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Synthesis, Crystal Structure, and Antiproliferative Activity of Novel 7-Arylaminopyrazolo[1,5-*a*]pyrimidine Derivatives Containing the Hydrazone Moiety

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Abstract—A series of novel 7-arylaminopyrazolo[1,5-*a*]pyrimidine derivatives containing the hydrazone moiety has been synthesized by a five-step procedure including cyclization, chlorination, amination, hydrazinolysis, and condensation. Structures of the products have been characterized by IR, ^{1}H NMR and MS spectra, and single-crystal X-ray diffraction. The bioassay results indicate most of the compounds as potentially antiproliferation agents against A549 and HT-29 cell lines. Among those, compounds **6e** and **6f** exhibit remarkable inhibitory activity against HT-29 cell lines, that are comparable with that of the positive control sorafenib. Preliminary structure–activity relationship is considered.

Keywords: pyrazolo[1,5-*a*]pyrimidine, synthesis, X-ray diffraction, antiproliferation activity

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INTRODUCTION

Pyrazolo[1,5-*a*]pyrimidine derivatives constitute an imperative class of compounds with diverse therapeutic and pharmacological properties such as antitumor [1–3], antifungal [4], antibacterial [5], analgesics [6], anti-inflammatory [7]. Insomnia agent indiplon [8], anticancer drug dinaciclib [9], Type 2 diabetes mellitus agent anagliptin [10] are all the approved drugs containing the pyrazolo[1,5-*a*]pyrimidine moiety. On the other hand, the hydrazone moiety has been widely applied in drug design due to its ability to act as H-bond donator and acceptor. Also it can impart some degree of flexibility to chemical structures [11, 12]. Previous literatures have not been fully considered to the compounds contain both pyrazolo[1,5-*a*]pyrimidine and hydrazone units, which may possess novel bio-activities for screening.

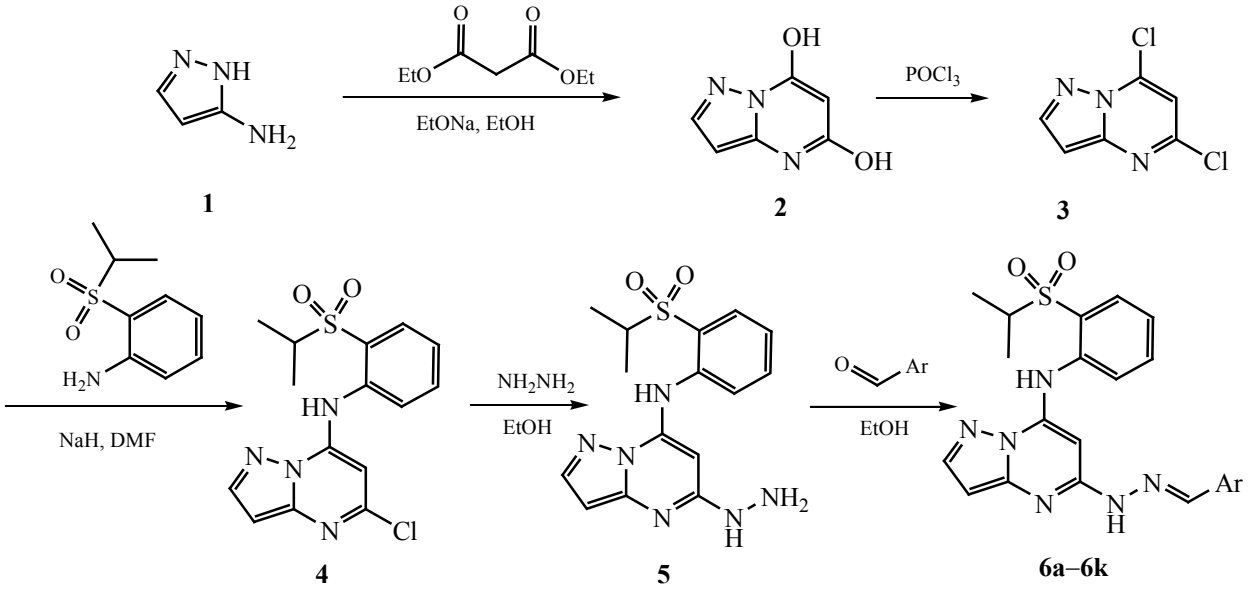
In continuation of our research on novel anticancer agents [13, 14], herein we report the synthesis of a series of substituted pyrazolo[1,5-*a*]pyrimidine–hydrazone hybrids by the five-step synthesis and evaluation of their in vitro antiproliferative activity against A549 and HT-29 cancer cell lines.

RESULTS AND DISCUSSION

The synthetic methods for compounds **6a**–**6k** were outlined in Scheme 1. The commercially available 3-aminopyrazole (**1**) was condensed with diethyl malonate in the presence of sodium ethanolate to give pyrazolo[1,5-*a*]pyrimidine-5,7-diol (**2**). Chlorination of compound **2** with phosphorus oxychloride proceeded smoothly with formation of 5,7-dichloropyrazolo[1,5-*a*]pyrimidine (**3**) as a yellow solid. A regioselective condensation of 2-(isopropylsulfonyl)aniline with the 7-position of 5,7-dichloropyrazolo [1,5-*a*]pyrimidine (**3**) in the presence of NaH in *N,N*-dimethylformamide provided the intermediate **4**, chlorine of which was substituted with hydrazine hydrate giving the key intermediate **5**, condensation of which with an appropriate aromatic aldehyde afforded the target compounds **6a**–**6k** with good yields. Their structures were supported by IR, ^{1}H NMR, and MS spectra. For the deeper insight into the structure of the products the crystal structure of **6j** was determined by X-ray single-crystal diffraction analysis.

The molecular structure of compound **6j** with atomic numbering scheme as presented in Figs. 1 and 2 depicts the molecular packing and hydrogen bonds in a unit cell.

Scheme 1. Synthetic route to the target compounds **6a–6k.**



Ar = phenyl (**6a**), 2-fluorophenyl (**6b**), 4-fluorophenyl (**6c**), 4-nitrophenyl (**6d**), 4-methoxyphenyl (**6e**), 4-(dimethylamino)phenyl (**6f**), 3-methoxyphenyl (**6g**), 2,4-difluorophenyl (**6h**), 2-chloro-4-fluorophenyl (**6i**), 2,3,4-trimethoxyphenyl (**6j**), 1*H*-3-indolyl (**6k**).

Configuration of the imino double bonds in the target compounds was confirmed to be the *E* isomer (Fig. 1). Generally, the average bond lengths and bond angles of pyrazole and phenyl rings were within normal ranges [3, 15]. The N^1–C^1 (1.325 Å) and N^3–C^4 (1.319 Å) bonds were longer than the general C=N double bond (1.27 Å), which indicated significant electron delocalization in the fused ring system. The bond C^5–N^7 (1.27 Å) belonged to the typical C=N double bond. In the crystal structure of compound **6j** the dihedral angles between the pyrazolo[1,5-*a*]pyrimidine ring, benzene ring (C^{18}, C^{19}, C^{20}) and benzene ring (C^8, C^9, C^{10}) were determined to be 40.877(8)° and 7.062°, respectively, which indicated the pyrazolo[1,5-*a*]pyrimidine ring to be almost coplanar with the benzene ring (C^8, C^9, C^{10}). The intermolecular hydrogen bonds C^7–$H^7\cdots O^{5\#1}$, C^{14}–$H^{14A}\cdots O^2$, and C^{20}–$H^{20}\cdots O^{1\#2}$ were formed (Table 1). The two adjacent molecules were stabilized via π···π stacking, van der Waals and intermolecular hydrogen forces with formation of complex accumulation space structures.

Antiproliferative activity and structure–activity relationship. Antiproliferative activity of all the newly synthesized compounds **6a–6k** was evaluated against human lung adenocarcinoma cell line A549 and human colon cancer cell line HT-29 using the standard MTT-based assay in vitro, with sorafenib used as the positive control (Table 2). All the compounds were more potent against HT-29 cell line than A549. Such results revealed

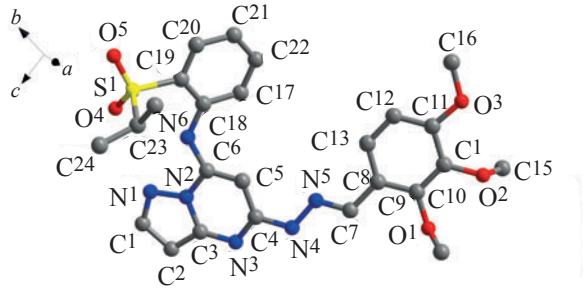


Fig. 1. The structure of $C_{25}H_{26}N_6O_5S$ with all non H-atom labeling scheme and ellipsoids drawn at the 30% probability level.

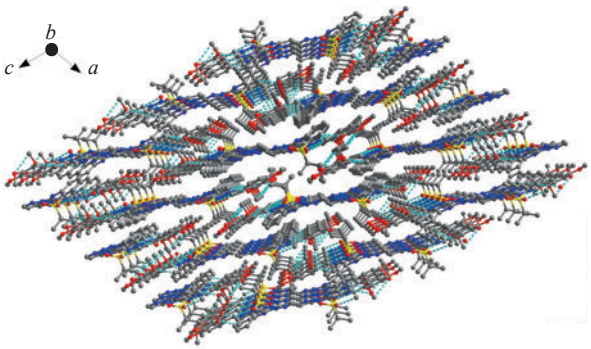


Fig. 2. Packing diagram of $C_{25}H_{26}N_6O_5S$.

that the compounds possessed selectivity against HT-29 cancer cell line, and demonstrated high potential as a specific drug for colon cancer. Results presented in Table 1 indicated that the presence of the electron-donating groups (EDGs) on the Ar had a positive effect on the antiproliferative activity, particularly of compounds **6e** (Ar = 4-OCH_3Ph) and **6f** [Ar = 4-$N(CH_3)_2$] that were more active than compound **6a** (Ar = Ph). However, attachment of the other groups to the Ar fragment (Ar = 2-FPh, 4-FPh, 4-NO_2Ph, 2,4-diFPh, and 2-Cl-4-FPh) reduced the antiproliferative activity. The bulky groups such as 2,3,4-trimethoxyphenyl (**6j**) or 1*H*-3-indolyl (**6k**) bonded with the Ar also reduced antiproliferative activity of the compounds.

EXPERIMENTAL

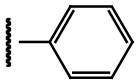
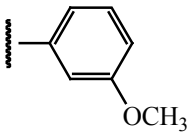
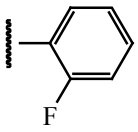
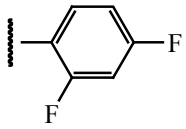
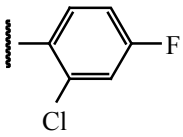
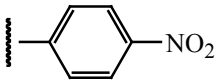
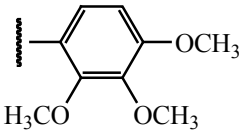
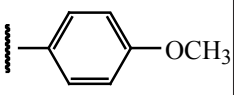
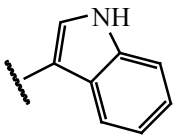
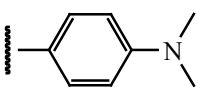
All starting materials and reagents were obtained from commercial suppliers without further purification, unless specified otherwise. Melting points were determined on a Beijing Taike X-4 microscopy melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured on a Bruker Biospin 600 MHz or Bruker Biospin 300 MHz spectrometers using TMS as the internal standard. IR spectra were recorded as KBr pellets on a Perkin-Elmer Spcetrum one FT-IR spectrophotometer. Mass spectra

Table 1. Hydrogen bond lengths and bond angles in compound **6j**[a]

D–H···A	*d*(D–H), Å	*d*(H···A), Å	*d*(D···A), Å	DHA angle, deg
C^7–H^7···$O^{5\#1}$	0.93	2.63	3.434(3)	144
C^{14}–H^{14A}···O^2	0.96	2.42	3.022(4)	120
C^{20}–H^{20}···$O^{1\#2}$	0.93	2.47	3.159(3)	131

[a] Symmetry codes: (#1) *x*, *y*–1, *z*; (#2) *x*, *y*+1, *z*.

Table 2. Inhibitory effect of target compounds against tumor cell lines[a]

Compound	Ar	IC_{50}±SD, μmol/L		Compound	Ar	IC_{50}±SD, μmol/L	
		A549	HT-29			A549	HT-29
6a		27.21±1.86	4.95±0.29	**6g**		27.47±1.76	8.02±1.27
6b		30.68±2.11	8.32±0.98	**6h**		25.44±2.03	6.75±0.56
6c		29.32±1.45	10.41±1.10	**6i**		22.35±1.38	7.86±0.62
6d		32.45±2.24	4.78±1.04	**6j**		42.38±2.73	8.95±0.36
6e		11.22±1.21	2.94±0.24	**6k**		32.81±1.74	11.631±0.99
6f		8.83±0.54	4.29±0.35	Sorafenib	–	3.43±0.15	4.02±0.19

[a] Each experiment was carried out in triplicate.

were measured on a Waters Quattro micro API mass spectrometer (ESI, direct injection). Elemental analysis of the newly synthesized compounds was carried out on a Carlo Erba 1108 analyzer.

Synthesis of pyrazolo[1,5-*a*]pyrimidine-5,7-diol (2). Metal sodium (7.62 g, 331.0 mmol) was reacted with 150 mL of ethanol at 0°C. 3-Aminopyrazole (11.00 g, 132.38 mmol) and diethyl malonate (21.20 g, 132.38 mmol) were added to the above solution of sodium ethoxide in ethanol. The reaction mixture was refluxed for 20 h, then cooled down to room temperature. The precipitate was filtered off and washed with EtOH. The dried solid was dissolved in water (400 mL), and the solution was acidified to pH 2 with 10 N HCl. The solid precipitate was collected by filtration and washed with water to give pyrazolo[1,5-*a*]pyrimidine-5,7-diol as a light yellow solid, yield 63.5%. Thus obtained crude product was used in the next step without further purification.

Synthesis of 5,7-dichloropyrazolo[1,5-*a*]pyrimidine (3). Solution of pyrazolo[1,5-*a*]pyrimidine-5,7-diol (6.50 g, 43.0 mmol) in phosphorus oxychloride (80 mL) was mixed with *N N*-dimethylaniline (5.0 mL), and the mixture was refluxed for 24 h. Excess phosphorus oxychloride was removed in vacuo. After cooling to room temperature, the residue was poured into ice water and extracted with EtOAc (3×100 mL). The combined organic phases were washed with 1 N HCl several times, until *N,N*-dimethylaniline was completely removed, and then washed with water and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography to afford the compound **3** as a yellow solid, yield 68.0%. ^{1}H NMR spectrum, δ, ppm: 8.23 d (1H, *J* = 3.0 Hz), 7.02 s (1H), 6.77 d (1H, *J* = 3.0 Hz). MS (ESI): *m/z*: 181.1 $[M + H]^+$.

Synthesis of 5-chloro-*N*-(2-(isopropylsulfonyl)-phenyl)pyrazolo[1,5-*a*] pyrimidin-7-amine (4). To the suspension of 60% NaH (1.36 g, 34 mol) in *N,N*-dimethylformamide (60 mL), 2-(isopropylsulfonyl)aniline (4.10 g, 20.6 mmol) was added at 0°C. The mixture was stirred for 30 min at 0°C, and then 5,7-dichloropyrazolo-[1,5-*a*]pyrimidine (3.20 g, 17.0 mmol) diluted by *N,N*-dimethylformamide (10 mL) was added slowly. The mixture was warmed up to room temperature and heated at 60°C for 8 h, then poured into water to give a dark red precipitate which was collected by filtration. The crude product was crystallized from isopropanol to afford compound **4** as a dark yellow solid, yield 68.7%. ^{1}H NMR spectrum, δ, ppm: 10.10 s (1H), 8.14 d (1H, *J* = 1.8 Hz), 8.04 d (1H, *J* = 7.8 Hz), 7.78 d (2H, *J* = 5.0 Hz), 7.48–7.40 m (1H), 6.67–6.48 m (2H), 3.26–3.08 m (1H), 1.28 d, (6H, *J* = 6.8 Hz). MS (ESI): *m/z*: 373.0 $[M + Na]^+$.

Synthesis of 5-hydrazinyl-*N*-(2-(isopropylsulfonyl)-phenyl)pyrazolo[1,5-*a*]pyrimidin-7-amine (5). A mixture of compound **4** (4.00 g, 0.012 mol) with 80% hydrazine monohydrate (15 mL) in ethanol (40 mL) was refluxed overnight upon vigorous agitation. Most of the solvent was evaporated under reduced pressure and white solid precipitated. After cooling down to 10°C, the precipitate was filtered off, washed with water, and dried under vacuum to afford the title compound **5** as a pale solid, yield 81.0%. ^{1}H NMR spectrum, δ, ppm: 9.79 s (1H), 8.00 d (1H, *J* = 7.9 Hz), 7.94 d (1H, *J* = 1.9 Hz), 7.75 d (1H, *J* = 8.1 Hz), 7.68 t (1H, *J* = 7.7 Hz), 7.32 t (1H, *J* = 7.6 Hz), 6.17 d (1H, *J* = 2.0 Hz), 6.08 d (2H, *J* = 6.8 Hz), 3.87 s (2H), 3.26–3.15 m (1H), 1.28 d (6H, *J* = 6.8 Hz). MS (ESI): *m/z*: 474.2 $[M + H]^+$.

General procedure for preparation of compounds 6a–6k. To a solution of compound **5** (0.20 g, 0.58 mmol) in EtOH (5 mL), 1.2 equiv of the appropriate aldehyde and acetic acid (1 drop) were added, and the mixture was refluxed for 10–12 h to completion of the process according to TLC. After cooling down to room temperature, the resulting precipitate was filtered off and dried under vacuum to afford the corresponding target compound **6a–6k** as a light yellow solid.

5-(2-Benzylidenehydrazinyl)-*N*-[2-(isopropylsulfonyl)phenyl]pyrazolo[1,5-*a*]pyrimidin-7-amine (6a). Yield 82%, mp 236–238°C. IR spectrum, ν, cm^{-1}: 3434.6 (NH_2), 1633.4 (C=N), 1553.2 ($C=C_{arom}$), 1466.1, 1360.2 (O=S=O), 1143.3. ^{1}H NMR spectrum, δ, ppm: 11.26 s (1H), 9.75 s (1H), 8.13–7.89 m (5H), 7.66–7.45 m (3H), 7.45–7.25 m (3H), 6.64 s (1H), 6.13 d (1H, *J* = 2.1 Hz), 3.53–3.37 m (1H), 1.13 d (6H, *J* = 6.8 Hz). MS (ESI): *m/z*: 435.1 $[M + H]^+$, 457.1 $[M + Na]^+$. Found, %: C 60.94; H 5.14; N 19.45. $C_{22}H_{22}N_6O_2S$. Calculated, %: C 60.81; H 5.10; N 19.34.

5-[2-(2-Fluorobenzylidene)hydrazinyl]-*N*-[2-(isopropylsulfonyl)phenyl]pyrazolo[1,5-*a*]pyrimidin-7-amine (6b). Yield 84%, mp 228–230°C. IR spectrum, ν, cm^{-1}: 3440.5 (NH), 2976.2, 1634.6 (C=N), 1553.0 ($C=C_{arom}$), 1448.9, 1420.8, 1319.8, 1275.5, 1242.9, 1201.7, 1088.2, 1051.8, 909.0. ^{1}H NMR spectrum, δ, ppm: 11.41 s (1H), 9.77 s (1H), 8.22 s (1H), 8.08–7.86 m (4H), 7.75 m (1H), 7.51 m (1H), 7.37 m (1H), 7.22 d (2H, *J* = 7.4 Hz), 6.64 br (1H), 6.15 s (1H), 3.58–3.37 m

(1H), 1.23–0.99 m (6H). MS (ESI): *m/z*: 453.1 $[M + H]^+$, 475.2 $[M + Na]^+$. Found, %: C 58.47; H 4.74; N 18.64. $C_{22}H_{21}FN_6O_2S$. Calculated, %: C 58.39; H 4.68; N 18.57.

5-[2-(4-Fluorobenzylidene)hydrazinyl]-*N*-[2-(isopropylsulfonyl)phenyl]pyrazolo[1,5-*a*]pyrimidin-7-amine (6c). Yield 83%, mp 230–232°C. IR spectrum, ν, cm^{-1}: 3428.0 (NH), 1634.6 (C=N), 1555.0 ($C=C_{arom}$), 1495.0, 1466.0, 1275.9 (O=S=O), 1232.9, 1143.2. ^1H NMR spectrum, δ, ppm: 11.26 s (1H), 9.76 s (1H), 8.16–7.83 m (5H), 7.70–7.57 m (2H), 7.55–7.41 m (1H), 7.24 t (2H, *J* = 8.9 Hz,), 6.63 s (1H), 6.13 d (1H, *J* = 2.1 Hz), 3.52–3.39 m (1H), 1.13 d (6H, *J* = 6.8 Hz). MS (ESI) *m/z*(%): 453.1 $[M + H]^+$, 475.2 $[M + Na]^+$. Found, %: C 58.48; H 4.72; N 18.62. $C_{22}H_{21}FN_6O_2S$. Calculated, % C 58.39; H 4.68; N 18.57.

***N*-[2-(Isopropylsulfonyl)phenyl]-5-[2-(4-nitrobenzylidene)-hydrazinyl]pyrazolo[1,5-*a*]pyrimidin-7-amine (6d).** Yield 76%, mp 232–234°C. IR spectrum, ν, cm^{-1}: 3441.9 (NH), 1636.7 (C=N), 1551.8 ($C=C_{arom}$), 1514.0, 1464.3, 1335.6 (O=S=O), 1312.2 ($C_{arom}–NO_2$), 1144.0. ^1H NMR spectrum, δ, ppm: 11.64 s (1H), 9.83 s (1H), 8.25 d (2H, *J* = 8.9 Hz), 8.12 s (1H), 8.03 d (1H, *J* = 2.2 Hz), 8.00–7.93 m (3H), 7.83 d (2H, *J* = 8.9 Hz) , 7.53 s (1H), 6.67 s (1H), 6.20 d (1H, *J* = 2.1 Hz), 3.52–3.40 m (1H), 1.13 d (6H, *J* = 6.8 Hz). MS (ESI): *m/z*: 480.1 $[M + H]^+$, 502.1 $[M + Na]^+$. Found, %: C 55.22; H 4.47; N 20.53. $C_{22}H_{21}N_7O_4S$. Calculated, %: C 55.11; H 4.41; N 20.45.

***N*-[2-(Isopropylsulfonyl)phenyl]-5-[2-(4-methoxybenzylidene)hydrazinyl]pyrazolo[1,5-*a*]pyrimidin-7-amine (6e).** Yield 81%, mp 223–225°C. IR spectrum, ν, cm^{-1}: 3435.7 (NH), 1634.6 (C=N), 1554.7 ($C=C_{arom}$), 1497.7, 1465.0, 1307.4 (O=S=O), 1253.8, 1142.8. ^1H NMR spectrum, δ, ppm: 11.11 s (1H), 9.73 s (1H), 7.96 m (5H), 7.52 d (3H, *J* = 8.5 Hz), 6.96 d (2H, *J* = 8.8 Hz), 6.62 s (1H), 6.10 d (1H, *J* = 2.0 Hz), 3.77 s (3H), 3.55–3.38 m (1H), 1.13 d (6H, *J* = 6.8 Hz). MS (ESI): *m/z*: 465.1 $[M + H]^+$, 487.1 $[M + Na]^+$. Found, %: C 59.59; H 5.26; N 18.14. $C_{23}H_{24}N_6O_3S$. Calculated, %: C 59.47; H 5.21; N 18.09.

5-{2-[4-(Dimethylamino)benzylidene]hydrazinyl}-*N*-[2-(isopropylsulfonyl)phenyl]pyrazolo[1,5-*a*]-pyrimidin-7-amine (6f). Yield 81%, mp 222–225°C. IR spectrum, ν, cm^{-1}: 3430.5 (NH), 1633.9 (C=N), 1555.7 ($C=C_{arom}$), 1489.7, 1464.5, 1360.8 (O=S=O), 1198.9, 1124.8, 1050,8. ^1H NMR spectrum, δ, ppm: 10.94 s (1H), 9.70 s (1H), 8.06–7.85 m (5H), 7.57–7.45 m (1H), 7.39 d (2H, *J* = 8.8 Hz), 6.70 d (2H, *J* = 8.8 Hz), 6.62 s (1H), 6.07 d (1H, *J* = 2.1 Hz), 3.55–3.38 m (1H), 2.93 s (6H), 1.13 d (6H, *J* = 6.8 Hz). MS (ESI): *m/z*: 478.2 $[M + H]^+$, 500.2 $[M + Na]^+$. Found, %: C 60.48; H 5.76; N 20.61. $C_{24}H_{27}N_7O_2S$. Calculated, %: C 60.36; H 5.70; N 20.53.

***N*-[2-(Isopropylsulfonyl)phenyl]-5-[2-(3-methoxybenzylidene)hydrazinyl]pyrazolo[1,5-*a*]pyrimidin-7-amine (6g).** Yield 80%, mp 224–226°C. IR spectrum, ν, cm^{-1}: 3444.3 (NH), 1635.8 (C=N), 1582.3 ($C=C_{arom}$), 1551.7, 1465.7, 1422.3, 1320.2 (O=S=O), 1273.8, 1141.1, 1046.6. ^1H NMR spectrum, δ, ppm: 11.29 s (1H), 9.73 s (1H), 8.07–7.80 m (5H), 7.51 t (1H, *J* = 7.5 Hz), 7.29 t (1H, *J* = 7.8 Hz), 7.20–7.05 m (2H), 6.89 d (1H, *J* = 7.5 Hz), 6.62 s (1H), 6.13 s (1H), 3.74 s (3H), 3.57–3.38 m (1H), 1.12 d (6H, *J* = 6.7 Hz). MS (ESI): *m/z*: 465.1 $[M + H]^+$, 487.1 $[M + Na]^+$. Found, %: C 59.55; H 5.25; N 18.16. $C_{23}H_{24}N_6O_3S$. Calculated, %: C 59.47; H 5.21; N 18.09.

5-[2-(2,4-Difluorobenzylidene)hydrazinyl]-*N*-(2-(isopropylsulfonyl)phenyl)pyrazolo[1,5-*a*]pyrimidin-7-amine (6h). Yield 83%, mp 231–233°C. IR spectrum, ν, cm^{-1}: 3419.3 (NH), 1634.5 (C=N), 1685.8, 1585.8 ($C=C_{arom}$), 1488.9, 1463.9, 1418.4, 1345.7 (O=S=O), 1277.0, 1142.4. ^1H NMR spectrum, δ, ppm: 11.39 s (1H), 9.78 s (1H), 8.16 s (1H), 8.04–7.85 m (4H), 7.87–7.73 m (1H), 7.51 t (1H, *J* = 7.3 Hz), 7.31 t (1H, *J* = 9.3 Hz), 7.16 t (1H, *J* = 8.4 Hz), 6.63 s (1H), 6.15 d (1H, *J* = 1.8 Hz), 3.54–3.39 m (1H), 1.13 d (6H, *J* = 6.7 Hz). MS (ESI): *m/z*: 471.1 $[M + H]^+$, 493.1 $[M + Na]^+$. Found, %: C 56.28; H 4.31; N 17.93. $C_{22}H_{20}F_2N_6O_2S$. Calculated, %: C 56.16; H 4.28; N 17.86.

5-[2-(2-Chloro-4-fluorobenzylidene)hydrazinyl]-*N*-[2-(isopropylsulfonyl)phenyl]pyrazolo[1,5-*a*]-pyrimidin-7-amine (6i). Yield 79%, mp 234–236°C. IR spectrum, ν, cm^{-1}: 3431.4 (NH), 3280.2, 2978.0, 1633.9 (C=N), 1555.4 ($C=C_{arom}$), 1465.0, 1316.9 (O=S=O), 1419.1, 1249.4, 1144.3. ^1H NMR spectrum, δ, ppm: 11.48 s (1H), 9.78 s (1H), 8.34 s (1H), 8.07–7.76 m (5H), 7.61–7.42 m (2H), 7.29 m (1H), 6.63 s (1H), 6.17 d (1H, *J* = 2.1 Hz), 3.51–3.38 m (1H), 1.20–1.04 m (6H). MS (ESI): *m/z*: 487.1 $[M + H]^+$, 509.1 $[M + Na]^+$. Found, %: C 54.34; H 4.17; N,17.33. $C_{22}H_{20}ClFN_6O_2S$. Calculated, %: C 54.26; H 4.14; N 17.26.

***N*-[2-(Isopropylsulfonyl)phenyl]-5-[2-(2,3,4-trimethoxybenzylidene)hydrazinyl]pyrazolo[1,5-*a*]pyrimidin-7-amine (6j).** Yield 78%, mp 235–237°C. IR spectrum, ν, cm^{-1}: 3443.7 (NH), 1635.7 (C=N), 1554.3 ($C=C_{arom}$), 1483.0, 1459.6, 1286.5 (O=S=O), 1143.2, 1094.8, 1028.0. ^1H NMR spectrum, δ, ppm: 11.13 s (1H),

Table 3. Crystal data for compound **6j**

Parameter	Value	Parameter	Value
Crystal size	0.18×0.15×0.12	α, deg	102.739(2)
Formula	$C_{25}H_{26}N_6O_5S$	β, deg	105.385(2)
Fw	522.58	γ, deg	104.187(2)
T, K	273(2) K	*V*, Å ^{3}	1277.4(2)
Crystal system	Triclinic	*Z*	2
Space group	*P*1	*Dc*, g/cm^3	1.359
a, Å	9.5142(11)	*F*(000)	548
b, Å	11.9857(13)	GOF on F^2	1.095
c, Å	12.5854(14)	Reflection/unique	6491/4459
		R_1, wR_2 [$I > 2(I)$]	0.0551, 0.1793
		R_1, wR_2 (all data)	0.0697, 0.1950

9.71 s (1H), 8.20 s (1H), 8.02–7.88 m (4H), 7.51 t (1H, *J* = 7.3 Hz), 7.40 d (1H, *J* = 8.8 Hz), 6.87 d (1H, *J* = 9.0 Hz), 6.60 s (1H), 6.11 d (1H, *J* = 2.1 Hz), 3.81 s (3H), 3.77 s (3H), 3.74 s (3H), 3.56–3.37 m (1H), 1.13 d (6H, *J* = 6.8 Hz). MS (ESI): *m/z*: 525.2 $[M + H]^+$, 547.1 $[M + Na]^+$. Found, %: C 57.37; H 5.42; N 16.11. $C_{25}H_{28}N_6O_5S$. Calculated, %: C 57.24; H 5.38; N 16.02.

5-{2-[(1*H*-Indol-3-yl)methylene]hydrazinyl}-*N*-[2-(isopropylsulfonyl)phenyl]pyrazolo[1,5-*a*]pyrimidin-7-amine (6k). Yield 82%, mp 226–228°C. IR spectrum, ν, cm^{-1}: 3348.6 (NH), 1634.2 (C=N), 1584.8 (C=C$_{arom}$), 1494.4, 1466.8, 1415.2, 1357.9 (O=S=O), 1274.0, 1131.7. ^{1}H NMR spectrum, δ, ppm: 11.43 s (1H), 10.92 s (1H), 9.60 s (1H), 8.21 s (1H), 8.11–7.86 m (5H), 7.73–7.57 m (2H), 7.40 d (1H, *J* = 8.1 Hz), 7.25–7.12 m (1H), 7.04–6.90 m (1H), 6.68 s (1H), 6.06 s (1H), 3.54–3.38 m (1H), 1.11 d, (6H, *J* = 6.5 Hz). MS (ESI): *m/z*: 474.2 $[M + H]^+$, 497.1 $[M + Na]^+$. Found, %: C 60.97; H 4.94; N 20.78. $C_{24}H_{23}N_7O_2S$. Calculated, %: C 60.87; H 4.90; N 20.70.

Crystal data structure determination of compound 6j. The yellow powder of compound **6i** was dissolved in ethanol–ethyl acetate–tetrahydrofuran (5 : 2 : 2 by v : v : v). Upon slow evaporation of the solvents for several days, some single crystals suitable for X-ray analysis were obtained. A yellow crystal ($C_{25}H_{26}N_6O_5S$) with dimensions of 0.18×0.15×0.12 mm was selected for data collection on a Bruker APEX-II CCD automatic diffractometer with graphite-monochromatized MoK_α radiation (λ = 0.71073 Å) using multi-scan mode at 273(2) K. A total of 6491 reflections were collected in the range of $2.4° < \theta < 25.0°$ (index ranges: $-11 < h < 11$, $-13 < k < 14$, $-14 < l < 8$) and 4459 were independent ($R_{int} = 0.013$), of which 3515 observed reflections with $I > 2\sigma(I)$ were used in the structure determination and refinements. The structure was solved by direct methods with SHELXS-97 program and expanded by Fourier technique. The non-hydrogen atoms were refined anisotropically [16]. The hydrogen atoms bound to carbon were determined with theoretical calculations, and those attached to nitrogen and oxygen were determined with successive difference Fourier syntheses. The structure was refined by full-matrix least-squares techniques on F^2 with SHELXL-97 [17]. The final refinement gave the final $R = 0.055$ and $wR = 0.195$ ($w = 1/[\sigma^2(Fo^2) + (0.140P)^2 + 0.0035P]$), where $P = (Fo^2 + 2Fc^2)/3$; $S = 1.10$, $(\Delta\sigma)_{max} < 0.001$, $(\Delta\rho)_{max} = 0.78$ and $(\Delta\rho)_{min} = -0.43$ *e*/Å^{-3}. All calculations were performed using the Crystal Structure crystallographic software package except for the refinement. Crystallographic data and experimental details of structural analyses for the compound are summarized in Table 3. CCDC 1948432 contains the supplementary data for this paper. The data can be obtained free of charge from the Cambridge Crystallographic Data Centre via https://summary.ccdc.cam.ac.uk/structure-summary-form.

In vitro anticancer activity test of the compounds 6a–6j on HT-29 and A549 cell lines. The anti-proliferative activity of the title compounds was evaluated against HT-29 and A549 cell lines using the standard MTT as-

say in vitro, with Foretinib as the positive control. The cancer cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS). Approximately 4×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37°C for 24 h. The tested compounds at the indicated final concentrations were added to the culture medium and the cell cultures were stored for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 μg/mL, and incubated with cells at 37°C for 4 h. The formazan crystals were dissolved in 100 mL DMSO each well, and the absorbance at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with an ELISA reader. All compounds were tested three times for each cell line. The results expressed as IC_{50} were the averages of three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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