 (CHK1) [3], cyclin-dependent kinase 2 (CDK2) [4], and c-Src kinase [5]. Moreover, pyrazolo[1,5-*a*]pyrimidine derivatives have significant antimicrobial [6], antitumor [7], and anti-inflammatory [8] activities.

On the other hand, Schiff bases are important classes of compounds in the medicinal field, which have biological applications including antimicrobial [9], antioxidant [10, 11], and antitumor [12–15] activities.

In view of these facts, we report herein the synthesis of a new series of substituted pyrazolo[1,5-*a*]pyrimidines and Schiff bases derived from 5-aminopyrazole derivatives for the examination of their antitumor activity.

Results and Discussion

Chemistry

The starting compounds, 5-amino-3-(phenylamino)-1*H*-pyrazole-4-carboxamide (**1a**) [16] and 5-amino-3-[(4-methoxyphenyl)amino]-1*H*-pyrazole-4-carboxamide **1b** were utilized in preparing the target compounds (Schemes 1 and 2).

Pyrazole derivative **1b** was obtained by the reaction of 2-cyano-3-[(4-methoxy–phenyl)amino]-3-(methylthio)acrylamide with hydrazine hydrate in boiling ethanol in the presence of triethylamine. Its structure was assigned upon compatible elemental analyses and spectral data. Correct analytical data and molecular weight determination (MS) corresponded to $C_{11}H_{13}N_5O_2$ (m/z=247 [M$^+$]). The IR spectrum (KBr/cm^{-1}) revealed the presence of strong absorption bands at 3439, 3277, and 3163 corresponding to -NH$_2$ and -NH. Bands at 1659 and 1624 were attributed to C=O and C=N group frequencies, respectively, and the band at 1558 was due to C=C (aromatic). The ^{1}H NMR spectrum (DMSO-d$_6$, δ ppm) revealed the presence of signals at 3.64 for -OCH$_3$ protons as a singlet, four singlets at 5.74, 6.60, 8.68, and 10.82 assigned for two -NH$_2$ and two -NH protons, which were D$_2$O exchangeable. The two doublets present at 6.75 (2H) and 7.24 (2H) were assigned for the four aromatic protons (AB system, J_{HH}=8.4 Hz), and its ^{13}C NMR spectrum (DMSO-d$_6$, δ ppm) showed signals at 55.6 (-OCH$_3$), 86.2 (C$_4$, pyrazole), 114.6 (2C, aromatic), 117.4 (2C, aromatic), 137.0 (C, aromatic), 148.2 (C$_5$, pyrazole), 152.2 (C$_3$, pyrazole), 152.8 (C, aromatic), and 167.2 (C=O, amide).

Compounds **1a,b** were reacted with acetylacetone **2** in boiling glacial acetic acid to afford the corresponding new pyrazolo[1,5-*a*]pyrimidines **4a,b** (Scheme 1). The structures of **4a,b** were confirmed on the basis of their analytical and spectral data. Compound **4a,** taken as a representative example, revealed the molecular formula $C_{15}H_{15}N_5O$ (m/z=281 [M$^+$]), and its IR spectrum (KBr/cm^{-1}) showed strong absorption bands at 3374 and 3142, corresponding to -NH$_2$ and -NH, a band at 1658 due to C=O, two bands at 1626 and 1596 for C=N, and a band at 1562 due to C=C (aromatic). Its ^{1}H NMR spectrum (DMSO-d$_6$, δ ppm) showed two singlets at 2.50 and 2.66 due to two -CH$_3$ group protons and a signal at 6.93 due to the H-6 proton of the pyrimidine nucleus. Two singlets present at 7.47 and 9.57 were assigned for the -NH$_2$ and -NH protons, which were D$_2$O exchangeable. Two triplets present at 6.90 (1H) and 7.30 (2H) were assigned for the three aromatic protons and one doublet at 7.66 (2H) for the two aromatic protons (J_{HH}=7.65 Hz), and their ^{13}C NMR spectrum (DMSO-d$_6$, δ ppm) showed signals at 17.1 (-CH$_3$), 24.6 (-CH$_3$), 86.9 (C$_3$, pyrazolopyrimidine), 109.3 (C$_6$, pyrazolopyrimidine), 117.5 (2C, aromatic), 121.2 (C, aromatic), 129.5 (2C, aromatic), 140.8 (C$_{3a}$, pyrazolopyrimidine), 146.4 (C, aromatic), 146.7 (C$_7$, pyrazolopyrimidine), 156.7 (C$_2$, pyrazolopyrimidine), 161.3 (C$_5$, pyrazolopyrimidine), and 166.4 (C=O, amide).

The formation of compounds **4a,b** was therefore assumed to proceed *via* initial attack of the exocyclic amino group of **1** on the keto group of the 1,3-dicarbonyl compound **2** followed by intramolecular cyclization *via* elimination of water.

Arylidenemalononitriles **5a–c** were reacted with **1a,b** in ethanol under reflux conditions to give 7-amino-5-aryl-2-(arylamino)-6-cyanopyrazolo[1,5-*a*]pyrimidine-3-carboxamides **7a–f** (Scheme 1). The structures of **7a–f** were established based on their analytical and spectral data. Thus, as an example, the mass spectrum of compound **7d** showed an ion peak at m/z 397, which corresponded to [M$^+$-2H], and its IR spectrum (KBr/cm^{-1}) showed bands at 3399, 3333, and 3151 for -NH$_2$ and -NH, 2211 for C≡N, 1646 for C=O, 1597 for C=N, and 1509 for C=C (aromatic) groups. Its ^{1}H NMR spectrum (DMSO-d$_6$, δ ppm) revealed the presence of a singlet at 3.71 corresponding to protons of the -OCH$_3$ group, three singlets at 7.58, 8.92, and 9.43 due to two -NH$_2$ and -NH protons which were D$_2$O exchangeable. Two doublets at 7.75 (2H) and 7.85 (2H) were assigned for four aromatic protons (J_{HH}=8.4 Hz). A multiplet appeared at 6.86–7.55 for five aromatic protons and its ^{13}C NMR spectrum (DMSO-d$_6$, δ ppm) showed signals at 55.6 (-OCH$_3$), 74.9 (C$_6$, pyrazolopyrimidine), 89.6 (C$_3$, pyrazolopyrimidine), 114.7 (2C, aromatic), 116.7 (C≡N), 119.3 (2C, aromatic), 128.8 (2C, aromatic), 129.0 (C, aromatic), 129.2 (2C, aromatic), 131.1 (C$_{3a}$, pyrazolopyrimidine), 133.9 (C, aromatic), 137.3 (C, aromatic), 149.8 (C, aromatic), 154.2 (C$_2$, pyrazolopyrimidine), 156.9 (C$_5$, pyrazolopyrimidine), 161.6 (C$_7$, pyrazolopyrimidine), and 166.2 (C=O, amide).

The formation of compounds **7a–f** was assumed to proceed *via* initial attack of the exocyclic amino function of the compounds **1a,b** on the α,β-unsaturated system in compound **5,** followed by intramolecular cyclization and spontaneous autooxidation through the loss of the H$_2$ molecule [17] (Scheme 1).

Condensation of **1a,b** with isatin **8** in boiling ethanol gave 3-(arylamino)-5-[(2-oxoindolin-3-ylidene)amino]-1*H*-pyrazole-4-carboxamides **9a,b** in excellent yields (Scheme 2). Structures of compounds **9a,b** were confirmed on the basis of elemental analysis and spectral data. As an example, the IR spectrum (KBr/cm^{-1}) of **9a** showed strong stretching

bands at 3427, 3317, and 3181 for –NH$_2$ and -NH, two strong absorption bands at 1687 and 1623 due to two C=O groups, and a band at 1597 for the C=N group. Its ^{1}H NMR spectrum (DMSO-d$_6$, δ ppm) showed a diffused multiplet at 6.89–7.53 due to aromatic and -NH$_2$ protons. Three singlets appeared at 9.14, 10.95, and 12.98 due to three -NH protons, which were D$_2$O exchangeable.

Sch. 1. Synthesis of pyrazolo[1,5-*a*]pyrimidine derivatives
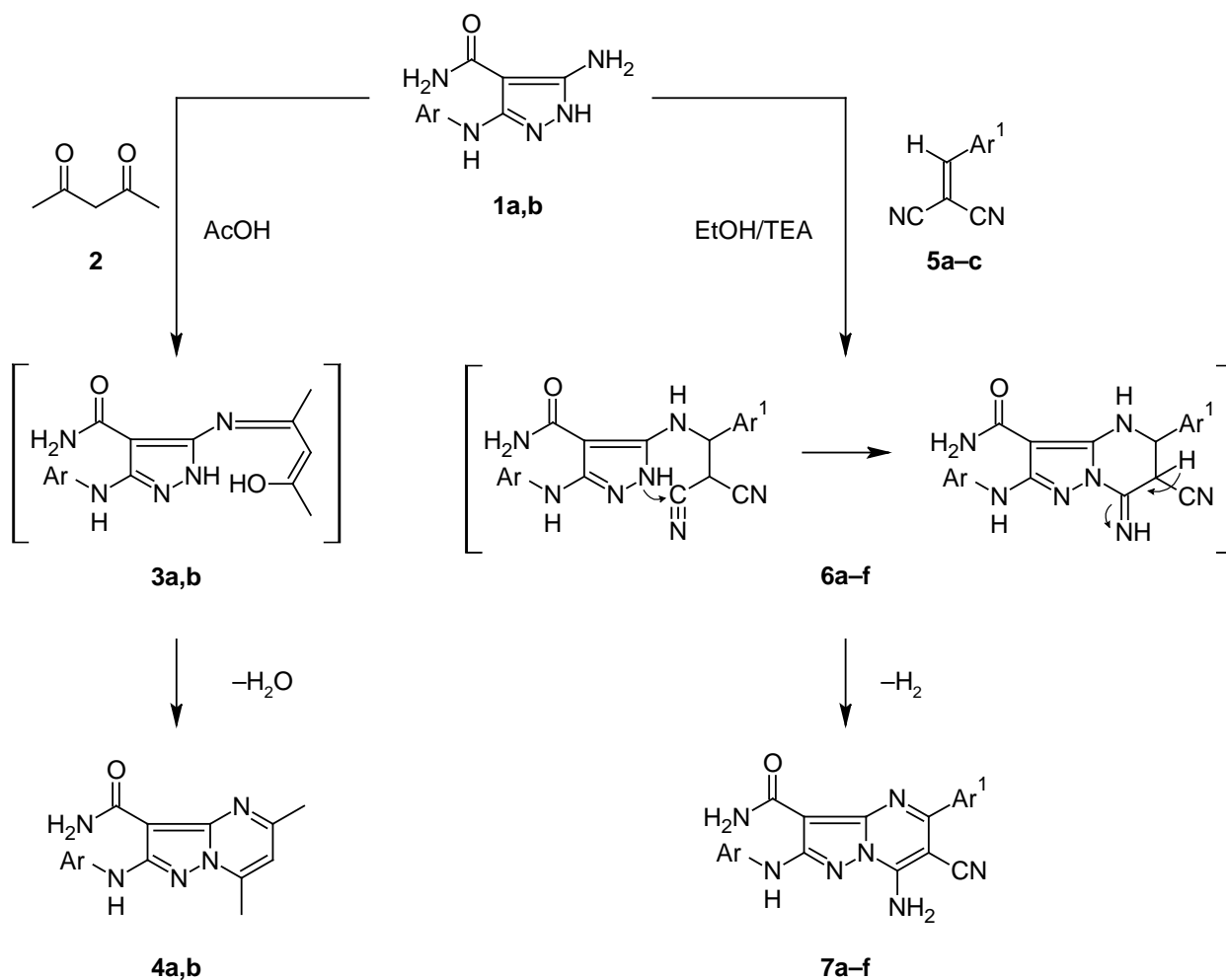
1,3,4   **a**: Ar = Ph   **b**: Ar = 4-MeO-C$_6$H$_4$

5   **a**: Ar^1 = Ph   **b**: Ar^1 = 4-MeO-C$_6$H$_4$ **c**: Ar^1 = 1-naphythyl

6,7   **a**: Ar = Ar^1 = Ph   **b**: Ar = Ph, Ar^1 = 4-MeO-C$_6$H$_4$ **c**: Ar = Ph, Ar^1 = 1-naphthyl

      d: Ar = 4-MeO-C$_6$H$_4$, Ar^1 = Ph   **e**: Ar = Ar^1 = 4-MeO-C$_6$H$_4$ **f**: Ar = 4-MeO-C$_6$H$_4$, Ar^1 = 1-naphthyl

A trial for ring closures of **9a,b** to give the spiro derivatives **10a,b** was unsuccessful, even after their prolonged boiling in glacial acetic acid.

Schiff bases **12a–j** were obtained by the reaction of 1*H*-pyrazolo-4-carboxamides **1a,b** with some selected aldehydes **11a–e** [e.g. aromatic- **11a–c**, heteroaromatic- **11d,** and

ferrocenecarboxaldehyde **11e**] in boiling ethanol using a catalytic amount of triethylamine (Scheme 2).

9,10   **a:** Ar = Ph   **b:** Ar = 4-MeO-C$_6$H$_4$

11   **a:** Ar^2 = Ph   **b:** Ar^2 = 4-MeO-C$_6$H$_4$ **c:** Ar^2 = 1-naphthyl
 d: 5-methyl-2-furyl   **e:** ferrocenyl

12   **a:** Ar = Ar^2 = Ph
 b: Ar = Ph, Ar^2 = 4-MeO-C$_6$H$_4$
 c: Ar = Ph, Ar^2 = 1-naphthyl
 d: Ar = Ph, Ar^2 = 5-methyl-2-furyl
 e: Ar = Ph, Ar^2 = ferrocenyl
 f: Ar = 4-MeO-C$_6$H$_4$, Ar^2 = Ph
 g: Ar = Ar^2 = 4-MeO-C$_6$H$_4$
 h: Ar = 4-MeO-C$_6$H$_4$, Ar^2 = 1-naphthyl
 i: Ar = 4-MeO-C$_6$H$_4$, Ar^2 = 1-naphthyl
 j: Ar = 4-MeO-C$_6$H$_4$, Ar^2 = ferrocenyl
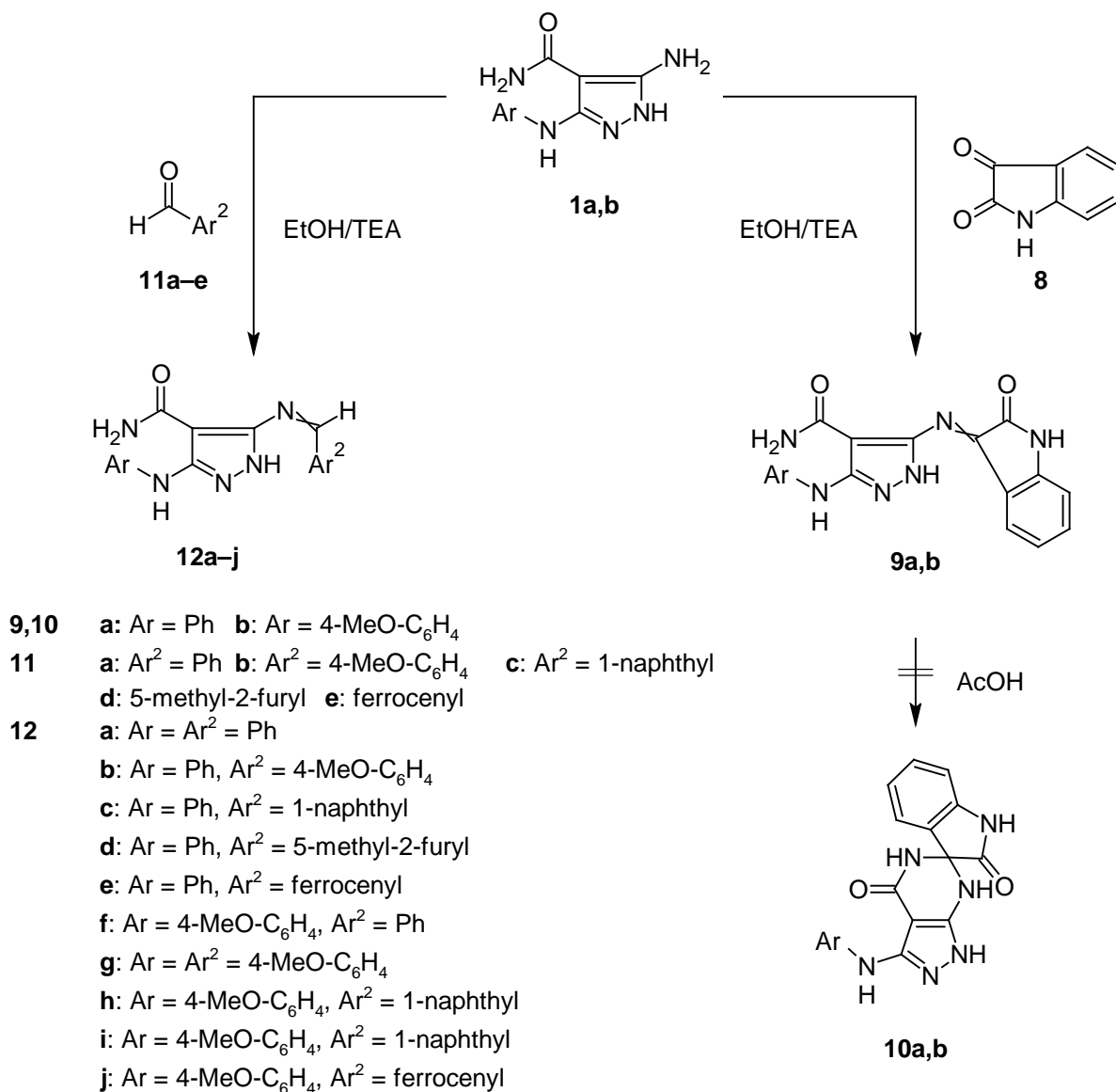
Sch. 2.   Reaction of 1*H*-pyrazolo-4-carboxamide derivatives with isatin and some selected aldehydes.

The structures of compounds **12a–j** were confirmed on the basis of their analytical and spectral data. As an example, the mass spectrum of compound **12e** exhibited a molecular ion peak at *m/z* 413 (C$_{21}$H$_{19}$FeN$_5$O), which was also the base peak. Its IR spectrum (KBr/cm^{-1}) showed stretching bands at 3359 and 3168 for -NH$_2$ and -NH, as well as bands at 1660, 1588, and 1563 for C=O, C=N, and C=C (aromatic) groups, respectively. Its ^{1}H NMR spectrum (DMSO-d$_6$, δ ppm) showed the 5H of the unsubstituted ferrocene ring at 4.29 as a singlet, the 4H of the monosubstituted ferrocene ring at 4.68 (2H) and 4.88 (2H) as two singlets, and a signal at 9.03 due to the 1H of the -N=CH– group, three singlets at 7.50, 8.72, and 12.55 due to the -NH$_2$ and two -NH protons which were D$_2$O

exchangeable, and a multiplet at 6.79-7.28 for five aromatic protons. The ^{13}C NMR spectrum (DMSO-d$_6$, δ ppm) of **12e** showed signals at 70.2 (5C, ferrocene ring), 73.3 (4C, ferrocenyl ring), 79.1 (C, ferrocenyl ring), 92.8 (C$_4$, pyrazole), 116.6 (2C, aromatic), 119.8 (C, aromatic), 129.5 (2C, aromatic), 142.0 (C, aromatic), 148.3 (-N=CH-), 153.2 (C$_3$ & C$_5$, pyrazole), and 167.0 (C=O, amide).

Biological evaluation

In vitro antitumor screening

Preliminary experiments were done to check the availability of the prepared compounds as antitumor agents. We selected different varieties of the newly synthesized compounds containing variable groups and then we evaluated their *in vitro* cytotoxic activities against the human breast cancer cell line (MCF7) where Doxorubicin was used as a standard drug [18]. The results were expressed as the IC$_{50}$ value, which corresponds to the concentration required for 50% inhibition of cell growth of the treated cells when compared to that of control cells.

From the results in Table 1, it was found that the IC$_{50}$ values of compounds **7f**, **12j,** and **12e** were 0.085 µM, 9.294 µM, and 28.48 µM, respectively, which exhibited the highest cytotoxic activities, followed by compound **4a** (IC$_{50}$=122.9 µM) which also showed better activity than the reference drug Doxorubicin (IC$_{50}$=96.41 µM), while compound **12d** (IC$_{50}$=280.0 µM) showed lower activity than the reference drug.

Tab. 1. The cytotoxicity of the tested compounds on the MCF-7 tumor cell line.

Cpd.	IC$_{50}$^{a} µM Tumor cell growth inhibition (%)
4a	122.9
7f	0.085*
12d	280.0
12e	28.48
12j	9.294
Doxorubicin	96.41

^{a} The concentration required for 50% inhibition of cell growth.
* The most potent compound.

The promising results obtained from screening against the MCF7 tumor cell line (Table 1) encouraged us to study the cytotoxicity of the tested compounds by using the MTT assay against different human cancer cell lines, including: cervical carcinoma (KB), ovarial carcinoma (SK OV-3), CNS cancer (SF-268), non-small cell lung cancer (NCl H460), colonadenocarcinoma (RKOP 27) (Table 2), anti-leukemia (HL60, U937, K562), melanoma (SK-MEL-28), and neuroblastoma (GOTO, NB-1) (Table 3). The cytotoxic effects of the tested compounds over the cell lines of HeLa (cervical), HT1080 (fibrosarcoma), and HepG2 (liver) were also investigated (Table 4).

Screening the cytotoxicity of the tested compounds on cervical carcinoma (KB), where Fluorouracil was used as a standard drug (IC$_{50}$=4.46 nM), showed that the seven tested compounds **7e, 12b–f,** and **12j** were more potent than the standard. The most potent one was **12e** (IC$_{50}$=0.54 nM).

On the ovarial carcinoma (SK OV-3) cell line, compounds **7e, 12b, 12d, 12e, 12f,** and **12h** (IC$_{50}$=0.30, 0.44, 3.30, 0.32, 0.90, and 2.20 nM respectively) were more potent than the standard drug Doxorubicin (IC$_{50}$=4.16 nM). The most potent one was found to be **7e** (IC$_{50}$=0.30 nM).

Studying the effects of cytotoxicity for the tested compounds on the CNS cancer (SF-268) cell line, using Cytarabine (IC$_{50}$=7.68 nM) as a standard drug, revealed that compounds **4a, 7e,** 7**f**, **12c, 12d, 12e, 12f**, **12h,** and **12j** were more active than the standard drug, where **12e** (IC$_{50}$=0.30 nM) was the most promising one.

On the non-small cell lung cancer (NCI H460) cell line, the tested compounds, except for **12e** and **12h** (IC$_{50}$=6.60 & 7.00 nM respectively), were more potent than the standard drug Gencitabine hydrochloride (IC$_{50}$=2.13 nM).

On the colonadenocarcinoma (RKOP 27) cell line, the tested compounds, except for **12c, 12d, 12e,** and **12h,** were found to be less active than the standard drug Capecitabine (IC$_{50}$=4.33 nM). Compounds **4b** and **12j** had a comparable activity to Capecitabine.

The study of the cytotoxicity on the leukemia (HL60) cell line indicated that compounds **4b** (IC$_{50}$=7.50 nM), **7f** (IC$_{50}$=3.57 nM), **9b** (IC$_{50}$=5.30 nM), **12h** (IC$_{50}$=5.50 nM), and **12j** (IC$_{50}$=5.60 nM) were less potent than Doxorubicin (IC$_{50}$=1.13 nM).

On the leukemia (U937) cell line, compound **12d** (IC$_{50}$=0.09 nM) was the most potent, but **7f** was the least bioactive (IC$_{50}$=55.0 nM).

On the leukemia (K562) cell line, compound **7f** (IC$_{50}$=0.17 nM) was the most potent among the tested compounds, followed by compound **12e** (IC$_{50}$=0.43 nM), but compound **7e** (IC$_{50}$=8.00 nM) was less potent than Doxorubicin (IC$_{50}$=6.66 nM)**.**

From the estimation of the cytotoxicity on the melanoma (SK-MEL-28) cell line, the tested compounds were less active than the standard drug Aldesleukin (IC$_{50}$=3.45 nM), except **12b** (IC$_{50}$=3.20 nM) was slightly more active.

On the neuroblastoma (GOTO) and (NB-1) cell lines, compound **12d** was the most potent (IC$_{50}$=0.45 nM) and (IC$_{50}$=0.64 nM), respectively, among the tested compounds. Moreover, it was more active than the standard drug Doxorubicin (IC$_{50}$=4.73 nM and IC$_{50}$=5.15 nM, respectively)**.**

The cytotoxicity of the tested compounds on the HeLa (cervical) cell line showed that Tamoxifen (IC$_{50}$=0.11 nM), the standard drug used, was more active than all of the tested compounds.

On the HT1080 (fibrosarcoma) cell line, compound **12d** (IC$_{50}$=0.54 nM) was the most potent one, but compound **12c** (IC$_{50}$=8.97 nM) was found to be less potent than Tamoxifen (IC$_{50}$=1.16 nM).

The cytotoxicity of the tested compounds on the HepG2 (liver) cell line showed that seven of the tested compounds were more bioactive than Tamoxifen (IC$_{50}$=1.31 nM) in a decreasing order of **12e>12d>12j>12c>12f>7e>12h.**

Tab. 2. The cytotoxicity of synthesized compounds was determined by using the MTT assay on different human cancer cell lines

Cpd.	IC_{50}^{a} nM Tumor cell growth inhibition (%)				
	KB	SK OV-3	SF-268	NCI H460	RKOP27
4a	7.70	6.50	0.90	0.80	6.00
4b	4.88	6.73	7.70	0.60	4.00
7e	0.67	0.30*	7.00	0.70	8.00
7f	4.50	7.80	0.60	0.70	7.00
9b	8.50	8.70	8.00	0.89	8.00
12b	0.65	0.44	8.00	0.77	5.00
12c	0.76	4.40	3.00	0.60	0.50*
12d	0.78	3.30	0.65	0.60	3.00
12e	0.54*	0.32	0.30*	6.60	0.60
12f	0.67	0.90	6.00	0.70	7.00
12h	4.40	2.20	6.00	7.00	0.70
12j	0.77	5.60	0.44	0.50*	4.00
Fluorouracil	4.46	–	–	–	–
Doxorubicin	–	4.16	–	–	–
Cytarabine	–	–	7.68	–	–
Gemcitabine HCl	–	–	–	2.13	–
Capecitabine	–	–	–	–	4.33

[a] The concentration required for 50% inhibition of cell growth.
* The most potent compound.

Tab. 3. The cytotoxicity of synthesized compounds was determined by using the MTT assay on different human cancer cell lines

Cpd.	IC_{50}^{a} nM Tumor cell growth inhibition (%)					
	Leukemia			Melanoma	Neuroblastoma	
	HL60	U937	K562	SK-MEL-28	GOTO	NB-1
4a	0.57	6.60	0.78	5.40	0.46	5.80
4b	7.50	7.80	0.50	7.90	5.60	6.43
7e	0.50	0.60	8.00	7.00	0.69	0.65
7f	3.57	55.0	0.17*	66.0	6.00	5.29
9b	5.30	7.00	4.30	6.00	5.00	2.59
12b	0.88	5.40	0.70	3.20*	0.60	5.45
12c	0.78	7.70	0.65	7.30	0.67	6.53
12d	0.43*	0.09*	4.00	8.40	0.45*	0.64*
12e	0.66	6.40	0.43	6.80	0.67	3.47
12f	0.55	0.50	6.50	66.0	0.60	0.69
12h	5.50	4.00	0.79	7.00	0.79	5.54
12j	5.60	8.80	7.80	6.90	5.47	4.27
Doxorubicin	1.13	4.45	6.66	–	4.73	5.15
Aldesleukin	–	–	–	3.45	–	–

[a] The concentration required for 50% inhibition of cell growth.
* The most potent compound.

Tab. 4. The cytotoxicity of synthesized compounds was determined by using the MTT assay on different human cancer cell lines

Cpd.	IC_{50}[a] nM Tumor cell growth inhibition (%)		
	HeLa (cervical)	HT1080 (fibrosarcoma)	HepG2 (liver)
4a	0.36*	0.96	8.70
4b	7.59	7.63	8.47
7e	0.87	0.59	0.96
7f	3.96	3.60	4.45
9b	9.50	8.50	8.00
12b	0.42	0.84	7.80
12c	0.73	8.97	0.66
12d	0.80	0.54*	0.35
12e	7.60	8.48	0.09*
12f	0.90	0.64	0.86
12h	0.75	0.65	0.99
12j	8.48	0.78	0.62
Tamoxifen	0.11	1.16	1.31

[a] The concentration required for 50% inhibition of cell growth.
* The most potent compound.

Based on these results, it is evident that there is a structure-activity relationship (SAR). Shown in Table 4, from the screening of the tested compounds against the HepG2 (liver) cell line, it was found that some derivatives, in which the amino group on the pyrazole ring is linked to a phenyl group, were more active than their respective analogues with a 4-methoxyphenyl group on that nitrogen atom. Thus, compounds **12c** (IC_{50}=0.66 nM) and **12e** (IC_{50}=0.09 nM) were found to be more potent than **12h** (IC_{50}=0.99 nM) and **12j** (IC_{50}=0.62 nM), respectively. However, compounds **4a** and **4b** were found to be of a comparable potency.

On the other hand, the investigation confirmed the prominent biological activity of the ferrocenyl moiety over other substituents, where among the tested Schiff bases, compound **12e** was found to be the most potent against the HepG2 (liver) cell line. The order of activity was **12e** (IC_{50}=0.09 nM)>**12d** (IC_{50}=0.35 nM)>**12c** (IC_{50}=0.66 nM). Similarly, **12j** (IC_{50}=0.62 nM) was more active than **12h** (IC_{50}=0.99 nM).

Experimental

Chemistry

All melting points were measured on a Gallenkamp melting point apparatus and were uncorrected. The IR spectra were recorded (KBr disk) on a Perkin Elmer 1650 FT-IR instrument. The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Varian spectrometer using DMSO-d_6 as a solvent and TMS as an internal standard. Chemical shifts were reported in ppm. Mass spectra were recorded on a Varian MAT 112 spectrometer at 70 eV. Elemental analyses were obtained from The Microanalytical Data Center at Cairo University, Egypt.

Progress of the reactions was monitored by thin-layer chromatography (TLC) using aluminum sheets coated with silica gel F_{254} (Merck), viewed by short-wavelength UV lamp detection. All evaporations were carried out under reduced pressure at 40 °C.

5-Amino-3-(arylamino)-1H-pyrazole-4-carboxamides (1a,b).

A mixture of cyanoacetamide (0.01 mol) and 4-(methoxyphenyl)isothiocyanate or phenylisothiocyanate (0.01 mol) was heated for 5-10 min in ethanol (25 mL) containing potassium hydroxide (0.01 mol). After cooling, methyl iodide (0.01 mol) was added. The reaction mixture was stirred at room temperature for 1 h then poured onto ice-water. The precipitated product [3-(4-arylamino)-2-cyano-3-(methylthio)acrylamide] was filtered off and recrystallized from ethanol, then its mixture with hydrazine hydrate (0.01 mol) was refluxed for 4 h in ethanol (30 mL) containing triethylamine as a catalyst. After evaporating the solvent under reduced pressure, the resulting solid product was collected by filtration and recrystallized from ethanol.

5-Amino-3-(phenylamino)-1H-pyrazole-4-carboxamide (1a)

Yield: 80%, white crystals, m.p. 179 °C [16].

5-Amino-3-[(4-methoxyphenyl)amino]-1H-pyrazole-4-carboxamide (1b)

Yield: 82%, white crystals, m.p. 200 °C. IR (KBr) v_{max}/cm^{-1} 3439, 3277, 3163 (NH, NH$_2$), 1659 (C=O), 1624 (C=N), 1558 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 3.64 (s, 3H, OCH$_3$), 5.74 (s, 2H, NH$_2$, D$_2$O exchangeable), 6.60 (s, 2H, NH$_2$, D$_2$O exchangeable), 6.75 (d, 2H, aromatic, *AB-system, J_{HH}=8.4* Hz), 7.24 (d, 2H, aromatic, *AB-system, J_{HH}=8.4* Hz), 8.68 (s, 1H, NH, D$_2$O exchangeable), 10.82 (s, 1H, NH, D$_2$O exchangeable). ^{13}C NMR (DMSO-d$_6$, δ ppm) 55.6 (-OCH$_3$), 86.2 (C$_4$, pyrazole), 114.6 (2C, aromatic), 117.4 (2C, aromatic), 137.0 (C, aromatic), 148.2 (C$_5$, pyrazole), 152.2 (C$_3$, pyrazole), 152.8 (C, aromatic), 167.2 (C=O, amide). MS *m/z* (%): 247 (59.90) [M$^+$]. Anal. Calcd. (%) for C$_{11}$H$_{13}$N$_5$O$_2$ (247.25): C, 53.43; H, 5.30; N, 28.32. Found: C, 53.35; H, 5.39; N, 28.21 %.

Synthesis of 2-(arylamino)-5,7-dimethylpyrazolo[1,5-a]pyrimidine-3-carboxamides (4a,b)

A mixture of compound **1a** or **1b** (0.01 mol) with acetylacetone **2** (0.01 mol) in glacial acetic acid (20 mL) was refluxed for 6 h, then poured onto crushed ice and the separated solid was filtered off, dried well, and recrystallized from ethanol to afford compounds **4a,b**.

5,7-Dimethyl-2-(phenylamino)pyrazolo[1,5-a]pyrimidine-3-carboxamide (4a)

Yield: 82%, white crystals, m.p. 275–277 °C. IR (KBr) v_{max}/cm^{-1} 3374, 3142 (NH, NH$_2$), 1658 (C=O), 1626, 1596 (C=N), 1562 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 2.50 (s, 3H, CH$_3$), 2.66 (s, 3H, CH$_3$), 6.90 (t, 1H, aromatic), 6.93 (s, 1H, pyrimidine H-6), 7.30 (t, 2H, aromatic), 7.47 (s, 2H, NH$_2$, D$_2$O exchangeable), 7.66 (d, 2H, aromatic, J_{HH}=7.65 Hz), 9.57 (s, 1H, NH, D$_2$O exchangeable). ^{13}C NMR (DMSO-d$_6$, δ ppm) 17.1 (-CH$_3$), 24.6 (-CH$_3$), 86.9 (C$_3$, pyrazolopyrimidine), 109.3 (C$_6$, pyrazolopyrimidine), 117.5 (2C, aromatic), 121.2 (C, aromatic), 129.5 (2C, aromatic), 140.8 (C$_{3a}$, pyrazolopyrimidine), 146.4 (C, aromatic), 146.7 (C$_7$, pyrazolopyrimidine), 156.7 (C$_2$, pyrazolopyrimidine), 161.3 (C$_5$, pyrazolopyrimidine), 166.4 (C=O, amide). MS m/z (%): 281 (6.59) [M$^+$]. Anal. Calcd. (%) for C$_{15}$H$_{15}$N$_5$O (281.31): C, 64.04; H, 5.37; N, 24.90. Found: C, 63.90; H, 5.45; N, 25.00 %.

2-[(4-Methoxyphenyl)amino]-5,7-dimethylpyrazolo[1,5-a]pyrimidine-3-carboxamide **(4b)**

Yield: 78%, white crystals, m.p. 260–262 °C. IR (KBr) v_{max}/cm^{-1} 3364, 3159 (NH, NH_2), 1655 (C=O), 1623, 1597 (C=N), 1564 (C=C, aromatic). ^1H NMR (DMSO-d_6, δ ppm) 2.50 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 6.89 (d, 2H, aromatic, J_{HH}=7.6 Hz), 6.90 (s, 1H, pyrimidine H-6), 7.47 (s, 2H, NH_2, D_2O exchangeable), 7.60 (d, 2H, aromatic, J_{HH}=7.6 Hz), 9.35 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ ppm) 17.2 (-CH_3), 24.6 (-CH_3), 55.7 (-OCH_3), 86.6 (C_3, pyrazolopyrimidine), 109.2 (C_6, pyrazolo-pyrimidine), 114.7 (2C, aromatic), 117.6 (2C, aromatic), 131.5 (C_{3a}, pyrazolopyrimidine), 133.8 (C, aromatic), 146.6 (C_7, pyrazolopyrimidine), 151.3 (C, aromatic), 156.9 (C_2, pyrazolopyrimidine), 161.4 (C_5, pyrazolopyrimidine), 166.4 (C=O, amide). MS m/z (%): 311 (47.17) [M$^+$]. Anal. Calcd. (%) for $C_{16}H_{17}N_5O_2$ (311.34): C, 61.72; H, 5.50; N, 22.49. Found: C, 61.83; H, 5.42; N, 22.40 %.

Synthesis of 7-amino-5-aryl-2-(arylamino)-6-cyanopyrazolo[1,5-a]pyrimidine-3-carboxamides (7a–f).

A mixture of compound **1a** or **1b** (0.01 mol) with arylidenemalononitriles **5a–c** (0.01 mol) and a catalytic amount of triethylamine (four drops) in absolute ethanol (30 mL) was refluxed for 6 h. The solvent was concentrated under reduced pressure and the solid obtained was collected and recrystallized from ethanol to give **7a–f**.

7-Amino-6-cyano-5-phenyl-2-(phenylamino)pyrazolo[1,5-a]pyrimidine-3-carboxamide **(7a)**

Yield: 78%, yellow crystals, m.p. > 300 °C. IR (KBr) v_{max}/cm^{-1} 3393, 3303, 3148 (NH, NH_2), 2212 (C≡N), 1658 (C=O), 1593 (C=N), 1564 (C=C, aromatic). ^1H NMR (DMSO-d_6, δ ppm) 6.94-7.55 (m, 6H, aromatic), 7.60 (s, 2H, NH_2, D_2O exchangeable), 7.81 (d, 2H, aromatic, J_{HH}=7.6 Hz), 7.86 (d, 2H, aromatic, J_{HH}=8.4 Hz), 8.97 (s, 2H, NH_2, D_2O exchangeable), 9.64 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ ppm) 74.6 (C_6, pyrazolopyrimidine), 89.9 (C_3, pyrazolopyrimidine), 116.5 (C≡N), 118.3 (2C, aromatic), 121.4 (C, aromatic), 128.5 (2C, aromatic), 129.2 (C, aromatic), 129.5 (2C, aromatic), 130.1 (2C, aromatic), 137.3 (C, aromatic), 140.6 (C_{3a}, pyrazolopyrimidine), 146.8 (C, aromatic), 150.2 (C_2, pyrazolopyrimidine), 156.7 (C_5, pyrazolopyrimidine), 161.9 (C_7, pyrazolo-pyrimidine), 166.3 (C=O, amide). MS m/z (%): 369 (29.30) [M$^+$]. Anal. Calcd. (%) for $C_{20}H_{15}N_7O$ (369.38): C, 65.03; H, 4.09; N, 26.54. Found: C, 65.17; H, 4.02; N, 26.44 %.

7-Amino-6-cyano-5-(4-methoxyphenyl)-2-(phenylamino)pyrazolo[1,5-a]pyrimidine-3-carboxamide **(7b)**

Yield: 65%, yellow crystals, m.p. > 300 °C. IR (KBr) v_{max}/cm^{-1} 3440, 3379, 3315 (NH, NH_2), 2217 (C≡N), 1662 (C=O), 1621, 1596 (C=N), 1560 (C=C, aromatic). ^1H NMR (DMSO-d_6, δ ppm) 3.82 (s, 3H, OCH_3), 6.94 (t, 1H, aromatic), 7.09 (d, 2H, aromatic, J_{HH}=8.4 Hz), 7.29 (2H, aromatic), 7.58 (s, 2H, NH_2, D_2O exchangeable), 7.80 (d, 2H, aromatic, J_{HH}=8.4 Hz), 7.86 (d, 2H, aromatic, J_{HH}=8.4 Hz), 8.90 (s, 2H, NH_2, D_2O exchangeable), 9.63 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ ppm) 55.9 (-OCH_3), 74.4 (C_6, pyrazolopyrimidine), 89.6 (C_3, pyrazolopyrimidine), 114.4 (2C, aromatic), 117.0 (C≡N), 118.0 (2C, aromatic), 121.4 (C, aromatic), 129.4 (C, aromatic), 129.5 (2C, aromatic), 130.9 (2C, aromatic), 140.4 (C_{3a}, pyrazolopyrimidine), 146.4 (C, aromatic), 150.0 (C_2, pyrazolopyrimidine), 156.8 (C, aromatic), 161.1 (C_5, pyrazolo-pyrimidine), 161.8 (C_7, pyrazolopyrimidine), 166.2 (C=O, amide). MS m/z (%): 401 (8.21)

[M$^+$+2H]. Anal. Calcd. (%) for $C_{21}H_{17}N_7O_2$ (399.41): C, 63.15; H, 4.29; N, 24.55. Found: C, 63.06; H, 4.35; N, 24.43 %.

7-Amino-6-cyano-5-(naphthalen-1-yl)-2-(phenylamino)pyrazolo[1,5-a]pyrimidine-3-carboxamide (7c)

Yield: 65%, orange crystals, m.p. 262–264 °C. IR (KBr) v_{max}/cm^{-1} 3413, 3358, 3275, 3165 (NH, NH$_2$), 2217 (C≡N), 1655 (C=O), 1593 (C=N), 1558 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 6.95-8.21 (m, 14H, aromatic and NH$_2$), 9.11 (s, 2H, NH$_2$, D$_2$O exchangeable), 9.65 (s, 1H, NH, D$_2$O exchangeable). Anal. Calcd. (%) for $C_{24}H_{17}N_7O$ (419.44): C, 68.72; H, 4.09; N, 23.38. Found: C, 68.85; H, 3.97; N, 23.45 %.

7-Amino-6-cyano-2-[(4-methoxyphenyl)amino]-5-phenylpyrazolo[1,5-a]pyrimidine-3-carboxamide (7d)

Yield: 70%, Yellow crystals, m.p. > 300 °C. IR (KBr) v_{max}/cm^{-1} 3399, 3333, 3151 (NH, NH$_2$), 2211 (C≡N), 1655 (C=O), 1597 (C=N), 1509 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 3.71 (s, 3H, OCH$_3$), 6.86-7.55 (m, 5H, aromatic), 7.58 (s, 2H, NH$_2$, D$_2$O exchangeable), 7.75 (d, 2H, aromatic, J_{HH}=8.4 Hz), 7.85 (d, 2H, aromatic, J_{HH}=8.4 Hz), 8.92 (s, 2H, NH$_2$, D$_2$O exchangeable), 9.43 (s, 1H, NH, D$_2$O exchangeable). ^{13}C NMR (DMSO-d$_6$, δ ppm) 55.6 (-OCH$_3$), 74.9 (C$_6$, pyrazolopyrimidine), 89.6 (C$_3$, pyrazolo-pyrimidine), 114.7 (2C, aromatic), 116.7 (C≡N), 119.3 (2C, aromatic), 128.8 (2C, aromatic), 129.0 (C, aromatic), 129.2 (2C, aromatic), 131.1 (C$_{3a}$, pyrazolopyrimidine), 133.9 (C, aromatic), 137.3 (C, aromatic), 149.8 (C, aromatic), 154.2 (C$_2$, pyrazolo-pyrimidine), 156.9 (C$_5$, pyrazolopyrimidine), 161.6 (C$_7$, pyrazolopyrimidine), 166.2 (C=O, amide). MS m/z (%): 397 (17.06) [M$^+$-2H]. Anal. Calcd. (%) for $C_{21}H_{17}N_7O_2$ (399.41): C, 63.15; H, 4.29; N, 24.55. Found: C, 63.24; H, 4.19; N, 24.60 %.

7-Amino-6-cyano-5-(4-methoxyphenyl)-2-[(4-methoxyphenyl)amino]pyrazolo[1,5-a]-pyrimidine-3-carboxamide (7e)

Yellow crystals, m.p. 259-261 °C, yield (73%). IR (KBr) v_{max}/cm^{-1} 3396, 3313, 3190 (NH, NH$_2$), 2210 (C≡N), 1646 (C=O), 1598 (C=N), 1568 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 3.71 (s, 3H, OCH$_3$), 3.83 (s, 3H, OCH$_3$), 6.85-7.09 (m, 4H, aromatic), 7.45 (s, 2H, NH$_2$, D$_2$O exchangeable), 7.75-7.86 (m, 4H, aromatic), 8.83 (s, 2H, NH$_2$, D$_2$O exchangeable), 9.43 (s, 1H, NH, D$_2$O exchangeable). ^{13}C NMR (DMSO-d$_6$, δ ppm) 55.6 (-2OCH$_3$), 74.1 (C$_6$, pyrazolopyrimidine), 89.8 (C$_3$, pyrazolopyrimidine), 114.7 (2C, aromatic), 114.9 (2C, aromatic), 117.2 (C≡N), 119.5 (2C, aromatic), 129.9 (C, aromatic), 130.2 (2C, aromatic), 133.6 (C, aromatic), 140.2 (C$_{3a}$, pyrazolopyrimidine), 149.6 (C, aromatic), 154.4 (C$_2$, pyrazolopyrimidine), 156.3 (C, aromatic), 161.5 (C$_5$, pyrazolo-pyrimidine), 161.9 (C$_7$, pyrazolopyrimidine), 166.3 (C=O, amide). Anal. Calcd. (%) for $C_{22}H_{19}N_7O_3$ (429.43): C, 61.53; H, 4.46; N, 22.83. Found: C, 61.40; H, 4.52; N, 22.90 %.

7-Amino-6-cyano-2-[(4-methoxyphenyl)amino]-5-(naphthalen-1-yl)pyrazolo[1,5-a]-pyrimidine-3-carboxamide (7f)

Yield: 70%, reddish-orange crystals, m.p. 252–254 °C. IR (KBr) v_{max}/cm^{-1} 3416, 3365, 3142 (NH, NH$_2$), 2210 (C≡N), 1641 (C=O), 1594 (C=N), 1563 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 3.68 (s, 3H, OCH$_3$), 6.84 (d, 2H, aromatic, J_{HH}=8.4 Hz), 7.16 (d, 1H, aromatic, J_{HH}=8.4 Hz), 7.29 (s, 1H, aromatic), 7.47 (s, 2H, NH$_2$, D$_2$O exchangeable), 7.64-7.72 (m, 3H, aromatic), 8.05 (d, 1H, aromatic, J_{HH}=7.6 Hz), 8.21 (d, 2H, aromatic, J_{HH}=8.4

Hz), 8.34 (d, 1H, aromatic, J_{HH}=*6.9 Hz*), 8.82 (s, 2H, NH$_2$, D$_2$O exchangeable), 9.65 (s, 1H, NH, D$_2$O exchangeable). MS m/z (%): 449 (0.39) [M$^+$]. Anal. Calcd. (%) for C$_{25}$H$_{19}$N$_7$O$_2$ (449.46): C, 66.81; H, 4.26; N, 21.81. Found: C, 66.95; H, 4.21; N, 21.74 %.

Synthesis of 3-(arylamino)-5-[(2-oxoindolin-3-ylidene)amino]-1H-pyrazole-4-carboxamides (9a,b).

A mixture of compound **1a** or **1b** (0.01 mol) with isatin **8** (0.01 mol) and triethylamine (3 drops) was refluxed in ethanol (30 mL) for 4 h. The precipitate obtained was filtered off, well-dried, and recrystallized from ethanol to give **9a,b**.

5-[(2-Oxo-1,2-dihydro-3H-indol-3-ylidene)amino]-3-(phenylamino)-1H-pyrazole-4-carboxamide (9a)

Yield: 82%, dark brown crystals, m.p. > 300 °C. IR (KBr) v_{max}/cm^{-1} 3427, 3317, 3181 (NH, NH$_2$), 1694, 1623 (C=O), 1597 (C=N), 1569 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 6.89-7.53 (m, 11H, aromatic and NH$_2$), 9.14 (s, 1H, NH, D$_2$O exchangeable), 10.95 (s, 1H, NH, D$_2$O exchangeable), 12.98 (s, 1H, NH, D$_2$O exchangeable). Anal. Calcd. (%) for C$_{18}$H$_{14}$N$_6$O$_2$ (346.34): C, 62.42; H, 4.07; N, 24.27. Found: C, 62.30; H, 4.14; N, 24.21 %.

3-[(4-Methoxyphenyl)amino]-5-[(2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino]-1H-pyrazole-4-carboxamide (9b)

Yield: 82%, dark green crystals, m.p. > 300 °C. IR (KBr) v_{max}/cm^{-1} 3414, 3308, 3162 (NH, NH$_2$), 1688, 1623 (C=O), 1599 (C=N), 1570 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 3.71 (s, 3H, OCH$_3$), 6.82-7.69 (m, 10H, aromatic and NH$_2$), 8.94 (s, 1H, NH, D$_2$O exchangeable), 10.94 (s, 1H, NH, D$_2$O exchangeable), 12.92 (s, 1H, NH, D$_2$O exchangeable). MS m/z (%): 376 (73.36) [M$^+$]. Anal. Calcd. (%) for C$_{19}$H$_{16}$N$_6$O$_3$ (376.37): C, 60.63; H, 4.28; N, 22.33. Found: C, 60.50; H, 4.33; N, 22.27 %.

Synthesis of 3-(arylamino)-5-(arylmethyleneamino)-1H-pyrazole-4-carboxamides (12a–j).

A mixture of compound **1a** or **1b** (0.01 mol) with an aromatic-, heteroaromatic aldehyde, or ferrocenecarboxaldehyde **11a–e** (0.01 mol) and triethylamine (four drops) was refluxed in absolute ethanol (30 mL) for 6 h. The volatile materials were removed under reduced pressure; the solid obtained was collected and recrystallized from ethanol to give **12a–j**.

5-(Benzylideneamino)-3-(phenylamino)-1H-pyrazole-4-carboxamide (12a)

Yield: 76%, yellow crystals, m.p. 269–271 °C. IR (KBr) v_{max}/cm^{-1} 3384, 3190 (NH, NH$_2$), 1653 (C=O), 1593 (C=N), 1562 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 6.83–7.38 (m, 4H, aromatic), 7.47 (s, 2H, NH$_2$, D$_2$O exchangeable), 7.56-7.96 (m, 6H, aromatic), 8.91 (s, 1H, NH, D$_2$O exchangeable), 9.07 (s, 1H, –N=CH–), 12.75 (s, 1H, NH, D$_2$O exchangeable). ^{13}C NMR (DMSO-d$_6$, δ ppm) 92.9 (C$_4$, pyrazole), 116.4 (2C, aromatic), 119.7 (C, aromatic), 127.9 (2C, aromatic), 129.0 (2C, aromatic), 129.5 (2C, aromatic), 130.8 (C, aromatic), 135.3 (C, aromatic), 141.9 (C, aromatic), 148.2 (-N=CH-), 153.4 (C$_3$ & C$_5$, pyrazole), 167.4 (C=O, amide). Anal. Calcd. (%) for C$_{17}$H$_{15}$N$_5$O (305.33): C, 66.87; H, 4.95; N, 22.94. Found: C, 66.94; H, 4.87; N, 22.89 %.

5-[(4-Methoxybenzylidene)amino]-3-(phenylamino)-1H-pyrazole-4-carboxamide (12b)

Yield: 65%, yellow crystals, m.p. 260 °C. IR (KBr) v_{max}/cm^{-1} 3399, 3317, 3203 (NH, NH$_2$), 1648 (C=O), 1596 (C=N), 1556 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 3.85 (s, 3H, OCH$_3$), 6.83 (t, 1H, aromatic), 7.12 (d, 2H, aromatic, J_{HH}=7.6 Hz), 7.25-7.38 (m, 4H, aromatic), 7.52 (s, 2H, NH$_2$, D$_2$O exchangeable), 7.94 (d, 2H, aromatic, J_{HH}=7.6 Hz), 8.81 (s, 1H, NH, D$_2$O exchangeable), 9.10 (s, 1H, –N=CH–), 12.62 (s, 1H, NH, D$_2$O exchangeable). ^{13}C NMR (DMSO-d$_6$, δ ppm) 56.1 (-OCH$_3$), 93.2 (C$_4$, pyrazole), 115.2 (2C, aromatic), 116.6 (2C, aromatic), 119.9 (C, aromatic), 128.1 (C, aromatic), 129.5 (2C, aromatic), 131.9 (2C, aromatic), 142.3 (C, aromatic), 147.8 (-N=CH-), 153.1 (C$_3$ & C$_5$, pyrazole), 163.7 (C, aromatic), 166.9 (C=O, amide). MS m/z (%): 335 (100) [M$^+$]. Anal. Calcd. (%) for C$_{18}$H$_{17}$N$_5$O$_2$ (335.36): C, 64.47; H, 5.11; N, 20.88. Found: C, 64.57; H, 5.06; N, 20.97 %.

5-[(Naphthalen-1-ylmethylidene)amino]-3-(phenylamino)-1H-pyrazole-4-carboxamide (12c)

Yield: 76%, buff crystals, m.p. 263–265 °C. IR (KBr) v_{max}/cm^{-1} 3357, 3144 (NH, NH$_2$), 1645 (C=O), 1592 (C=N), 1558 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 6.83-8.83 (m, 14H, aromatic and NH$_2$), 9.10 (s, 1H, –N=CH–), 9.66 (s, 1H, NH, D$_2$O exchangeable), 12.89 (s, 1H, NH, D$_2$O exchangeable). Anal. Calcd. (%) for C$_{21}$H$_{17}$N$_5$O (355.39): C, 70.97; H, 4.82; N, 19.71. Found: C, 70.88; H, 4.88; N, 19.79 %.

5-{[(5-Methylfuran-2-yl)methylidene]amino}-3-(phenylamino)-1H-pyrazole-4-carboxamide (12d)

Yield: 80%, yellow crystals, m.p. 235–238 °C. IR (KBr) v_{max}/cm^{-1} 3367, 3181 (NH, NH$_2$), 1651 (C=O), 1594 (C=N), 1565 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 2.40 (s, 3H, CH$_3$), 6.43-7.35 (m, 7H, aromatic, furan H-4 and furan H-3), 7.48 (s, 2H, NH$_2$, D$_2$O exchangeable), 8.56 (s, 1H, NH, D$_2$O exchangeable), 9.04 (s, 1H, –N=CH–), 12.59 (s, 1H, NH, D$_2$O exchangeable). ^{13}C NMR (DMSO-d$_6$, δ ppm) 14.3 (-CH$_3$), 93.8 (C$_4$, pyrazole), 110.9 (C$_4$, furan), 116.6 (C$_3$, furan), 120.0 (2C, aromatic), 123.7 (C, aromatic), 129.5 (2C, aromatic), 141.9 (C, aromatic), 147.4 (-N=CH-), 149.6 (C$_2$, furan), 150.1 (C$_5$, furan), 153.4 (C$_3$ & C$_5$, pyrazole), 166.8 (C=O, amide). MS m/z (%): 309 (21.84) [M$^+$]. Anal. Calcd. (%) for C$_{16}$H$_{15}$N$_5$O$_2$ (309.32): C, 62.13; H, 4.89; N, 22.64. Found: C, 62.00; H, 4.94; N, 22.55 %.

5-[(Ferrocene-1-ylmethylidene)amino]-3-(phenylamino)-1H-pyrazole-4-carboxamide (12e)

Yield: 83%, red-pirck crystals, m.p. 255 °C. IR (KBr) v_{max}/cm^{-1} 3359, 3168 (NH, NH$_2$), 1660 (C=O), 1588 (C=N), 1563 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 4.29 (s, 5H, C$_5$H$_5$, ferrocene ring protons), 4.68 (s, 2H, C$_5$H$_4$, ferrocene ring protons), 4.88 (s, 2H, C$_5$H$_4$, ferrocene ring protons), 6.79-7.28 (m, 5H, aromatic), 7.50 (s, 2H, NH$_2$, D$_2$O exchangeable), 8.72 (s, 1H, NH, D$_2$O exchangeable), 9.03 (s, 1H, –N=CH–), 12.55 (s, 1H, NH, D$_2$O exchangeable). ^{13}C NMR (DMSO-d$_6$, δ ppm) 70.2 (5C, ferrocene ring), 73.3 (4C, ferrocenyl ring), 79.1 (C, ferrocenyl ring), 92.8 (C$_4$, pyrazole), 116.6 (2C, aromatic), 119.8 (C, aromatic), 129.5 (2C, aromatic), 142.0 (C, aromatic), 148.3 (-N=CH-), 153.2 (C$_3$ & C$_5$, pyrazole), 167.0 (C=O, amide). MS m/z (%): 413 (100%) [M$^+$]. Anal. Calcd. (%) for C$_{21}$H$_{19}$FeN$_5$O (413.25): C, 61.03; H, 4.63; N, 16.95. Found: C, 60.90; H, 4.69; N, 17.05 %.

5-(Benzylideneamino)-3-[(4-methoxyphenyl)amino]-1H-pyrazole-4-carboxamide (12f)

Yield: 79%, orange crystals, m.p. 260–262 °C. IR (KBr) v_{max}/cm^{-1} 3396, 3181 (NH, NH$_2$), 1655 (C=O), 1594 (C=N), 1565 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 3.68 (s, 3H, OCH$_3$), 6.85-7.37 (m, 5H, aromatic), 7.55 (s, 2H, NH$_2$, D$_2$O exchangeable), 7.59 (d, 2H, aromatic, J_{HH}=7.6 Hz), 7.95 (d, 2H, aromatic, J_{HH}=7.6 Hz), 8.82 (s, 1H, NH, D$_2$O exchangeable), 8.91 (s, 1H, –N=CH–), 12.58 (s, 1H, NH, D$_2$O exchangeable). ^{13}C NMR (DMSO-d$_6$, δ ppm) 55.6 (-OCH$_3$), 92.8 (C$_4$, pyrazole), 114.9 (2C, aromatic), 118.2 (2C, aromatic), 128.2 (2C, aromatic), 129.5 (2C, aromatic), 131.3 (C, aromatic), 133.6 (C, aromatic), 135.1 (C, aromatic), 135.7 (C, aromatic), 148.1 (-N=CH-), 153.3 (C$_3$ & C$_5$, pyrazole), 167.3 (C=O, amide). Anal. Calcd. (%) for C$_{18}$H$_{17}$N$_5$O$_2$ (335.36): C, 64.47; H, 5.11; N, 20.88. Found: C, 64.56; H, 5.06; N, 20.75 %.

5-[(4-Methoxybenzylidene)amino]-3-[(4-methoxyphenyl)amino]-1H-pyrazole-4-carboxamide (12g)

Yield: 81%, yellow crystals, m.p. >300 °C. IR (KBr) v_{max}/cm^{-1} 3395, 3194 (NH, NH$_2$), 1651 (C=O), 1598 (C=N), 1511 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 3.68 (s, 3H, OCH$_3$), 3.83 (s, 3H, OCH$_3$), 6.83-7.26 (m, 6H, aromatic), 7.43 (s, 2H, NH$_2$, D$_2$O exchangeable), 7.91 (d, 2H, aromatic, J_{HH}=8.4 Hz), 8.80 (s, 1H, NH, D$_2$O exchangeable), 8.82 (s, 1H, –N=CH–), 12.50 (s, 1H, NH, D$_2$O exchangeable). ^{13}C NMR (DMSO-d$_6$, δ ppm) 55.6 (-2OCH$_3$), 92.7 (C$_4$, pyrazole), 114.9 (2C, aromatic), 115.4 (2C, aromatic), 117.8 (2C, aromatic), 128.2 (C, aromatic), 131.6 (2C, aromatic), 133.5 (C, aromatic), 135.8 (C, aromatic), 147.9 (-N=CH-), 153.2 (C$_3$ & C$_5$, pyrazole), 163.9 (C, aromatic), 166.9 (C=O, amide). Anal. Calcd. (%) for C$_{19}$H$_{19}$N$_5$O$_3$ (365.39): C, 62.46; H, 5.24; N, 19.17. Found: C, 62.58; H, 5.18; N, 19.10 %.

3-[(4-Methoxyphenyl)amino]-5-[(naphthalen-1-ylmethylidene)amino]-1H-pyrazole-4-carboxamide (12h)

Yield: 68%, reddish-orange crystals, m.p. 250–252 °C. IR (KBr) v_{max}/cm^{-1} 3365, 3149 (NH, NH$_2$), 1643 (C=O), 1595 (C=N), 1563 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 3.69 (s, 3H, OCH$_3$), 6.86-8.32 (m, 13H, aromatic and NH$_2$), 8.85 (s, 1H, NH, D$_2$O exchangeable), 9.66 (s, 1H, –N=CH–), 12.78 (s, 1H, NH, D$_2$O exchangeable). ^{13}C NMR (DMSO-d$_6$, δ ppm) 55.7 (-OCH$_3$), 93.1 (C$_4$, pyrazole), 114.9 (2C, aromatic), 118.3 (2C, aromatic), 123.7 (C, aromatic), 126.2 (C, aromatic), 127.1 (2C, aromatic), 128.4 (C, aromatic), 129.6 (2C, aromatic), 130.6 (2C, aromatic), 131.8 (2C, aromatic), 134.0 (C, aromatic), 148.6 (-N=CH-), 153.2 (C$_3$ & C$_5$, pyrazole), 166.9 (C=O, amide). Anal. Calcd. (%) for C$_{22}$H$_{19}$N$_5$O$_2$ (385.42): C, 68.56; H, 4.97; N, 18.17. Found: C, 68.41; H, 5.04; N, 18.07 %.

3-[(4-Methoxyphenyl)amino]-5-{[(5-methylfuran-2-yl)methylidene]amino}-1H-pyrazole-4-carboxamide(12i)

Yield: 70%, buff crystals, m.p. 232–234 °C. IR (KBr) v_{max}/cm^{-1} 3346, 3157 (NH, NH$_2$), 1652 (C=O), 1588 (C=N), 1512 (C=C, aromatic). ^{1}H NMR (DMSO-d$_6$, δ ppm) 2.39 (s, 3H, CH$_3$), 3.68 (s, 3H, OCH$_3$), 6.42-7.35 (m, 6H, aromatic, furan H-4 and furan H-3), 7.42 (s, 2H, NH$_2$, D$_2$O exchangeable), 8.56 (s, 1H, NH, D$_2$O exchangeable), 8.78 (s, 1H, –N=CH–), 12.45 (s, 1H, NH, D$_2$O exchangeable). ^{13}C NMR (DMSO-d$_6$, δ ppm) 14.2 (-CH$_3$), 55.6 (-OCH$_3$), 93.6 (C$_4$, pyrazole), 110.8 (C$_4$, furan), 116.3 (C$_3$, furan), 120.1 (2C, aromatic), 123.7 (2C, aromatic), 132.2 (C, aromatic), 147.1 (-N=CH-), 149.0 (C$_2$, furan), 150.5 (C$_5$,

furan), 151.4 (C, aromatic), 153.0 (C_3 & C_5, pyrazole), 167.1 (C=O, amide). Anal. Calcd. (%) for $C_{17}H_{17}N_5O_3$ (339.35): C, 60.17; H, 5.05; N, 20.64. Found: C, 60.06; H, 5.11; N, 20.73 %.

5-[(Ferrocene-1-ylmethylidene)amino]-3-[(4-methoxyphenyl)amino]-1H-pyrazole-4-carboxamide (12j)

Yield: 76%, brown crystals, m.p. 230–232 °C. IR (KBr) v_{max}/cm^{-1} 3378, 3202 (NH, NH_2), 1631 (C=O), 1589 (C=N), 1562 (C=C, aromatic). ^{1}H NMR (DMSO-d_6, δ ppm) 3.67 (s, 3H, OCH_3), 4.28 (s, 5H, C_5H_5, ferrocene ring protons), 4.67 (s, 2H, C_5H_4, ferrocene ring protons), 4.87 (s, 2H, C_5H_4, ferrocene ring protons), 6.83–7.22 (m, 4H, aromatic), 7.43 (s, 2H, NH_2, D_2O exchangeable), 8.70 (s, 1H, NH, D_2O exchangeable), 8.78 (s, 1H, –N=CH–), 12.49 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ ppm) 55.7 (-OCH_3), 70.1 (5C, ferrocene ring), 73.3 (4C, ferrocenyl ring), 79.1 (C, ferrocenyl ring), 92.6 (C_4, pyrazole), 114.8 (2C, aromatic), 117.7 (2C, aromatic), 133.8 (C, aromatic), 135.8 (C, aromatic), 148.4 (-N=CH-), 153.2 (C_3 & C_5, pyrazole), 167.1 (C=O, amide). MS m/z (%): 443 (10.10%) [M$^+$]. Anal. Calcd. (%) for $C_{22}H_{21}FeN_5O_2$ (443.28): C, 59.61; H, 4.78; N, 15.80. Found: C, 59.71; H, 4.71; N, 15.70 %.

Biological experiments

In vitro antitumor screening

The tested compounds were subjected to *in vitro* disease-oriented primary antitumor screening. The different cell lines of tumor cell lines were utilized. The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96-well microtiter plates in 100 mL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to the addition of the experimental drugs. After 24 h, two plates of each cell line were fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition. Experimental drugs were solubilized in DMSO at 400-fold of the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 mg/ml Gentamicin. Additional four 10-fold or 1/2 log serial dilutions were made to provide a total of five drug concentrations plus the control. Aliquots of 100 mL of these different drug dilutions were added to the appropriate microtiter wells already containing 100 mL of medium, resulting in the required final drug concentrations. Following drug addition, the plates were incubated for an additional 48 h at 37°C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 mL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4°C. The supernatant layer was discarded, and the plates were washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (100 mL) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air-dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm.

For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 mL of 80% TCA (final concentration, 16% TCA). The parameter used here was GI_{50}, which is the log10 concentration at which PG is 50, and was calculated for each cell line [19–21].

Conclusion

In conclusion, 5-amino-1*H*-pyrazole-4-carboxamide derivatives **1a,b** were used as starting materials for the synthesis of some new pyrazolo[1,5-*a*]pyrimidine derivatives and Schiff bases. The new synthesized compounds were characterized by analytical and spectroscopic data. Some selected new compounds were screened for their potential antitumor activities. The results of the cytotoxicity for the tested compounds against different human cancer cell lines indicated that most of them exhibit a high cytotoxicity at very low concentrations in comparison with the reference drugs used, and pyrazolo[1,5-*a*]-pyrimidine derivatives **4a**, **7e,** and **7f** were found to have the most potent growth inhibitory activity against MCF7, ovarial carcinoma (SK OV-3), leukemia (K562), and HeLa (cervical) human tumor cell lines. On the other hand, Schiff bases **12b–e** and **12j** were found to be the most potent against cervical carcinoma (KB), CNS cancer (SF-268), non-small cell lung cancer (NCl H460), colonadenocarcinoma (RKOP 27), anti-leukemia (HL60, U937), melanoma (SK-MEL-28), neuroblastoma (GOTO, NB-1), HT1080 (fibrosarcoma), and HepG2 (liver) human tumor cell lines.

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Authors' Statement

Competing Interests

The authors declare no conflict of interest.

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