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Synthesis, SAR study and biological evaluation of novel pyrazolo[1,5-*a*]pyrimidin-7-yl phenyl amides as anti-proliferative agents

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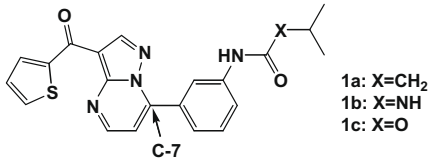
ABSTRACT

Checkpoint deficiency of malignant cells can be exploited in cancer drug discovery. Compounds that selectively kill checkpoint-deficient cells versus checkpoint-proficient cells can be utilized to preferentially target tumor cells, while sparing normal cells. The protein $p21^{Wafl/Cipl/Sdi1}$ (hereafter referred to as p21) inhibits progression of the cell cycle by inhibiting the activity of G1 kinases (cyclin D/cdk4 and cyclin E-cdk2) and the G2 kinase (cyclin B/cdkl) in response to DNA damage or abnormal DNA content. The expression of p21 is often low in human cancer cells due to frequent loss of the upstream activator, p53, and is associated with poor prognosis in some cancer patients. Using an isogenic pair of cell lines, HCT116 (p21+/+) and 80S14 (p21−/−), we have disclosed previously a novel series of pyrazolo[1,5-*a*]pyrimidines that preferentially kill the p21-deficient cells. We will present the synthesis, biological activities and SAR study of a series of pyrazolo[1,5-*a*]pyrimidines with an optimized phenyl amide moiety at the C-7 position. The mechanism of action of these compounds will also be discussed.

1. Introduction

Cell cycle checkpoints are signal transduction pathways that monitor the orderly progression of the cell division cycle. Loss of checkpoint control is a hallmark of tumor cells, as it increases the mutation rate and allows a more rapid progression to the tumorigenic state. Checkpoint deficiency in malignant cells can be exploited in cancer drug discovery.[1] Compounds that selectively kill checkpoint-deficient cells versus checkpoint-proficient cells can be in principle utilized to preferentially target tumor cells.[2,3]

The p53 tumor suppressor gene is the major regulator of the G1/S checkpoint and one of the most commonly mutated genes in human cancer (50–70%). As an important downstream effector of p53, p21 inhibits the cyclin-dependent kinases (CDKs) and arrests cell cycle progression in response to genotoxic stress. The protein p21 blocks progression of the cell cycle by inhibiting the activity of G1 (cyclin D/cdk4 and cyclin E-cdk2) and G2 kinases (cyclin B/cdkl) in response to DNA damage or abnormal DNA content.[4–6] The expression of p21 is often low in human cancer cells due to frequent loss of the upstream activator p53, and this low expression is associated with poor prognosis in some cancer patients.[7,8] Disruption of the checkpoint by deletion of the p21 gene results in failure of the cells to arrest in response to DNA damage and other environmental stressors, followed by endoreduplication and apoptosis.[9,10] It was shown that the absence of p21 renders cells remarkably sensitive to apoptosis following treatment with several cancer therapeutics. Vogelstein et al reported[1–3] a human colorectal cancer cell line, 80S14, in which the p21 genes have been inactivated by targeted gene deletion. These cells show increased chemosensitivity compared with the isogenic p21-proficient parental cells (HCT 116), to various antineoplastic drugs and gamma irradiation *in vitro* and in vivo, validating the role of checkpoints in determining chemosensitivity.



Using an isogenic pair of cell lines, HCT116 (p21+/+) and 80S14 (p21−/−), we have identified a novel series of pyrazolo[1,5-*a*]pyrimidines, including **1a–1c,** that preferentially inhibit the p21−/− cells, as described previously.[11–13] For example, pyrazolo[1,5-*a*]-pyrimidin-7-yl phenyl amide **1a** exhibited excellent activity in p21−/− cells (IC_{50} = 0.14 μM) and LoVo cells (IC_{50} = 0.021 μM). It was also found that the aryl ketone moiety and the pyrazolo[1,5-*a*]pyrimidine core are the essential compo-

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nents for their anti-proliferative activities. We present here the synthesis and SAR study of a series of pyrazolo[1,5-*a*]pyrimidines with an optimized phenyl amide moiety at the C-7 position along with a group of derivatives with reversed amide and amine side chains.

2. Chemistry

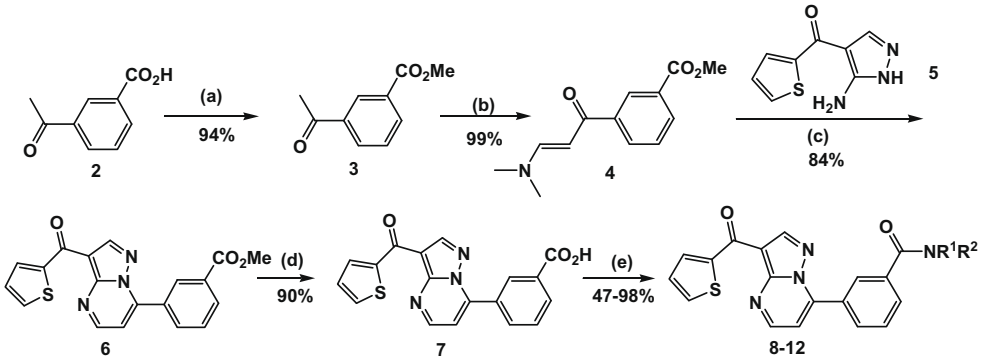
To investigate whether the amide moiety is part of the essential pharmacophore, compounds **8–12**, pyrazolopyrimidines with reverse phenyl amide groups, were prepared as shown in Scheme 1. Esterification of *m*-acetylbenzoic acid provided **3**. Treatment of **3** with *N*,*N*-dimethylformamide dimethyl acetal (DMF–DMA) gave enamine **5** which was readily coupled with **4**[14] in HOAc to afford **6**. Compound **6** was hydrolyzed in aqueous KOH to give acid **7**, which was subsequently treated with various amines to provide reverse amide products **8**–**12**.

To further explore the SAR of the amide side chain, compounds **14–17**, wherein various alkyl groups replaced the alkyl carbonyl group of **1a**, were prepared by reductive amination of compound **13**[11] with a variety of aldehydes in the presence of $NaBH(OAc)_3$ (Scheme 2).

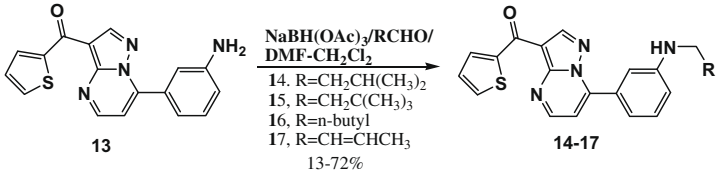
As illustrated in Scheme 3, pyrazolo[1,5-*a*]pyrimidines with substituted phenyl amides at C-7 (compounds **22–30**) were prepared starting from substituted *m*-nitroacetophenone (**18a–18e**). In general, substituted *m*-nitroacetophenones were converted to enamines (**19a**–**19d**) by reaction with DMF–DMA. However, the reaction between 4-fluoro-3-nitroacetophenone (**18e**) and DMF–DMA resulted in a substantial amount of by-product due to the replacement of fluoro by dimethylamine. To circumvent this problem, 4-fluoro-3-nitroacetophenone was treated with *N*,*N*-dimethylformamide di-*tert*-butyl acetal at ambient temperature to produce enamine **19e** cleanly.

All enaminones (**19a–19e**) reacted smoothly with **5** in HOAc to give **20a–20e**. Reduction of most nitro intermediates (**20a–20d**) proceeded readily using Fe in HOAc/MeOH, while a milder reaction condition ($SnCl_2$/THF) was required for the reduction of **20e** in order to minimize the undesired substitution of fluoro by methanol. Anilines **21a–21e** were thus converted to amide **22–28** (RCH_2COCl/Et_3N/THF), urea **29** (RNCO) or carbamate **30** (*p*-nitrophenyl chloroformate/ROH) analogs.

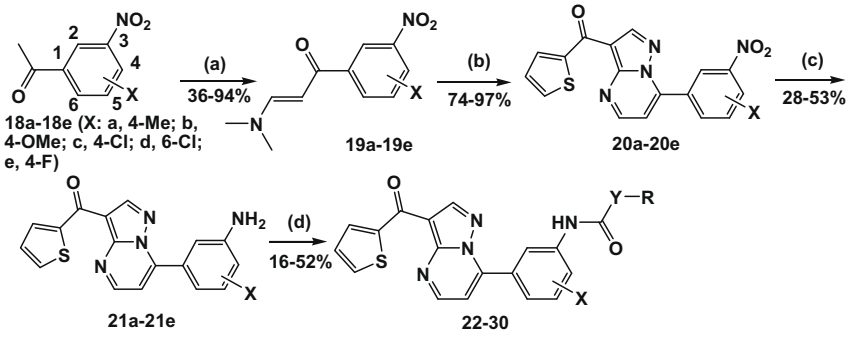
Using an analogous approach, pyrazolo[1,5-*a*]pyrimidines with a pyridine or a thiophene moiety (**35a–35c**, Scheme 4) at C-7 were prepared starting from the appropriate nitro-substituted hetero-



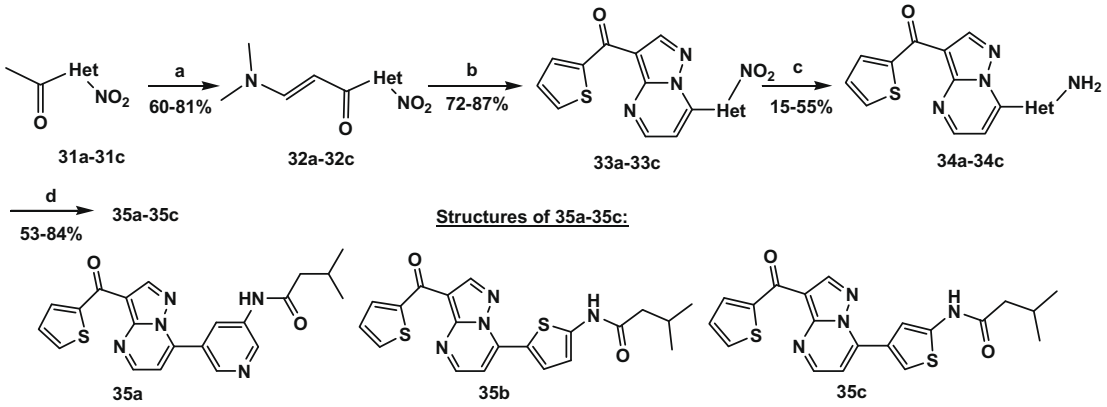
Scheme 1. Reagents and conditions: (a) $MeOH/H_2SO_4$, reflux; (b) $Me_2NCH(OMe)_2$, reflux; (c) **5**[14], acetic acid, reflux; (d) KOH (aq), heat; (e) R^1R^2NH/Py-BOP/CH_2C.



Scheme 2.



Scheme 3. Reagents and conditions: (a) $Me_2NCH(OMe)_2$, reflux; (b) **5**, acetic acid, reflux; (c) Fe, NH_4Cl, MeOH or $SnCl_2$/THF; (d) RCOCl/THF, RNCO, or *p*-nitrophenyl chloroformate/ROH.



Scheme 4. Reagents and conditions: (a) $Me_2NCH(OMe)_2$, reflux; (b) **4**, HOAc, reflux; (c) Fe, NH_4Cl, MeOH; (d) $(CH_3)_2CHCH_2CO_2H$/*iso*butyl chloroformate/*N*-methylmorpholine/THF.

aryl methyl ketones, via a reaction sequence of enamine formation, cyclocondensation, reduction and amidation.

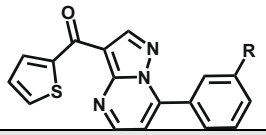
3. Results and discussion

3.1. Antiproliferative activity in P21 isogenic cells

All pyrazolo[1,5-*a*]pyrimidines were evaluated for their inhibitory activity in the p21 isogenic cells. Their activities and the selectivity ratios between p21−/− (80s14) and p21+/+ (HCT116) were shown in Tables 1 and 2. Pyrazolo[1,5-*a*]pyrimidine **8**, which has a reversed *iso*butyl amide was about 3 fold less active in p21−/− cells than **1a**. As seen from the data in Table 1, small alkyl groups in the amide moiety were well tolerated (**9, 10** and **11**), while a reversed phenyl amide analog showed good inhibitory activity against p21−/− cells (**12**). This is similar to what we observed with the amide series (compound **1a** and its analogs).[11] All reversed amide analogs showed selectivity ratios of 12–19 fold for p21−/− cells over p21+/+ cells.

It was also observed that the replacement of amide carbonyl group with methylene (–CH_2–) resulted in loss of activity. For example, **14**, the corresponding analog of **1a**, had a IC_{50} of 0.77 μM in p21−/− cells, comparing to 0.14 μM of **1a**. Small alkyl or alkenyl groups are generally tolerated (**15**, **16**, and **17**), showing IC_{50} range from 0.43 to 0.84 μM in p21−/− cells. The amine analogs (compounds **14–17**) were in general less selective than **1a** and reversed amide analogs (compounds **8–12**).

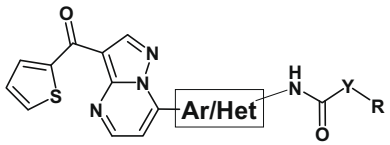
Table 1
Anti-proliferative activity (IC_{50}, μM): reverse amide and amines derivatives[a]



compd	R	80s14 (p21−/−)[b]	HCT116 (p21+/+)[c]	Selectivity ratio
1a	NHCO–*iso*butyl	0.14	6.4	46
8	CONH–*iso*butyl	0.40	5.7	12
9	CONH–butyl	0.34	5.3	16
10	CONH–cyclopentyl	0.39	6.8	17
11	CONH–$CH(CH_3)_2$	0.68	>13	>19
12	CONH–Ph	0.31	5.1	16
14	$NH(CH_2)_2CH(CH_3)_2$	0.77	5.7	7.6
15	$NH(CH_2)_2C(CH_3)_3$	0.55	4.8	8.9
16	NH–pentyl	0.84	7.9	9.5
17	$NHCH_2CH{=}CHCH_3$	0.43	3.8	8.7

[a] The IC_{50} values are average of multiple determinations except compound **14**.
[b] The standard deviations, on average, were 25% of the IC_{50} value.
[c] The standard deviations, on average, were 36% of the IC_{50} value.

Table 2
Anti-proliferative activity: substituted phenyls and heteroaryls[a]



compd	Ar/Het	R	Y	p21−/− IC_{50} (μM)[b]	p21+/+ IC_{50} (μM)[c]	Ratio
1a[9]	1,3-Ph	*iso*propyl	CH_2	0.14	6.4	46
1b[9]	1,3-Ph	*iso*propyl	NH	0.089	2.7	30
1c[9]	1,3-Ph	*iso*propyl	O	0.051	1.1	22
22	1,3-Ph-4-Me	*iso*propyl	CH_2	1.0	9.2	9.2
23	1,3-Ph-4-OMe	*iso*propyl	CH_2	6.4	19	3.0
24	1,3-Ph-4-Cl	*iso*propyl	CH_2	0.43	8.8	20
25	1,3-Ph-6-Cl	*iso*propyl	CH_2	2.8	20	7.1
26	1,3-Ph-4-F	*iso*propyl	CH2	0.089	3.2	36
27	1,3-Ph-4-F	$CH(CH_3)(CF_3)$	CH_2	0.12	2.5	21
28	1,3-Ph-4-F	*tert*-butyl	CH_2	0.19	3.7	19
29	1,3-Ph-4-F	*i*-Pr	NH	0.041	5.3	130
30	1,3-Ph-4-F	*i*-Pr	O	0.035	2.3	65
35a	2,5-Thiophene	*i*-Pr	CH_2	2.0	>20	>10
35b	2,4-Thiophene	*i*-Pr	CH_2	7.8	>20	>2.6
35c	3,5-Pyridine	*i*-Pr	CH_2	7.6	>2.6	>3.8

[a] The IC_{50} values are average of multiple determinations.
[b] The standard deviations, on average, were 33% of the IC_{50} value.
[c] The standard deviations, on average, were 25% of the IC_{50} value.

As shown in Table 2, introducing substitution onto the phenyl ring has substantial impact on the activity of pyrazol[1,5-*a*]pyrimidine compounds. Compared to **1a**, reduced activity in p21−/− cells was seen with 4-methyl derivative **22** and the 4-methoxy derivatives **23** as well as lessened selectivity over P21+/+ cells. While a chloro substitution at C-4 position (**24**) of the phenyl led to better activity than methyl and methoxy substitutions, a chloro substitution at-C-6 (**25**) was unfavorable to the activity in p21−/− cells and its selectivity ratio over P21+/+ cells.

Interestingly, 4-fluoro derivative **26** showed improved activity, with an IC_{50} of 0.089 μM in p21−/− cells. The fluorinated derivative **27** and *tert*-butyl analog **28** were comparably active to **1a**. Both urea and carbamate analogs (**29** and **30**) of **26** were more potent in p21−/− cells compared to the corresponding non-fluorinated com-

pounds (**1b** and **1c**). We also observed a significant improvement of selectivity ratio against p21+/+ cells for both **29** and **30** (Table 2).

Compounds **35a–35c**, having heteroaryl groups (pyridine or thiophenes) instead of the phenyl group in **1a**, were much less active in p21−/− and less selective against p21+/+ cells, indicating that the phenyl ring is a crucial component of the anti-proliferative pharmacophore.

3.2. Antiproliferative activity in colon cells

To study the activity of the compounds against non-engineered colon tumor cell lines, a group of selected compounds were further evaluated against a panel of 4 human colon cell lines (DLD1, HT29, SW620 and LoVo)[15,16] and their activities are shown in Table 3. In general, IC_{50} values observed for the pyrazolo[1,5-*a*]pyrimidine derivatives in the colon cell lines are fairly correlated with their activities in p21−/− cells. The reversed amide derivative **8** had an IC_{50} of 0.06–0.15 μM across the panel, while the amine analog **14** showed IC_{50} values of 0.081–0.77 μM compared to 0.021–0.14 μM for compound **1a**. All fluoro derivatives (**26**, **29** and **30**), which showed improved potency in p21−/− cells over compounds **1a**, had IC_{50} values ranging from 0.011 to 0.015 μM in LoVo cells compared to 0.021 μM for **1a**. Pyridine derivative **35a** showed moderate activity in the colon cell lines.

3.3. Microtubulin binding studies

We have previously determined that pyrazolo[1,5-*a*]pyrimidines, exemplified by **1a**, bind to the colchicines site of tubulin,[12,13] suggesting that tubulin binding may be the underlying mechanism or a contributing mechanism for their potent anti-proliferative cell activity. Several selected compounds were evaluated both for their binding affinity and effect on the oligomerization-state of tubulin. Their binding affinities ($K_{D\ values}$) were determined based on the fluorescence change of compound and tryptophan residues in tubulin upon binding.[17] In the binding assays, unlabeled tubulin was used as a substrate, in which the intrinsic fluorescence of the protein due to tryptophan residues served as a reporter group for interactions of inhibitor with tubulin. Upon binding to compounds, the fluorescence of tryptophan residues is quenched significantly due to either electronic interactions or local conformation/domain changes in the protein such that the quantum yield of the fluorescence is decreased. The changes in the fluorescence were used to estimate the binding affinity.

It was found that compounds **1a** and **26** bind to tubulin stoichiometrically, with K_D values of 4.3 μM and 2.5 μM, respectively. In a separate experiment, the oligomerization state of tubulin in the absence and presence of the p21 inhibitor (**26**) was evaluated using static multi-angle light scattering.[17] It was shown that both compound **1a** and **26** did not induce oligomerization of tubulin even though they bind tubulin saturably. We also found that the compounds' binding affinities are correlated with their cellular activities in p21−/− cells and colon cell lines.

Table 3
Anti-proliferative activity (IC_{50}, μM) of selected compounds in colon cell lines

compd	p21−/−	LoVo	SW620	DLD1	HT-29
1a[9]	0.14	0.021	0.045	0.042	0.036
8	0.40	0.15	0.15	0.06	0.15
14	0.77	0.081	0.11	0.12	0.45
26	0.089	0.015	0.015	0.016	0.016
29	0.041	0.011	0.012	0.019	0.017
30	0.045	0.012	0.015	0.015	0.015
35a	2.0	0.68	0.76	0.62	0.67

3.4. Pharmacokinetic assessment

To select a compound for in vivo study, the plasma levels of 3 most potent compounds (**26**, **29**, **30**) were evaluated. An oral dose of 50 mg/kg of **26** and **30** was administered to nude mice, and blood samples were taken after 1 and 4 h. The plasma levels of compound **30** were determined in a cassette-dosing format at 20 mg/kg po at 1 and 4 h time points. As shown in Table 4, the plasma levels at both 1 h and 4 h time points for amide analog **26** were substantially higher than those of **29** and **30**, even with the adjustment from the fact that **30** was dosed lower than **26**.

3.5. In vivo xenograft studies

Based on its plasma exposure level, compound **26** was chosen for evaluation in an in vivo LoVo xenograft model. Tumors were implanted 3 days prior to dosing and a 30 mg/kg dose of **26** administered po for 20 days produced a T/C of 57% at day 21 (Fig. 1). A more effective inhibition of tumour growth was achieved at 50 mg/kg (T/C value: 24%) with statistic significance.

In conclusion, replacement of C-7 phenyl amide group with reversed amides or amines led to a reduction in cell growth inhibition. Fluoro substitution at C-4 position improved the potency and selectivity while a larger group (Cl, Me or OMe) reduced activity. Replacement of the phenyl moiety with a heteroaryl group (phenyl or thiophene) led to a major reduction in activity. Tubulin binding activity was measured for **26** and may be the underlying mechanism or a contributing mechanism for anti-proliferative activity. Compound **26** effectively inhibit the tumor growth in an in vivo LoVo xenograft model.

4. Experimental

4.1. General methods

Melting points were determined in open capillary tubes on a Meltemp melting point apparatus and are uncorrected. ^{1}H NMR

Table 4
Plasma concentration of **26**, **29**, **30**

Compd	Dosage (PO)	Plasma concn at 1 h (μg/mL)	Plasma concn at 4 h (μg/mL)
26	50 mg/kg	0.11	0.04
29	50 mg/kg	0.014	0.008
30	20 mg/kg	0.038	0.006

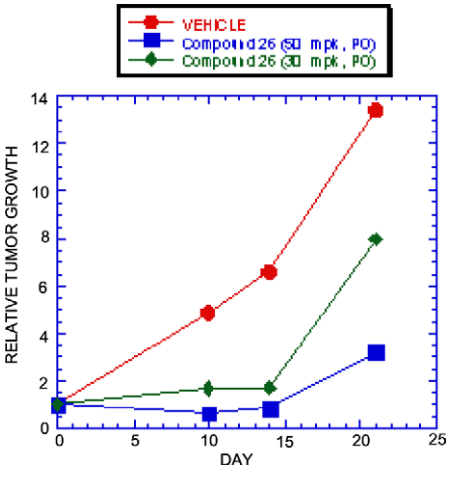


Figure 1. Antitumor activity of 26 in LoVo xenografts.

spectra were recorded using a NT-300 WB spectrometer. Chemical shifts (δ) are in parts per million referenced to Me_4Si. Electrospray (ES) mass spectra were recorded in positive mode on a Micromass Platfor spectrometer. Electron impact (EI) and high-resolution mass spectra were obtained on a Finnigan MAT-90 spectrometer. Flash chromatography was performed with Baker 40 μM silica gel. Reactions were carried out under an inert atmosphere, either nitrogen or argon, if not otherwise specified.

4.2. Methyl 3-acetylbenzoate (3)

3-Acetylbenzoic acid (5.1 g, 31 mmol) was dissolved in anhydrous methanol (250 mL) and concentrated sulfuric acid (1.0 mL) was added. The reaction mixture was heated at reflux overnight, concentrated and partitioned between water and methylene chloride. The combined organics were dried over $MgSO_4$, concentrated to yield 5.25 g (94%) of the title compound as a light yellow liquid, which was used in next step without further purification, mp 43–45°C; ^{1}H NMR ($CDCl_3$) δ 8.60 (S, 1H), 8.23 (d, J = 6 Hz, 1H), 8.16 (d, J = 6 Hz, 1H), 7.56 (t, J = 6 Hz, 1H), 3.96 (s, 3H), 2.66 (S, 3h); MS (ES) m/z 179.1 (M+H).

Anal. ($C_{10}H_{10}O_3$) C, H.

4.3. Methyl 3-[3-(2-thienylcarbonyl)pyrazolo[1,5-*a*]pyrimidin-7-yl]benzoate (6)

A mixture of **3** (5.28 g, 29.6 mmol) and DMF–DMA (75 mL) was heated at reflux for 2 h and evaporated to remove volatiles to give a yellow oil, which crystallized on standing. The crude solid was dissolved in methylene chloride. The solution was passed through a short pad of magnesol, washed with 5% MeOH/methylene chloride. The filtrate was concentrated to give 6.8 g (99%) of enamine **4** intermediate as a yellow solid, which was used for next step without further purification.

A mixture of enamine intermediate (131 mg, 0.6 mmol) and **5** (120 mg, 0.62 mmol) in HOAc (2.0 mL) was heated at reflux for 5 h, cooled to room temperature and diluted with hexane. The precipitates were collected by filtration, washed thoroughly with water and ether, and dried to give 177 mg (87%) of **6** as a tan solid; ^{1}H NMR (DMSO-d_6) δ 8.91 (d, J = 3 Hz, 1H), 8.85 (s, 1H), 8.69 (t, J = 1 Hz, 1H), 8.33 (d, J = 6 Hz, 1H), 8.24–8.21 (m, 2H), 8.06 (d, J = 4 Hz, 1H), 7.81 (t, J = 6 Hz, 1H), 7.59 (d, J = 3 Hz, 1H), 7.31 (dd, J = 4, 3 Hz, 1H), 3.92 (s, 3H); MS (ES) m/z 364.2 (M+H).

Anal. ($C_{10}H_{10}O_3$-0.5H_2O) C, H, N.

4.4. 3-[3-(2-Thienylcarbonyl)pyrazolo[1,5-*a*]pyrimidin-7-yl]benzoic acid (7)

A mixture of **6** (177 mg, 0.49 mmol), potassium hydroxide (1.0 M, 5.0 mL) and methanol (0.3 mL) was stirred at room temperature overnight and then heated at 50 °C for 15 min. The reaction mixture was cooled to room temperature and solid precipitated. The resulting suspension was diluted with water until a solution and acidified with concentrated hydrochloric acid. The precipitates were collected, washed with water and dried to give 154 mg (90%) of **7** as a light yellow solid: mp 174–177 °C; ^{1}H NMR (DMSO-d_6) δ 13.3 (s, br, 1H), 8.91 (d, J = 3 Hz, 1H), 8.85 (s, 1H), 8.69 (t, J = 1 Hz, 1H), 8.32 (d, J = 6 Hz, 1H), 8.23 (dd, J = 1, 3 Hz, 1H), 8.20 (d, J = 6 Hz, 1H), 8.06 (d, J = 4 Hz, 1H), 7.79 (t, J = 6 Hz, 1H), 7.59 (d, J = 3 Hz, 1H), 7.31 (dd, J = 3, 4 Hz, 1H); MS (ES) m/z 348.0 (M+H).

4.5. *N-iso*butyl-3-[3-(2-thienylcarbonyl)pyrazolo[1,5-a]pyrimidin-7-yl]benzamide (8)

A mixture of **7** (150 mg, 0.43 mmol), diisopropylethylamine (167 mg, 1.3 mmol), benzotriazole-1-yloxy-tripyrrolidinophosphonium hexafluorophosphate (290 mg, 0.56 mmol) and *iso*butylamine (31 mg, 0.43 mmol) in methylene chloride (4 mL) was stirred at room temperature for 5 h. The reaction mixture was partitioned between saturated aqueous sodium bicarbonate and methylene chloride. The combined organics were dried over sodium sulfate, concentrated and purified by flash column chromatography (eluting with a gradient of methanol and methylene chloride) to give 170 mg (98%) of the title compound as a white solid; ^{1}H NMR (DMSO-d_6) δ 8.93 (d, J = 3 Hz, 1H), 8.84 (s, 1H), 8.64 (t, J = 4 Hz, 1H), 8.51 (t, J = 1 Hz, 1H), 8.27 (dd, J = 1, 6 Hz, 1H), 8.23 (dd, J = 1, 3 Hz, 1H), 8.10 (dd, J = 1, 6 Hz, 1H), 8.06 (d, J = 4 Hz, 1H), 7.79 (t, J = 6 Hz, 1H), 7.59 (d, J = 3 Hz, 1H), 7.31 (dd, J = 4, 3 Hz, 1H), 3.13 (t, J = 5 Hz, 2H), 1.91–1.82 (m, 1H), 0.91 (d, J = 5 Hz, 6H); MS (ES) m/e 405.2 (M+H).

Anal. ($C_{22}H_{20}N_4O_2S$–1.0H_2O) C, H, N.

4.6. *N*-Butyl-3-[3-(2-thienylcarbonyl)pyrazolo[1,5-*a*]pyrimidin-7-yl]benzamide (9)

According to the procedure used to prepare **8**, reaction of **7** and *n*-butylamine provided **9** in 55% yield as an off-white solid: mp 172–174 °C; ^{1}H NMR (DMSO-d_6) δ 8.93 (d, J = 3 Hz, 1H), 8.84 (s, 1H), 8.62 (t, J = 4 Hz, 1H), 8.50 (s, 1H), 8.27 (d, J = 6 Hz, 1H), 8.23 (d, J = 3 Hz, 1H), 8.10 (d, J = 6 Hz, 1H), 8.06 (dd, J = 1, 4 Hz, 1H), 7.73 (t, J = 6 Hz, 1H), 7.59 (d, J = 3 Hz, 1H), 7.31 (dt, J = 1, 3 Hz, 1H), 3.32–3.28 (m, 2H), 1.57–1.50 (m, 2H), 1.40–1.31 (m, 2H), 0.91 (t, J = 5 Hz, 3H); MS (ES) m/e 405.2 (M+H).

Anal. ($C_{22}H_{20}N_4O_2S$–0.3H_2O) C, H, N.

4.7. *N*-Cyclopentyl-3-[3-(2-thienylcarbonyl)pyrazolo[1,5-*a*]pyrimidin-7-yl]benzamide (10)

According to the procedure used to prepare **8**, reaction of **7** and cyclopentylamine provided **10** in 58% yield as an off-white solid: mp 207–209 °C; ^{1}H NMR (DMSO-d_6) δ 8.93 (d, J = 3 Hz, 1H), 8.84 (s, 1H), 8.48 (t, J = 1 Hz, 1H), 8.45 (d, J = 5 Hz, 1H), 8.25 (dd, J = 1, 6 Hz, 1H), 8.23 (dd, J = 1, 3 Hz, 1H), 8.10 (d, J = 6 Hz, 1H), 8.06 (dd, J = 1, 4 Hz, 1H), 7.73 (t, J=6 Hz, 1H), 7.59 (d, J = 3 Hz, 1H), 7.31 (dd, J = 3, 4 Hz, 1H), 4.29–4.24 (m, 1H), 1.93–1.90 (m, 2H), 1.71–1.68 (m, 2H), 1.65–1.50 (m, 4H); MS (ES) m/e 461.1 (M+HCOO$^-$).

Anal. ($C_{23}H_{20}N_4O_2S$–0.2H_2O) C, H, N.

4.8. *N-iso*Propyl-3-[3-(2-thienylcarbonyl)pyrazolo[1,5-*a*]pyrimidin-7-yl]benzamide (11)

According to the procedure used to prepare **8**, reaction of **7** and *iso*propylamine provided **11** in 47% yield as a white solid: mp 218–220 °C; ^{1}H NMR (DMSO-d_6) δ 8.93 (d, J = 3 Hz, 1H), 8.84 (s, 1H), 8.48 (t, J = 1 Hz, 1H), 8.40 (d, J = 5 Hz, 1H), 8.26 (dd, J = 1, 6 Hz, 1H), 8.23 (dd, J = 1, 3 Hz, 1H), 8.10 (d, J = 6 Hz, 1H), 8.06 (dd, J = 1, 4 Hz, 1H), 7.73 (t, J = 6 Hz, 1H), 7.59 (d, J = 3 Hz, 1H), 7.31 (dd, J = 3, 4 Hz, 1H), 4.17–4.10 (m, 1H), 1.20 (d, J = 5 Hz, 4H); MS (ES) m/e 391.1 (M+H).

Anal. ($C_{21}H_{18}N_4O_2S$–0.1H_2O) C, H, N.

4.9. *N*-Phenyl-3-[3-(2-thienylcarbonyl)pyrazolo[1,5-*a*]pyrimidin-7-yl]benzamide (12)

According to the procedure used to prepare **8**, reaction of **7** and aniline provided **12** in 51% yield as a white solid: mp 211–212 °C; ^{1}H NMR (DMSO-d_6) δ 10.42 (s, 1H), 8.95 (d, J = 3 Hz, 1H), 8.86 (s, 1H), 8.62 (t, J = 1 Hz, 1H), 8.34 (dd, J = 1, 5 Hz, 1H), 8.24 (dd, J = 1, 3 Hz, 1H), 8.22 (d, J = 6 Hz, 1H), 8.06 (dd, J = 1, 4 Hz, 1H), 7.83–7.80 (m, 3H), 7.65 (d, J = 3 Hz, 1H), 7.39 (d, J = 5 Hz, 1H), 7.38 (d, J = 6 Hz, 1H), 7.13 (t, J = 5 Hz, 1H); MS (ES) m/e 425.1 (M+H).

Anal. ($C_{24}H_{16}N_4O_2S$–0.5H_2O) C, H, N.

4.10. {7-[3-(*iso*Pentylamino)phenyl]pyrazolo[1,5-*a*]pyrimidin-3-yl}(thien-2-yl)methanone (14)

The mixture of **13** (100 mg, 0.312 mmol) and 3-metylbutyraldehyde (35 mg, 0.328 mmol) in methylene chloride (5.0 mL and DMF (1.0 mL) was stirred at room temperature for 15 min and $Na(OAc)_3BH$ (330 mg, 1.56 mmol) was added in portions. The reaction mixture was stirred at room temperature for 3 h, concentrated to dryness, dissolved in methanol (4.0 mL) and purified by HPLC (eluting with a gradient of acetonitrile/H_2O) to give 86 mg (72%) of **14** as a yellow solid: mp 75–77 °C; ^{1}H NMR (DMSO-d_6) δ 8.85 (d, J = 4 Hz, 1H), 8.80 (s, 1H), 8.24 (t, J = 1 Hz, 1H), 8.04 (d, J = 3 Hz, 1H), 7.43 (d, J = 4 Hz, 1H), 7.33–7.29 (m, 2H), 7.25 (s, 1H), 7.20 (d, J = 6 Hz, 1H), 6.83 (d, J = 5 Hz, 1H), 6.1–5.7 (s, br, 1H), 3.08 (t, J = 5 Hz, 2H), 1.75–1.67 (m, 1H), 1.49 (q, J = 5 Hz, 2H), 0.92 (d, J = 5 Hz, 6H); MS (ES) *m/e* 391.2 (M+H).

4.11. (7-{3-[(3,3-Dimethylbutyl)amino]phenyl}pyrazolo[1,5-*a*]pyrimidin-3-yl)(thien-2- yl)methanone (15)

According to the procedure used to prepare **14**, reaction of **13** and 3, 3-dimethylbutyraldehyde provided **15** in 45% yield as a yellow solid: mp 127–130 °C; ^{1}H NMR (DMSO-d_6) δ 8.85 (d, J = 3 Hz, 1H), 8.79 (s, 1H), 8.24 (dd, J = 1, 3 Hz, 1H), 8.05 (dd, J = 1, 4 Hz, 1H), 7.33–7.29 (m, 2H), 7.23 (d, J = 1 Hz, 1H), 7.18 (d, J = 3 Hz, 1H), 6.81 (dd, J = 1, 6 Hz, 1H), 5.86 (t, J = 4 Hz, 1H), 3.11–3.05 (m, 2H), 1.55–1.49 (m, 2H), 0.95 (s, 9H); MS (ES) *m/e* 405.2 (M+H).

Anal. ($C_{21}H_{20}N_4OS$–0.25H_2O) C, H, N.

4.12. {7-[3-(Butylamino)phenyl]pyrazolo[1,5-*a*]pyrimidin-3-yl}(thien-2-yl)methanone (16)

According to the procedure used to prepare **14**, reaction of **13** and n-butyraldehyde provided **16** in 13% yield as a yellow solid: mp 87–91 °C; ^{1}H NMR (DMSO-d_6) δ 8.85 (d, J = 3 Hz, 1H), 8.80 (s, 1H), 8.24 (dd, J = 1, 3 Hz, 1H), 8.04 (dd, J = 1, 4 Hz, 1H), 7.43 (d, J = 3 Hz, 1H), 7.32–7.28 (m, 2H), 7.23 (d, J = 1 Hz, 1H), 7.18 (d, J = 3 Hz, 1H), 6.82 (dd, J = 1, 6 Hz, 1H), 5.94 (t, J = 4 Hz, 1H), 3.07 (q, J = 5 Hz, 2H), 1.61–1.53 (m, 2H), 1.45–1.36 (m, 2H), 0.92 (t, J = 5 Hz, 3H); MS (ES) *m/e* 377.1 (M+H).

Anal. ($C_{21}H_{20}N_4OS$–0.95H_2O) C, H, N.

4.13. (7-{3-[(2*E*)-But-2-enylamino]phenyl}pyrazolo[1,5-*a*]pyrimidin-3-yl)(thien-2- yl)methanone (17)

According to the procedure used to prepare **14**, reaction of **13** and crotonaldehyde provided **17** in 32% yield as a yellow solid: mp 110–113 °C; ^{1}H NMR (DMSO-d_6) δ 8.85 (d, J = 3 Hz, 1H), 8.79 (s, 1H), 8.23 (dd, J = 1, 3 Hz, 1H), 8.05 (dd, J = 1, 4 Hz, 1H), 7.41 (d, J = 4 Hz, 1H), 7.32–7.28 (m, 2H), 7.23 (t, J = 1 Hz, 1H), 7.20 (d, J = 6 Hz, 1H), 6.83 (dd, J = 1, 6 Hz, 1H), 6.12 (t, J = 4 Hz, 1H), 5.75–5.50 (m, 2H), 3.68 (t, J = 5 Hz, 2H), 1.67 (d, J = 4 Hz, 3H); MS (ES) *m/e* 375.1 (M+H).

Anal. ($C_{21}H_{18}N_4OS$) C, H, N·H, N.

4.14. (2*E*)-3-(Dimethylamino)-1-(4-methyl-3-nitrophenyl)prop-2-en-1-one (19a)

According to the procedure used to prepare **4**, reaction of 4-methyl-3-nitroacetophenone and DMF–DMA provided **19a** in 73% yield as a brown solid: mp 123–125 °C; ^{1}H NMR (DMSO-d_6) δ 8.41 (d, J = 1 Hz, 1H), 8.14 (dd, J = 1, 6 Hz, 1H), 7.79 (d, J = 9 Hz, 1H), 7.56 (d, J = 6 Hz, 1H), 5.90 (d, J = 9 Hz, 1H), 3.17 (s, 3H), 2.95 (s, 3H), 2.55 (s, 3H); MS (ES) *m/e* 235.1 (M+H).

Anal. ($C_{12}H_{14}N_2O_3$–0.2H_2O) C, H, N.

4.15. (2*E*)-3-(Dimethylaino)-1-(4-methoxy-3-nitrophenyl)prop-2-en-1-one (19b)

According to the procedure used to prepare **4**, reaction of 4-methoxy-3-nitroacetophenone and DMF–DMA provided **19b** in 84% yield as an orange solid: mp 165–167 °C; ^{1}H NMR ($CDCl_3$) δ 8.38 (d, J = 2 Hz, 1H), 8.18 (dd, J = 2, 7 Hz, 1H), 7.84 (d, J = 9 Hz, 1H), 7.11 (d, J = 7 Hz, 1H), 5.66 (d, J = 9 Hz, 1H), 4.01 (s, 3H), 3.18 (s, 3H), 2.96 (s, 3H); MS (ES) *m/e* 251.2 (M+H).

Anal. ($C_{12}H_{14}N_2O_4$) C, H, N.

4.16. (2*E*)-3-(Dimethylaino)-1-(4-chloro-3-nitrophenyl)prop-2-en-1-one (19c)

According to the procedure used to prepare **4**, reaction of 4-chloro-3-nitroacetophenone and DMF–DMA provided **19c** in 36% yield as an orange solid: mp 150–152 °C; ^{1}H NMR (DMSO-d_6) δ 8.51 (s, 1H, 8.19 (d, J = 6 Hz, 1H), 7.85–7.81 (m, 2H), 5.75 (d, J = 9 Hz, 1H), 3.19 (s, 3H), 2.96 (s, 3H); MS (ES) *m/e* 255.1 (M+H).

Anal. ($C_{11}H_{11}ClN_2O_3$–0.1H_2O) C, H, N.

4.17. (2*E*)-3-(Dimethylaino)-1-(2-chloro-5-nitrophenyl)prop-2-en-1-one (19d)

According to the procedure used to prepare **4**, reaction of 2-chloro-5-nitroacetophenone and DMF–DMA provided **19d** in 65% yield as an orange solid: mp 123–125 °C; ^{1}H NMR (DMSO-$d_{6)\ \delta\ 8.21}$ (dd, J = 6, 9 Hz, 1H), 8.20–8.00 (m, 2H), 7.76 (d, J = 9 Hz, 1H), 5.29 (d, J = 9 Hz, 1H), 3.09 (s, 3), 2.86 (s, 3H); MS (ES) *m/e* 255.1 (M+H).

4.18. (2*E*)-3-(Dimethylamino)-1-(4-fluoro-3-nitrophenyl)prop-2-en-1-one (19e)

To 4-Acetyl-2-aminofluorobenzene[18] (15.0 g, 81.0 mmol) in THF (300 mL) was added *N*,*N*-dimethylformamide di-*tert*-butyl acetal (29 mL, 243 mmol) in portions. The reaction mixture was stirred at room temperature overnight and evaporated. The residual solids were collected by filtration, washed with water and ether to give 17.8 g (94%) of **19e** as an orange solid: mp 131–136 °C; ^{1}H NMR (DMSO-d_6) δ 8.57 (dd, J = 2, 5 Hz, 1H), 8.36–8.32 (m, 1H), 7.82 (d, J = 9 Hz, 1H), 7.65 (dd, J = 7, 8 Hz, 1H), 5.96 (d, J = 9 Hz, 1H), 3.18 (s, 3H), 2.97 (s, 3H); MS (ES) *m/e* 239.2 (M+H).

Anal. ($C_{11}H_{11}FN_2O_3$) C, H, N.

4.19. [7-(4-Methyl-3-nitrophenyl)pyrazolo[1,5-*a*]pyrimidin-3-yl](thien-2-yl)methanone (20a)

According to the procedure used to prepare **6**, reaction of **19a** and **5** provided **20a** in 86% yield as an orange solid: mp 217–219 °C; ^{1}H NMR (DMSO-d_6) δ 8.93 (d, J = 3 Hz, 1H), 8.87 (s, 1H), 8.82 (d, J = 1 Hz, 1H), 8.36 (dd, J = 1, 6 Hz, 1h), 8.21 (dd, J = 1, 3 Hz, 1H), 8.06 (dd, J = 1, 4 Hz, 1H), 7.79 (d, J = 6 Hz, 1H), 7.65 (d, J = 3 Hz, 1H), 7.30 (dd, J = 1, 6 Hz, 1H), 2.66 (s, 3H); MS (ES) *m/e* 365.2 (M+H).

Anal. ($C_{18}H_{12}N_4O_3S$) C, H, N.

4.20. [7-(4-Methoxy-3-nitrophenyl)pyrazolo[1,5-*a*]pyrimidin-3-yl](thien-2-yl)methanone (20b)

According to the procedure used to prepare **6**, reaction of **19b** and **5** provided **20b** in 97% yield as a light yellow solid: mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 8.90 (d, J = 3 Hz, 1H), 8.87 (s, 1H), 8.80 (d, J = 2 Hz, 1H), 8.48 (dd, J = 2, 7 Hz, 1H), 8.22 (d, J = 5 Hz, 1H), 8.06 (d, J = 4 Hz, 1H), 7.65 (d, J = 5 Hz, 1H), 7.63 (s, 1H), 7.30 (t, J = 3 Hz, 1H), 4.07 (s, 3H); MS (ES) *m/e* 351.1 (M+H).

Anal. ($C_{18}H_{14}N_4O_4S$–0.2H_2O) C, H, N.

4.21. [7-(4-Chloro-3-nitrophenyl)pyrazolo[1,5-*a*]pyrimidin-3-yl](thien-2-yl)methanone (20c)

According to the procedure used to prepare **6**, reaction of **19c** and **5** provided **20c** in 90% yield as a yellow solid: mp 220–222 °C; ^{1}H NMR (DMSO-d_6) δ 8.90 (d, J = 3 Hz, 1H), 8.87 (s, 1H), 8.86 (d, J = 1 Hz, 1H), 8.44 (dd, J = 2, 6 Hz, 1H), 8.20 (dd, J = 1, 3 Hz, 1H), 8.09 (d, J = 6 Hz, 1H), 8.07 (dd, J = 1, 4 Hz, 1H), 7.67 (d, J = 3 Hz, 1H), 7.31 (dd, J = 1, 4 Hz, 1H); MS (ES) *m/e* 385.2 (M+H).

Anal. ($C_{17}H_9ClN_4O_3S$–0.2H_2O) C, H, N.

4.22. [7-(2-Chloro-5-nitrophenyl)pyrazolo[1,5-*a*]pyrimidin-3-yl](thien-2-yl)methanone (20d)

According to the procedure used to prepare **6**, reaction of **19d** and **5** provided **20d** in 75% yield as a reddish solid: mp 162–166 °C; ^{1}H NMR (DMSO-d_6) δ 9.00 (d, J = 3 Hz, 1H), 8.79 (s, 1H), 8.69 (d, J = 2 Hz, 1H), 8.50 (dd, J = 2, 6 Hz, 1H), 8.23 (dd, J = 1, 3 Hz, 1H), 8.07 (dd, J = 2, 2 Hz, 1H), 8.05 (s, 1H), 7.57 (d, J = 3 Hz, 1H), 7.31 (dd, J = 1, 4 Hz, 1H); MS (ES) *m/e* 385.2 (M+H).

Anal. ($C_{17}H_9ClN_4O_3S$–1.0H_2O) C, H, N.

4.23. [7-(4-Fluoro-3-nitrophenyl)pyrazolo[1,5-*a*]pyrimidin-3-yl](thien-2-yl)methanone (20e)

According to the procedure used to prepare **6**, reaction of **19e** and **5** provided **20e** in 74% yield as an orange solid: mp 205–208 °C; ^{1}H NMR (DMSO-d_6) δ 8.99 (dd, J = 2, 5 Hz, 1H), 8.94 (d, J = 4 Hz, 1H), 8.87 (s, 1H), 8.60–8.55 (m, 1H), 8.21 (dd, J = 3, 3 Hz, 1H), 8.07 (dd, J = 3, 4 Hz, 1H), 7.90 (dd, J = 2, 9 Hz, 1H), 7.66 (d, J = 3 Hz, 1H), 7.31 (dd, J = 3, 4 Hz, 1H); MS (ES) *m/e* 369.2 (M+H).

Anal. ($C_{17}H_9FN_4O_3S$) C, H, N.

4.24. [7-(3-Amino-4-methylphenyl)pyrazolo[1,5-*a*]pyrimidin-3-yl](thien-2-yl)methanone (21a)

A mixture of **20a** (1.95 g, 5.32 mmol), acetic acid (17.8 mL), iron powder (2.97 g, 53.2 mmol) in methanol (54 mL) was heated at reflux for 1 h, cooled to room temperature, filtered through a pad of celite. The filtrate was concentrated and the residue was partitioned between EtOAc and aqueous sodium carbonate. The combined organics were dried over sodium sulfate and filtered through a pad of magnesol. The filtrate was concentrated to give 845 mg (48%) of **21a** as a yellow solid: mp 193–195 °C; ^{1}H NMR (DMSO-d_6) δ 8.83 (d, J = 4 Hz, 1H), 8.80 (s, 1H), 8.24 (dd, J = 1, 3 Hz, 1H), 8.04 (dd, J = 1, 4 Hz, 1H), 7.38 (d, J = 1 Hz, 1H), 7.37 (s, 1H), 7.31 (dd, J = 1, 4 Hz, 1H), 7.21–7.15 (m, 2H), 5.20 (S, 2H), 2.17 (S, 3h); MS (ES) *m/e* 335.2 (M+H).

Anal. ($C_{18}H_{14}N_4OS$–0.05EtOAc) C, H, N.

4.25. [7-(3-Amino-4-methoxyphenyl)pyrazolo[1,5-*a*]pyrimidin-3-yl](thien-2-yl)methanone (21b)

According to the procedure used to prepare **21a**, reaction of **20b** provided **21b** in 53% yield as a yellow solid: mp 210–213 °C; ^{1}H NMR (DMSO-d_6) δ 8.81 (s, 1H), 8.82 (d, J = 3 Hz, 1H), 8.25 (dd, J = 1, 4 Hz, 1H), 8.04 (dd, J = 1, 3 Hz, 1H), 7.48 (d, J = 2 Hz, 1H), 7.40 (dd, J = 2, 6 Hz, 1H), 7.38 (d, J = 3 Hz, 1H), 7.30 (dd, J = 1, 4 Hz, 1H), 7.03 (d, J = 6 Hz, 1H), 5.06 (s, 2H), 3.89 (s, 3H); MS (ES) *m/e* 351.1 (M+H).

Anal. ($C_{18}H_{14}N_4O_2S$–0.2H_2O) C, H, N.

4.26. [7-(3-Amino-4-chlorophenyl)pyrazolo[1,5-*a*]pyrimidin-3-yl](thien-2-yl)methanone (21c)

According to the procedure used to prepare **21a**, reaction of **20c** provided **21c** in 28% yield as a reddish solid: mp 231–233 °C; ^{1}H NMR (DMSO-d_6) δ 8.86 (d, J = 4 Hz, 1H), 8.82 (s, 1H), 8.22 (dd, J = 1, 3 Hz, 1H), 8.05 (dd, J = 1, 4 Hz, 1H), 7.53 (d, J = 2 Hz, 1H), 7.44 (d, J = 6 Hz, 1H), 7.42 (d, J = 3 Hz, 1H), 7.30 (dd, J = 1, 4 Hz, 1H), 7.22 (dd, J = 2, 6 Hz, 1H), 5.72 (s, 2H); MS (ES) *m/e* 355.2 (M+H).

Anal. ($C_{17}H_{11}ClN_4OS$–0.4H_2O) C, H, N.

4.27. [7-(5-Amino-2-chlorophenyl)pyrazolo[1,5-*a*]pyrimidin-3-yl](thien-2-yl)methanone (21d)

According to the procedure used to prepare **21a**, reaction of **20d** provided **21d** in 46% yield as a reddish solid: mp 162–168 °C; ^{1}H NMR (DMSO-d_6) δ 8.91 (d, J = 3 Hz, 1H), 8.76 (s, 1H), 8.25 (d, J = 1 Hz, 1H), 8.05 (d, J = 3 Hz, 1H), 7.38 (d, J = 3 Hz, 1H), 7.30–7.26 (m, 2H), 6.79 (d, J = 6 Hz, 1H). 6.78 (s, 1H), 5.59 (s, 2H); MS (ES) *m/e* 355.2 (M+H).

Anal. ($C_{17}H_{11}ClN_4OS$–3.2H_2O) C, H, N.

4.28. [7-(3-Amino-4-fluorophenyl)pyrazolo[1,5-*a*]pyrimidin-3-yl](thien-2-yl)methanone (21e)

According to the procedure used to prepare **21a**, reaction of **20e** provided **21e** in 52% yield as a yellow solid: mp 241–242 °C; ^{1}H NMR (DMSO-d_6) δ 8.84 (d, J = 4 Hz, 1H), 8.81 (s, 1H), 8.23 (dd, J = 2, 3 Hz, 1H), 8.05 (dd, J = 1, 4 Hz, 1H), 7.54 (dd, J = 1, 6 Hz, 1H), 7.40 (d, J = 3 Hz, 1H), 7.30 (dd, J = 1, 4 Hz, 1H), 7.24 (dd, J = 4, 6 Hz, 1H), 5.52 (s, 2H); MS (ES) *m/e* 339.1 (M+H).

Anal. ($C_{17}H_{11}FN_4OS$–0.1H_2O) C, H, N.

4.29. 3-Methyl-N-{2-methyl-5-[3-(2-thienylcarbonyl)-pyrazolo[1,5-a]pyrimidin-7-yl]phenyl}butanamide (22)

To a solution of isovalaeric acid (80 mg, 0.783 mmol) and *N*-methylmorpholine (118 mg, 1.18 mmol) in THF (2.0 mL) was added *iso*butyl chloroformate (107 mg, 0.783 mmol) dropwise at 0–5 °C. The above mixture was stirred at 0–5 °C for 5 min; **21a** (130 mg, 0.389 mmol) in THF (2.0 mL) was added. The resulting mixture was stirred at room temperature for 22 h and diluted with methylene chloride. The organic phase was washed with saturated aqueous sodium bicarbonate and brine, dried over sodium sulfate, concentrated and purified by preparative TLC (eluting with 90% EtOAc/Hexanes) to give 75 mg (46%) of **22** as a yellow solid: mp 185–187 °C; ^{1}H NMR (DMSO-d_6) δ 9.51 (s, 1H), 8.86 (d, J = 3 Hz, 1H), 8.81 (s, 1H), 8.23 (dd, J = 1, 3 Hz, 1H), 8.16 (d, J = 1 Hz, 1H), 8.05 (dd, J = 1, 4 Hz, 1H), 7.86 (dd, J = 1, 6 Hz, 1H), 7.48 (d, J = 6 Hz, 1H), 7.46 (d, J = 3 Hz, 1H), 7.30 (dd, J = 1, 6 Hz, 1H), 2.33 (s, 3H), 2.26 (d, J = 5 Hz, 2H), 2.17–2.05 (m, 1H), 0.97 (d, J = 5 Hz, 6H); MS (ES) *m/e* 419.2 (M+H).

Anal. ($C_{23}H_{22}N_4O_2S$–0.3H_2O) C, H, N.

4.30. N-{2-Methoxy-5-[3-(2-thienylcarbonyl)pyrazolo[1,5-a]pyrimidin-7-yl]phenyl}-3-methylbutanamide (23)

According to the procedure used to prepare **22**, reaction of **21b** and isovalaeric acid provided **23** in 44% yield as a yellow solid: mp 192–194 °C; ^{1}H NMR (DMSO-d_6) δ9.30 (s, 1H), 8.83 (d, J = 3 Hz, 1H), 8.81 (s, 1H), 8.72 (s, 1H), 8.24 (d, J = 3 Hz, 1H), 8.04 (d, J = 4 Hz, 1H), 7.96 (d, J = 6 Hz, 1H), 7.43 (d, J = 3 Hz, 1H), 7.30 (d, J = 4 Hz, 1H), 7.28 (s, 1H), 3.96 (s, 3H), 2.31 (d, J = 5 Hz, 2H), 2.12–2.03 (m, 1H), 0.94 (d, J = 5 Hz, 6H); MS (ES) *m/e* 435.1 (M+H).

Anal. ($C_{23}H_{22}N_4O_3S$–0.3H_2O) C, H, N.

4.31. *N*-{2-Chloro-5-[3-(2-thienylcarbonyl)pyrazolo[1,5-*a*]-pyrimidin-7-yl]phenyl}-3-methylbutanamide (24)

According to the procedure used to prepare **22**, reaction of **21c** and isovalaeric acid provided **24** in 16% yield as a yellow solid: mp

172–175 °C; ^{1}H NMR (DMSO-d_6) δ 9.75 (s, 1H), 8.88 (d, J = 3 Hz, 1H), 8.82 (s, 1H), 8.39 (d, J = 2 Hz, 1H), 8.22 (dd, J = 1, 3 Hz, 1H), 8.05 (dd, J = 1, 4 Hz, 1H), 7.93 (dd, J = 2, 6 Hz, 1H), 7.77 (d, J = 6 Hz, 1H), 7.51 (d, J = 3 Hz, 1H), 7.30 (dd, J = 1, 4 Hz, 1H), 2.31 (d, J = 5 Hz, 2H), 2.12–2.05 (m, 1H), 0.97 (d, J = 5 Hz, 6H); MS (ES) *m/e* 439.2 (M+H).

Anal. ($C_{22}H_{19}ClN_4O_2S$–0.7H_2O) C, H, N.

4.32. *N*-{4-Chloro-3-[3-(2-thienylcarbonyl)pyrazolo[1,5-*a*]-pyrimidin-7-yl]phenyl}-3-methylbutanamide (25)

According to the procedure used to prepare **22**, reaction of **21d** and isovalaeric acid provided **25** in 50% yield as a beige solid: mp 162–165 °C; ^{1}H NMR (DMSO-d_6) δ 10.25 (s, 1H), 8.95 (d, J = 3 Hz, 1H), 8.77 (s, 1H), 8.25 (d, J = 1 Hz, 1H), 8.06 (d, J = 3 Hz, 1H), 8.01 (s, 1H), 7.79 (d, J = 3 Hz, 1H), 7.64 (d, J = 6 Hz, 1H), 7.47 (d, J = 3 Hz, 1H), 7.31 (t, J = 3 Hz, 1H), 2.11 (d, J = 5 Hz, 2H), 2.09–2.05 (m, 1H), 0.93 (d, J = 5 Hz, 6H); MS (ES) *m/e* 439.1 (M+H).

Anal. ($C_{22}H_{19}ClN_4O_2S$–0.8H_2O) C, H, N.

4.33. *N*-{2-Fluoro-5-[3-(2-thienylcarbonyl)pyrazolo[1,5-*a*]-pyrimidin-7-yl]phenyl}-3-methylbutanamide (26)

To a mixture of **21e** (1.0 g, 2.96 mmol) and 4-dimethylaminopyridine (36 mg, 0.294 mmol) in methylene chloride (20 mL) was added isovaleryl chloride (71 mg, 0.591 mmol) in methylene chloride (5 mL) dropwise followed by triethylamine (450 mg, 4.45 mmol). The resulting mixture was stirred at room temperature for 90 min and filtered. The filtrate was washed with aqueous sodium bicarbonate, dried over sodium sulfate and concentrated. The residue was purified by column chromatography (eluting with 1–5% methanol in methylene chloride) to give 619 mg (52%) of **246** as a yellow solid: mp 192–194 °C; ^{1}H NMR (DMSO-d_6) δ 9.93 (s, 1H), 8.88 (d, J = 3 Hz, 1H), 8.82 (s, 1H), 8.59 (dd, J = 2, 6 Hz, 1H), 8.23 (dd, J = 2, 3 Hz, 1H), 8.05 (dd, J = 3, 4 Hz, 1H), 7.54 (dd, J = 2, 8 Hz, 1H), 7.48 (d, J = 3 Hz, 1H), 7.30 (dd, J = 1, 4 Hz, 1H), 2.30 (d, J = 5 Hz, 2H), 2.12–2.03 (m, 1H), 0.95 (d, J = 5 Hz, 6H); MS (ES) *m/e* 423.1 (M+H).

Anal. ($C_{22}H_{19}FN_4O_2S$–0.1H_2O) C, H, N.

4.34. 4,4,4-Trifluoro-*N*-{2-fluoro-5-[3-(thien-2-ylcarbonyl)-pyrazolo[1,5-*a*]pyrimidin-7- yl]phenyl}-3-methylbutanamide (27)

According to the procedure used to prepare **22**, reaction of **21e** and 4,4,4-trifluoro-3-methylbutanoic acid provided **27** in 33% yield as an off-white solid: mp 185–186 °C; ^{1}H NMR (DMSO-d_6) δ 10.21 (s, 1H), 8.89 (d, J = 3 Hz, 1H), 8.82 (s, 1H), 8.63 (dd, J = 1, 6 Hz, 1H), 8.23 (dd, J = 1, 3 Hz, 1H), 8.06 (dd, J = 3, 6 Hz, 1H), 8.00–7.95 (m, 1H), 7.57 (dd, J = 3, 9 Hz, 1H), 7.48 (d, J = 3 Hz, 1H), 7.31 (dd, J = 3, 6 Hz, 1H), 2.93–2.86 (m, 1H), 2.80 (dd, J = 4, 11 Hz, 1H), 2.57 (dd, J = 5, 11 Hz, 1H), 1.14 (d, J = 5 Hz, 3H); MS (ES) *m/e* 477.1 (M+H).

Anal. ($C_{22}H_{16}FN_4O_2S$–0.05CH_2Cl_2) C, H, N.

4.35. *N*-{2-Fluoro-5-[3-(thien-2-ylcarbonyl)pyrazolo[1,5-*a*]pyrim-idin-7-yl]phenyl}-3,3-dimethylbutanamide (28)

According to the procedure used to prepare **24**, reaction of **19e** and 3,3-dimethylbutanoyl chloride provided **26** in 27% yield as a yellow solid: mp 180–181 °C; ^{1}H NMR (DMSO-d_6) δ 9.86 (s, 1H), 8.88 (d, J = 3 Hz, 1H), 8.82 (s, 1H), 8.55 (dd, J = 2, 5 Hz, 1H), 8.23 (dd, J = 1, 3 Hz, 1H), 8.05 (dd, J = 1, 4 Hz, 1H), 7.95–7.90 (m, 1H), 7.54 (dd, J = 2, 8 Hz, 1H), 7.48 (d, J = 3 Hz, 1H), 7.30 (dd, J = 1, 4 Hz, 1H), 2.31 (s, 2H), 1.04 (s, 9H); MS (ES) *m/e* 437.2 (M+H).

Anal. ($C_{23}H_{21}FN_4O_2S$–0.15CH_2Cl_2) C, H, N.

4.36. *N*-{2-Fluoro-5-[3-(thien-2-ylcarbonyl)pyrazolo[1,5-*a*]-pyrimidin-7-yl]phenyl}-*N*′-*iso*propylurea (29)

To a mixture of **21e** (100 mg, 0.0.296 mmol) and 4-dimethylaminopyridine (catalytic amount) in 1,4-dioxane (10 mL) was added *iso*propyl *iso*cyanate (50 mg, 0.588 mmol). The resulting mixture was heated at reflux for 24 h and cooled to room temperature. Additional *iso*propyl *iso*cyanate (250 mg, 2.94 mmol) was added and the reaction mixture was stirred for 6 days at room temperature and partitioned between ethyl acetate and water. The combined organics were dried over sodium sulfate, concentrated and purified by column chromatographyl (eluting with 1–5% methanol/methylene chloride) to give 24 mg (19%) of **29** as a yellow solid: mp >200 °C; ^{1}H NMR (DMSO-d_6) δ 8.88–8.86 (m, 2H), 8.81 (t, J = 2 Hz, 1H), 8.41 (s, 1H), 8.23 (t, J = 2 Hz, 1H), 8.04 (d, J = 3 Hz, 1H), 7.67 (d, J = 3 Hz, 1), 7.45 (dd, J = 1, 8 Hz, 1H), 7.42 (d, J = 3 Hz, 1H), 7.30 (d, J = 2 Hz, 1H), 6.62 (s, 1H), 5.47 (s, 1H), 3.85–3.60 (m, 2H), 1.11 (d J = 5 Hz, 3H), 1.00 (d, J = 5 Hz, 3H); MS (ES) *m/e* 424.0 (M+H).

Anal. ($C_{21}H_{18}FN_5O_2S$–0.40H_2O) C, H, N.

4.37. *iso*Propyl 2-fluoro-5-[3-(thien-2-ylcarbonyl)pyrazolo[1,5-*a*]pyrimidin-7- yl]phenylcarbamate (30)

To **21e** (300 mg, 0.887 mmol) in THF (20 mL) was added triethylamine (97 mg, 0.958 mmol). After stirring for 20 min at room temperature, *iso*propyl chloroformate (1.0 M in methylene chloride, 0.97 mL, 0.97 mmol) was added dropwise. The reaction mixture was stirred at room temperature overnight and additional *iso*propyl chloroformate (0.97 mL, 0.97 mmol) and triethylamine (97 mg, 0.958 mmol) were added. The resulting mixture was heated at reflux for 2 h, cooled to room temperature, filtered to collect the solids. The crude solids were washed with hexanes and further purified by HPLC (eluting with a gradient of MeCN/water) to give 112 mg (30%) of **30** as a yellow solid: mp 172–175 °C; ^{1}H NMR (DMSO-d_6) δ 9.54 (s, 1H), 8.88 (d, J = 3 Hz, 1H), 8.82 (s, 1H), 8.42 (dd, J = 2, 6 Hz, 1H), 8.23 (dd, J = 2, 3 Hz, 1H), 8.06 (dd, J = 3, 4 Hz, 1H), 7.95–7.88 (m, 1H), 7.50 (dd, J = 2, 8 Hz, 1H), 7.49 (d, J = 3 Hz, 1H), 7.30 (dd, J = 1, 4 Hz, 1H), 4.94–4.87 (m, 1H), 1.27 (d, J = 5 Hz, 6H); MS (ES) *m/e* 425.1 (M+H).

Anal. ($C_{21}H_{17}FN_4O_3S$) C, H, N.

4.38. (2*E*)-3-(Dimethylamino)-1-(5-nitro-3-pyridinyl)-2-propen-1-one (32a)

According to the procedure used to prepare **4**, reaction of 3-acetyl-5-nitropyridine[19] and DMF–DMA provided **32a** in 81% yield as an orange solid: mp 170–172 °C; ^{1}H NMR ($CDCl_3$) δ 9.48 (d, J = 2 Hz, 1H), 9.36 (d, J = 1 Hz, 1H), 8.91 (t, J = J = 2 Hz, 1H), 7.94 (d, J = 9 Hz, 1H), 5.68 (d, J = 9 Hz, 1H), 3.24 (s, 3H), 3.02 (s, 3H); MS (ES) *m/e* 222.1 (M+H).

Anal. ($C_{10}H_{11}N_3O_3$–0.15H_2O) C, H, N.

4.39. [7-(5-Nitro-3-pyridinyl)pyrazolo[1,5-*a*]pyrimidin-3-yl](2-thienyl)methanone (33a)

According to the procedure used to prepare **6**, reaction of **32a** and **5** provided **33a** in 87% yield as a yellow solid: mp 170–172 °C; ^{1}H NMR (DMSO-d_6) δ 9.61 (t, J = 2 Hz, 1H), 9.39 (dd, J = 1, 2 Hz, 1H), 8.98 (d, J = 3 Hz, 1H), 8.90 (s, 1H), 8.21 (dd, J = 1, 3 Hz, 1H), 8.08 (dd, J = 1, 4 Hz, 1H), 7.81 (d, J = 3 Hz, 1H), 7.31 (dd, J = 1, 4Hz, 1H); MS (ES) *m/e* 352.1 (M+H).

Anal. ($C_{16}H_9N_5O_3S$) C, H, N.

4.40. [7-(5-Amino-3-pyridinyl)pyrazolo[1,5-*a*]pyrimidin-3-yl]-(2-thienyl)methanone (34a)

According to the procedure used to prepare **21a**, reaction of **33a** provided **34a** in 55% yield as a yellow solid: mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 8.87 (d, J = 3 Hz, 1H), 8.82 (s, 1H), 8.31 (d, J = 1, 3 Hz, 1H), 8.23 (dd, J = 1, 3 Hz, 1H), 8.15 (d, J = 2 Hz, 1H), 8.05 (dd, J = 1, 4 Hz, 1H), 7.65 (t, J = 2 Hz, 1H), 7.51 (d, J = 3 Hz, 1H), 7.30 (dd, J = 1, 4 Hz, 1H), 5.71 (s, 1H); MS (ES) *m/e* 322.1 (M+H).

Anal. ($C_{16}H_{11}N_5OS$) C, H, N.

4.41. 3-Methyl-*N*-{5-[3-(2-thienylcarbonyl)pyrazolo[1,5-*a*]pyrimidin-7-yl}-3-pyridinyl}butanamide (35a)

According to the procedure used to prepare **22**, reaction of **33a** and isovalaerid acid provided **34a** in 53% yield as a beige solid: mp 217–219 °C; ^{1}H NMR (DMSO-d_6) δ 10.40 (s, 1H), 8.99 (d, J = 2 Hz, 1H), 8.92 (d, J = 3 Hz, 1H), 8.86 (d, J = 2 Hz, 1H), 8.84 (s, 1H), 8.80 (dd, J = 1, 3 Hz, 1H), 8.22 (d, J = 3 Hz, 1H), 7.31 (dd, J = 1, 3 Hz, 1H), 2.29 (d, J = 5 Hz, 2H), 2.16–2.06 (m, 1H), 0.96 (d, J = 5 Hz, 6H); MS (ES) *m/e* 406.1 (M+H).

Anal. ($C_{21}H_{19}N_5O_2S0$–$0.4H_2O$–$0.1Et_2O$) C, H, N.

4.42. (*E*)-3-(Dimethylamino)-1-(5-nitrothien-2-yl)prop-2-en-1-one (32b)

According to the procedure used to prepare **4**, reaction of 5-acetyl-2-nitrothiophene and DMF–DMA provided **32b** in 60% yield as an orange solid: mp 202–203 °C; ^{1}H NMR (DMSO-d_6) δ 8.12 (d, J = 3 Hz, 1H), 7.85 (d, J = 3 Hz, 1H), 7.82 (d, J = 9 Hz, 1H), 5.90 (d, J = 9 Hz, 1H), 3.19 (s, 3H), 2.98 (s, 3H); MS (ES) *m/e* 227.1 (M+H).

Anal. ($C_9H_{10}N_2O_3S$) C, H, N.

4.43. [7-(5-Nitro-2-thienyl)pyrazolo[1,5-*a*]pyrimidin-3-yl](2-thienyl)methanone (33b)

According to the procedure used to prepare **6**, reaction of **32b** and **5** provided **33b** in 72% yield as a yellow solid: mp 213–215 °C; ^{1}H NMR (DMSO-d_6) δ 9.02 (s, 1H), 8.97 (d, J = 5 Hz, 1H), 8.59 (d, J = 5 Hz, 1H), 8.35 (d, J = 5 Hz, 1H), 8.30 (d, J = 5 Hz, 1H), 8.22 (dd, J = 1, 4 Hz, 1H), 8.08 (dd, J = 1, 5 Hz, 1H), 7.31 (dd, J = 1, 5 Hz, 1H).

4.44. [7-(5-Aminothien-2-yl)pyrazolo[1,5-*a*]pyrimidin-3-yl]-(thien-2-yl)methanone (34b)

According to the procedure used to prepare **21a**, reaction of **33b** provided **34b** in 55% yield as a reddish solid: mp 232–235 °C; ^{1}H NMR (DMSO-d_6) δ 8.77 (s, 1H), 8.48 (d, J = 4 Hz, 1H), 8.38 (dd, J = 1, 3 Hz, 1H), 8.28 (d, J = 3 Hz, 1H), 8.01 (dd, J = 1, 4 Hz, 1H), 7.53 (d, J = 4 Hz, 1H), 7.28 (dd, J = 1, 4 Hz, 1H), 7.26 (s, 1H), 6.22 (d, J = 3 Hz, 1H), 5.71 (s, 2H); MS (ES) *m/e* 327.1 (M+H).

Anal. ($C_{15}H_{10}N_4OS_2$–0.5EtOAc) C, H, N.

4.45. 3-Methyl-*N*-{5-[3-(thien-2-ylcarbonyl)pyrazolo[1,5-*a*]-pyrim-idin-7-yl]thien-2-yl}butanamide (35b)

According to the procedure used to prepare **22**, reaction of **34b** and valaeryl chloride provided **35b** in 59% yield as a light yellow solid: mp 268–270 °C; ^{1}H NMR (DMSO-d_6) δ 11.95 (s, 1H), 8.93 (s, 1H), 8.77 (d, J = 3 Hz, 1H), 8.43 (d, J = 3 Hz, 1H), 8.29 (dd, J = 1, 3 Hz, 1H), 8.04 (dd, J = 1, 3 Hz, 1H), 7.88 (d, J = 3 Hz, 1H), 7.30 (dd, J = 1, 3 Hz, 1H), 6.92 (d, J = 3 Hz, 1H), 2.35 (d, J = 5 Hz, 2H), 2.16–2.09 (m, 1H), 0.90 (d, J = 5 Hz, 6H); MS (ES) *m/e* 411.1 (M+H).

Anal. ($C_{20}H_{18}N_4O_2S_2$–$0.3H_2O$) C, H, N.

4.46. (*E*)-3-(Dimethylamino)-1-(5-nitrothien-3-yl)prop-2-en-1-one (32c)

According to the procedure used to prepare **4**, reaction of 4-acetyl-2-nitrothiophene and DMF–DMA provided **32c** in 63% yield as a yellow solid: mp 165–166 °C; ^{1}H NMR (DMSO-d_6) δ 8.53 (d, J = 1 Hz, 1H), 8.46 (d, J = 1 Hz, 1H), 7.74 (d, J = 9 Hz, 1H), 5.82 (d, J = 9 Hz, 1H), 3.16 (s, 3H), 2.94 (s, 3H); MS (ES) *m/e* 227.1 (M+H).

Anal. ($C_9H_{10}N_2O_3S$) C, H, N.

4.47. [7-(5-Nitro-3-thienyl)pyrazolo[1,5-*a*]pyrimidin-3-yl](2-thienyl)methanone (33c)

According to the procedure used to prepare **6**, reaction of **32c** and **5** provided **33c** in 80% yield as an orange solid: mp 225–226 °C; ^{1}H NMR (DMSO-d_6) δ 9.44 (d, J = 1 Hz, 1H), 9.12 (d, J = 1 Hz, 1H), 8.94 (s, 1H), 8.93 (d, J = 3 Hz, 1H), 8.21 (dd, J = 1, 3 Hz, 1H), 8.07 (dd, J = 1, 3 Hz, 1H), 7.99 (d, J = 3 Hz, 1H), 7.30 (dd, J = 1, 3 Hz, 1H); MS (ES) *m/e* 357.1 (M+H).

Anal. ($C_{15}H_8N_4O_3S_2$) C, H, N.

4.48. [7-(5-Aminothien-3-yl)pyrazolo[1,5-*a*]pyrimidin-3-yl]-(thien-2-yl)methanone (34c)

According to the procedure used to prepare **21a**, reaction of **33c** provided **34c** in 15% yield as a light brown solid: mp 174–176 °C; ^{1}H NMR (DMSO-d_6) δ 8.86 (s, 1H), 8.79 (d, J = 4 Hz, 1H), 8.23 (dd, J = 1, 3 Hz, 1H), 8.21 (d, J = 1 Hz, 1H), 8.04 (dd, J = 1, 3 Hz, 1H), 7.58 (d, J = 4 Hz, 1H), 7.29 (dd, J = 1, 3 Hz, 1H), 6.76 (d, J = 1 Hz, 1H), 5.90 (s, br, 2H); MS (ES) *m/e* 327.1 (M+H).

Anal. ($C_{15}H_{10}N_4OS_2$–$0.3H_2O$) C, H, N.

4.493-Methyl-*N*-{4-[3-(thien-2-ylcarbonyl)pyrazolo[1,5-*a*]pyrimidin-7-yl]thien-2-yl}butanamide (35c)

According to the procedure used to prepare **22**, reaction of **34c** and valaeryl chloride provided **35c** in 84% yield as a light yellow solid: mp 234–235 °C; ^{1}H NMR (DMSO-d_6) δ 11.46 (s, 1H), 8.88 (d, J = 1 Hz, 1H), 8.85 (d, J = 1 Hz, 1H), 8.56 (d, J = 1 Hz, 1H), 8.24 (d, J = 1 Hz, 1H), 8.04 (dd, J = 1, 3 Hz, 1H), 7.68 (dd, J = 1, 3 Hz, 1H), 7.61 (dd, J = 1, 3 Hz, 1H), 7.23 (dd, J = 1, 3 Hz, 1H), 2.27 (t, J = 5 Hz, 2H), 2.14–2.09 (m, 1H), 0.94 (d, J = 5 Hz, 6H); MS (ES) *m/e* 411.1 (M+H).

Anal. ($C_{20}H_{18}N_4O_2S_2$–$0.25H_2O$) C, H, N.

4.50. Biological evaluation

The protocol for p21 isogenic cell and colon cell proliferation assays were previously reported.[9]

4.51. Microtubulin binding assay

The protocol for microtubulin binding assay was previously reported.[15]

4.52. LoVo xenograft assay

Compounds were evaluated in vivo using standard pharmacological test procedures which measure the ability to inhibit the growth of human tumor xenografts. Human Colon Carcinoma LoVo cells (American Type Culture Collection, Manassas, Maryland) were grown in tissue culture in RPMI (Gibco/InVitrogen, Gaithersburg, MD) supplemented with 10% FBS (Gemini Bio-Products Inc., Calabasas, CA). Athymic nu/nu female mice (Charles River, Wilmington, MA) were injected SC in the flank area with 1×10^7 LoVo cells. When tumors attained a mass of between 90 and 120 mg, the mice

were randomized into treatment groups (day zero), 5 animals per group. Animals were treated PO once a day on days 1 through 20 post staging (day zero) with either compound prepared in 0.5% Methocel/2.0% Tween 80 or 0.5% Methocel/0.4% Tween 80 or vehicle alone. Tumor mass was determined every 7 days [(length $\times$ width^2)/2] for up to 28 days post staging. Relative tumor growth (Mean tumor mass on days 7, 14, 21 and 28 divided by the mean tumor mass on day zero) was determined for each treatment group. Statistical analysis (Student-t-test) of log relative tumor growth was used to compare treated verses control group in each experiment. A p-value ($p \leqslant 0.05$) indicates a statistically significant reduction in relative tumor growth of treated group compared to the vehicle control.

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