Full Paper

Synthesis and Anti-tumor Activities of Novel Pyrazolo[1,5-*a*]pyrimidines

Juan Li, Yan Fang Zhao, Xiang Lin Zhao, Xiao Ye Yuan, and Ping Gong

School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang, P. R. China

A series of novel pyrazolo[1,5-*a*]pyrimidines were designed and synthesized in order to find novel potent anti-tumor compounds. The structures of all the compounds were confirmed by IR, ^{1}H-NMR, elemental analysis, and MS. Their anti-tumor activities against cancer cell lines were tested by the MTT method *in vitro*. Compound **19** displayed potent anti-tumor activity.

Keywords: Anti-tumor activities / Pyrazolo[1,5-*a*]pyrimidines / Synthesis

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Introduction

Cancer is the second leading cause of death in the world. In the last few years, a large number of compounds, for instance 4-anilinoquinazolines, 4-anilinophthalazines, 4-4-anilinopyrozolo[4,3-*d*]pyrimidines, indolin-2-ones, and purines were reported as anticancer agents used in clinics or in clinical trials [1, 2]. 3, 6-Diarylpyrazolo[1,5-*a*]pyrimidine **1** was another template reported as anti-tumor compound, which targeted vascular endothelial growth factor receptor-2 [3, 4]. This series of compounds had been hindered by poor physical properties, such as low solubility and high protein binding. Our chemistry efforts began with the goal of improving these limitations and we discovered a new series of pyrazolo[1,5-*a*]pyrimidine derivatives with anti-tumor activities. Herein, we replaced the aryl group with a cyano at position C-3 (Fig. 1). Since small substitution changes of structures might affect the biological activity and drug properties, we were intrigued to determine the effect on anti-tumor activity resulting from substitution at the C-5 and C-7 positions of the pyrazolo[1,5-*a*]pyrimidine core structure. Thus, various anilines and amino alkoxy groups, which were extensively used in the design of tyrosine kinase inhibitors as anticancer agents, were introduced.

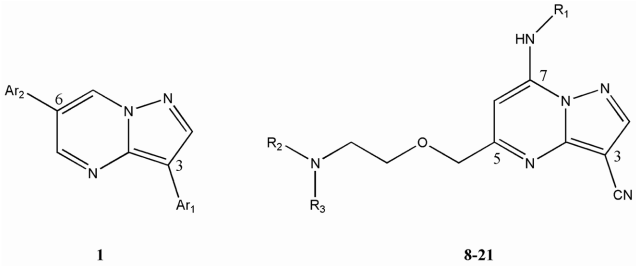


Figure 1. Structures of 3-cyano-5,7-disubstituted pyrazolo[1,5-*a*]pyrimidine derivatives **8**–**21**.

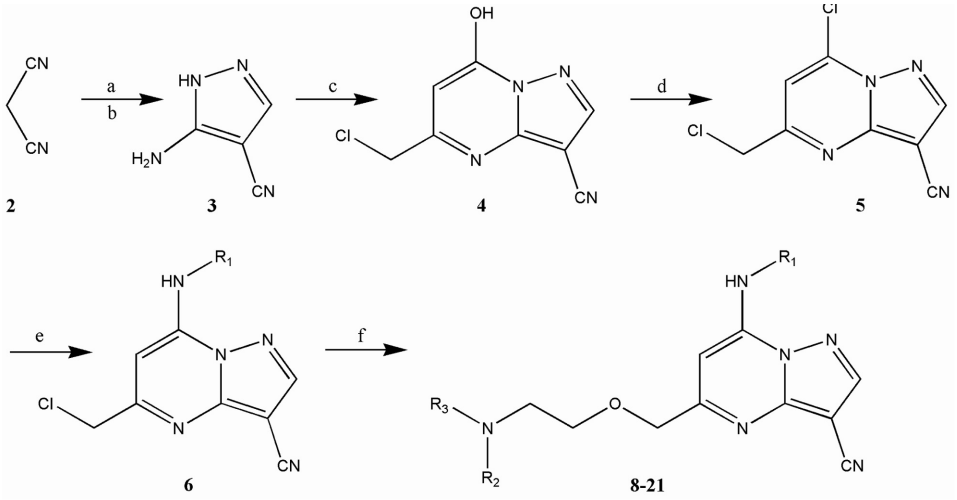
In this present work, we would like to report the synthesis and anti-tumor activities of a series of novel 3-cyano-5,7-disubstituted pyrazolo[1,5-*a*]pyrimidine derivatives represented by the general formula of **8**–**21** in Fig. 1.

Results and Discussion

We developed an efficient and facile approach to prepare a variety of pyrazolo[1,5-*a*]pyrimindine derivatives with various C-5 and C-7 substituents. As shown in Scheme 1, the straightforward five-step synthetic route allowed us to diversify positions C-5 and C-7 of the pyrazolo[1,5-*a*]pyrimidine moiety via the key intermediate **5**.

The synthesis of 3-amino-4-cyanopyrazole **3** has been reported by treatment of malononitrile **2** with triethyl orthoformate and aniline in methanol firstly, and the intermediate obtained exchanged with hydrazine hydrate [6]. In our modified approach, intermediate **3**

Correspondence: Ping Gong, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenhe District, 110016 Shenyang, Liaoning, P. R. China.
E-mail: gongpinggp@126.com
Fax: +86 24 2388-2925



Reagents and conditions: a: $HC(OC_2H_5)_3$, Ac_2O, reflux, 2 h; b: $H_2NNH_2 \times H_2O$, r.t., 18 h; c: $ClCH_2COCH_2COOC_2H_5$, HAc, reflux, 4 h; d: $POCl_3$, Py, 120°C, 1 h; e: R_1NH_2, $^{i}PrOH$, 60°C, 2 h; f: NaH, $R_2R_3NCH_2CH_2OH$ (**7**), 40°C, 2 h.

Scheme 1. Synthesis route of compounds **8**–**21**.

Table 1. The substituents and anti-tumor effect of compounds **8**–**14**.

Compound	IC_{50} (μM)			
	R_1	NR_2R_3	Bel-7402	HT-1080
8	3,4-dichlorophenyl	dimethylamino	27.2	22.2
9	3,4-dichlorophenyl	diethylamino	78.5	198.6
10	3,4-dichlorophenyl	4-methylpiperazinyl	26.1	52.2
11	3-chlorophenyl	dimethylamino	156.8	113.5
12	3-chlorophenyl	diethylamino	157.9	130.3
13	3-chlorophenyl	pyrrolidinyl	143.6	98.2
14	3-chlorophenyl	4-methylpiperazinyl	122.0	204.2
Cisplatin			76.7	63.3

Table 2. The substituents and anti-tumor effect of compounds **15**–**21**.

Compound	IC_{50} (μM)			
	R_1	NR_2R_3	Bel-7402	HT-1080
15	3-fluro-4-bromophenyl	pyrrolidinyl	126.4	154.7
16	3-fluro-4-bromophenyl	4-methylpiperazinyl	59.4	129.1
17	3,4-methylenedioxyphenyl	pyrrolidinyl	19.7	44.3
18	3,4-methylenedioxyphenyl	4-methylpiperazinyl	178.9	200.0
19	3,5-di(trifluoromethyl)phenyl	piperdinyl	13.7	17.6
20	3,5-di(trifluoromethyl)phenyl	morpholinyl	79.8	46.7
21	3,5-di(trifluoromethyl)phenyl	4-methylpiperazinyl	81.6	119.5
Cisplatin			75.2	58.2

can be synthesized easily in one pot. Moreover, the yield was about 25% higher than that reported [6].

Intermediate **3** was then cyclized with ethyl 4-chloroaceto-acetate to obtain the intermediate **4**. Chlorination of **4** with phosphorus oxychloride in the presence of pyridine gave **5** [7, 8]. Substitution of 7-Cl in **5** with various anilines provided the 7-anilinopyrazolo[1,5-*a*]pyrimidine **6**. Treatment of **6** with various alcohols in basic DMF solution generated the desired final products **8**–**21** (Tables 1 and 2). The pharmacological anti-tumor activities of com-

pounds **8**–**21** are shown in Tables 1 and 2. The data in Table 1 indicated that the di-substituted anilino group at position 7 of pyrazolo[1,5-*a*]pyrimidine core structure enhanced the anti-tumor activity. In the best case, potency was almost improved six-fold over the corresponding 3-chloroaniline substituted compound (**8** *vs.* **11**). Introduction of an ethyl group to the amino alkoxy moiety of C-5 decreased the anti-tumor activity, indicating non-tolerance for longer chains in this region. With the discovery of the di-substituted anilino group at position 7 as an advance in the anti-tumor activity, we next synthesized a second set of analogues, **15**–**21**, by varying the substituent at the 7-position and examined the effect of extension from the basic nitrogen of the amino alkoxy moiety. From the data in Table 2, compound **19** was the most potent one against two cancer cell lines. The pyrazolo[1,5-*a*]pyrimidine derivative **17** possessed potent anti-tumor effect against Bel-7402, and their IC_{50} were three times less than that of the positive control. It could be concluded that incorporation of the bis(trifluoromethyl)aniline functionality enhanced the anticancer activity. Comparing the activity of compounds **15**–**21** with various cyclic basic groups at the 2′-position of the alkoxy moiety, a 4-methylpiperzinyl group was detrimental to the activity, although compound **16** was active against the cancer cell lines Bel-7402.

Experimental

Chemistry

Melting points were determined with capillary tube method, and the thermometer was uncorrected. Mass spectra were obtained with a Finnigan LCQ HPLC-MS instrument (Thermo Electron Corporation, Bremen, Germany). IR spectra were collected on a Bruker IFS 55 instrument (Bruker, Rheinstetten, Germany). ^{1}H-NMR and ^{13}C-NMR spectra were run on a Bruker ARX-300 instrument, TMS as the internal standard. Elemental analysis was performed by a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy). Compound **7** was prepared according to the literature [9].

*3-Amino-4-cyano-pyrazole **3***

A mixture of malononitrile (16.5 g, 0.25 mol), triethyl orthoformate (50 mL, 0.25 mol), and acetic anhydride (51 mL, 0.54 mol) was heated to reflux. After 2 h., the mixture was cooled to 20°C, then hydrazine hydrate (19.4 g, 0.31 mol, 80%) was added dropwise at 20–25°C. The resulting mixture was stirred at 25–30°C for 18 h, and neutralized with aqueous sodium hydroxide. Filtration and washing with water gave 19.2 g (71%) of **3** as white powder, m.p.171–172°C, (lit. 166–169°C [6]). MS [MH$^+$] (*m/z*): 109.1; IR (KBr) cm^{-1}: 3308.5 (υ_{NH2}), 2240.3 ($\upsilon_{C=N}$), 1645.0 1569.6 1523.3 ($\upsilon_{C=C}$, $\upsilon_{C=N}$); Anal. calcd. for $C_4H_4N_4$: C 44.44, H 3.73, N 51.83; Found: C 44.45, H 3.73, N 51.84.

*5-Chloromethyl-3-cyano-7-hydroxypyrazolo[1,5-a]pyrimidine **4***

A mixture of **3** (10.8 g, 0.1 mol), ethyl 4-chloroacetoacetate (20 mL, 0.15 mol), and glacial acetic acid (100 mL) was refluxed for 4 h. After cooling to room temperature, the pale white solid was filtered and washed with ethanol, dried with suction, and recrystallized from ethyl acetate to obtain the solid product (19.7 g, 95%) [7, 8]. MS [MH$^+$] (*m/z*): 209.6; IR (KBr) cm^{-1}: 3450.1(υ_{OH}), 2230.3 ($\upsilon_{C=N}$), 1639.7, 1590.6 ($\upsilon_{C=C}$, $\upsilon_{C=N}$); ^{1}H-NMR (DMSO-d_6) d: 4.71 (s, 2H, CH_2), 6.77 (s, 1H, C_6-H), 8.85 (s, 1H, C_2-H), 11.83 (s, 0.78H, OH); ^{13}C-NMR (DMSO-d_6) δ: 55.8, 75.2, 99.5, 112.7, 145.3, 149.6, 155.2, 174.1; Anal. calcd. for $C_8H_5ClN_4O$: C 46.07, H 2.42, N 26.86; Found: C 46.16, H 2.34, N 26.55.

*5-Chloromethyl-3-cyano-7-chloropyrazolo[1,5-a]-pyrimidine **5***

Phosphorus oxychloride (55 mL, 0.59 mol) was added dropwise into a solution of **3** (82.0 g, 0.39 mol) in pyridine (35 mL, 0.43 mol). The mixture was heated to 85°C slowly, and then stirred at 120°C for 1 h. After it was cooled to 60°C, chloroform (500 mL) was added and stirred for 1 h. Cold water (300 mL) was added to the solution. The insoluble materials was filtered off, the organic layer of the filtrate was washed with cold water to pH 7, concentrated, and recrystallized from ethyl acetate/cyclohexane to give **4** (70 g, 78%) [7, 8]. MS [MH$^+$] (*m/z*): 228.0; IR (KBr) cm^{-1}: 2232.3 ($\upsilon_{C=N}$), 1612.7, 1541.5, 1499.8 ($\upsilon_{C=C}$, $\upsilon_{C=N}$); ^{1}H-NMR (DMSO-d_6) δ: 4.72 (s, 2H, CH_2), 6.83 (s, 1H, C_6-H), 8.86 (s, 1H, C_2-H); ^{13}C-NMR (DMSO-d_6) δ: 53.8, 78.2, 103.5, 116.7, 151.3, 159.6, 165.2, 169.1; Anal. calcd. for $C_8H_4Cl_2N_4$: C 42.32, H 1.78, Cl 31.23, N 24.68; Found: C 42.23, H 1.67, Cl 31.31, N 24.55.

General procedure for the synthesis of 5-Chloromethyl-3-cyano-7-subsituted-pyrazolo[1,5-a]-pyrimidine 6

A mixture of **5** (8.0 g, 35 mmol), R_1NH_2 (45 mmol), and isopropanol (50 mL) was heated at 60°C for 2 h. After cooling to room temperature, a massive solid precipitated. Recrystallization from methol gave **6** as slight yellow powder (80–86%).

General procedure for the synthesis of 8–21

NaH (0.3 g, 6.2 mmol, 60%) was added to the solution of **7** (6.2 mmol) in dried DMF (20 mL). The mixture was stirred for 30 min. Then **6** (2.84 mmol) was added and the resulting mixture was stirred for 2 h at 40°C. It was then poured into water and recrystallized to afford **8**–**21**.

*7-(3,4-Dichlorophenylamino)-3-cyano-5-((2-(dimethyl-amino)ethoxy)methyl)pyrazolo[1,5-a]pyrimidine **8***

Yield: 85%; m. p. 187–188°C; MS [MH$^+$] (*m/z*): 406.2; IR (KBr) cm^{-1}: 3440.5 (υ_{NH}), 2928.1 (υ_{CH3}), 2229.6 ($\upsilon_{C=N}$), 1612.7, 1576.9, 1542.4 ($\upsilon_{C=C}$, $\upsilon_{C=N}$). ^{1}H-NMR (DMSO-d_6) δ: 2.81 (s, 6H, $N(CH_3)_2$), 3.31 (t, J = 5.7 Hz, 2H, NCH_2CH_2O), 3.84 (t, J = 5.7 Hz, 2H, NCH_2CH_2O), 4.63 (s, 2H, CH_2), 6.64 (s, 1H, C_6-H), 7.51 (m, J = 8.7 Hz, 1H, ph-H), 7.55–7.59 (m, 2H, ph-2H), 8.81 (s, 1H, C_2-H); Anal. calcd. for $C_{18}H_{18}Cl_2N_6O$: C 53.34, H 4.48, N 20.74; Found: C 53.21, H 4.55, N 20.59.

7-(3,4-Dichlorophenylamino)-3-cyano-5-((2-(diethylamino)ethoxy)methyl)pyrazolo[1,5-a]pyrimidine ***9***

Yield: 81%; m. p. 181-183°C; MS [MH^+] (*m/z*): 434.0; IR (KBr) cm^{-1}: 3443.5 (υ_{NH}), 2963.1 (υ_{CH2}), 2230.9 ($\upsilon_{C=N}$), 1590.0, 1556.5, 1473.4 ($\upsilon_{C=C}$, $\upsilon_{C=N}$). ^{1}H-NMR (DMSO-d_6) δ: 0.82 (t, *J* = 7.0 Hz, 6H, 2 × CH_2CH_3), 2.40 (q, *J* = 7.0 Hz, 4H, 2 x CH_2CH_3), 2.55 (t, *J* = 6.0 Hz, 2H, NCH_2CH_2O), 3.48 (t, *J* = 6.0 Hz, 2H, NCH_2CH_2O), 4.31(s, 2H, CH_2), 6.06 (s, 1H, C_6-H), 7.55 – 7.58 (m, 3H, ph-3H), 8.26 (s, 1H, C_2-H); Anal. calcd. for $C_{20}H_{22}Cl_2N_6O$: C 55.43, H 5.12, N 19.39; Found: C 55.29, H 5.18, N 19.27.

7-(3,4-Dichlorophenylamino)-3-cyano-5-((2-(4-methylpiperazin-1-yl)ethoxy)methyl)pyrazolo[1,5-a]pyrimidine ***10***

Yield: 78%; m. p. 170 – 173°C; MS [MH^+] (*m/z*): 461.0; IR (KBr) cm^{-1}: 3443.0 (υ_{NH}), 2923.7 (υ_{CH2}), 2228.8 ($\upsilon_{C=N}$), 1586.5, 1557.2, 1472.8 ($\upsilon_{C=C}$, $\upsilon_{C=N}$). ^{1}H-NMR (DMSO-d_6) δ: 2.51 (s, 3H, CH_3), 2.73 (b, 4H, piperazinyl-3,5-2CH_2), 2.87 (brs, 4H, piperaziny-2,6-2CH_2), 3.04 (t, *J* = 5.7 Hz, 2H, NCH_2CH_2O), 3.67 (t, *J* = 5.7 Hz, 2H, NCH_2CH_2O), 4.57 (s, 2H, CH_2), 6.59 (s, 1H, C_6-H), 7.51 (d, *J* = 6.6 Hz, 1H, ph-H), 7.77 – 7.80 (m, 2H, ph-2H), 8.79 (s, 1H, C_2-H); Anal. calcd. for $C_{21}H_{23}Cl_2N_7O$: C 54.79, H 5.04, N 21.30; Found: C 54.87, H 4.98, N 21.46.

7-(3-Chlorophenylamino)-3-cyano-5-((2-(dimethylamino)ethoxy)methyl)pyrazolo[1,5-a]pyrimidine ***11***

Yield: 84%; m. p. 180 – 181°C; MS [MH^+] (*m/z*): 371.1; IR (KBr) cm^{-1}: 3426.9 (υ_{NH}), 2925.1 (υ_{CH2}), 2228.2 ($\upsilon_{C=N}$), 1622.9 1566.9 1537.0 1478.5 ($\upsilon_{C=C}$, $\upsilon_{C=N}$). ^{1}H-NMR (DMSO-d_6) δ: 2.79 (s, 6H, 2 × CH_3), 3.29 (t, *J* = 5.4 Hz, 2H, NCH_2CH_2O), 3.82 (t, *J* = 5.4 Hz, 2H, NCH_2CH_2O), 4.64 (s, 2H, CH_2), 6.59 (s, 1H, C_6-H), 7.40 – 7.58 (m, 4H, ph-4H), 8.81 (s, 1H, C_2-H); Anal. calcd. for $C_{18}H_{19}ClN_6O_2$: C 58.30, H 5.16, N 22.66; Found: C 58.43, H 5.29, N 22.76.

7-(3-Chlorophenylamino)-3-cyano-5-((2-(diethylamino)ethoxy)methyl)pyrazolo[1,5-a]pyrimidine ***12***

Yield: 81%; m. p. 176 – 177°C; MS [M^+] (*m/z*): 399.0; IR (KBr) cm^{-1}: 3443.5 (υ_{NH}), 2926.7 (υ_{CH2}), 2226.3 ($\upsilon_{C=N}$), 1622.8, 1588.5, 1476.8 ($\upsilon_{C=C}$, $\upsilon_{C=N}$). ^{1}H-NMR (DMSO-d_6) δ: 1.14 (t, *J* = 7.2 Hz, 6H, 2 × CH_2CH_3), 3.14 (q, *J* = 7.2 Hz, 4H, 2 × CH_2CH_3), 3.31 (t, *J* = 5.7 Hz, 2H, NCH_2CH_2O), 3.83 (t, *J* = 5.7 Hz, 2H, NCH_2CH_2O), 4.64 (s, 2H, CH_2), 6.54 (s, 1H, C_6-H), 7.41 – 7.57 (m, 4H, ph-4H), 8.81 (s, 1H, C_2-H); Anal. calcd. for $C_{20}H_{23}ClN_6O_2$: C 60.22, H 5.81, N 21.07; Found: C 60.18, H 5.72 N 21.16.

7-(3-Chlorophenylamino)-3-cyano-5-((2-(pyrrolidin-1-yl)ethoxy)methyl)pyrazolo[1,5-a]pyrimidine ***13***

Yield: 85%; m. p. 190 – 191°C; MS [M^+] (*m/z*): 397.1; IR (KBr) cm^{-1}: 3441.7 (υ_{NH}), 2930.6 (υ_{CH2}), 2226.7 ($\upsilon_{C=N}$), 1623.8, 1586.4, 1535.7, 1477.2 ($\upsilon_{C=C}$, $\upsilon_{C=N}$). ^{1}H-NMR (DMSO-d_6) δ: 1.89 (b, 4H, pyrrolidinyl-3,4-2CH_2), 3.23 (b, 4H, pyrrolidinyl-2,5-2CH_2), 3.34 (t, *J* = 5.7 Hz, 2H, NCH_2CH_2O), 3.79 (t, *J* = 5.7 Hz, 2H, NCH_2CH_2), 4.63 (s, 2H, CH_2), 6.57 (s, 1H, C_6-H), 7.41 – 7.58 (m, 4H, ph-4H), 8.80 (s, 1H, C_2-H); Anal. calcd. for $C_{20}H_{21}ClN_6O$: C 60.53, H 5.33, N 21.18; Found: C 60.67, H 5.44 N 21.29.

7-(3-Chlorophenylamino)-3-cyano-5-((2-(4-methylpiperazin-1-yl)ethoxy)methyl)pyrazolo[1,5-a]pyrimidine ***14***

Yield: 79%; m. p. 180 – 181°C; MS [M^+] (*m/z*): 426.0; IR (KBr) cm^{-1}: 3444.4 (υ_{NH}), 2931.7 (υ_{CH2}), 2222.4 ($\upsilon_{C=N}$), 1623.4, 1585.3, 1559.4, 1473.0 ($\upsilon_{C=C}$, $\upsilon_{C=N}$). ^{1}H-NMR (DMSO-d_6) δ: 2.51 (s, 3H, CH_3), 2.71 (b, 4H, piperazinyl-3,5-1CH_2), 2.82 (b, 4H, piperazinyl-2,6-2CH_2), 3.14 (t, *J* = 5.4 Hz, 2H, NCH_2CH_2O), 3.57 (t, *J* = 5.4 Hz, 2H, NCH_2CH_2O), 4.57 (s, 2H, CH_2), 6.59 (s, 1H, C_6-H), 7.41 – 7.58 (m, 4H, ph-4H), 8.80 (s, 1H, C_2-H); Anal. calcd. for $C_{21}H_{24}ClN_7O$: C 59.22, H 5.68, N 23.02; Found: C 59.37, H 5.74 N 23.26.

7-(4-Bromo-3-cholrophenylanimo)-3-cyano-5-((2-(pyrrolidin-1-yl)ethoxy)methyl)pyrazolo[1,5-a]pyrimidine ***15***

Yield: 77%; m. p. 175 – 176°C; MS [M^+] (*m/z*): 459.0; IR (KBr) cm^{-1}: 3445.4 (υ_{NH}), 2923.7 (υ_{CH2}), 2221.5 ($\upsilon_{C=N}$), 1624.0, 1575.0, 1483.3 ($\upsilon_{C=C}$, $\upsilon_{C=N}$). ^{1}H-NMR (DMSO-d_6) δ: 3.23 (b, 4H, pyrrolinyl-3,5-2CH_2), 3.34 (b, 4H, pyrrolinyl-2,6-2CH_2), 3.39 (t, *J* = 5.7 Hz, 2H, NCH_2CH_2O), 3.79 (t, *J* = 5.7 Hz, 2H, NCH_2CH_2O), 4.63 (s, 2H, CH_2), 6.67 (s, 1H, C_6-H), 7.34 (d, *J* = 8.7 Hz, 1H, ph-H), 7.56 (d, *J* = 8.4 Hz, 1H, ph-H), 7.83 (t, *J* = 8.7 Hz, 1H, ph-H), 8.79 (s, 1H, C_2-H); Anal. calcd. for $C_{20}H_{20}BrFN_6O$: C 52.30, H 4.39, N 18.30; Found: C 52.19, H 4.26 N 18.29.

7-(4-Bromo-3-cholrophenylanimo)-3-cyano-5-((2-(4-methylpiperazin-1-yl)ethoxy)methyl)pyrazolo[1,5-a]pyrimidine ***16***

Yield: 75%; m. p. 194 – 196°C; MS [M^+] (*m/z*): 488.0; IR (KBr) cm^{-1}: 3440.4 (υ_{NH}), 2924.0 (υ_{CH2}), 2227.0 ($\upsilon_{C=N}$), 1615.2, 1566.9, 1483.1 ($\upsilon_{C=C}$, $\upsilon_{C=N}$). ^{1}H-NMR (DMSO-d_6) δ: 2.67 (s, 3H, CH_3), 2.75 (b, 4H, piperazinyl-3,5-2CH_2), 2.69 (b, 4H, piperazinyl-2,6-2CH_2), 3.24 (t, *J* = 5.4 Hz, 2H, NCH_2CH_2O), 3.83 (t, *J* = 5.4 Hz, 2H, NCH_2CH_2O), 4.58 (s, 2H, CH_2), 6.65 (s, 1H, C_6-H), 7.33 (d, *J* = 8.7 Hz, 1H, ph-H), 7.57 (d, *J* = 8.4 Hz, 1H, ph-H), 7.85 (t, *J* = 8.7 Hz, 1H, ph-H), 8.79 (s, 1H, C_2-H); Anal. calcd. for $C_{21}H_{23}BrFN_7O$: C 51.65, H 4.75, N 20.08; Found: C 51.49, H 4.74, N 20.18.

7-(Benzo[d][1,3]dioxol-5-yl-amino)-3-cyano-5-((2-pyrrolidin-1-yl)ethoxy)methyl)pyrazolo[1,5-a]pyrimidine ***17***

Yield: 84%; m. p. 201 – 202°C; MS [MH^+] (*m/z*): 407.1; IR (KBr) cm^{-1}: 3442.8 (υ_{NH}), 2961.1 (υ_{CH3}), 2227.0 ($\upsilon_{C=N}$), 1623.0, 1584.7, 1485.7 ($\upsilon_{C=C}$, $\upsilon_{C=N}$). ^{1}H-NMR (DMSO-d_6) δ: 3.23 (b, 4H, pyrrolinyl-3,5-2CH_2), 3.34 (b, 4H, pyrrolinyl-2,6-2CH_2), 3.39 (t, *J* = 5.7 Hz, 2H, NCH_2CH_2O), 3.79 (t, *J* = 5.7 Hz, 2H, NCH_2CH_2O), 4.63 (s, 2H, CH_2), 6.12 (s, 2H, OCH_2O), 6.36 (s, 1H, C_6-H), 6.92 (d, *J* = 8.4 Hz, 1H, ph-H), 7.04 – 7.07 (m, 2H, ph-2H), 8.74 (s, 1H, C_2-H); Anal. calcd. for $C_{21}H_{22}N_6O_3$: C 62.06, H 5.46, N 20.68; Found: C 62.15, H 5.33, N 20.52.

7-(Benzo[d]-[1,3]dioxol-5-yl-amino)-3-cyano-5-((2-(4-methylpiperazin-1-yl)ethoxy)methyl)pyrazolo[1,5-a]pyrimidine ***18***

Yield: 82%; m. p. 191 – 192°C; MS [MH^+] (*m/z*): 436.0; IR (KBr) cm^{-1}: 3443.5 (υ_{NH}), 2962.1 (υ_{CH3}), 2227.1 ($\upsilon_{C=N}$), 1629.5, 1585.8, 1538.4, 1486.5 ($\upsilon_{C=C}$, $\upsilon_{C=N}$). ^{1}H-NMR (DMSO-d_6) δ: 2.71 (s, 3H, CH_3), 3.13 (b, 4H, piperazinyl-3,5-2CH_2), 3.36 (b, 4H, piperazinyl-2,6-2CH_2), 3.29

(t, *J* = 5.7 Hz, 2H, N*CH*$_2$CH$_2$O), 3.76 (t, *J* = 5.7 Hz, 2H, NCH$_2$*CH*$_2$O), 4.30 (s, 2H, CH$_2$), 6.1 1(s, 2H, OCH$_2$O), 6.57 (s, 1H, C$_6$-H), 6.93 (d, *J* = 7.5 Hz, 1H, ph-H), 7.02 – -7.05 (m, 2H, ph-2H), 8.82 (s, 1H, C$_2$-H); Anal. calcd. for $C_{22}H_{25}N_7O_3$: C 60.68, H 5.79, N 22.51; Found: C 60.80, H 5.84, N 22.52.

7-(3,5-Bis(trifluoromethyl)phenylamino)-3-cyano-5-((2-(piperidin-1-yl)ethoxy)methyl)pyrazolo[1,5-a]pyrimidine ***19***

Yield: 81%; m. p. 170 – 173°C; MS [MH$^+$] (*m/z*): 513.0; IR (υ, KBr, cm^{-1}): 3409.6 (υ_{NH}), 2924.1 (υ_{CH2}), 2214.4 ($\upsilon_{C=N}$), 1581.7, 1543.9, 1466.4 ($\upsilon_{C=C}$, $\upsilon_{C=N}$). ^{1}H-NMR δ: 1.39 (b, 6H, piperidinyl-3,4,5-3*CH*$_2$), 2.33 (b, 4H, piperidinyl-2,6-2*CH*$_2$), 2.73 (t, *J* = 5.4 Hz, 2H, N*CH*$_2$CH$_2$O), 3.62 (t, *J* = 5.4 Hz, 2H, NCH$_2$*CH*$_2$O), 4.53 (s, 2H, CH$_2$), 6.56 (s, 1H, C$_6$-H), 7.93 (1H, ph-H), 8.06 (2H, ph-2H), 8.67 (s, 1H, C$_2$-H); Anal. calcd. for $C_{22}H_{22}F_6N_6O$: C 53.91, H 4.33, N 16.40; Found: C 53.77, H 4.32, N 16.29.

7-(3,5-Bis(trifluoromethyl)phenylamino)-3-cyano-5-((2-morpholinoethyl)methyl)pyrazolo[1,5-a]pyrimidine ***20***

Yield: 75%; m. p. 166 – 167°C; MS [MH$^+$] (*m/z*): 515.0; IR (υ, KBr, cm^{-1}): 3442.3. (υ_{NH}), 2925.1 (υ_{CH2}), 2225.4 ($\upsilon_{C=N}$), 1663.1, 1594.4, 1541.4, 1494.1 ($\upsilon_{C=C}$, $\upsilon_{C=N}$). ^{1}H-NMR δ: 2.17 (brs, 4H, piperidinyl-2,6-2*CH*$_2$), 2.74 (brs, 4H, piperidinyl-3,5-2*CH*$_2$), 3.34 (t, *J* = 5.4 Hz, 2H, N*CH*$_2$CH$_2$O), 3.53 (t, *J* = 5.4 Hz, 2H, NCH$_2$*CH*$_2$O), 4.28 (s, 2H, CH$_2$), 5.99 (s, 1H, C$_6$-H), 7.52 (1H, ph-H), 7.98 (2H, ph-2H), 8.19 (s, 1H, C$_2$-H); Anal. calcd. for $C_{22}H_{20}F_6N_6O_2$: C 51.37, H 3.92, N 16.34; Found: C 51.23, H 3.81, N 16.29.

7-(3,5-Bis(trifluoromethyl)phenylamino)-3-cyano-5-((2-(4-methylpiperazin-1-yl)ethoxy)methyl)pyrazolo[1,5-a]pyrimidine ***21***

Yield: 83%; m. p. 176 – 177°C; MS [MH$^+$] (*m/z*): 528.0; IR (υ, KBr, cm^{-1}): 3440.1 (υ_{NH}), 2924.5 (υ_{CH2}), 2224.1 ($\upsilon_{C=N}$), 1597.6, 1543.1, 1460.6 ($\upsilon_{C=C}$, $\upsilon_{C=N}$). ^{1}H-NMR δ: 2.09 (s, 3H, CH$_3$), 2.17 (brs, 4H, piperidinyl-3,5-2*CH*$_2$), 2.20 (brs, 4H, piperidinyl-3,5-2*CH*$_2$), 2.42 (t, *J* = 5.7 Hz, 2H, N*CH*$_2$CH$_2$O), 3.53 (t, *J* = 5.7 Hz, 2H, NCH$_2$*CH*$_2$O), 4.35 (s, 2H, CH$_2$), 6.17 (s, 1H, C$_6$-H), 7.61 (1H, ph-H), 7.69 (2H, ph-2H), 8.33 (s, 1H, C$_2$-H); Anal. calcd. for $C_{23}H_{23}F_6N_7O_2$: C 52.37, H 4.40, N 18.59; Found: C 52.22, H 4.28 N 18.72.

Pharmacology

The anticancer activities of compounds **8 – 21** were evaluated with Bel-7402 (Human Liver Cancer Cell Lines) and HT-1080 (Human Fibro Sarcoma Cell Lines) by the MTT method *in vitro* [10], with cisplatin as the positive control. The cells were seeded in 96-well plate at the concentration of 4000 cells per well in 100 mL RPM I 1640 medium. After cultured for 12 h at 37°C and 5% CO_2, cells were incubated with various concentrations of samples for 24 h. MTT was added at a terminal concentration of 5 μg/mL and incubated with cells for 4 h. The formazan crystals were dissolved in 100 μL DMSO each well, and the optical density was measured at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength). The IC_{50} was calculated by Bliss method.

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