Tetrahedron 68 (2012) 9658–9664

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

One-pot synthesis and antiproliferative evaluation of pyrazolo[3,4-*d*]pyrimidine derivatives

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ARTICLE INFO

Article history:
Received 11 July 2012
Received in revised form 3 September 2012
Accepted 11 September 2012
Available online 16 September 2012

Keywords:
Pyrazolo[3,4-*d*]pyrimidines
5-Aminopyrazoles
Antiproliferative activity
One-pot synthesis

ABSTRACT

An efficient one-pot methodology for the synthesis of pyrazolo[3,4-*d*]pyrimidines was developed by using 5-aminopyrazoles with formamide in presence of PBr_3 as the coupling agent. Among the examples presented in this work, compounds **41** and **54**–**56** with phenyl or 2-quinolinyl groups at *N*-1 and *p*-Me–Ph, *p*-Cl–Ph, or *p*-OMe–Ph group at C-3 position in the pyrazole ring possessed better potency against NCI-H226 and NPC-TW01 cancer cells with GI_{50} values between 18 μM and 39 μM.

1. Introduction

Pyrazolopyrimidines and related fused heterocycles have been found to possess significant pharmacological activities,[1] such as antibacterial,[2] antifungal,[3,4] antitumor,[5] herbicidal,[6] antivirus,[7] and as the effective inhibitors of inflammatory mediators in intact cells.[8] Owing to the structural similarity of pyrazolo[3,4-*d*]pyrimidines with purines,[9] they are also known to function as CNS (Central Nervous System) depressants,[10] neuroleptic agents,[11] and as tuberculostatic.[12] Moreover, some novel pyrazolo[3,4-*d*]pyrimidine derivatives determine the antioxidant properties to play an important role in numerous degenerative or chronic diseases, such as atherosclerosis and cancer.[13]

Most of synthesis approach of pyrazolopyrimidines were performed through heterocyclization reaction using 5-amino-4-formypyrazoles,[14] 5-amino-1*H*-4-pyrazolcarbonitriles,[2] 5-amino-1*H*-4-pyrazolcarboxamides[2] or other similar chemical reagents as the starting substrates.[15–17] Herein, we developed an efficient one-pot synthesis of pyrazolo[3,4-*d*]pyrimidines by treating 5-aminopyrazoles, formamide using PBr_3 as the coupling agent. Furthermore, the cytotoxicities of these pyrazolo[3,4-*d*]pyrimidine derivatives were explored for realizing the structure–activity relationship and identifying the structural fragments responsible for the biological activity. Among the compounds, pyrazolo[3,4-*d*]pyrimidines **41** and **54**–**56** possessed better refficacy to inhibit the growth of NCI-H226 and NPC-TW01 cancer cells with GI_{50} values of 18–39 μM.

2. Result and discussion

2.1. Synthesis of pyrazolo[3,4-d]pyrimidine derivatives

Scheme 1 shows the newly developed one-pot methodology for synthesis of pyrazolo[3,4-*d*]pyrimidines. Variation of 5-amino-1,3-disubstituted pyrazole substrates **1**–**28** were prepared as the starting materials by following our previous developed efficient synthesis methodology.[18] The reliable model procedure involved the treatment of 5-aminopyrazoles with 3.0 equiv of PBr_3 in formamide at 60 °C for 0.5–1.0 h. After aqueous worked-up and purified by column chromatography on silica gel, the corresponding pyrazolo[3,4-*d*]pyrimidine products **29**–**58** was isolated in good to excellent yields (>76%, see Scheme 1 and Table 1).

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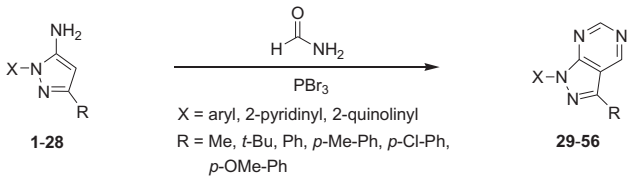
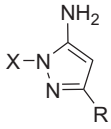
http://dx.doi.org/10.1016/j.tet.2012.09.054



Scheme 1.

Table 1
The results of the one-pot synthesis of pyrazolo[3,4-*d*]pyrimidines from 5-aminopyrazoles, formamide, and PBr_3

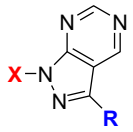


Pyrazolo[3,4-*d*]pyrimidine **29**–**56**

Substrates	X	R	No.	Yields (%)
1	Ph	Ph	**29**	96
2	*o*-Me–Ph	Ph	**30**	93
3	*o*-Cl–Ph	Ph	**31**	91
4	*m*-Me–Ph	Ph	**32**	92
5	*m*-Cl–Ph	Ph	**33**	96
6	*m*-NO_2–Ph	Ph	**34**	87
7	*p*-Cl–Ph	Ph	**35**	91
8	*p*-Br–Ph	Ph	**36**	95
9	*p*-OMe–Ph	Ph	**37**	89
10	Ph	Me	**38**	93
11	Ph	*t*-Bu	**39**	91
12	Ph	*p*-Me–Ph	**40**	93
13	Ph	*p*-Cl–Ph	**41**	91
14	Ph	*p*-OMe–Ph	**42**	94
15	*o*-Cl–Ph	*p*-Cl–Ph	**43**	93
16	*p*-Cl–Ph	*p*-Cl–Ph	**44**	95
17	2-Pyridinyl	Me	**45**	97
18	2-Pyridinyl	*t*-Bu	**46**	93
19	2-Pyridinyl	Ph	**47**	91
20	2-Pyridinyl	*p*-Me–Ph	**48**	92
21	2-Pyridinyl	*p*-Cl–Ph	**49**	94
22	2-Pyridinyl	*p*-OMe–Ph	**50**	89
23	2-Quinolinyl	Me	**51**	84
24	2-Quinolinyl	*t*-Bu	**52**	86
25	2-Quinolinyl	Ph	**53**	92
26	2-Quinolinyl	*p*-Me–Ph	**54**	89
27	2-Quinolinyl	*p*-Cl–Ph	**55**	87
28	2-Quinolinyl	*p*-OMe–Ph	**56**	76

To evaluate the one-pot synthesis method for the preparation of pyrazolo[3,4-*d*]pyrimidines, 5-amino-1-3-diphenylpyrazole (**1**) was used as the model case. Various coupling agents with various equivalents were used to provide the optimal condition. These reagents include benzoyl chloride (PhCOCl), oxalyl chloride (ClCO-COCl), phosphorous tribromide (PBr_3), thionyl chloride ($SOCl_2$), and *p*-toluenesulfonic acid (TsOH). We found the use of 3.0 equiv of PBr_3 can be provide the best yield of desired pyrazolo[3,4-*d*]pyrimidine (**29**, 96% yield). Employing the same reaction condition to 5-aminopyrazole substrates **2**–**9** bearing *N*1 aryl group with various substituents, including *o*-Me, *o*-Cl, *m*-Me, *m*-Cl, *m*-NO_2, *p*-Cl, and *p*-OMe, the one-pot heterocyclization methodology also smoothly gave the corresponding pyrazolo[3,4-*d*]pyrimidines **30**–**37** in 87–97% yields (see Scheme 1 and Table 2). Furthermore, we extended this new method toward 5-amino-1-aryl-3-substituted pyrazoles **10**–**16**, which contain methyl, *tert*-butyl, *p*-Me–Ph, position *p*-OMe–Ph groups at C-3 or Ph, *m*-Cl–Ph, *p*-OMe–Ph groups at *N*1-substituted position in pyrazolic ring. The corresponding **38**–**44** were obtained in 91–95% isolated yields (see Scheme 1 and Table 1). This same reaction condition was also applied to 5-amino-1-(2-pyridinyl)-3-arylpyrazole **17**–**22**, or 5-amino-1-(2-quinolinyl)-3-arylpyrazole **23**–**28**. The corresponding pyrazolo[3,4-*d*]pyrimidines **45**–**50**, and **51**–**56** were obtained in 89–94% and 76–92% yields, respectively (see Scheme 1 and Table 1). All of pyrazolo[3,4-*d*]pyrimidines **29**–**56** were fully characterized by spectroscopic methods. For example, compound **29** presented two singlet peaks at δ 9.02 and 9.31 ppm for pyrimidine ring in ^{1}H NMR. In ^{13}C NMR spectrum, compound **29** possessed characterization absorptions at δ 155.0 ppm for pyrimidine carbon N=^{13}CH–N=C, at δ 34.4 ppm for –^{13}C(CH$_3$)$_3$, and at δ 30.0 ppm for –C (^{13}CH$_3$)$_3$.

Table 2
The inhibitory activity of the pyrazolo[3,4-*d*]pyrimidine derivatives in NCI-H226, NPC-TW01, and Jurkat



Compounds	Pyrazolo[3,4-*d*]pyrimidines **29**–**56**		GI_{50} (μM)[a,b]		
	X (*N*-1)	R (C-3)	NCI-H226	NPC-TW01	Jurkat
29	Ph	Ph	46	41	41
30	*o*-Me–Ph	Ph	85	95	82
31	*o*-Cl–Ph	Ph	44	58	57
32	*m*-Me–Ph	Ph	75	85	72
33	*m*-Cl–Ph	Ph	50	79	>100
34	*p*-NO_2–Ph	Ph	60	62	99
35	*p*-Cl–Ph	Ph	45	59	58
36	*p*-Br–Ph	Ph	47	42	45
37	*p*-OMe–Ph	Ph	46	55	55
38	Ph	Me	75	78	>100
39	Ph	*t*-Bu	67	54	39
40	Ph	*p*-Me–Ph	35	49	48
41	Ph	*p*-Cl–Ph	18	23	36
42	Ph	*p*-OMe–Ph	46	83	90
43	*o*-Cl–Ph	*p*-Cl–Ph	>100	>100	>100
44	*p*-Cl–Ph	*p*-Cl–Ph	>100	>100	>100
45	2-Pyridinyl	Me	>100	>100	>100
46	2-Pyridinyl	*t*-Bu	>100	>100	>100
47	2-Pyridinyl	Ph	>100	>100	>100
48	2-Pyridinyl	*p*-Me–Ph	>100	>100	>100
49	2-Pyridinyl	*p*-Cl–Ph	68	>100	>100
50	2-Pyridinyl	*p*-OMe–Ph	65	>100	61
51	2-Quinolinyl	Me	>100	>100	>100
52	2-Quinolinyl	*t*-Bu	>100	>100	92
53	2-Quinolinyl	Ph	67	>100	>100
54	2-Quinolinyl	*p*-Me–Ph	29	30	54
55	2-Quinolinyl	*p*-Cl–Ph	39	35	69
56	2-Quinolinyl	*p*-OMe–Ph	37	36	>100

[a] NCI-H226: human lung carcinoma; NPC-TW01: human nasopharyngeal carcinoma; Jurkat: human T-cell leukemia.

[b] All tested compounds were dissolved in 100% DMSO at a concentration of 20 mM as the stock solution. Cells were cultured without or in the presence of the methnimidamide derivatives at different concentrations for 72 h. Cell survival was determined by MTT assay. Drug molar concentration causing 50% cell growth inhibition (GI_{50}) was calculated. Each value represents the mean±SD of three independent experiments.

2.2. Antiproliferative activities of pyrazolo[3,4-*d*]pyrimidine derivatives

The growth inhibitory activities of all pyrazolo[3,4-*d*]pyrimidines **29**–**56** were evaluated against a panel of human cancer cell lines in vitro, including lung carcinoma (NCI-H226), nasopharyngeal (NPC-TW01), and T-cell leukemia (Jurkat) cells. The GI_{50} value indicates the concentration of the compound that results in a 50% decrease in the cell growth relative to the vehicle. The results are presented in Table 2 and indicated that pyrazolo[3,4-*d*]pyrimidines

29–**56** compounds showed the better inhibitory potency against nasopharyngeal (NPC-TW01) and lung carcinoma (NCI-H226).

Compound **29** was selected as the compared model for the inhibitory activity study. Their GI_{50} values are 46 μM (NCI-H226), 41 μM (NPC-TW01), and 41 μM (Jurkat), respectively. In comparison with compounds **30**–**37** containing various substituents, including H, *o*-Me, *o*-Cl, *m*-Me, *m*-Cl, *p*-NO_2, *p*-Cl, *p*-Br, and *p*-OMe, at *N*-1 of the phenyl ring, the results showed that pyrazolo[3,4-*d*]pyrimidines **31**, **35**–**37** possessed similar inhibitory activity against NCI-H226, NPC-TW01 and Jurkat with GI_{50} values between 42 μM and 59 μM. In particular, compounds **31** and **36** with *o*-Cl or *p*-Br at *N*-1 of the phenyl ring showed the better inhibitory potency against NCI-H226 near 44 μM and 47 μM and compound **36** also presented better against NPC-TW01 and Jurkat (42 μM and 45 μM, see Table 2). However, compound **31** with *m*-Cl–Ph at *N*-1 on pyrazole ring displayed poor inhibitory activity (see Table 2).

For further structure–activity relationship study, we modified pyrazolo[3,4-*d*]pyrimidine **29** to compounds **38**–**42** with various substituents at C-3 position in pyrazole ring, such as methyl (**38**), *tert*-butyl (**39**), *p*-Me–Ph (**40**), *p*-Cl–Ph (**41**), and *p*-OMe–Ph (**42**). Their antiproliferative activities were presented in Table 2. Most of the modified pyrazolo[3,4-*d*]pyrimidine **38**–**42** exhibited similar potency. Particularly, compounds **41** showed the best antiproliferative activity against NCI-H226 (18 μM), NPC-TW01 (23 μM), and Jurkat (36 μM, see Table 2), which possess *p*-Cl–Ph group at C-3 position on the pyrazole ring.

Based on the results presented in Table 2, we believed that the *o*-Cl and *p*-Cl at *N*-1 of the phenyl ring (compounds **29** and **33**) and *p*-Cl–Ph group at C-3 position on pyrazolic ring (compounds **39**) are essential for promoting the inhibitory activity. As a result, we designed and synthesized compounds **43** and **44** for testing the growth inhibitory activity. Unfortunately, compounds **43** and **44** were indicated the poor potent activity with GI_{50} values >100 μM for three cancer cell lines, which might come from to their poor solubility property.

Furthermore, 1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidines **38**–**44** were modified at the *N*-1-aryl groups to provide compounds **45**–**50** and **51**–**56** with different methyl, *tert*-butyl, *p*-Me–Ph, *p*-Cl–Ph, and *p*-OMe–Ph substituents at C-3. Compounds **45**–**50** displayed poor potency the cancer cells that might be due to their poor solubility. However, 1-(quinolin-2-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidines **51**–**56** showed better biological activity than compounds **45**–**50**, especially compounds **54**–**56** with *p*-Me, *p*-Cl and *p*-OMe at *N*-1 phenyl ring. They inhibited the growth of NCI-H226 and NPC-TW01 with GI_{50} values between 29 μM and 37 μM.

In conclusion, we developed a one-pot method to prepare a series of pyrazolo[3,4-*d*]pyrimidines by treating 5-aminopyrazoles in presence of PBr_3 coupling agent in formamide. Following the structure–activity relationship study, we found that introducing *o*-Cl–Ph or *p*-Br–Ph groups at *N*-1 and *p*-Me–Ph or *p*-Cl–Ph group at C-3 position in the pyrazole are necessary for the improved bioactivity. Further SAR indicated the better potency of 1-(quinolin-2-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidines than 1-(pyridin-2-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidines, compound **54**–**56** with *p*-Me, *p*-Cl and *p*-OMe at *N*-1 phenyl ring were the examples. Their inhibit values of the growth of NCI-H226 and NPC-TW01 two cells between 29 μM and 37 μM. Based on the antiproliferative evaluation of pyrazolo[3,4-*d*]pyrimidines, compounds **41** possessed the best efficacy against NCI-H226 (18 μM) and NPC-TW01 (23 μM) cancer cells.

3. Experimental section

3.1. General procedure

All chemicals were reagent grade and used as purchased. All reactions were carried out under nitrogen atmosphere and monitored by TLC analysis. Flash column chromatography was carried out on silica gel (230–400 mesh). Infrared (IR) spectra were measured on a Bomem Michelson Series FT-IR spectrometer. The wavenumbers reported are referenced to the polystyrene 1601 cm^{-1} absorption. Absorption intensities are recorded by the following abbreviations: s, strong; m, medium; w, weak. Proton NMR spectra were obtained on a Bruker (200, 400, or 500 MHz) spectrometer by use of $CDCl_3$ and DMSO-d_6 as solvent. Carbon-13 NMR spectra were obtained on a Bruker (50, 100, or 125 MHz) spectrometer by used of $CDCl_3$ as solvent. Carbon-13 chemical shifts are referenced to the center of the $CDCl_3$ triplet (δ 77.0 ppm). Multiplicities are recorded by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; *J*, coupling constant (hertz). Elemental analyses were carried out on a Heraeus CHN–O RAPID element analyzer. ESI-MS spectra were obtained from an Applied Biosystems API 300 mass spectrometer. High-resolution mass spectra were obtained by means of a JEOL JMS-HX110 mass spectrometer.

3.2. Standard procedure for synthesis of pyrazolo[3,4-*d*]pyrimidines (29–56)

The reliable procedure involved the treatment of 5-aminopyrazoles (**1**–**28**, 1.0 equiv) with catalytic amount of PBr_3 (~3 equiv) in formamide solution (2 mL) at 50–60 °C within 0.5–1 h. When the reaction was completed, the reaction mixture was added to saturate sodium bicarbonate (15 mL) and extracted with dichloromethane (15 mL). The organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue solution was purified by column chromatography on silica gel to give the corresponding pyrazolo[3,4-*d*] pyrimidine products (**29**–**56**) in 76–96% yields.

*3.2.1. 1,3-Diphenyl-1H-pyrazolo[3,4-d]pyrimidine (**29**).* Mp (purified by column chromatography on silica gel) 157–158 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.29–7.36 (m, 1H, ArH), 7.41–7.48 (m, 1H, ArH), 7.48–7.56 (m, 4H, ArH), 7.97–8.07 (m, 2H, ArH), 8.25–8.35 (m, 2H, ArH), 9.09 (s, 1H), 9.45 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 114.1, 121.2 (2× CH), 126.7, 127.2 (2× CH), 129.0 (2× CH), 129.1 (2× CH), 129.5, 131.4, 138.5, 144.8, 152.7, 153.2, 155.5; IR (KBr) 2364 (m), 2331 (m), 1589 (m), 1498 (m), 1417 (m), 1369 (s), 1219 (m), 1093 (m), 950 (m), 759 (m), 690 (m), 590 (m) cm^{-1}; EIMS *m*/*z* (relative intensity) 272 (100), 271 (M^+, 34), 244 (5), 218 (5), 195 (6), 169 (4), 142 (12), 115 (4), 91 (2), 77 (24); HRMS (EI) calcd for $C_{17}H_{12}N_4$ 272.1062, found 272.1059; Anal. Calcd for $C_{17}H_{12}N_4$; C: 74.98; H: 4.44; N: 20.58, found: C: 74.96; H: 4.45; N: 20.61.

*3.2.2. 1-(2-Methylphenyl)-3-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**30**).* Mp (purified by column chromatography on silica gel) 142–143 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.21 (s, 3H, CH_3), 7.33–7.43 (m, 3H, ArH), 7.44–7.50 (m, 2H, ArH), 7.51–7.57 (m, 2H, ArH), 8.02–8.08 (m, 2H, ArH), 9.05 (s, 1H), 9.53 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.2 (CH_3), 112.8, 126.7, 127.2 (2× CH), 127.6, 129.2 (2× CH), 129.5 (2× CH), 131.4, 131.7, 135.5, 136, 144.9, 152.8, 154.1, 155.8; IR (KBr) 3053 (w), 2922 (w), 2360 (m), 2339 (m), 1579 (m), 1556 (s), 1498 (m), 1222 (m), 1085 (m), 952 (m), 759 (m), 829 (m), 692 (m) cm^{-1}; EIMS *m*/*z* (relative intensity) 286 (100), 285 (M^+, 34), 244 (5), 218 (5), 195 (6), 169 (4), 142 (12), 115 (4), 91 (2), 77 (23); HRMS (EI) calcd for $C_{18}H_{14}N_4$ 286.1218, found 286.1219; Anal. Calcd for $C_{18}H_{14}N_4$; C: 75.50; H: 4.93; N: 19.57, found: C: 75.53; H: 4.92; N: 19.58.

*3.2.3. 1-(2-Chlorophenyl)-3-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**31**).* Mp (purified by column chromatography on silica gel) 142–143 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.43–7.49 (m, 3H, ArH), 7.50–7.56 (m, 2H, ArH), 7.57–7.65 (m, 2H, ArH), 8.02–8.08 (m, 2H, ArH), 9.07 (s, 1H), 9.52 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 122.9,

127.3 (2× CH), 127.6, 129.2 (2× CH), 129.6 (2× CH), 130.7 (2× CH), 131.4, 132.1, 134.7, 145.6, 152.8, 154.5, 155.9; IR (KBr) 3061 (w), 2924 (w), 2370 (w), 2349 (w), 1581 (m), 1557 (m), 1516 (m), 1497 (m), 1304 (m), 1223 (m), 1088 (m), 955 (m), 760 (m), 684 (m) cm^{-1}; EIMS *m*/*z* (relative intensity) 308 (M+2, 23), 306 (89), 305 (M^+, 3), 271 (100), 244 (5), 195 (15), 176 (5), 168 (14), 141 (4), 114 (6), 77 (22); HRMS (EI) calcd for $C_{17}H_{11}ClN_4$ 306.0672, found 306.0674; Anal. Calcd for $C_{17}H_{11}ClN_4$; C: 66.56; H: 3.61; N: 18.26, found: C: 66.59; H: 3.58; N: 18.22.

*3.2.4. 1-(3-Methylphenyl)-3-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**32**).* Mp (purified by column chromatography on silica gel) 75–76 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.47 (s, 3H, CH_3), 7.12–7.18 (m, 1H, ArH), 7.38–7.42 (m, 1H, ArH), 7.43–7.49 (m, 1H, ArH), 7.50–7.56 (m, 2H, ArH), 8.00–8.06 (m, 2H, ArH), 8.07–8.11 (m, 2H, ArH), 9.10 (s, 1H), 9.46 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.6 (CH_3), 114.1, 118.6, 122.0, 127.3 (2× CH), 127.6 (2× CH), 129.0, 129 (2× CH), 129.5, 131.5, 138.3, 139.2, 144.7, 152.7, 153.2, 155.5; IR (KBr) 3053 (w), 2920 (w), 1611 (m), 1582 (m), 1557 (m), 1495 (m), 1386 (m), 1229 (m), 1094 (m), 959 (m), 783 (m), 764 (m), 692 (m) cm^{-1}; EIMS *m*/*z* (relative intensity) 286 (100), 285 (M^+, 22), 271 (4), 232 (3), 209 (4), 156 (6), 128 (2), 91 (5), 77 (7), 65 (4); HRMS (EI) calcd for $C_{18}H_{14}N_4$ 286.1218, found 286.1215; Anal. Calcd for $C_{18}H_{14}N_4$; C: 75.50; H: 4.93; N: 19.57, found: C: 75.54; H: 4.96; N: 19.53.

*3.2.5. 1-(3-Chlorophenyl)-3-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**33**).* Mp (purified by column chromatography on silica gel) 188–189 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.27–7.33 (m, 1H, ArH), 7.43–7.48 (m, 1H, ArH), 7.48–7.52 (m, 1H, ArH), 7.53–7.59 (m, 2H, ArH), 8.01–8.07 (m, 2H, ArH), 8.32–8.36 (m, 1H, ArH), 8.42–8.44 (m, 1H, ArH), 9.14 (s, 1H), 9.50 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 114.5, 118.9, 121.1, 126.6, 127.4 (2× CH), 129.3 (2× CH), 129.9, 130.2, 131.2, 135.0, 139.6, 145.4, 152.9, 153.6, 155.8; IR (KBr) 3099 (w), 3062 (w), 2922 (w), 2851 (w), 1585 (m), 1491 (m), 1406 (m), 1215 (m), 951 (m), 866 (m), 779 (m), 758 (m), 691 (m) cm^{-1}; EIMS *m*/*z* (relative intensity) 308 (M+2, 36), 306 (100), 305 (M^+, 23), 286 (4), 271 (4), 252 (4), 229 (4), 176 (7), 111 (5), 91 (5), 77 (15); HRMS (EI) calcd for $C_{17}H_{11}ClN_4$ 306.0672, found 306.0671; Anal. Calcd for $C_{17}H_{11}ClN_4$; C: 66.56; H: 3.61; N: 18.26, found: C: 66.58; H: 3.62; N: 18.29.

*3.2.6. 1-(3-Nitrophenyl)-3-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**34**).* Mp (purified by column chromatography on silica gel) 183–184 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.50–7.61 (m, 3H, ArH), 7.67–7.73 (m, 1H, ArH), 8.02–8.10 (m, 2H, ArH), 8.14–8.20 (m, 1H, ArH), 8.81–8.87 (m, 1H, ArH), 9.18 (s, 1H), 9.31–9.35 (m, 1H, ArH), 9.52 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 114.7, 115.6, 120.8, 126.0, 127.5 (2× CH), 129.3 (2× CH), 130.1, 130.1, 130.8, 139.6, 146.0, 148.8, 153.1, 154.0, 156.0; IR (KBr) 2924 (w), 2348 (w), 1531 (m), 1431 (m), 1416 (m), 1343 (m), 1215 (m), 1092 (m), 954 (m), 762 (m), 739 (m) cm^{-1}; EIMS *m*/*z* (relative intensity) 317 (100), 271 (11), 243 (6), 194 (4), 168 (10), 141 (8), 114 (5), 104 (8), 90 (4), 77 (15); HRMS (EI) calcd for $C_{17}H_{11}N_5O_2$ 317.0913, found 317.0916; Anal. Calcd for $C_{17}H_{11}N_5O_2$; C: 64.35; H: 3.49; N: 22.07, found: C: 64.37; H: 3.51; N: 22.03.

*3.2.7. 1-(4-Chlorophenyl)-3-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**35**).* Mp (purified by column chromatography on silica gel) 144–145 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.46–7.52 (m, 3H, ArH), 7.52–7.58 (m, 2H, ArH), 8.00–8.06 (m, 2H, ArH), 8.29–8.35 (m, 2H, ArH), 9.12 (s, 1H), 9.49 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 114.3, 122.2 (2× CH), 127.4 (2× CH), 129.2 (2× CH), 129.3 (2× CH), 129.8, 131.3, 132.1, 137.2, 145.2, 152.9, 153.4, 155.7; IR (KBr) 3051 (w), 2922 (w), 2851 (w), 2384 (w), 2349 (w), 1589 (m), 1554 (m), 1500 (m), 1406 (m), 1367 (m), 1215 (m), 955 (m), 822 (m) cm^{-1}; EIMS *m*/*z* (relative intensity) 308 (M+2, 33), 306 (100), 305 (M^+, 18), 286 (4), 271 (8), 252 (5), 229 (4), 176 (9), 111 (5), 91 (3), 77 (16); HRMS (EI) calcd for $C_{17}H_{11}ClN_4$ 306.0672, found 306.0669; Anal. Calcd for $C_{17}H_{11}ClN_4$; C: 66.56; H: 3.61; N: 18.26, found: C: 66.53; H: 3.58; N: 18.23.

*3.2.8. 1-(4-Bromophenyl)-3-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**36**).* Mp (purified by column chromatography on silica gel) 176–177 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.45–7.57 (m, 3H, ArH), 7.60–7.66 (m, 2H, ArH), 7.98–8.06 (m, 2H, ArH), 8.21–8.29 (m, 2H, ArH), 9.10 (s, 1H), 9.47 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 114.3, 120.0, 122.4 (2× CH), 127.3 (2× CH), 129.2 (2× CH), 130.0, 131.2, 132.2 (2× CH), 137.6, 145.2, 152.8, 153.4, 155.6; IR (KBr) 2374 (w), 2347 (w), 1585 (m), 1495 (m), 1398 (m), 1389 (m), 1215 (m), 1072 (m), 951 (m), 822 (m), 760 (m), 689 (m), 588 (m) cm^{-1}; EIMS *m*/*z* (relative intensity) 352 (M+2, 98), 350 (100), 349 (M^+, 11), 298 (3), 270 (13), 243 (4), 217 (3), 194 (11), 168 (6), 140 (4), 114 (11), 90 (6), 77 (43); HRMS (EI) calcd for $C_{17}H_{11}BrN_4$ 350.0167, found 350.0166; Anal. Calcd for $C_{17}H_{11}BrN_4$; C: 58.14; H: 3.16; N: 15.95, found: C: 58.16; H: 3.18; N: 15.91.

*3.2.9. 1-(4-Methoxylphenyl)-3-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**37**).* Mp (purified by column chromatography on silica gel) 149–150 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.84 (s, 3H, CH_3), 7.00–7.06 (m, 2H, ArH), 7.42–7.48 (m, 1H, ArH), 7.50–7.54 (m, 2H, ArH), 7.99–8.05 (m, 2H, ArH), 8.09–8.13 (m, 2H, ArH), 9.10 (s, 1H), 9.45 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.5 (CH_3), 113.8, 114.3 (2× CH), 123.1 (2× CH), 127.2 (2× CH), 129.1 (2× CH), 129.4, 131.5, 131.6, 144.5, 152.7, 152.8, 155.4, 158.4; IR (KBr) 3053 (w), 2836 (w), 2347 (w), 1580 (m), 1555 (m), 1514 (m), 1416 (m), 1366 (m), 1250 (m), 1219 (m), 1177 (m), 1088 (m), 1034 (m) cm^{-1}; EIMS *m*/*z* (relative intensity) 302 (100), 301 (M^+, 34), 244 (5), 218 (5), 195 (6), 169 (4), 142 (12), 115 (4), 91 (2), 77 (17); HRMS (EI) calcd for $C_{18}H_{14}N_4O$ 302.1168, found 302.1165; Anal. Calcd for $C_{18}H_{14}N_4O$; C: 71.51; H: 4.67; N: 18.53, found: C: 75.49; H: 4.71; N: 18.51.

*3.2.10. 3-Methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**38**).* Mp (purified by column chromatography on silica gel) 79–80 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.63 (s, 3H, CH_3), 7.23–7.29 (m, 1H, ArH), 7.42–7.50 (m, 2H, ArH), 8.13–8.19 (m, 2H, ArH), 9.02 (s, 1H), 9.10 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.4 (CH_3), 115.6, 120.8 (2× CH), 126.3, 129.1 (2× CH), 138.4, 143.2, 151.6, 152.6, 155.5; IR (KBr) 3051 (w), 2922 (w), 2852 (w), 1593 (m), 1562 (m), 1508 (m), 1440 (m), 1355 (m), 1211 (m), 1066 (m), 754 (m), 691 (m), 592 (m) cm^{-1}; EIMS *m*/*z* (relative intensity) 210 (100), 209 (M^+, 27), 195 (13), 168 (4), 142 (16), 115 (4), 91 (6), 77 (24); HRMS (EI) calcd for $C_{12}H_{10}N_4$ 210.0905, found 210.0903; Anal. Calcd for $C_{12}H_{10}N_4$; C: 68.56; H: 4.79; N: 26.65, found: C: 68.58; H: 4.76; N: 26.61.

*3.2.11. 3-tert-Butyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**39**).* Mp (purified by column chromatography on silica gel) 48–49 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.55 (s, 9H, 3× CH_3), 7.23–7.29 (m, 1H, ArH), 7.44–7.52 (m, 2H, ArH), 8.19–8.12 (m, 2H, ArH), 9.02 (s, 1H), 9.31 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 30.3 (3× CH), 34.4, 114.0, 121.0 (2× CH), 126.2, 129.0 (2× CH), 138.6, 152.8, 153.1, 154.8, 155.0; IR (KBr) 3049 (w), 2968 (w), 2666 (w), 1599 (m), 1578 (m), 1555 (m), 1508 (m), 1427 (m), 1366 (m), 1261 (m), 1098 (m), 961 (m), 756 (m) cm^{-1}; EIMS *m*/*z* (relative intensity) 252 (44), 251 (M^+, 1), 237 (100), 195 (5), 168 (2), 142 (2), 116 (1), 77 (14); HRMS (EI) calcd for $C_{15}H_{16}N_4$ 252.1375, found 252.1377; Anal. Calcd for $C_{15}H_{16}N_4$; C: 71.40; H: 6.39; N: 22.21, found: C: 71.43; H: 6.35; N: 22.17.

*3.2.12. 3-(4-Methylphenyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**40**).* Mp (purified by column chromatography on silica gel) 139–140 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.43 (s, 3H, CH_3), 7.32–7.42 (m, 3H, ArH), 7.51–7.61 (m, 2H, ArH), 7.86–8.02 (m, 2H, ArH), 8.24–8.38 (m, 2H, ArH), 9.11 (s, 1H), 9.47 (s, 1H); ^{13}C NMR

(125 MHz, $CDCl_3$) δ 21.4 (CH_3), 114.2, 121.3 (2× CH), 126.7, 127.2 (2× CH), 128.7, 129.2 (2× CH), 129.9 (2× CH), 138.5, 139.8, 145.0, 152.8, 153.3, 155.5; IR (KBr) 3030 (w), 2920 (w), 2374 (w), 2349 (w), 2313 (w), 1584 (m), 1503 (m), 1430 (m), 1368 (m), 1220 (m), 953 (m), 785 (m), 756 (m) cm^{-1}; EIMS *m/z* (relative intensity) 286 (100), 285 (M^+, 34), 244 (5), 218 (5), 195 (6), 169 (4), 142 (12), 115 (4), 91 (2), 77 (18); HRMS (EI) calcd for $C_{18}H_{14}N_4$ 286.1218, found 286.1216; Anal. Calcd for $C_{18}H_{14}N_4$; C: 75.50; H: 4.93; N: 19.57, found: C: 75.49; H: 4.91; N: 19.55.

*3.2.13. 3-(4-Chlorophenyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**41**).* Mp (purified by column chromatography on silica gel) 196–197 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.34–7.38 (m, 1H, ArH), 7.48–7.58 (m, 4H, ArH), 7.96–8.02 (m, 2H, ArH), 8.24–8.30 (m, 2H, ArH), 9.12 (s, 1H), 9.46 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 114.0, 121.5 (2× CH), 127.0, 128.5 (2× CH), 129.3 (2× CH), 129.5 (2× CH), 130.0, 135.7, 138.4, 143.8, 152.6, 153.3, 155.7; IR (KBr) 3043 (w), 2926 (w), 2848 (w), 1585 (m), 1555 (m), 1503 (m), 1368 (m), 1219 (m), 1093 (m), 954 (m), 831 (m), 789 (m), 745 (m) cm^{-1}; EIMS *m/z* (relative intensity) 308 (M+2, 36), 306 (100), 305 (M^+, 21), 286 (5), 271 (7), 252 (4), 229 (3), 176 (9), 111 (6), 91 (3), 77 (18); HRMS (EI) calcd for $C_{17}H_{11}ClN_4$ 306.0672, found 306.0671; Anal. Calcd for $C_{17}H_{11}ClN_4$; C: 66.56; H: 3.61; N: 18.26, found: C: 66.53; H: 3.62; N: 18.29.

*3.2.14. 3-(4-Methoxylphenyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**42**).* Mp (purified by column chromatography on silica gel) 175–176 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.87 (s, 3H, CH_3), 7.08–7.84 (m, 2H, ArH), 7.32–7.36 (m, 1H, ArH), 7.50–7.56 (m, 2H, ArH), 7.95–8.01 (m, 2H, ArH), 8.28–8.32 (m, 2H, ArH), 9.10 (s, 1H), 9.45 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.4 (CH_3), 114.2, 114.6 (2× CH), 121.3 (2× CH), 124.1, 126.7, 128.7 (2× CH), 129.2 (2× CH), 138.6, 144.8, 152.8, 153.2, 155.5, 160.8; IR (KBr) 3047 (w), 2924 (w), 1612 (m), 1582 (m), 1503 (m), 1431 (m), 1356 (m), 1254 (m), 1219 (m), 1092 (m), 962 (m), 835 (m), 758 (m) cm^{-1}; EIMS *m/z* (relative intensity) 302 (100), 301 (M^+, 6), 287 (25), 265 (4), 259 (8), 233 (3), 195 (2), 151 (4), 91 (5), 77 (12); HRMS (EI) calcd for $C_{18}H_{14}N_4O$ 302.1168, found 302.1169; Anal. Calcd for $C_{18}H_{14}N_4O$; C: 71.51; H: 4.67; N: 18.53, found: C: 71.48; H: 4.68; N: 18.50.

*3.2.15. 1-(2-Chlorophenyl)-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d] pyrimidine (**43**).* Mp (purified by column chromatography on silica gel) 178–179 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.34–7.38 (m, 1H, ArH), 7.48–7.58 (m, 4H, ArH), 7.96–8.02 (m, 2H, ArH), 8.24–8.30 (m, 2H, ArH), 9.12 (s, 1H), 9.46 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 114.0, 121.5 (2× CH), 127.0, 128.5 (2× CH), 129.3 (2× CH), 129.5 (2× CH), 130.0, 135.7, 138.4, 143.8, 152.6, 153.3, 155.7; IR (KBr) 3043 (w), 2926 (w), 2848 (w), 1585 (m), 1555 (m), 1503 (m), 1368 (m), 1219 (m), 1093 (m), 954 (m), 831 (m), 789 (m), 745 (m) cm^{-1}; HRMS (EI) calcd for $C_{17}H_{10}Cl_2N_4$ 340.0283, found 340.0287; Anal. Calcd for $C_{17}H_{10}Cl_2N_4$; C: 59.84; H: 2.95; N: 16.42, found: C: 59.79; H: 2.93; N: 16.39.

*3.2.16. 1-(4-Chlorophenyl)-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d] pyrimidine (**44**).* Mp (purified by column chromatography on silica gel) 153–154 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.34–7.38 (m, 1H, ArH), 7.48–7.58 (m, 4H, ArH), 7.96–8.02 (m, 2H, ArH), 8.24–8.30 (m, 2H, ArH), 9.12 (s, 1H), 9.46 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 114.0, 121.5 (2× CH), 127.0, 128.5 (2× CH), 129.3 (2× CH), 129.5 (2× CH), 130.0, 135.7, 138.4, 143.8, 152.6, 153.3, 155.7; IR (KBr) 3043 (w), 2926 (w), 2848 (w), 1585 (m), 1555 (m), 1503 (m), 1368 (m), 1219 (m), 1093 (m), 954 (m), 831 (m), 789 (m), 745 (m) cm^{-1}; HRMS (EI) calcd for $C_{17}H_{10}Cl_2N_4$ 341.0283, found 341.0281; Anal. Calcd for $C_{17}H_{10}Cl_2N_4$; C: 59.84; H: 2.95; N: 16.42, found: C: 59.86; H: 2.98; N: 16.38.

*3.2.17. 3-Methyl-1-(pyridin-2-yl)-1H-pyrazolo[3,4-d]pyrimidine (**45**).* Mp (purified by column chromatography on silica gel) 78–79 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.65 (s, 3H, CH_3), 7.16–7.22 (m, 1H, ArH), 7.82–7.86 (m, 1H, ArH), 8.57–8.61 (m, 1H, ArH), 9.08–9.12 (m, 2H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.5, 29.5, 115.5, 116.1, 121.6, 138.4, 144.5, 148.9, 150.5, 151.7, 153.2, 156.1; IR (KBr) 2920 (w), 2853 (w), 1730 (w), 1589 (m), 1506 (m), 1317 (m), 1213 (m), 1124 (m), 1067 (m), 779 (m), 738 (m), 690 (m) cm^{-1}; Anal. Calcd for $C_{11}H_9N_5$; C: 62.55; H: 4.29; N: 33.16, found: C: 62.53; H: 4.27; N: 33.17.

*3.2.18. 3-tert-Butyl-1-(pyridin-2-yl)-1H-pyrazolo[3,4-d]pyrimidine (**46**).* Mp (purified by column chromatography on silica gel) 64–65 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.50 (s, 9H, CH_3), 7.15–7.19 (m, 1H, ArH), 7.77–7.81 (m, 1H, ArH), 8.05–8.07 (m, 1H, ArH), 8.60–8.62 (m, 1H, ArH), 9.07 (s, 1H), 9.28 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 29.7, 34.4, 114.2, 116.1, 121.5, 138.2, 148.8, 151.1, 152.8, 153.6, 155.7, 155.7; IR (KBr) 2970 (w), 2227 (w), 1595 (m), 1556 (m), 1498 (m), 1450 (m), 1369 (m), 1192 (m), 1112 (m), 9100 (m), 779 (m) cm^{-1}; EIMS *m/z* (relative intensity) 253 (23), 252 (M^+, 2), 238 (100), 198 (12), 171 (2), 105 (3), 78 (31), 57 (5), 51 (7); Anal. Calcd for $C_{14}H_{15}N_5$; C: 66.38; H: 5.97; N: 27.65, found: C: 66.40; H: 5.99; N: 27.63.

*3.2.19. 3-Phenyl-1-(pyridin-2-yl)-1H-pyrazolo[3,4-d]pyrimidine (**47**).* Mp (purified by column chromatography on silica gel) 206–207 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.22–7.30 (m, 1H, ArH), 7.41–7.55 (m, 3H, ArH), 7.83–7.91 (m, 1H, ArH), 7.99–8.05 (m, 2H, ArH), 8.20–8.26 (m, 1H, ArH), 8.66–8.70 (m, 1H, ArH), 9.19 (s, 1H), 9.47 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 114, 116, 122, 128 (2× CH), 129 (2× CH), 130, 131, 139, 146, 149, 151, 153, 154, 156; IR (KBr) 3049 (w), 2920 (w), 2851 (w), 2380 (w), 2349 (w), 1593 (m), 1481 (m), 1452 (m), 1368 (m), 1265 (m), 1219 (m), 1080 (m), 957 (m), 736 (m), 592 (m) cm^{-1}; EIMS *m/z* (relative intensity) 273 (100), 272 (M^+, 17), 230 (16), 196 (10),170 (22), 143 (27), 91 (20), 78 (35), 51 (17); Anal. Calcd for $C_{16}H_{11}N_5$; C: 70.32; H: 4.06; N: 25.63, found: C: 70.28; H: 4.09; N: 25.66.

*3.2.20. 3-(4-Methylphenyl)-1-(pyridin-2-yl)-1H-pyrazolo[3,4-d]pyrimidine (**48**).* Mp (purified by column chromatography on silica gel) 127–128 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.42 (s, 1H, CH_3), 7.28–7.34 (m, 3H, ArH), 7.89–7.95 (m, 3H, ArH), 8.26–8.28 (m, 1H, ArH), 8.71–8.72 (m, 1H, ArH), 9.21 (s, 1H), 9.49 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.4, 114.7, 116.4, 122.0, 127.5 (2× CH), 128.2, 129.8 (2× CH), 138.5, 140.1, 146.2, 149.0, 151.1, 152.9, 153.9, 156.2; IR (KBr) 2922 (W), 2853 (W), 1591 (m), 1481 (m), 1445 (m), 1366 (m), 1317 (m), 1221 (m), 1080 (m), 957 (m), 793 (m) cm^{-1}; EIMS *m/z* (relative intensity) 287 (100), 286 (M^+, 22), 259 (7), 196 (6), 170 (18), 154 (9), 143 (21), 91 (9), 78 (32), 65 (7), 51 (10); Anal. Calcd for $C_{17}H_{13}N_5$; C: 71.06; H: 4.56; N: 24.37, found: C: 71.08; H: 4.54; N: 24.39.

*3.2.21. 3-(4-Chlorophenyl)-1-(pyridin-2-yl)-1H-pyrazolo[3,4-d]pyrimidine (**49**).* Mp (purified by column chromatography on silica gel) 164–165 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.28–7.34 (m, 1H, ArH), 7.48–7.52 (m, 2H, ArH), 7.88–8.02 (m, 3H, ArH), 8.25–8.29 (m, 1H, ArH), 8.70–8.74 (m, 1H, ArH), 9.22 (s, 1H), 9.48 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 114.5, 116.5, 122.3, 128.5 (2× CH), 128.8, 129.5 (2× CH), 136.0, 138.7, 145.0, 150.9, 152.7, 153.9, 156.4; IR (KBr) 2920 (w), 2851 (w), 1587 (m), 1481 (m), 1217 (m), 1094 (m), 957 (m), 833 (m), 770 (m) cm^{-1}; EIMS *m/z* (relative intensity) 309 (M+2, 30), 307 (100), 306 (M^+, 13), 279 (5), 196 (9), 174 (8), 170 (27), 143 (30), 111 (6), 78 (39), 75 (6), 51 (13); Anal. Calcd for $C_{16}H_{10}ClN_5$; C: 62.45; H: 3.28; N: 22.76, found: C: 62.43; H: 3.30; N: 22.75.

*3.2.22. 3-(4-Methoxyphenyl)-1-(pyridin-2-yl)-1H-pyrazolo[3,4-d] pyrimidine (**50**).* Mp (purified by column chromatography on silica gel) 167–168 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.84 (s, 1H, CH_3), 6.99–7.05 (m, 2H, ArH), 7.22–7.28 (m, 1H, ArH), 7.87–7.99

(m, 3H, ArH), 8.22–8.26 (m, 1H, ArH), 8.66–8.70 (m, 1H, ArH), 9.18 (s, 1H), 9.44 (s, 1H); ^{13}C NMR (125 MHz, CDCl3) δ 55.4, 114.0 (2× CH), 114.6, 116.3, 122.0, 123.6, 129.0 (2× CH), 138.5, 146.0, 149.0, 151.1, 152.9, 153.8, 156.2, 161.0; IR (KBr) 3460 (w), 3057 (w), 2960 (w), 2924 (w), 2850 (w), 1614 (m), 1529 (m), 1367 (m), 1253 (m), 1219 (m), 1178 (m), 1080 (m) cm^{-1}; Anal. Calcd for $C_{17}H_{13}N_5O$; C: 67.32; H: 4.32; N: 23.09, found: C: 67.35; H: 4.30; N: 23.11.

*3.2.23. 3-Methyl-1-(quinolin-2-yl)-1H-pyrazolo[3,4-d]pyrimidine (**51**).* Mp (purified by column chromatography on silica gel) 158–159 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.78 (s, 3H, CH_3), 7.51–7.59 (m, 1H, ArH), 7.71–7.87 (m, 2H, ArH), 8.22–8.38 (m, 2H, ArH), 8.58–8.62 (m, 1H, ArH), 9.19 (s, 1H), 9.21 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 29.7, 115.0, 116.5, 126.6, 127.0, 127.5, 129.4, 130.3 139.0, 145.3, 147.0, 149.4, 151.9, 154.0, 156.3; IR (KBr) 2924 (w), 2855 (w), 2361 (w), 1597 (m), 1504 (m), 1427 (m), 1327 (m), 1211 (m), 1134 (m), 1080 (m) cm^{-1}; Anal. Calcd for $C_{15}H_{11}N_5$; C: 68.95; H: 4.24; N: 26.80, Found: C: 68.98; H: 4.27; N: 26.83.

*3.2.24. 3-tert-Butyl-1-(quinolin-2-yl)-1H-pyrazolo[3,4-d]pyrimidine (**52**).* Mp (purified by column chromatography on silica gel) 144–145 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.58 (s, 3H, CH_3), 7.49–7.53 (m, 1H, ArH), 7.70–7.72 (m, 1H, ArH), 7.80–7.82 (m, 1H, ArH), 8.18–8.20 (m, 1H, ArH), 8.29–8.31 (m, 1H, ArH), 9.18 (s, 1H), 9.38 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 29.8, 34.6, 114.6, 115.5, 126.5, 126.9, 127.4, 129.3, 130.1, 138.8, 146.9, 150.0, 152.9, 154.3, 154.7, 156.2; IR (KBr) 2964 (w), 2922 (w), 2851 (w), 1622 (m), 1600 (m), 1583 (m), 1502 (m), 1433 (m), 1367 (m), 1190 (m), 1047 (m) cm^{-1}; Anal. Calcd for $C_{18}H_{17}N_5$; C: 71.27; H: 5.65; N: 23.09, found: C: 71.29; H: 5.62; N: 23.11.

*3.2.25. 3-Phenyl-1-(quinolin-2-yl)-1H-pyrazolo[3,4-d]pyrimidine (**53**).* Mp (purified by column chromatography on silica gel) 194–195 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.49–7.59 (m, 4H, ArH), 7.75–7.79 (m, 1H, ArH), 7.87–7.89 (m, 1H, ArH), 8.11–8.13 (m, 2H, ArH), 8.37–8.39 (m, 1H, ArH), 8.47–8.49 (m, 1H, ArH), 9.26 (s, 1H), 9.55 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 114.9, 115.6, 126.8, 127.2, 127.5, 127.8 (2× CH), 129.2 (2× CH), 129.5, 130.4, 131.2, 139.0, 146.6, 147.0, 149.8, 153.0, 154.4, 156.4; IR (KBr) 3055 (w), 2376 (w), 2347 (w), 1582 (m), 1557 (m), 1502 (m), 1477 (m), 1409 (m), 1368 (m), 1325 (m), 1219 (m), 1090 (m), 962 (m), 825 (m), 761 (m), 694 (m) cm^{-1}; EIMS *m*/*z* (relative intensity) 323 (100), 295 (M^+, 21) 246 (17), 220 (29), 193 (39), 140 (12), 128 (46), 101 (18), 77 (21); Anal. Calcd for $C_{20}H_{13}N_5$; C: 74.29; H: 4.05; N: 21.66, found: C: 74.31; H: 4.07; N: 21.69.

*3.2.26. 3-(4-Methylphenyl)-1-(quinolin-2-yl)-1H-pyrazolo[3,4-d]pyrimidine (**54**).* Mp (purified by column chromatography on silica gel) 163–164 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.42 (s, 3H, CH_3), 7.32–7.36 (m, 2H, ArH), 7.50–7.58 (m, 1H, ArH), 7.70–7.86 (m, 2H, ArH), 7.96–8.00 (m, 2H, ArH), 8.22–8.48 (m, 3H, ArH), 9.23 (s, 1H), 9.51 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.4, 114.8, 115.4, 126.7, 127.0, 127.4, 127.5 (2× CH), 128.3, 129.4, 129.8 (2× CH), 130.3, 138.8, 140.1, 146.5, 146.9, 149.7, 152.9, 154.3, 156.3; IR (KBr) 3473 (m), 3048 (w), 2924 (w), 2855 (w), 1557 (m), 1504 (m), 1477 (m), 1431 (m), 1367 (m), 1325 (m), 1217 (m), 1089 (m), 1022 (m) cm^{-1}; Anal. Calcd for $C_{21}H_{15}N_5$; C: 74.46; H: 4.48; N: 20.76, found: C: 74.48; H: 4.50; N: 20.73.

*3.2.27. 3-(4-Chlorophenyl)-1-(quinolin-2-yl)-1H-pyrazolo[3,4-d]pyrimidine (**55**).* Mp (purified by column chromatography on silica gel) 127–128 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.50–7.60 (m, 4H, ArH), 7.69–7.89 (m, 3H, ArH), 8.01–8.07 (m, 2H, ArH), 8.22–8.26 (m, 1H, ArH), 8.33–8.49 (m, 2H, ArH), 9.24 (s, 1H), 9.50 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 114.6, 115.5, 126.9, 127.2, 127.6, 128.9 (3× CH), 129.5 (3× CH), 130.5, 136.1, 139.1, 145.3, 147.0, 149.5, 152.8, 154.4, 156.4; IR (KBr) 3455 (m), 3051 (w), 2922 (w), 1602 (m), 1581 (m), 1554 (m), 1477 (m), 1431 (m), 1219 (m), 1089 (m) cm^{-1}; EIMS *m*/*z* (relative intensity) 359 (M+2, 34), 357 (100), 356 (M^+, 17), 246 (13), 220 (26), 193 (36), 128 (42), 101 (15), 77 (7); Anal. Calcd for $C_{20}H_{12}ClN_5$; C: 67.14; H: 3.38; N: 19.57, found: C: 67.12; H: 3.41; N: 19.57.

*3.2.28. 3-(4-Methoxyphenyl)-1-(quinolin-2-yl)-1H-pyrazolo[3,4-d]pyrimidine (**56**).* Mp (purified by column chromatography on silica gel) 153–154 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.86 (s, 3H, CH_3), 7.05–7.07 (m, 2H, ArH), 7.53–7.55 (m, 1H, ArH), 7.72–7.76 (m, 1H, ArH), 7.83–7.85 (m, 1H, ArH), 8.02–8.04 (m, 2H, ArH), 8.23–8.25 (m, 1H, ArH), 8.32–8.34 (m, 1H, ArH), 8.45–8.47 (m, 1H, ArH), 9.21 (s, 1H), 9.49 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.5, 114.7 (2× CH), 114.9, 115.5, 123.7, 126.7, 127.1, 127.5, 129.1 (2× CH), 129.4, 130.4, 139.0, 146.4, 147.0, 149.8, 153.0, 156.3, 161.1, 167.8; IR (KBr) 2922 (w), 2853 (w), 1589 (m), 1481 (m), 1447 (m), 1366 (m), 1219 (m), 1080 (m), 957 (m), 791 (m) cm^{-1}; Anal. Calcd for $C_{21}H_{15}N_5O$; C: 71.38; H: 4.28; N: 19.82, found: C: 71.39; H: 4.25; N: 19.81.

3.3. Cell lines

Human non-small cell lung carcinoma (NCI-H226) was purchased from American Type Culture Collection (ATCC; Rockville, MD). T-cell leukemia (MT-2) was obtained from Japanese Collection of Research Bioresources (JCRB) and nasopharyngeal carcinoma (NPC-TW01) was purchased from Bioresource Collection and Research Center (BCRC, Taiwan). All the tumor cell lines were maintained in either RPMI-1640 or Modified essential medium (MEM) supplied with 10% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2/95% air in the present of penicillin and streptomycin.

3.4. Growth inhibition assay

Logarithmic phase cells were seeded in a 96-well plate and incubated overnight prior to addition of the designated compounds. After incubation with different concentrations of the tested compounds for 72 h, cells were incubated with MEM containing 0.4 mg/mL MTT for 2 h. The conversion of MTT to formazan by metabolically viable cells was measured by the absorbance at 570 nm in a 96-well microtiter plate reader. The percentage conversion by mock-treated control cells was used to evaluate the effect of the chemicals on cell growth and to determine the concentration that inhibited 50% of growth (GI_{50}).

Acknowledgements

We are grateful to the China Medical University (CMU100-ASIA–17 and CMU-101-S-23), Tsuzuki Institute for Traditional Medicine, and the National Science Council of Republic of China for financial support (NSC-99-2320-B-039-014-MY3). This study is also supported in part by Taiwan Department of Health Clinical Trial and Research Center of Excellence (DOH100-TD-B-111-004).

Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.tet.2012.09.054.

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