Discovery of Novel Thieno[2,3-*d*]pyrimidin-4-yl Hydrazone-Based Cyclin-Dependent Kinase 4 Inhibitors: Synthesis, Biological Evaluation and Structure–Activity Relationships

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The design, synthesis, and evaluation of novel thieno[2,3-*d*]pyrimidin-4-yl hydrazone analogues as cyclin-dependent kinase 4 (CDK4) inhibitors are described. In continuing our program aim to search for potent CDK4 inhibitors, the introduction of a thiazole group at the hydrazone part has led to marked enhancement of chemical stability. Furthermore, by focusing on the optimization at the C-4′ position of the thiazole ring and the C-6 position of the thieno[2,3-*d*]pyrimidine moiety, compound 35 has been identified with efficacy in a xenograft model of HCT116 cells. In this paper, the potency, selectivity profile, and structure–activity relationships of our synthetic compounds are discussed.

Key words cyclin-dependent kinase 4 inhibitor; thieno[2,3-*d*]pyrimidine-4-yl hydrazone

Cyclin-dependent kinases (CDKs), a family of serine/threonine kinases, are responsible for coordinating the events by which cells progress through the cell cycle and become active at specific phases: G1, S, G2, and M.[1—6] In the G1 phase, cyclin D1 increases to trigger the activation of CDK4/6 in early G1, and then cyclin E/CDK2 is activated. CDK4/6 activities are negatively regulated by the tumor suppressor p16, a cyclin-dependent kinase inhibitor of the INK4 family. However, many tumors have been reported to contain mutations, deletions, or silencing of p16.[7—9] Moreover, mutations in CDKs and abnormal expressions of their regulators have been found in a large percentage of melanoma patients.[10] In addition, Malumbres and Barbacid have reported that knockdown of CDK4 in mammary tumor cells prevents tumor formation,[11] and McCormick and Tetsu have found that cancer cells proliferate despite CDK2 inhibition.[12] These results suggest that selective inhibition of CDK4 may restore normal cell activity and could be a more valuable approach to cancer therapy than CDK2, especially for those who have lost the INK4 family such as p16.

In our previous paper, we reported the discovery of the potent CDK4 (IC_{50}=0.038 μg/ml) inhibitor (**1**), which had moderate selectivity for CDK2 with strong cytotoxic activity[13,14] (Fig. 1). The cell cycle distribution analysis in HTC116 cells was carried out with selected compounds, same as **1**, and the DNA content of the cells was assessed by flow cytometry. After treating the cells with the compounds, significant increases in G0/G1 population were observed. Furthermore, apoptosis was induced in some compounds, which is indicated by the sub-G1 fraction. However, during our studies to improve selectivity and the physical profile of **1**, we found that thieno[2,3-*d*]pyrimidine hydrazones with a phenyl ring had a common feature of poor chemical stability under acidic conditions. Since we aimed to investigate orally available drugs, we had to prevent the degradation of compounds by gastric acid. Therefore, we tried to change the electronic environment around the hydrazone moiety by introducing heteroaryl groups such as imidazoles, oxazoles, and thiazoles instead of the phenyl ring. We found that thiazole compounds were well tolerated in the acidic condition and they also possessed potent CDK4 inhibitory activity. To further explore the potential of this class of compounds, we introduced various substituted thiazole at the hydrazone moiety and the alkyl group at the C-6 position of thieno[2,3-*d*]pyrimidine scaffold.

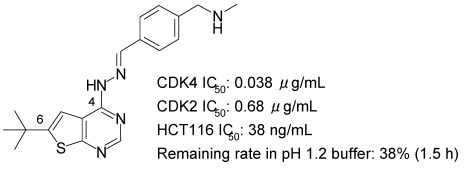
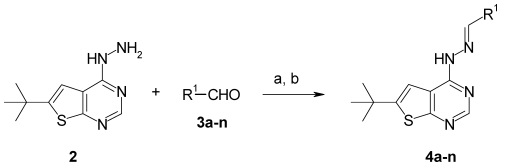


Fig. 1. Structure of Compound **1**

Herein, we report the chemical stabilities of compound **1**, and describe the design, synthesis of novel analogues of 1,3-thiazole-2-carbaldehyde thieno[2,3-*d*]pyrimidin-4-yl hydrazone with marked enhancement of chemical stability, and their inhibitory activities for CDK4 with selectivity against CDK2. We also report their cytotoxic potential against human cancer cell lines.

Chemistry and Chemical Stability Preparation of **1** and **4a**—**n** was accomplished using a general synthetic route as shown in Chart 1. The hydrazone analogues **4a**—**n** were produced by a condensation reaction with **2** and appropriate aldehydes **3a**—**n** in benzene, followed by deprotection in the case of **4d**, **4e**, and **4g**—**n**.

In our recent studies, the chemical stability of compound **1** was examined in pH 1.2 and pH 6.8 phosphate buffers at



Reagents and conditions: (a) aldehydes **3a**—**n**, benzene, reflux; (b) deprotection with 4 N HCl in 1,4-dioxane in the case of **4d**, **e**, **g**—**n**, 21—88% from **2**.

Chart 1. Synthesis of Compounds **4a**—**n**

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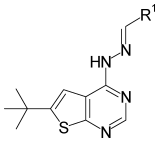
Table 1. Enzyme Inhibitory Activity and Chemical Stability of (6-*tert*-Butylthieno[2,3-*d*]pyrimidine-4-yl)hydrazone Analogues



Compd.	R^1	CDK4 IC_{50} (μg/ml)[a]	CDK2 IC_{50} (μg/ml)[a]	CDK4 selectivity[b]	Remaining rate (%)[c] in pH 1.2 buffer
1	4-[(Methylamino)methyl]phenyl	0.038	0.68	17.9	38 (1.5 h)
4a	1,3-Thiazol-2-yl	0.041	0.20	4.9	79 (3 h)
4b	1,3-Thiazol-4-yl	0.89	2.2	2.5	75 (1 h)
4c	1,3-Thiazol-5-yl	0.052	0.059	1.1	86 (1.5 h)

a) Concentration (μg/ml) needed to inhibit the Rb phosphorylation by 50%, as determined from the dose–response curve. Values are the means of at least two determinations. *b*) The values are calculated by using the following equation: (CDK2 IC_{50})/(CDK4 IC_{50}). *c*) Remaining rate of the substrate after incubation with pH 1.2 buffer at 37 °C. Numbers in parentheses indicate the incubation time.

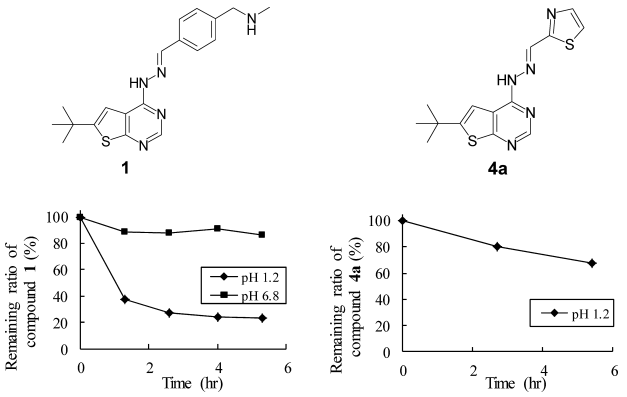
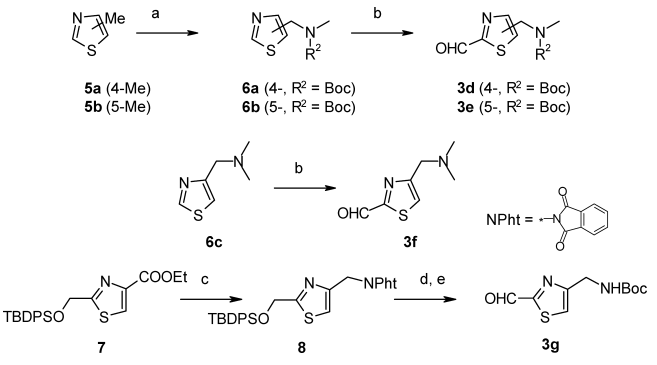


Fig. 2. Plots of the Remaining Ratio of Compounds **1** and **4a** *versus* Time in Buffers



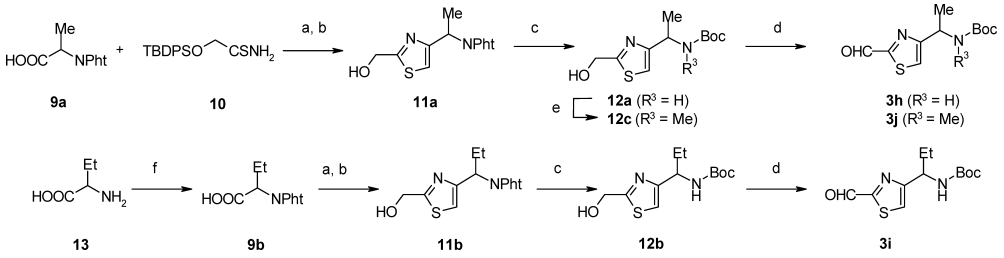
Reagents and conditions: (a) NBS, AIBN, CCl_4, 90 °C; $MeNH_2$, THF, 0 °C; $(Boc)_2O$, Et_3N, THF, 35% from **5a**, 57% from **5b**; (b) *n*-BuLi, *N*-formylmorpholine, THF, −78 °C, 35% from **6a**, 32% from **6b**, 44% from **6c**; (c) LAH, THF, 0 °C; phthalimide, PPh_3, DEAD, 49% for two steps; (d) $NH_2NH_2 \cdot 1H_2O$, EtOH; $(Boc)_2O$, $NaHCO_3$, CH_2Cl_2, 44% for two steps; (e) TBAF, THF; MnO_2, CCl_4, reflux, 20% for two steps.

Chart 2. Synthesis of Aldehydes **3d**—**g**

37 °C, and the corresponding remaining ratios are given in Fig. 2. Compound **1** is reasonably stable in neutral solution, but a rapid decrease of **1** was observed in acidic conditions. We then evaluated the acidic hydrolysis of **1** by HPLC analysis. As a result, an inverse correlation was observed between the production of hydrazine **2** and the decomposition of **1**. In order to overcome this problem, we tried to change the electronic environment around the hydrazone moiety by introducing heteroaryl groups (R^1) as imidazoles, oxazoles, and thiazoles instead of a benzene ring. We found that a series of compounds **4a**—**c**, bearing a non-substituted thiazole group, was well tolerated in the acidic condition and they also possessed potent CDK4 inhibitory activity as shown in Table 1. Among these compounds, 1,3-thiazole-2-yl compound **4a** showed excellent CDK4 inhibitory activity with some selectivity against CDK2 (4.9-fold). In contrast, 1,3-thiazole-5-yl compound **4c** had poor selectivity. The plot of the remaining ratio of compound **4a** in pH 1.2 is shown in Fig. 2, with no data in pH 6.8 because **4a** was insoluble under the condition. From these results, we selected **4a** to initiate our study to improve CDK4/CDK2 selectivity and cytotoxicity for further investigation.

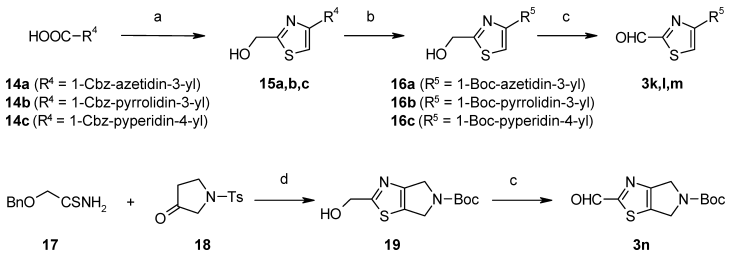
The synthesis of aldehydes was described as follows. The thiazole aldehydes **3a**—**c** were commercially available. The substituted thiazole aldehydes **3d**—**f** were prepared using the reported method of Dority Jr. *et al.*[15] as shown in Chart 2. After bromination of **5a**, **b** and amination with methylamine, followed by *N*-Boc protection, **6a**, **b** were produced, respectively. **6a** was converted to aldehyde **3d** with *n*-BuLi and *N*-formylmorpholine. Aldehydes **3e** and **3f** were obtained from **6b** and commercial dimethylamine **6c** in the same manner. Aldehyde **3g** was prepared from **7**. Thiazole **7** was synthesized using the reported method of Subhash *et al.*[16] After reduction of ethyl ester using lithium aluminum hydride (LAH), Mitsunobu reaction was carried out to introduce the amino methyl unit at the C-4 position. Deprotection of the phthalimide group of **8** with hydrazine monohydrate gave the corresponding amine, followed by protection with the Boc group. After deprotection of the *tert*-butyldiphenylsilyl (TBDPS) group with tetrabutylammonium fluoride (TBAF), oxidation of the resulting alcohol with MnO_2 afforded aldehyde **3g**.

The substituted thiazole aldehydes **3h**—**j** were synthesized as shown in Chart 3. As reported by Illig *et al.*,[17] **11a** was obtained from commercially available compound **9a** using the reaction of thioamide **10**[16] and bromomethyl ketone intermediate, which was made by diazoketone and hydrogen bromide. After the protection of amine **13** with phthalic anhydride, **11b** was synthesized from **9b** in a similar manner. **12a**, **b** were obtained from **11a**, **b** by the procedure for **3g**. **12c** was prepared from **12a** by deprotection of *N*-Boc and methylation of resulting amine, followed by *N*-Boc protection. Conversion of **12a**—**c** to **3h**—**j** was carried out by the procedure utilized for the preparation of **3g**.



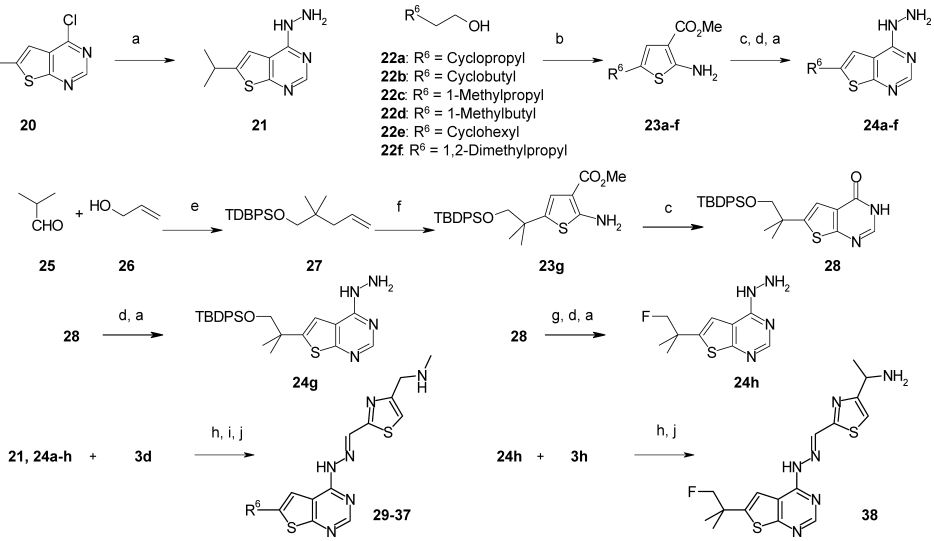
Reagents and conditions: (a) $SOCl_2$, cat. DMF, reflux; $TMSCHN_2$, THF, 0 °C; (b) 47% HBr, Et_2O; thioamide **10**, EtOH, reflux, 67% from **9a**, 60% from **9b**; (c) $NH_2NH_2 \cdot 1H_2O$, EtOH; $(Boc)_2O$, $NaHCO_3$, CH_2Cl_2, 89% from **11a**, quant. from **11b**; (d) MnO_2, CCl_4, reflux, 82% from **12a**, 93% from **12b**, 95% from **12c**; (e) HCl, EtOH; TFAA, Et_3N, CH_2Cl_2; MeI, K_2CO_3, DMF; 1 N NaOH, THF; $(Boc)_2O$, 11%; (f) phthalic anhydride, 120 °C, 99%.

Chart 3. Synthesis of Aldehydes **3h**—**j**



Reagents and conditions: (a) $(COCl)_2$, cat. DMF, reflux; $TMSCHN_2$, THF, 0 °C; 47% HBr, Et_2O; thioamide **10**, EtOH, reflux, 26% from **14a**, 44% from **14b**, 70% from **14c**; (b) BCl_3, CH_2Cl_2; $(Boc)_2O$, Et_3N, THF, 11% from **15a**, 64% from **15b**, 69% from **15c**; (c) MnO_2, $CHCl_3$, reflux, 68% from **16a**, 83% from **16b**, 60% from **16c**, 81% from **19**; (d) **18**, Br_2, AcOH; **17**, DMF; MsCl, Et_3N; 47% HBr, phenol; $(Boc)_2O$, NaOH aq, CH_2Cl_2, 9% for five steps.

Chart 4. Synthesis of Aldehydes **3k**—**n**



Reagents and conditions: (a) $NH_2NH_2 \cdot 1H_2O$, EtOH, reflux, 95% from **20**, 31—58% from **23a**—**g**; (b) PCC, CH_2Cl_2; methyl cyanoacetate, S_8, Et_3N, DMF, 5—37% from **22a**—**f**; (c) $HCONH_2$, 210 °C; (d) $POCl_3$, 110 °C; (e) *p*-cymene, *p*-TsOH; LAH, Et_2O, TBDPSCl, imidazole, THF, 38% for three steps; (f) OsO_4, $NaIO_4$, THF·H_2O; methyl cyanoacetate, S_8, Et_3N, DMF, 38% for two steps; (g) TBAF, THF, 70 °C; DAST, CH_2Cl_2; (h) aldehyde **3d** or **3h**, benzene, reflux; (i) TBAF, THF, 70 °C in case of **24g**; (j) 4 N HCl in 1,4-dioxane or 1 N HCl in EtOH, 2—97% from **21**, **24a**—**h**.

Chart 5. Synthesis of Compounds **29**—**38**

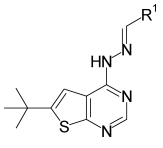
The substituted thiazole aldehydes **3k**—**n**, containing a cyclic alkyl group or fused ring, were synthesized as shown in Chart 4. **15a**—**c** were obtained from **14a**—**c** in a similar manner to **11a**, respectively. *N*-Cbz deprotection of **15a**—**c** followed by *N*-Boc protection afforded **16a**—**c**. Finally, **16a**—**c** were converted to **3k**—**m** by the procedure for **3h**. Bicyclic compound **3n** was synthesized from thioamide **17** and pyrrolidin-3-one **18**. After α-bromination of **18** and cyclization with **17**, dehydration was performed by treatment with methanesulfonyl chloride, followed by deprotection of *O*-benzyl and *N*-Ts with HBr, and then *N*-Boc protection afforded **19**. Conversion to aldehyde **3n** from **19** was performed as previously described.

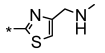
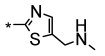
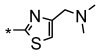
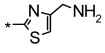
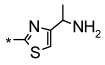
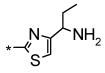
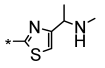
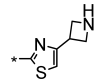
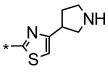
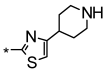
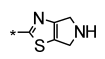
The hydrazine **21** was prepared from **20** with hydrazine monohydrate in ethanol under reflux. The thiophenes **23a**—**f** were synthesized from appropriate alcohols **22a**—**f** with

methyl cyanoacetate in the presence of sulfur by the method of Tinney *et al.*[18] **27** was obtained from **25** and **26** *via* 2,2-dimethyl-4-pentenal,[19] followed by reduction of aldehyde, and protection of hydroxide with TBDPSCl. Thiophene **23g** was synthesized in the same manner as **23a**—**f** after Lemieux-Johnson oxidation of **27**. Cyclization of **23a**—**g** with formamide gave 4-oxothieno[2,3-*d*]pyrimidines. The hydrazines **24a**—**h** were prepared from chlorination of 4-oxothieno[2,3-*d*]pyrimidines with phosphorus oxychloride, followed by treatment with hydrazine monohydrate according to the reported procedure.[20] For hydrazine **24h**, intermediate **28** was fluorinated with (dimethylamino)sulfur trifluoride after removal of TBDPS protection. The hydrazone analogues **29**—**38** were produced by a condensation reaction with hydrazines **21**, **24a**—**h**, and aldehyde **3d** or **3h** in benzene, followed by deprotection (Chart 5).

Biological Evaluation Biological activities of the novel thieno[2,3-*d*]pyrimidin-4-yl hydrazone analogues were evaluated in two assay systems: enzyme inhibitory activity against CDK4/CDK2 and cytotoxicity against two cell lines

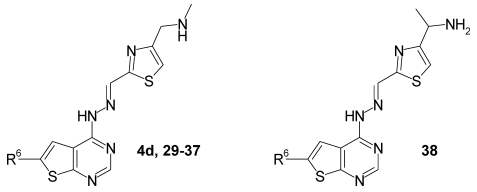
Table 2. Enzyme Inhibitory and Cytotoxic Activity for Substituted Thiazole-2-carbaldehyde (6-*tert*-Butylthieno[2,3-*d*]pyrimidine-4-yl)hydrazones



Compd.	R^1	CDK4 IC_{50} (μg/ml)[a]	CDK2 IC_{50} (μg/ml)[a]	CDK4 selectivity[b]	HCT116 IC_{50} (μg/ml)[c]	PC-6 IC_{50} (μg/ml)[c]	Remaining rate (%)[d]
4a	1,3-Thiazol-2-yl	0.041	0.20	4.9	0.419	0.381	80
4d		0.034	0.98	28.8	0.343	0.214	85
4e		0.042	0.35	8.3	0.156	0.108	NT[e]
4f		0.043	1.4	32.6	0.328	0.241	80
4g		0.090	0.38	4.2	0.730	0.490	89
4h		0.019	0.83	43.7	0.177	0.103	81
4i		0.058	1.4	24.1	0.211	0.107	84
4j		0.037	1.1	29.7	0.320	0.171	85
4k		0.073	1.0	13.7	0.768	0.706	81
4l		0.070	1.8	25.7	0.812	0.635	NT[e]
4m		0.014	1.7	121.4	0.929	NT[e]	NT[e]
4n		0.025	0.20	8.0	0.458	0.240	90

a) Concentration (μg/ml) needed to inhibit the Rb phosphorylation by 50%, as determined from the dose–response curve. Values are the means of at least two determinations. *b*) The values are calculated by using the following equation: (CDK2 IC_{50})/(CDK4 IC_{50}). *c*) Dose–response curves were determined at ten concentrations. The IC_{50} values are the concentrations needed to inhibit cell growth by 50%, as determined from these curves. *d*) Remaining rate of the substrate after 3 h incubation with pH 1.2 buffer at 37 °C. *e*) NT=not tested.

Table 3. Enzyme Inhibitory and Cytotoxic Activity for Substituted Thieno[2,3-*d*]pyrimidines Focused on the C-6 (R^6) Position



Compd.	R^6	CDK4 IC_{50} (μg/ml)[a]	CDK2 IC_{50} (μg/ml)[a]	CDK4 selectivity[b]	HCT116 IC_{50} (μg/ml)[c]	PC-6 IC_{50} (μg/ml)[c]	Remaining rate (%)[d]
4d		0.034	0.98	28.8	0.343	0.214	85
29		0.058	1.50	25.9	0.380	0.221	85
30		0.260	>2.0		0.421	0.271	83
31		0.045	1.4	31.1	0.521	0.393	80 (4 h)
32		0.029	0.44	15.2	0.203	0.114	80 (4 h)
33		0.030	1.40	46.7	0.431	0.201	NT[e]
34		0.020	0.81	40.5	0.340	0.166	NT[e]
35		0.022	0.88	40.0	0.315	0.108	83
36		0.069	1.8	26.1	1.32	0.681	NT[e]
37		0.140	1.8	12.9	0.184	0.078	80
38		0.046	0.68	14.7	0.061	0.029	81

a) Concentration (μg/ml) needed to inhibit the Rb phosphorylation by 50%, as determined from the dose–response curve. Values are the means of at least two determinations. *b*) The values are calculated by using the following equation: (CDK2 IC_{50})/(CDK4 IC_{50}). *c*) Dose–response curves were determined at ten concentrations. The IC_{50} values are the concentrations needed to inhibit cell growth by 50%, as determined from these curves. *d*) Remaining rate of the substrate after 3 h incubation with pH 1.2 buffer at 37 °C. *e*) NT=not tested.

(HCT116 human colon carcinoma and PC-6 human non-small cell lung carcinoma).

Previously, we reported that the presence of an ionizable nitrogen atom at the C-4′ position on the benzene plays an important role in the cytotoxic activity.[14] Therefore, we introduced a series of substituent groups with alkyl amine at the C-4′ or C-5′ position on the thiazole ring. All the derivatives in Table 2 maintained fairly good CDK4 inhibitory activity and chemical stability. 4-[(Methylamino)methyl]-1,3-thiazole-2-yl compound **4d** and 5-[(methylamino)methyl]-1,3-thiazole-2-yl compound **4e** sustained good CDK4 activity as **4a**, and **4d** had improved selectivity (28.8-fold *vs.* CDK2). On the other hand, **4e** was less selective. Therefore, we undertook replacements with a variety of alkyl amine analogues at the C-4′ position. Primary amine compound **4g** diminished cytotoxic activity and selectivity against CDK2. In contrast, tertiary amine compound **4f** exhibited similar IC_{50} values for **4d**. Then we noted the steric factors around a nitrogen atom of alkyl amine. Among the branched alkyl amines **4h**—**j**, compound **4h** improved its CDK4 inhibitory activity with good selectivity (43.7-fold *vs.* CDK2), and **4h** exhibited an IC_{50} value of 0.103 μg/ml against PC-6 cells. Conversely, we found that cyclic alkyl amino groups, as exemplified by compounds **4k**—**n**, markedly decreased cytotoxic activities. These results showed that the presence of the substituent alkyl amino group at the C-4′ position of the thiazole ring is very important and its steric factor influences cytotoxic activity.

For further improvement of cytotoxic activity, our effort was then focused on determining the effect of the alkyl group at the C-6 position. The biological activity and chemical stability data for **29**—**38** are shown in Table 3. Branched alkyl derivatives **29**, **31**—**35** maintained potent CDK4 inhibitory activity with selectivity (15.2 to 46.7-fold *vs.* CDK2). Among these analogues, compounds **32** and **35** had moderately good cytotoxicity against HCT116 and PC-6 (IC_{50} range: 0.108 to 0.315 μg/ml) with sufficient chemical stability. Meanwhile, cyclic alkyl compounds **30**, **31**, and **33** were less potent in cytotoxic activity. Next, we assessed the branched alkyl derivatives containing a heteroatom at the C-6 position. Hydroxyl compound **36** retained CDK4 inhibitory activity, but reduced cytotoxic activity. By contrast, hydrophobic fluorinated analogue **37** improved cytotoxic activities with weaker activity of CDK4 inhibition than that of **36**. Moreover, **38** had potent kinase inhibitory activity, and cytotoxic activity was nearly equivalent to that of **1**. These results suggest that modification of side chains at the C-6 position probably plays a crucial role for the improvement of pharma-

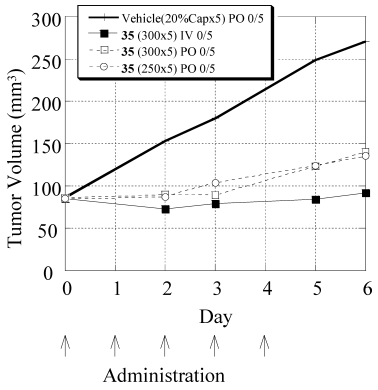


Fig. 3. Tumor Growth Inhibition Curves of **35**

cokinetics as well.

To evaluate the antitumor effects *in vivo*, HCT116 cells were subcutaneously transplanted into nude mice. Compound **35** was selected as a candidate because it possesses potent CDK4 inhibitory activity, good selectivity, and cytotoxic activity against HCT116 cells. In addition, after treating the cells with **35**, a marked increase in G0/G1 population (51%) was observed by the cell cycle distribution analysis. Therefore, **35** was administered intravenously (i.v.) and orally (*per os* (*p.o.*)). As a result, we found that **35** exhibited a tumor growth inhibition of 52% (i.v., 300 mg/kg) and 45% (*p.o.*, 300 mg/kg), respectively (Fig. 3).

Conclusion

In summary, we achieved the conversion into thiazole from the phenyl ring at the heteroaryl moiety of the C-4 hydrazone with adequate chemical stability. Next, we will focus on the optimization of the thiazole ring at the hydrazone and the C-6 position of the thieno[2,3-*d*]pyrimidine moiety. Also, compounds **4h**, **32**, **35**, and **38** have been identified as potent, selective CDK4 inhibitors with marked chemical stability and cytotoxic activity. Moreover, **35** showed efficacy in a xenograft model of the HCT116 cells. These results provide valuable information for the design of a potent CDK4 inhibitor and they also hold great promise for cancer therapy. We plan to evaluate the medical safety and pharmacokinetics using **35** for more optimization of this analogue, and further work will be reported elsewhere on the improvement of cytotoxic activity, CDK4 selectivity, and the physical profile in due course.

Experimental

Chemical Stability Assay For the chemical stability assay in aqueous solution, a 1 ml aliquot of 0.5 mg/ml stock solution of substrate in MeOH was added to 50 ml of the appropriate buffer solution. The prepared solution was incubated at 37 °C in the dark. At regular intervals, samples of the solution were analyzed by HPLC using the following conditions: a Waters Xterra RP18, 2.1 mm×10 cm, 3.5 μm column; mobile phase, 25 mM pH 6.8 phosphate buffer/acetonitrile=65/35; flow rate 0.3 ml/min; detector wavelength, 210 nm and 340 nm. Samples were also analyzed by LC-MS using the following conditions: (LC) a Waters Xterra RP18, 2.1 mm×5 cm, 3.5 μm column; mobile phase, 10 mM pH 6.8 sodium acetate-acetic acid buffer/acetonitrile=60/40; flow rate 0.4 ml/min; detector wavelength, 340 nm; (MS) a ThermoQuest LC-Q; ionization condition, Electron spray ionization (ESI); scanning method, positive scan (M.W. 100—400).

Chemistry Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. The solvent and reagent names are abbreviated as follows: acetic acid (AcOH), *tert*-butyldiphenylsilyl chloride (TBDPSCl), 1,1′-carbonylbis-1*H*-imidazole (CDI), di-*tert*-butyl dicarbonate $(Boc)_2O$, diethyl azodicarboxylate (DEAD), *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), lithium aluminum hydride (LAH), methanesulfonyl chloride (MsCl), *N*-methylmorpholine-*N*-oxide (NMO), pyridinium chlorochromate (PCC), TBAF, trifluoroacetic acid (TFA), trifluoroacetic anhydride (TFAA), tetrahydrofuran (THF). Column chromatography was performed with Merck silica gel 60 (particle size 0.060—0.200 or 0.040—0.063 mm). Flash column chromatography was performed with Biotage FLASH Si packed columns. Thin layer chromatography (TLC) was performed on Merck pre-coated TLC glass sheets with silica gel 60 F_{254}, and compound visualization was effected with a 5% solution of phosphomolybdic acid in ethanol, UV lamp, iodine, or Wako ninhydrin spray. ^{1}H-NMR spectra were recorded on a JEOL JNM EX400, and chemical shifts are given in ppm (δ) from tetramethylsilane as an internal standard. Significant ^{1}H-NMR data are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), coupling constant(s) in hertz. Infrared (IR) spectra were recorded on a Hitachi 270-30 spectrometer and a JASCO FT/IR-6100. Electron spray ionization condition (ESI) mass spectra were recorded on an Agilent 1100 and SCIEX API-150EX spectrometer. Fast atom bombardment ionization condition (FAB) mass spectra were recorded on a JEOL JMS-HX110 spectrometer. Electron impact ionization condition (EI) mass spectra were recorded on a JEOL JMS-AX505W. High-resolution mass spectra were obtained on a JEOL JMS-100LP AccuTOF LC-plus spectrometer (ESI) or a JEOL JMS-700 mass spectrometer (EI). Elemental analysis was performed using a PerkinElmer CHNS/O 2400II or a Yokokawa Analysis IC7000RS, and analytical results were within ±0.4% of the theoretical values unless otherwise noted.

1,3-Thiazole-2-carbaldehyde (6-*tert*-Butylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (4a) A mixture of 6-*tert*-butyl-4-hydrazinothieno[2,3-*d*]-pyrimidine **2** (60 mg, 0.27 mmol) and 1,3-thiazole-2-carbaldehyde **3a** (26 μl, 0.30 mmol) in benzene (2 ml) was stirred under reflux for 1 h and cooled to room temperature. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel eluted with $CHCl_3$/MeOH (50 : 1). The eluate was concentrated under reduced pressure, and the residue was recrystallized from *n*-hexane, EtOAc, and MeOH to afford title compound **4a** (35.4 mg, 41%) as a yellow solid. ^{1}H-NMR (CD_3OD) δ: 1.52 (9H, s), 7.66 (1H, d, J=3.4 Hz), 7.87 (1H, br), 7.99 (1H, d, J=3.4 Hz), 8.35 (1H, s), 8.42 (1H, s); IR (ATR) cm^{-1}: 2968, 1552, 1431, 1354, 1124; MS (EI) *m*/*z*: 317 (M^+); HR-MS (ESI) *m*/*z*: 318.08234 (Calcd for $C_{14}H_{16}N_5S_2$: 318.08471); *Anal.* Calcd for $C_{14}H_{15}N_5S_2$: C, 52.97; H, 4.76; N, 22.06; S, 20.20. Found: C, 52.77; H, 4.83; N, 21.83; S, 20.33.

1,3-Thiazole-4-carbaldehyde (6-*tert*-Butylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (4b) Compound **4b** was obtained from **2** and 1,3-thiazole-4-carbaldehyde **3b** as a pale yellow solid (59%) by following the procedure described for **4a**. ^{1}H-NMR ($CDCl_3$) δ: 1.50 (9H, s), 7.74 (1H, s), 7.87 (1H, s), 8.13(1H, s), 8.51 (1H, s), 8.88 (1H, s), 8.98 (1H, br); IR (ATR) cm^{-1}: 2960, 1562, 1442; MS (FAB) *m*/*z*: 318 (M^++H); HR-MS (ESI) *m*/*z*: 318.08587 (Calcd for $C_{14}H_{16}N_5S_2$: 318.08471); *Anal.* Calcd for $C_{14}H_{15}N_5S_2$: C, 52.97; H, 4.76; N, 22.06; S, 20.20. Found: C, 53.06; H, 4.68; N, 21.90; S, 20.17.

1,3-Thiazole-5-carbaldehyde (6-*tert*-Butylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (4c) Compound **4c** was obtained from **2** and 1,3-thiazole-5-carbaldehyde **3c** as an orange solid (85%) by following the procedure described for **4a**. ^{1}H-NMR ($CDCl_3$) δ: 1.52 (9H, s), 7.77 (1H, s), 8.03 (1H, s), 8.11 (1H, s), 8.50 (1H, s), 8.84 (1H, s); IR (ATR) cm^{-1}: 1550, 1427, 1109; MS (FAB) *m*/*z*: 318 (M^+); HR-MS (ESI) *m*/*z*: 318.08159 (Calcd for $C_{14}H_{16}N_5S_2$: 318.08471); *Anal.* Calcd for $C_{14}H_{15}N_5S_2$: C, 52.97; H, 4.76; N, 22.06; S, 20.20. Found: C, 53.22; H, 4.67; N, 22.07; S, 20.28.

***tert*-Butyl Methyl(1,3-thiazol-4-ylmethyl)carbamate (6a)** To a solution of 4-methyl-1,3-thiazole **5a** (3.0 g, 30.25 mmol) in CCl_4 (60 ml) were added *N*-bromosuccinimide (5.6 g, 31.77 mmol) and 2,2′-azobisisobutyronitrile (250 mg, 1.51 mmol, 5 mol%). The reaction mixture was refluxed for 30 min under an atmosphere of nitrogen and cooled to room temperature. The resulting white precipitate was filtered. The filtrate was diluted with THF and partially evaporated to afford crude 4-bromomethyl-1,3-thiazole as a THF solution, which was used in the next step without purification. Methylamine (2 m THF solution, 45 ml, 90.75 mmol) was added successively to the THF (15 ml) solution of 4-(bromomethyl)thiazole. The resulting mixture was stirred for 18 h at room temperature. After completion of the reaction, the insoluble matter was filtered off and the filtrate was evaporated. The residue was dissolved in THF (60 ml) and the solution was cooled to 0 °C. Boc_2O (6.9 ml, 30.25 mmol) and Et_3N (4.6 ml, 33.28 mmol) were added. The solution was allowed to warm to room temperature and stirred for 5 h. The resulting precipitate was filtered. The filtrate was concentrated under reduced pressure, and the residue was chromatographed on silica gel eluted

with *n*-hexane/EtOAc (3 : 1) to afford title compound **6a** (2.4 g, 35%) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 2.94 (3H, s), 4.58 (2H, s), 7.12 (1H, s), 8.74 (1H, d, J=2.2 Hz); IR (ATR) cm^{-1}: 2976, 1686, 1390, 1142; MS (ESI) *m/z*: 251 (M^++Na); MS (FAB) *m/z*: 229 (M^++H); HR-MS (ESI) *m/z*: 229.10345 (Calcd for $C_{10}H_{17}N_2O_2S$: 229.10107).

***tert*-Butyl [(2-Formyl-1,3-thiazol-4-yl)methyl]methylcarbamate (3d)** To a solution of *n*-butyllithium (1.52 m in *n*-hexane, 3.02 ml, 4.60 mmol) in Et_2O (10 ml) under an atmosphere of argon at −78 °C was added dropwise a solution of **6a** (1.0 g, 4.38 mmol) in Et_2O (10 ml). The mixture was stirred at −78 °C for 1 h, and then 4-formylmorpholine (462 μl, 4.60 mmol) was added. The mixture was stirred for 1 h at −78 °C, and then at −5 °C overnight. The reaction mixture was then separated with satd $NaHCO_3$ aq. and EtOAc. The aqueous layer was extracted with EtOAc and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel eluted with *n*-hexane/EtOAc (4 : 1) to afford title compound **3d** (392.7 mg, 35%) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s), 2.98 (3H, s), 4.62 (2H, s), 7.48—7.58 (1H, br), 9.97 (1H, s); IR (ATR) cm^{-1}: 2975, 2931, 2847, 1683, 1390, 1365, 1141; MS (EI) *m/z*: 256 (M^+); HR-MS (EI) *m/z*: 256.08964 (Calcd for $C_{11}H_{16}N_2O_3S$: 256.08816).

4-[(Methylamino)methyl]-1,3-thiazole-2-carbaldehyde (6-*tert*-Butylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (4d) A mixture of **2** (82.6 mg, 0.37 mmol) and **3d** (100 mg, 0.39 mmol) in benzene (2 ml) was stirred under reflux for 0.5 h and cooled to room temperature. The reaction mixture was concentrated under reduced pressure. The residue was chromatographed on silica gel eluted with $CHCl_3$/MeOH (50 : 1) to afford *tert*-butyl [(2-{[(6-*tert*-butylthieno[2,3-*d*]pyrimidin-4-yl)hydrazono]methyl}-1,3-thiazol-4-yl)-methyl]methylcarbamate (112.5 mg, 66%) as a pale yellow solid. To a solution of hydrazone (76.9 mg, 0.17 mmol) in 1,4-dioxane (1.0 ml) was added 4 N HCl in 1,4-dioxane (1.0 ml) at 0 °C. The mixture was stirred at room temperature for 11.5 h and concentrated under reduced pressure. The residue was recrystallized from EtOAc and *n*-hexane to afford title compound **4d** (62.2 mg, 85%) as a pale yellow solid. ^{1}H-NMR (DMSO-d_6) δ: 1.47 (9H, s), 2.61 (3H, br), 4.28 (2H, d, J=4.4 Hz), 7.83 (1H, s), 7.92 (1H, s), 8.49 (1H, s), 8.54 (1H, s), 9.25 (1H, br); IR (ATR) cm-1: 2359, 1801, 1560, 1417; MS (FAB) *m/z*: 361 (M^++H); HR-MS (ESI) *m/z*: 361.12640 (Calcd for $C_{16}H_{21}N_6S_2$: 361.12691); *Anal.* Calcd for $C_{16}H_{20}N_6S_2\cdot 2HCl\cdot 0.25H_2O$: C, 43.88; H, 5.18; Cl, 16.19; N, 19.19; S, 14.64. Found: C, 43.67; H, 5.05; Cl, 15.99; N, 18.95; S, 14.56.

***tert*-Butyl Methyl(1,3-thiazol-5-yl)carbamate (6b)** Compound **6b** was obtained from **5b** as a yellow oil (57%) by following the procedure described for **6a**. ^{1}H-NMR ($CDCl_3$) δ: 1.50 (9H, s), 2.85 (3H, s), 4.5 (2H, s), 7.73 (1H, s), 8.73 (1H, s); MS (FAB) *m/z*: 229 (M^++H).

***tert*-Butyl [(2-Formyl-1,3-thiazol-5-yl)methyl]methylcarbamate (3e)** Compound **3e** was obtained from **6b** as a yellow oil (32%) by following the procedure described for **3d**. ^{1}H-NMR ($CDCl_3$) δ: 1.50 (9H, s), 2.90 (3H, s), 4.63 (2H, s), 7.93 (1H, s), 9.94 (1H, s); MS (EI) *m/z*: 256 (M^+).

5-[(Methylamino)methyl]-1,3-thiazole-2-carbaldehyde (6-*tert*-Butylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (4e) Compound **4e** was obtained from **2** and **3e** as a yellow solid (81%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 1.47 (9H, s), 2.58 (3H, br), 4.48 (2H, br), 7.82 (1H, s), 8.07 (1H, s), 8.50 (1H, br), 8.53 (1H, s), 9.44 (1H, br), 9.52 (1H, br); IR (ATR) cm^{-1}: 2665, 1637, 1591, 1101; MS (ESI) *m/z*: 361 (M^++H); HR-MS (ESI) *m/z*: 361.12789 (Calcd for $C_{16}H_{21}N_6S_2$: 361.12691); *Anal.* Calcd for $C_{16}H_{20}N_6S_2\cdot 2HCl\cdot 0.5H_2O$: C, 43.44; H, 5.24; Cl, 16.03; N, 18.99; S, 14.50. Found: C, 43.71; H, 5.14; Cl, 15.74; N, 18.74; S, 14.37.

4-[(Dimethylamino)methyl]-1,3-thiazole-2-carbaldehyde (3f) Compound **3f** was obtained from **6c** as a brown solid (44%) by following the procedure described for **3d**. ^{1}H-NMR ($CDCl_3$) δ: 2.32 (6H, s), 3.69 (2H, s), 7.58 (1H, s), 10.00 (1H, s); MS (FAB) *m/z*: 171 (M^++H).

4-[(Dimethylamino)methyl]-1,3-thiazole-2-carbaldehyde (6-*tert*-Butylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (4f) Compound **4f** was obtained from **2** and **3f** as a yellow solid (21%) by following the procedure described for **4a**. ^{1}H-NMR ($CDCl_3$) δ: 1.52 (9H, s), 2.33 (6H, s), 3.63 (2H, s), 7.20 (1H, s), 3.75 (2H, s), 7.84 (1H, s), 8.14 (1H, s), 8.52 (1H, s); IR (ATR) cm^{-1}: 2964, 1549, 1421, 1169; MS (FAB) *m/z*: 375 (M^++H); HR-MS (ESI) *m/z*: 375.14159 (Calcd for $C_{17}H_{23}N_6S_2$: 375.14256); *Anal.* Calcd for $C_{17}H_{22}N_6S_2$: C, 54.52; H, 5.92; N, 22.44; S, 17.12. Found: C, 54.66; H, 5.89; N, 22.44; S, 17.09.

2-{[2-({[*tert*-Butyl(diphenyl)silyl]oxy}methyl)-1,3-thiazol-4-yl]methyl}-1*H*-isoindole-1,3(2*H*)-dione (8) To a solution of ethyl 2-(*tert*-butyldiphenylsilyloxymethyl)thiazole-4-carboxylate **7** (748.3 mg, 1.76 mmol) in THF (14 ml) was added LAH (67 mg, 1.76 mmol) at 0 °C, and stirred at room temperature for 5 h. After cooling at 0 °C, MeOH (0.2 ml), water (0.1 ml), 15% NaOH aq. (0.1 ml), and water (0.3 ml) were added to a reaction mixture successively. The precipitate was filtered through Celite. The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel eluted with *n*-hexane/EtOAc (2 : 1—1 : 1). The eluate was concentrated under reduced pressure and the residue was dissolved in THF (20 ml). To this solution were added phthalimide (154.4 mg, 1.05 mmol), triphenylphosphine (275.3 mg, 1.05 mmol), and DEAD (165 μl, 1.05 mmol) under an atmosphere of nitrogen. The mixture was stirred at room temperature for 4.5 h. The reaction mixture was concentrated under reduced pressure, and the residue was chromatographed on silica gel eluted with *n*-hexane/EtOAc (2 : 1) to afford title compound **8** (441.9 mg, 49%) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 1.10 (9H, s), 4.91 (2H, s), 4.94 (2H, s), 7.17 (1H, s), 7.34—7.42 (6H, m), 7.64—7.78 (8H, m), 7.85—7.88 (2H, m); MS (FAB) *m/z*: 513 (M^++H).

***tert*-Butyl (2-Formyl-1,3-thiazol-5-yl)carbamate (3g)** To a solution of **8** (441.9 mg, 0.86 mmol) in EtOH (4.4 ml) was added hydrazine monohydrate (0.95 ml, 1.89 mmol) at room temperature. After 2 h, Boc_2O (0.65 ml, 2.84 mmol), CH_2Cl_2 (4.4 ml), and satd $NaHCO_3$ aq. (4.4 ml) were added to the reaction mixture, and stirred for a further 2 h. The solution was partitioned between CH_2Cl_2 and water. The aqueous layer was washed several times with CH_2Cl_2 and the combined organic fractions were dried over Na_2SO_4. The reaction mixture was concentrated under reduced pressure and the residue was chromatographed on silica gel eluted with *n*-hexane/EtOAc (4 : 1) to afford *tert*-butyl {[2-({[*tert*-butyl(diphenyl)silyl]oxy}methyl)-1,3-thiazol-4-yl]methyl}carbamate. To a solution of the afforded compound (181.1 mg, 0.38 mmol) in THF (1.8 ml) was added TBAF (1 M THF solution, 0.37 ml, 0.37 mmol) at 0 °C, and stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure, and the residue was chromatographed on silica gel eluted with $CHCl_3$/MeOH (20 : 1). The eluate was concentrated under reduced pressure and the residue was dissolved in CCl_4 (1.0 ml). To this solution was added MnO_2 (160 mg) and stirred under reflux for 4.5 h. The reaction mixture was filtered through Celite. The filtrate was concentrated under reduced pressure to afford title compound **3g** (41.3 mg, 20%) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 4.52 (2H, d, J=6.1 Hz), 5.19 (1H, br), 7.59 (1H, s), 9.96 (1H, s); MS (EI) *m/z*: 242 (M^+).

4-(Aminomethyl)-1,3-thiazole-2-carbaldehyde (6-*tert*-Butylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (4g) Compound **4g** was obtained from **2** and **3g** as a pale yellow solid (49%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 1.47 (9H, s), 4.18 (2H, br), 7.87 (1H, br), 7.88 (1H, s), 8.49 (3H, br), 8.53 (1H, s); IR (ATR) cm^{-1}: 2615, 1637, 1508, 1107; MS (FAB) *m/z*: 347 (M^++H); HR-MS (ESI) *m/z*: 347.11089 (Calcd for $C_{15}H_{19}N_6S_2$: 347.11126); *Anal.* Calcd for $C_{15}H_{18}N_6S_2\cdot 2HCl\cdot 0.5H_2O$: C, 42.05; H, 4.94; Cl, 16.55; N, 19.62; S, 14.97. Found: C, 42.27; H, 4.65; Cl, 16.74; N, 19.32; S, 14.69.

2-{1-[2-(Hydroxymethyl)-4,5-dihydro-1,3-thiazol-4-yl]ethyl}-1*H*-isoindole-1,3(2*H*)-dione (11a) A mixture of *N*-phtahloyl-DL-alanine **9a** (2.19 g, 10 mmol), a catalytic amount of DMF (2 drops) and thionyl chloride (1.09 ml, 15 mmol), was stirred under reflux for 30 min and cooled to room temperature. The solvent was removed under reduced pressure. The residue was dissolved in THF (10 ml), and (trimethylsilyl)diazomethane (2 M *n*-hexane solution, 12.5 ml, 25 mmol) in THF (25 ml) was added at 0 °C. The reaction mixture was stirred at 0 °C for 18 h. After removal of the solvent under reduced pressure, the residue was separated with satd $NaHCO_3$ aq. and Et_2O. The aqueous layer was extracted with Et_2O and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel eluted with *n*-hexane/EtOAc (2 : 1) to afford 1-diazo-3-phthalimidobutan-2-one (2.35 g, 97%) as an orange oil. To the resulting oil (0.49 g, 2.0 mmol) in Et_2O (20 ml) was added hydrobromic acid (47%, 0.28 ml, 2.4 mmol) at 0 °C, and stirred at room temperature for 50 min. The reaction mixture was neutralized with $NaHCO_3$ aq, the aqueous layer was extracted with Et_2O, and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was dissolved in EtOH (20 ml). To this solution was added 2-{[*tert*-butyl(diphenyl)silyl]oxy}ethanethioamide **10** (0.66 g, 2.0 mmol), and the reaction mixture was stirred under reflux for 12 h. After removal of the solvent under reduced pressure, the residue was separated with water and EtOAc. The aqueous layer was extracted with EtOAc and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel eluted with *n*-hexane/EtOAc (2 : 3) to afford title compound **11a** (0.4 g, 69%) as an orange oil. ^{1}H-NMR ($CDCl_3$) δ: 1.92 (3H, d, J=7.3 Hz), 2.60

(1H, br), 4.85 (2H, s), 5.66 (1H, q, J=7.3 Hz), 7.24 (1H, s), 7.69—7.72 (2H, m), 7.81—7.84 (2H, m).

***tert*-Butyl {1-[2-(Hydroxymethyl)-4,5-dihydro-1,3-thiazol-4-yl]ethyl}-carbamate (12a)** To a solution of **11a** (0.4 g, 1.39 mmol) in EtOH (10 ml) was added hydrazine monohydrate (0.2 ml, 4.17 mmol). The mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the residue was dissolved in $CHCl_3$. The precipitate was filtered through Celite. The filtrate was concentrated under reduced pressure and the residue was dissolved in THF (5 ml). To this solution was added Boc_2O (0.33 g, 1.5 mmol) and stirred for 1 h. The reaction mixture was concentrated under reduced pressure and the residue was chromatographed on silica gel eluted with $CHCl_3$/MeOH (40 : 1) to afford title compound **12a** (0.32 g, 89%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (9H, s), 1.48 (3H, d, J=6.9 Hz), 2.90 (1H, br), 4.92 (3H, m), 5.09 (1H, br), 7.05 (1H, s).

***tert*-Butyl [1-(2-Formyl-4,5-dihydro-1,3-thiazol-4-yl)ethyl]carbamate (3h)** To a solution of **12a** (0.32 g, 1.24 mmol) in CCl_4 (10 ml) was added MnO_2 (500 mg) and the mixture was stirred under reflux for 18.5 h. The reaction mixture was filtered through Celite. The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel eluted with $CHCl_3$/MeOH (100 : 1) to afford title compound **3h** (0.26 g, 82%) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (9H, s), 1.56 (3H, d, J=6.8 Hz), 5.01 (1H, br), 5.09 (1H, br), 7.53 (1H, s), 9.95 (1H, d, J=1.3 Hz).

4-(1-Aminoethyl)-1,3-thiazole-2-carbaldehyde (6-*tert*-Butylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (4h) Compound **4h** was obtained from **2** and **3h** as a yellow solid (47%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 1.46 (9H, s), 1.57 (3H, d, J=6.8 Hz), 4.57—4.61 (1H, m), 7.87 (2H, br), 8.52 (4H, br); IR (KBr) cm^{-1}: 3338, 1631, 1587, 1552, 1504, 1421, 1367, 1338; MS (FAB) m/z: 361 (M^++1); HR-MS (ESI) m/z: 361.12668 (Calcd for $C_{16}H_{21}N_6S_2$: 361.12691); *Anal.* Calcd for $C_{16}H_{20}N_6S_2\cdot 2.4HCl\cdot 1.75H_2O$: C, 40.08; H, 5.44; Cl, 17.74; N, 17.59; S, 13.37. Found: C, 40.34; H, 5.25; Cl, 17.92; N, 17.74; S, 13.43.

2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)butanoic Acid (9b) A mixture of 2-aminobutyric acid **13** (10.3 g, 100 mmol) and phthalic anhydride (14.8 g, 100 mmol) was heated at 120 °C for 4 h. After cooling to room temperature, $CHCl_3$ (50 ml) and 1 N HCl aq. (50 ml) were added to the reaction mixture. The aqueous layer was extracted with $CHCl_3$ and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford title compound **9b** (23 g, 99%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, t, J=7.4 Hz), 2.25—2.32 (2H, m), 4.81—4.85 (1H, m), 7.73—7.75 (2H, m), 7.86—7.88 (2H, m).

2-{1-[2-(Hydroxymethyl)-4,5-dihydro-1,3-thiazol-4-yl]propyl}-1*H*-isoindole-1,3(2*H*)-dione (11b) Compound **11b** was obtained from **9b** as a brownish oil (60%) by following the procedure described for **11a**. ^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, t, J=7.4 Hz), 2.32—2.39 (1H, m), 2.47—2.55 (1H, m), 2.63 (1H, br), 4.85 (2H, s), 5.44 (1H, q, J=4.2 Hz), 7.26 (1H, s), 7.70—7.72 (2H, m), 7.82—7.84 (2H, m).

***tert*-Butyl {1-[2-(Hydroxymethyl)-4,5-dihydro-1,3-thiazol-4-yl]ethyl}-carbamate (12b)** Compound **12b** was obtained from **11b** as a brownish oil (quant.) by following the procedure described for **12a**. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=7.3 Hz), 1.47 (9H, s), 1.81—1.90 (2H, m), 2.82 (1H, br), 4.67 (1H, d, J=7.1 Hz), 4.91 (2H, s), 5.12 (1H, br), 7.05 (1H, s).

***tert*-Butyl [1-(2-Formyl-4,5-dihydro-1,3-thiazol-4-yl)ethyl]carbamate (3i)** Compound **3i** was obtained from **12b** as a yellow oil (93%) by following the procedure described for **3h**. ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, t, J=7.3 Hz), 1.47 (9H, s), 1.87—1.99 (2H, m), 4.79 (1H, br), 5.13 (1H, br), 7.52 (1H, s), 9.96 (1H, d, J=1.2 Hz).

4-(1-Aminopropyl)-1,3-thiazole-2-carbaldehyde (6-*tert*-Butylthieno-[2,3-*d*]pyrimidin-4-yl)hydrazone (4i) Compound **4i** was obtained from **2** and **3i** as a yellow solid (57%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 0.82 (3H, t, J=7.3 Hz), 1.44 (9H, s), 1.88—2.03 (2H, m), 4.36 (1H, m), 7.95 (1H, s), 8.56 (1H, s), 8.66 (4H, br); IR (ATR) cm^{-1}: 2960, 2069, 1943, 1731, 1633, 1577, 1504, 1482, 1421; MS (FAB) m/z: 375 (M^++H); HR-MS (ESI) m/z: 375.13947 (Calcd for $C_{17}H_{23}N_6S_2$: 375.14256); *Anal.* Calcd for $C_{17}H_{22}N_6S_2\cdot 2HCl\cdot 1.75H_2O$: C, 43.45; H, 5.68; Cl, 15.09; N, 17.88: S, 13.65. Found: C, 43.65; H, 5.60; Cl, 14.80; N, 18.23; S, 13.40.

***tert*-Butyl {1-[2-(Hydroxymethyl)-4,5-dihydro-1,3-thiazol-4-yl]ethyl}-methylcarbamate (12c)** To a solution of **12a** (1.5 g, 5.8 mmol) in EtOH (5.0 ml) was added 1 N HCl in EtOH (5.0 ml) at 0 °C. The mixture was stirred at room temperature for 17 h and concentrated under reduced pressure. To a suspension of the residue in CH_2Cl_2 (30 ml) were added Et_3N (4.1 ml, 29.1 mmol) and TFAA (2.1 ml, 14.5 mmol) at 0 °C, and stirred at room temperature for 1 h. The reaction mixture was then separated with 10% citric acid aq. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layer was washed with satd $NaHCO_3$ aq. and brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was dissolved in DMF (20 ml). To this solution, K_2CO_3 (1.9 g, 13.7 mmol) and methyl iodide (0.43 ml, 6.9 mmol) were added, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was separated with EtOAc and 10% sodium hydrosulfite aq. The aqueous layer was extracted with EtOAc and the combined organic layer was washed with water and brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was dissolved in THF (10 ml) and 1 N NaOH aq. (10 ml). After stirring at room temperature for 1 h, Boc_2O (1.5 g, 0.9 mmol) in THF (5 ml) was added to the reaction mixture and stirred for a further 30 min. The solution was partitioned between EtOAc and water. The aqueous layer was washed with EtOAc and the combined organic layer was dried over Na_2SO_4. The reaction mixture was concentrated under reduced pressure and the residue was chromatographed on silica gel eluted with $CHCl_3$/MeOH (40 : 1) to afford title compound **12c** (0.17 g, 11%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s), 1.53 (3H, d, J=7.1 Hz), 2.69 (3H, s), 2.95 (1H, br), 4.90 (2H, s), 7.00 (1H, s).

***tert*-Butyl [1-(2-Formyl-4,5-dihydro-1,3-thiazol-4-yl)ethyl]methylcarbamate (3j)** Compound **3j** was obtained from **12c** as a pale yellow oil (95%) by following the procedure described for **3h**. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (9H, s), 1.62 (3H, d, J=7.3 Hz), 2.75 (3H, br), 5.62 (1H, br), 7.48 (1H, s), 9.97 (1H, d, J=1.2 Hz).

4-[1-(Methylamino)ethyl]-1,3-thiazole-2-carbaldehyde (6-*tert*-Butylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (4j) Compound **4j** was obtained from **2** and **3j** as a yellow solid (88%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 1.45 (9H, s), 1.60 (3H, d, J=6.8 Hz), 2.44 (3H, t, J=5.4 Hz), 4.52—4.57 (1H, m), 7.90 (1H, br), 7.97 (1H, s), 8.53 (1H, s), 8.58 (1H, br), 9.25—9.26 (1H, br), 9.61 (1H, br); IR (ATR) cm^{-1}: 2965, 2468, 1735, 1641, 1592, 1546, 1508, 1469, 1428; MS (FAB) m/z: 375 (M^++H); HR-MS (ESI) m/z: 375.14256 (Calcd for $C_{17}H_{23}N_6S_2$: 375.1444); *Anal.* Calcd for $C_{17}H_{22}N_6S_2\cdot 2HCl\cdot 0.5H_2O$: C, 44.73; H, 5.52; Cl, 15.53; N, 18.41; S, 14.05. Found: C, 44.41; H, 5.20; Cl, 15.20; N, 18.39; S, 14.05.

Benzyl 3-[2-(Hydroxymethyl)-1,3-thiazol-4-yl]azetidine-1-carboxylate (15a) A mixture of 1-benzyloxycarbonylazetidine-3-carboxylic acid **14a** (1.2 g, 5.0 mmol), a catalytic amount of DMF (2 drops), and oxalyl chloride (0.63 ml, 7.5 mmol), was stirred at 0 °C for 15 min and then stirred at room temperature for 1 h. The solvent was removed under reduced pressure. The residue was dissolved in THF (30 ml), and (trimethylsilyl)diazomethane (2 M *n*-hexane solution, 6.25 ml, 12.5 mmol) was added at 0 °C. The reaction mixture was stirred at 0 °C for 15 h. After removal of the solvent under reduced pressure, the residue was separated with satd $NaHCO_3$ aq. and EtOAc. The aqueous layer was extracted with EtOAc and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure. To the resulting residue in Et_2O (20 ml) was added hydrobromic acid (47%, 0.7 ml, 6.0 mmol) at 0 °C, and stirred at 0 °C for 1 h. The reaction mixture was neutralized with $NaHCO_3$ aq., the aqueous layer was extracted with EtOAc, and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was dissolved in EtOH (30 ml). To this solution was added 2-{[*tert*-butyl(diphenyl)silyl]oxy}ethanethioamide **10** (1.65 g, 5.0 mmol), and the reaction mixture was stirred under reflux for 38 h. After removal of the solvent under reduced pressure, the residue was separated with water and EtOAc. The aqueous layer was extracted with EtOAc and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel eluted with *n*-hexane/EtOAc (1 : 2) to afford title compound **15a** (0.4 g, 26%) as a brownish oil. ^{1}H-NMR ($CDCl_3$) δ: 2.58 (1H, br s), 3.92—3.95 (1H, m), 4.19 (2H, dd, J=6.1, 8.8 Hz), 4.36 (1H, t, J=8.5 Hz), 4.94 (2H, s), 5.12 (2H, s), 7.01 (1H, s), 7.35—7.37 (5H, m).

***tert*-Butyl 3-[2-(Hydroxymethyl)-1,3-thiazol-4-yl]azetidine-1-carboxylate (16a)** To the solution of **15a** (0.4 g, 1.3 mmol) in CH_2Cl_2 (20 ml) was added boron trichloride (1 M CH_2Cl_2 solution, 4.0 ml, 4.0 mmol) at 0 °C under N_2 atmosphere, and stirred at room temperature for 3 d. The reaction mixture was neutralized with $NaHCO_3$ aq. and the solvent was removed under reduced pressure. To the resulting residue were added Boc_2O (0.43 g, 1.97 mmol) in THF (10 ml) and 1 N NaOH aq. (10 ml), and stirred at room temperature for 30 min. To the reaction mixture EtOAc and water were added, and the two layers were separated. The aqueous layer was extracted with EtOAc and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel eluted with $CHCl_3$/MeOH (40 : 1) to afford title compound **16a** (40 mg, 11%) as a brownish oil. ^{1}H-NMR

($CDCl_3$) δ: 1.45 (9H, s), 3.21 (1H, br), 3.85—3.91 (1H, m), 4.07 (1H, dd, J=6.4, 8.3 Hz), 4.27 (1H, t, J=8.5 Hz), 4.93 (2H, s), 7.01 (1H, s).

***tert*-Butyl 3-(2-Formyl-1,3-thiazol-4-yl)azetidine-1-carboxylate (3k)** To a solution of **16a** (40 mg, 0.148 mmol) in $CHCl_3$ (10 ml) was added MnO_2 (0.12 g), and stirred under reflux for 21 h. The reaction mixture was filtered through Celite. The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel eluted with *n*-hexane/EtOAc (2 : 1) to afford title compound **3k** (27 mg, 68%) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s), 3.96—4.03 (1H, m), 4.14 (2H, dd, J=6.1, 8.3 Hz), 4.34 (2H, t, J=8.5 Hz), 7.47 (1H, s), 9.98 (1H, d, J=0.8 Hz).

4-Azetidin-3-yl-1,3-thiazole-2-carbaldehyde (6-*tert*-Butylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (4k) Compound **4k** was obtained from **2** and **3k** as a yellow solid (85%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 1.45 (9H, s), 4.15 (2H, m), 4.25 (3H, m), 7.74 (1H, s), 7.88 (1H, br), 8.52 (1H, s), 9.05 (1H, br), 9.37 (1H, br); IR (ATR) cm^{-1}: 3380, 2962, 1633, 1583, 1560, 1508, 1423, 1365; MS (FAB) *m*/*z*: 373 (M^++H); MS (ESI) *m*/*z*: 373 (M^++H); HR-MS (ESI) *m*/*z*: 373.12682 (Calcd for $C_{17}H_{21}N_6S_2$: 373.12691); *Anal.* Calcd for $C_{17}H_{20}N_6S_2\cdot 2.25HCl\cdot 1.5H_2O$: C, 42.40; H, 5.28; Cl, 16.56; N, 17.45: S, 13.32. Found: C, 42.24; H, 5.32; Cl, 16.35; N, 17.31; S, 13.15.

Benzyl 3-[2-(Hydroxymethyl)-1,3-thiazol-4-yl]pyrrolidine-1-carboxylate (15b) Compound **15b** was obtained from **14b** as a brownish oil (44%) by following the procedure described for **15a**. ^{1}H-NMR ($CDCl_3$) δ: 2.10—2.17 (1H, m), 2.28—2.30 (1H, m), 2.96 (1H, br), 3.44—3.69 (4H, m), 3.84—3.89 (1H, m), 4.91 (2H, s), 5.14 (2H, d, J=2.4 Hz), 6.93 (1H, d, J=2.2 Hz), 7.32—7.36 (5H, m).

***tert*-Butyl 3-[2-(Hydroxymethyl)-1,3-thiazol-4-yl]pyrrolidine-1-carboxylate (16b)** Compound **16b** was obtained from **15b** as a brownish oil (64%) by following the procedure described for **16a**. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 2.06—2.14 (1H, m), 2.25 (1H, br), 2.76 (1H, br), 3.41—3.59 (4H, m), 3.74—3.77 (1H, m), 4.92 (2H, s), 6.94 (1H, s).

***tert*-Butyl 3-(2-Formyl-1,3-thiazol-4-yl)pyrrolidine-1-carboxylate (3l)** Compound **3l** was obtained from **16b** as a yellow oil (83%) by following the procedure described for **3k**. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (9H, s), 2.15—2.20 (1H, m), 2.33 (1H, br), 3.46—3.63 (4H, m), 3.81—3.86 (1H, m), 7.41 (1H, s), 9.96 (1H, s).

4-Pyrrolidin-3-yl-1,3-thiazole-2-carbaldehyde (6-*tert*-Butylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (4l) Compound **4l** was obtained from **2** and **3l** as a yellow solid (84%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 1.45 (9H, s), 2.07—2.10 (1H, m), 2.32—2.36 (1H, m), 3.25—3.27 (2H, m), 3.35 (1H, m), 3.58—3.64 (1H, m), 3.66—3.70 (1H, m), 7.70 (1H, s), 7.87 (1H, br), 8.51 (2H, s), 9.32 (2H, br); IR (ATR) cm^{-1}: 2960, 1735, 1633, 1587, 1556, 1504, 1402; MS (FAB) *m*/*z*: 387 (M^++H); HR-MS (ESI) *m*/*z*: 387.14215 (Calcd for $C_{18}H_{23}N_6S_2$: 387.14256); *Anal.* Calcd for $C_{18}H_{22}N_6S_2\cdot 2.7HCl\cdot H_2O$: C, 42.98; H, 5.35; Cl, 19.03; N, 16.71: S, 12.75. Found: C, 43.14; H, 5.35; Cl, 18.83; N, 16.52; S, 12.66.

Benzyl 4-[2-(Hydroxymethyl)-1,3-thiazol-4-yl]piperidine-1-carboxylate (15c) Compound **15c** was obtained from **14c** as a brownish oil (30%) by following the procedure described for **15a**. ^{1}H-NMR ($CDCl_3$) δ: 1.56—1.64 (4H, m), 2.02—2.04 (1H, m), 2.53 (1H, br), 2.89—2.96 (3H, m), 4.27 (1H, br), 4.91 (2H, s), 5.14 (2H, s), 6.86 (1H, s), 7.31—7.37 (5H, m).

***tert*-Butyl 4-[2-(Hydroxymethyl)-1,3-thiazol-4-yl]piperidine-1-carboxylate (16c)** Compound **16c** was obtained from **15c** as a yellow oil (69%) by following the procedure described for **16a**. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s), 1.59—1.66 (2H, m), 1.99—2.03 (2H, m), 2.67 (1H, br), 2.802.92 (3H, m), 4.20 (2H, br), 4.92 (2H, s), 6.86 (1H, s).

***tert*-Butyl 4-(2-Formyl-1,3-thiazol-4-yl)piperidine-1-carboxylate (3m)** Compound **3m** was obtained from **16c** as an orange oil (60%) by following the procedure described for **3k**. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (9H, s), 1.63—1.73 (2H, m), 2.04—2.07 (2H, m), 2.88 (1H, br), 2.99—3.05 (1H, m), 3.49 (2H, d, J=4.6 Hz), 4.22 (1H, br), 7.34 (1H, s), 9.96 (1H, d, J=1.2 Hz).

4-Piperidin-4-yl-1,3-thiazole-2-carbaldehyde (6-*tert*-Butylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (4m) Compound **4m** was obtained from **2** and **3m** as a yellow solid (48%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 1.46 (9H, s), 1.82—1.98 (2H, m), 2.12—2.16 (2H, m), 2.98—3.10 (3H, m), 3.32—3.35 (2H, m), 7.55 (1H, s), 7.86 (1H, s), 8.47 (1H, br), 8.51 (1H, s), 8.71 (1H, br), 8.95 (1H, br); IR (ATR) cm^{-1}: 2962, 1864, 1631, 1587, 1552, 1504, 1400, 1367; MS (FAB) *m*/*z*: 401 (M^++H); MS (ESI) *m*/*z*: 401 (M^++H); HR-MS (ESI) *m*/*z*: 401.15792 (Calcd for $C_{19}H_{25}N_6S_2$: 401.15821); *Anal.* Calcd for $C_{19}H_{24}N_6S_2\cdot 2.75HCl\cdot 1.25H_2O$: C, 43.60; H, 5.63; Cl, 18.63; N, 16.06: S, 12.25. Found: C, 43.20; H, 5.36; Cl, 19.02; N, 15.91; S, 12.17.

***tert*-Butyl 2-(Hydroxymethyl)-4,6-dihydro-5*H*-pyrrolo[3,4-*d*][1,3]thiazole-5-carboxylate (19)** To a solution of 1-[(4-methylphenyl)sulfonyl]-pyrrolidin-3-one **18** (1.4 g, 5.86 mmol) in AcOH (15 ml) was added bromine (0.33 ml, 6.4 mmol) in AcOH (15 ml). The mixture was stirred at 50 °C for 10 min. The reaction mixture was separated with water and $CHCl_3$. The aqueous layer was extracted with $CHCl_3$ and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was dissolved with DMF (15 ml). To this solution was added 2-(benzyloxy)ethanethioamide **17** (1.2 g, 6.6 mmol), and the reaction mixture was stirred at 50 °C for 7 h under N_2 atmosphere. After completion of the reaction, $NaHCO_3$ (0.5 g, 5.86 mmol) was added, and the solvent was removed under reduced pressure. To the residue, EtOAc and water were added, and the two layers were separated. The aqueous layer was extracted with EtOAc and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (50 ml). To this solution were added Et_3N (4.1 ml, 29.3 mmol) and MsCl (0.91 ml, 11.7 mmol) at 0 °C under N_2 atmosphere, and stirred at room temperature for 22 h. To the reaction mixture, $CHCl_3$ and water were added, and the two layers were separated. The aqueous layer was extracted with $CHCl_3$ and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel eluted with $CHCl_3$/MeOH (100 : 1). The eluate was concentrated under reduced pressure. To the resulting residue were added phenol (2.0 g) and hydrobromic acid (47%, 3.0 ml) at 0 °C, and stirred under reflux for 1 h. After cooling to ambient temperature, Et_2O and water were added to the reaction mixture. The two layers were separated, and the organic layer was extracted with 1 N HCl aq. The combined aqueous layer was alkalized with 10 N NaOH aq. To the basic solution, Boc_2O (0.55 g, 2.5 mmol) in THF (20 ml) was added, and stirred at room temperature for 23 h. To the reaction mixture, EtOAc and water were added, and the two layers were separated. The aqueous layer was extracted with EtOAc and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel eluted with $CHCl_3$/MeOH (50 : 1) to afford title compound **19** (0.13 g, 9%) as a brownish solid. ^{1}H-NMR ($CDCl_3$) δ: 1.52 (s, 9H), 2.48 (br, 1H), 4,52—4.53 (m, 1H), 4.58 (br, 1H), 4.66—4.67 (m, 2H), 4.94 (s, 2H); MS (EI) *m*/*z*: 257 (M^++H).

***tert*-Butyl 2-Formyl-4,6-dihydro-5*H*-pyrrolo[3,4-*d*][1,3]thiazole-5-carboxylate (3n)** Compound **3n** was obtained from **19** as a brownish solid (81%) by following the procedure described for **3k**. ^{1}H-NMR ($CDCl_3$) δ: 1.53 (s, 9H), 4.64—4.66 (m, 1H), 4.69 (br, 1H), 4.77—4.88 (m, 2H), 9.93 (s, 1H).

5,6-Dihydro-4*H*-pyrrolo[3,4-*d*][1,3]thiazole-2-carbaldehyde (6-*tert*-Butylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (4n) Compound **4n** was obtained from **2** and **3n** as a brownish solid (40%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 1.47 (9H, s), 4,43 (2H, br), 4.62 (2H, br), 7.77 (1H, s), 8.42 (1H, br), 8.52 (1H, s), 10.26 (1H, br); IR (ATR) cm^{-1}: 2962, 1635, 1581, 1515, 1425, 1365; MS (FAB) *m*/*z*: 359 (M^++H); MS (ESI) *m*/*z*: 359 (M^++H); HR-MS (ESI) *m*/*z*: 359.11144 (Calcd for $C_{16}H_{19}N_6S_2$: 359.11126); *Anal.* Calcd for $C_{16}H_{18}N_6S_2\cdot 1.9HCl\cdot 2H_2O$: C, 41.35; H, 5.40; Cl, 14.49; N, 18.08; S, 13.80. Found: C, 41.27; H, 5.06; Cl, 14.68; N, 17.88; S, 13.77.

4-Hydrazino-6-isopropylthieno[2,3-*d*]pyrimidine (21) To a solution of **20** (13.8 g, 64.9 mmol) in EtOH (250 ml) was added hydrazine monohydrate (140 ml). The mixture was stirred under reflux for 3 h and cooled to room temperature. The precipitate was collected by filtration and washed with *n*-hexane to afford title compound **21** (12.9 g, 95%) as a pale yellow solid. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (6H, d, J=6.8 Hz), 3.15—3.21 (1H, m), 7.35 (1H, br), 8.28 (1H, s); IR (KBr) cm^{-1}: 3195, 2957, 1576, 1504, 1345, 1306, 1142; MS (ESI) *m*/*z*: 209 (M^++H); HR-MS (ESI) *m*/*z*: 209.08350 (Calcd for $C_9H_{13}N_4S$: 209.08609); *Anal.* Calcd for $C_9H_{12}N_4S$: C, 51.90; H, 5.81; N, 26.90; S, 15.39. Found: C, 51.71; H, 5.84; N, 26.77; S, 15.41.

4-[(Methylamino)methyl]-1,3-thiazole-2-carbaldehyde (6-Isopropylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (29) Compound **29** was obtained from **3d** and **21** as a yellow solid (45%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 1.41 (6H, d, J=6.6 Hz), 2.59—2.61 (3H, br), 4.29 (2H, br), 7.80 (1H, s), 7.93 (1H, s), 8.52 (2H, s), 9.21 (2H, br); IR (ATR) cm^{-1}: 2696, 1637, 1587; MS (ESI) *m*/*z*: 347 (M^++H); MS (ESI) *m*/*z*: 347 (M^++H); HR-MS (ESI) *m*/*z*: 347.10962 (Calcd for $C_{15}H_{19}N_6S_2$: 347.11126); *Anal.* Calcd for $C_{15}H_{18}N_6S_2\cdot 13/7HCl\cdot 2.5H_2O$: C, 39.23; H, 5.46; Cl, 14.34; N, 18.30; S, 13.96; Found: C, 39.28; H, 5.20; Cl, 14.12; N, 18.00; S, 13.84.

Methyl 2-Amino-5-cyclopropylthiophene-3-carboxylate (23a) To a suspension of pyridinium chlorochromate (7.50 g, 34.8 mmol) in CH_2Cl_2 (50 ml) was added 2-cyclopropylethanol **22a** (2.0 g, 23.2 mmol), and stirred

vigorously at room temperature under N_2 atmosphere. After 1.5 h of stirring, Et_2O (180 ml) was added and stirred for 30 min. The reaction mixture passed through a Florisil® column to afford cyclopropylacetaldehyde (1.59 g) as a colorless oil. The resulting aldehyde was added to a solution of methyl cyanoacetate (1.50 ml, 17 mmol), Et_3N (2.40 ml, 17 mmol), and DMF (2.65 ml, 34 mmol) under N_2 atmosphere. After 10 min of stirring, S_8 (545 mg, 17 mmol) was added and stirred at room temperature for 40 h. The reaction mixture was poured into water and extracted with EtOAc. The aqueous layer was extracted with EtOAc and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel eluted with *n*-hexane/EtOAc (9 : 1) and recrystallized from *n*-pentane to afford title compound **23a** (580 mg, 13%) as a pale yellow solid. ^{1}H-NMR ($CDCl_3$) δ: 0.60—0.67 (2H, m), 0.80—0.86 (2H, m), 1.77—1.83 (1H, m), 3.78 (3H, s), 5.78 (2H, br), 6.59 (1H, s); IR (KBr) cm^{-1}: 3423, 3311, 1662, 1602; MS (EI) *m/z*: 197 (M^+); *Anal.* Calcd for $C_9H_{11}NO_2S$: C, 54.80; H, 5.62; N, 7.10; S, 16.26. Found: C, 55.10; H, 5.62; N, 7.02; S, 16.18.

6-Cyclopropyl-4-hydrazinothieno[2,3-*d*]pyrimidine (24a) A mixture of **23a** (550 mg, 2.79 mmol) in formamide (5.5 ml) was stirred at 210 °C for 2.5 h. After cooling, precipitate formed, which was separated from the solution by filtration and washed with water. The crude product was chromatographed on silica gel eluted with *n*-hexane/EtOAc (2 : 1) and recrystallized from EtOAc and *n*-hexane to afford 6-cyclopropylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (285 mg, 53%) as a colorless solid. The resulting compound (255 mg, 1.32 mmol) in phosphorous oxychloride (3.0 ml) was stirred at 110 °C for 6 h. The reaction mixture was concentrated under reduced pressure. The residue was poured into ice water, and sat. $NaHCO_3$ aq. was added and extracted with EtOAc. The aqueous layer was extracted with EtOAc and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel eluted with *n*-hexane/EtOAc (9 : 1) to afford 4-chloro-6-cyclopropylthieno[2,3-*d*]pyrimidine (275 mg, 99%) as a colorless solid. To a solution of the resulting compound (250 mg, 1.18 mmol) in EtOH (5.0 ml) was added hydrazine monohydrate (2.5 ml). The mixture was stirred under reflux for 1.5 h and cooled to room temperature. The reaction mixture was concentrated under reduced pressure. After EtOAc and water were added, the two layers were separated. The aqueous layer was extracted with EtOAc and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was recrystallized from EtOAc and *n*-hexane to afford title compound **24a** (190 mg, 78%) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 0.81—0.86 (2H, m), 1.08—1.12 (2H, m), 2.11—2.18 (1H, m), 6.39 (1H, br), 6.85 (1H, d, J=1.0 Hz), 8.48 (1H, s); IR (KBr) cm^{-1}: 3243, 3197, 1585; EI *m/z*: 206 (M^+). MS (ESI) *m/z*: 207 (M^++H); HR-MS (ESI) *m/z*: 207.06637 (Calcd for $C_9H_{11}N_4S$: 207.07044); *Anal.* Calcd for $C_9H_{10}N_4S$: C, 52.41; H, 4.89; N, 27.16; S, 15.54. Found: C, 52.32; H, 4.85; N, 27.17; S, 15.67.

4-[(Methylamino)methyl]-1,3-thiazole-2-carbaldehyde (6-Cyclopropylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (30) Compound **30** was obtained from **3d** and **24a** as a yellow solid (49%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 0.84—0.88 (2H, m), 1.15—1.23 (2H, m), 4.29 (2H, t, J=5.4 Hz), 7.77 (1H, br), 7.98 (1H, s), 8.53 (1H, s), 8.66 (1H, br), 9.83 (2H, br); IR (ATR) cm^{-1}: 2657, 1630, 1585, 1099; MS (FAB) *m/z*: 345 (M^++H); MS (ESI) *m/z*: 345 (M^++H); HR-MS (ESI) *m/z*: 345.09333 (Calcd for $C_{15}H_{17}N_6S_2$: 345.09561); *Anal.* Calcd for $C_{15}H_{16}N_6S_2\cdot 1.9HCl\cdot 0.75H_2O$: C, 42.17; H, 4.58; Cl, 15.77; N, 19.67; S, 15.01. Found: C, 42.31; H, 4.44; Cl, 16.17; N, 19.43; S, 14.66.

Methyl 2-Amino-5-cyclobutylthiophene-3-carboxylate (23b) Compound **23b** was obtained from **22b** as a pale yellow oil (37%) by following the procedure described for **23a**. ^{1}H-NMR ($CDCl_3$) δ: 1.79—2.34 (6H, m), 3.40—3.49 (1H, m), 3.79 (3H, s), 5.79 (2H, br), 6.62 (1H, s).

6-Cyclobutyl-4-hydrazinothieno[2,3-*d*]pyrimidine (24b) Compound **24b** was obtained from **23b** as a pale yellow oil (41%) by following the procedure described for **24a**. ^{1}H-NMR ($CDCl_3$) δ: 1.90—2.12 (2H, m), 2.19—2.29 (2H, m), 2.42—2.50 (2H, m), 3.71—3.80 (1H, m), 6.43 (1H, br), 6.86 (1H, d, J=1.0 Hz), 8.50 (1H, s); IR (KBr) cm^{-1}: 3234, 3193, 1585; MS (ESI) *m/z*: 221 (M^++H); HR-MS (ESI) *m/z*: 221.08765 (Calcd for $C_{10}H_{12}N_4S_2$: 221.08609); *Anal.* Calcd for $C_{10}H_{12}N_4S$: C, 52.52; H, 5.49; N, 25.43; S, 14.55. Found: C, 52.26; H, 5.41; N, 25.58; S, 14.68.

4-[(Methylamino)methyl]-1,3-thiazole-2-carbaldehyde (6-Cyclobutylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (31) Compound **31** was obtained from **3d** and **24b** as a yellow solid (55%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 1.89—1.97 (1H, m), 2.01—2.12 (1H, m), 2.19—2.29 (2H, m), 2.45—2.49 (2H, m), 2.58—2.61 (3H, t, J=5.8 Hz), 3.86 (3H, td, J=8.5, 16.8 Hz), 4.29 (2H, m), 7.83 (1H, s), 7.96 (1H, s), 8.54 (1H, s), 8.61 (1H, br), 9.33 (2H, br); IR (ATR) cm^{-1}: 2663, 1630, 1589, 1101; MS (FAB) *m/z*: 359 (M^++H); MS (ESI) *m/z*: 359 (M^++H); HR-MS (ESI) *m/z*: 359.10991 (Calcd for $C_{16}H_{19}N_6S_2$: 359.11126); *Anal.* Calcd for $C_{16}H_{18}N_6S_2\cdot 2HCl\cdot 0.5H_2O$: C, 43.63; H, 4.81; Cl, 16.10; N, 19.08; S, 14.56; Found: C, 43.54; H, 4.65; Cl, 16.07; N, 19.13; S, 14.56.

Methyl 2-Amino-5-(1-methylpropyl)thiophene-3-carboxylate (23c) Compound **23c** was obtained from **22c** as a pale yellow oil (36%) by following the procedure described for **23a**. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7.0 Hz), 1.22 (3H, d, J=7.0 Hz), 1.50—1.60 (2H, m), 2.62—2.69 (1H, m), 3.79 (3H, s), 5.79 (2H, br), 6.62 (1H, s); MS (ESI) *m/z*: 254 (M^++MeCN).

6-*sec*-Butyl-4-hydrazinothieno[2,3-*d*]pyrimidine (24c) Compound **24c** was obtained from **23c** as a pale yellow oil (56%) by following the procedure described for **24a**. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, t, J=7.0 Hz), 1.36 (3H, d, J=7.0 Hz), 1.64—1.72 (2H, m), 2.91—2.99 (1H, m), 6.57 (1H, br), 6.89 (1H, s), 8.50 (1H, s); IR (KBr) cm^{-1}: 3270, 2954, 1583; MS (ESI) *m/z*: 223 (M^++H); HR-MS (ESI) *m/z*: 223.10034 (Calcd for $C_{10}H_{15}N_4S$: 223.10174); *Anal.* Calcd for $C_{10}H_{14}N_4S$: C, 54.03; H, 6.35; N, 25.20; S, 14.42. Found: C, 53.99; H, 6.39; N, 25.52; S, 14.56.

4-[(Methylamino)methyl]-1,3-thiazole-2-carbaldehyde (6-*sec*-Butylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (32) Compound **32** was obtained from **3d** and **24c** as a yellow solid (45%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 0.92 (3H, t, J=7.3 Hz), 1.38 (3H, d, J=6.8 Hz), 1.68—1.76 (2H, m), 2.58—2.61 (3H, m), 3.06—3.13 (1H, m), 4.29 (2H, m), 7.83 (1H, s), 7.95 (1H, s), 8.53 (1H, s), 8.59 (1H, br), 9.29 (2H, br); IR (ATR) cm^{-1}: 2692, 1639, 1099; MS (FAB) *m/z*: 361 (M^++H); HR-MS (ESI) *m/z*: 361.12685 (Calcd for $C_{16}H_{21}N_6S_2$: 361.12691); *Anal.* Calcd for $C_{16}H_{20}N_6S_2\cdot 2HCl\cdot 0.5H_2O$: C, 43.44; H, 5.24; Cl, 16.03; N, 18.99; S, 14.50. Found: C, 43.70; H, 5.07; Cl, 16.04; N, 19.18; S, 14.53.

Methyl 2-Amino-5-(1-methylbutyl)thiophene-3-carboxylate (23d) Compound **23d** was obtained from **22d** as a yellow oil (24%) by following the procedure described for **23a**. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, t, J=7.3 Hz), 1.21 (3H, d, J=7.1 Hz), 1.26—1.35 (2H, m), 1.41—1.53 (2H, m), 2.75 (1H, td, J=13.9, 7.1 Hz), 3.78 (3H, s), 5.77 (2H, br), 6.61 (1H, s); IR (ATR) cm^{-1}: 3332, 2954, 1664, 1584, 1500, 1439, 1263; MS (ESI) *m/z*: 228 (M^++H); HR-MS (ESI) *m/z*: 228.10266 (Calcd for $C_{11}H_{18}NO_2S$: 228.10582).

4-Hydrazino-6-(1-methylbutyl)thieno[2,3-*d*]pyrimidine (24d) Compound **24d** was obtained from **23d** as a colorless solid (52%) by following the procedure described for **24a**. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, t, J=7.3 Hz), 1.27—1.36 (2H, m), 1.36 (3H, d, J=6.8 Hz), 1.56—1.69 (2H, m), 2.50 (1H, m), 3.06 (1H, td, J=13.9, 6.8 Hz), 6.48 (1H, br), 6.88 (1H, s), 8.50 (1H, s); IR (ATR) cm^{-1}: 3260, 2958, 2925, 1579, 1516, 1355; MS (ESI) *m/z*: 237 (M^++H); HR-MS (ESI) *m/z*: 237.11384 (Calcd for $C_{11}H_{17}N_4S$: 237.11739); *Anal.* Calcd for $C_{11}H_{16}N_4S$: C, 55.90; H, 6.82; N, 23.71; S, 13.57. Found: C, 55.96; H, 6.79; N, 23.72; S, 13.41.

4-[(Methylamino)methyl]-1,3-thiazole-2-carbaldehyde [6-(1-Methylbutyl)thieno[2,3-*d*]pyrimidin-4-yl]hydrazone (33) Compound **33** was obtained from **3d** and **24d** as a colorless solid (79%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 0.91 (3H, t, J=7.3 Hz), 1.30—1.36 (2H, m), 1.38 (3H, d, J=6.8 Hz), 1.63—1.71 (2H, m), 2.59 (3H, t, J=5.3 Hz), 3.17 (1H, q, J=6.6 Hz), 4.28 (2H, t, J=5.4 Hz), 7.81 (1H, s), 7.95 (1H, s), 8.52 (1H, s), 8.56 (1H, br), 9.27 (2H, br); IR (ATR) cm^{-1}: 1643, 1596, 1550, 1511, 1463, 1423, 1382, 1242, 1101; MS (FAB) *m/z*: 375 (M^++H); MS (ESI) *m/z*: 375 (M^++H); HR-MS (ESI) *m/z*: 375.14012 (Calcd for $C_{17}H_{23}N_6S_2$: 375.14256); *Anal.* Calcd for $C_{17}H_{22}N_6S_2\cdot 1.8HCl$: C, 46.39; H, 5.45; Cl, 14.50; N, 19.09; S, 14.57. Found: C, 46.40; H, 5.34; Cl, 14.24; N, 19.12; S, 14.51.

Methyl 2-Amino-5-cyclohexylthiophene-3-carboxylate (23e) Compound **23e** was obtained from **22e** as a yellow oil (5%) by following the procedure described for **23a**. ^{1}H-NMR ($CDCl_3$) δ: 1.16—1.25 (1H, m), 1.28—1.37 (4H, m), 1.67—1.71 (1H, m), 1.77—1.81 (2H, m), 1.93—1.98 (2H, m), 2.51—2.56 (1H, m), 3.78 (3H, s), 5.78 (2H, br), 6.61 (1H, d, J=1.1 Hz); IR (ATR) cm^{-1}: 3299, 2918, 2851, 1657, 1500, 1439, 1260; MS (ESI) *m/z*: 240 (M^++H); HR-MS (ESI) *m/z*: 240.10716 (Calcd for $C_{12}H_{18}NO_2S$: 240.10582).

6-Cyclohexyl-4-hydrazinothieno[2,3-*d*]pyrimidine (24e) Compound **24e** was obtained from **23e** as a colorless solid (31%) by following the procedure described for **24a**. ^{1}H-NMR (DMSO-d_6) δ: 1.21—1.24 (1H, m), 1.35—1.43 (4H, m), 1.67—1.70 (1H, m), 1.76—1.80 (2H, m), 2.00—2.04 (2H, m), 2.81—2.86 (1H, m), 4.49 (2H, br), 7.34 (1H, br), 8.28 (1H, s), 8.81 (1H, br); IR (ATR) cm^{-1}: 3286, 2917, 2849, 1581, 1510; MS (ESI) *m/z*: 249 (M^++H); HR-MS (ESI) *m/z*: 249.11937 (Calcd for $C_{12}H_{17}N_4S$: 249.11739);

Anal. Calcd for $C_{12}H_{16}N_4S$: C, 58.04; H, 6.49; N, 22.56; S, 12.91. Found: C, 57.76; H, 6.83; N, 22.51; S, 12.68.

4-[(Methylamino)methyl]-1,3-thiazole-2-carbaldehyde (6-Cyclohexylthieno[2,3-*d*]pyrimidin-4-yl)hydrazone (34) Compound **34** was obtained from **3d** and **24e** as a yellow solid (58%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 1.23—1.34 (1H, m), 1.38—1.57 (4H, m), 1.72 (1H, d, J=12.9 Hz), 1.83 (2H, d, J=12.9 Hz), 2.11 (2H, d, J=10.3 Hz), 2.60 (3H, t, J=2.9 Hz), 2.95 (1H, m), 4.29 (2H, t, J=4.6 Hz), 7.79 (1H, s), 7.94 (1H, s), 8.52 (1H, s), 8.55 (1H, br), 9.23 (2H, br); IR (ATR) cm^{-1}: 2701, 1633, 1593, 1099; MS (FAB) *m/z*: 387 (M^++H); HR-MS (ESI) *m/z*: 387.13763 (Calcd for $C_{18}H_{23}N_6S_2$: 387.14256); *Anal.* Calcd for $C_{18}H_{22}N_6S_2\cdot 2.1HCl\cdot 0.25H_2O$: C, 46.23; H, 5.30; Cl, 15.92; N, 17.97; S, 13.71. Found: C, 45.83; H, 5.30; Cl, 15.92; N, 18.34; S, 13.34.

Methyl 2-Amino-5-(1,2-dimethylpropyl)thiophene-3-carboxylate (23f) Compound **23f** was obtained from **22f** as a yellow oil (29%) by following the procedure described for **23a**. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, d, J=6.9 Hz), 0.89 (3H, d, J=6.9 Hz), 1.19 (3H, d, J=7.2 Hz), 1.67—1.73 (1H, m), 2.56 (1H, dt, J=6.9, 14.9 Hz), 3.79 (3H, s), 5.78 (2H, s), 6.60 (1H, s); IR (ATR) cm^{-1}: 3439, 3334, 2957, 1666, 1583, 1501, 1439, 1265; MS (ESI) *m/z*: 228 (M^++H); HR-MS (ESI) *m/z*: 228.10542 (Calcd for $C_{11}H_{18}NO_2S$: 228.10582).

6-(1,2-Dimethylpropyl)-4-hydrazinothieno[2,3-*d*]pyrimidine (24f) Compound **24f** was obtained from **23f** as a colorless solid (58%) by following the procedure described for **24a**. ^{1}H-NMR (DMSO-d_6) δ: 0.88 (3H, d, J=6.9 Hz), 0.91 (3H, d, J=6.9 Hz), 1.27 (3H, d, J=6.9 Hz), 1.84 (1H, td, J=13.5, 6.9 Hz), 2.85 (1H, dt, J=13.5, 6.9 Hz), 4.51 (2H, br), 7.34 (1H, s), 8.29 (1H, s), 8.82 (1H, br s); IR (ATR) cm^{-1}: 3289, 2956, 1579, 1508, 1355; MS (ESI) *m/z*: 237 (M^++H); HR-MS (ESI) *m/z*: 237.11924 (Calcd for $C_{11}H_{17}N_4S$: 237.11739); *Anal.* Calcd for $C_{11}H_{16}N_4S$: C, 55.90; H, 6.82; N, 23.71; S, 13.57. Found: C, 56.19; H, 6.99; N, 23.63; S, 13.30.

4-[(Methylamino)methyl]-1,3-thiazole-2-carbaldehyde [6-(1,2-Dimethylpropyl)thieno[2,3-*d*]pyrimidin-4-yl]hydrazone (35) Compound **35** was obtained from **3d** and **24f** as a yellow solid (82%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 0.92 and 0.93 (each 3H, d, J=6.6 Hz), 1.35 (3H, d, J=7.1 Hz), 1.91 (1H, td, J=13.2, 6.6 Hz), 2.59 (3H, t, J=5.4 Hz), 2.99 (1H, q, J=6.8 Hz), 4.29 (2H, t, J=5.6 Hz), 7.89 (1H, br), 7.99 (1H, s), 8.57 (1H, s), 8.68 (1H, br), 9.41 (2H, br); IR (ATR) cm^{-1}: 1635, 1579, 1508, 1460, 1419, 1373, 1247, 1147, 1099; MS (FAB) *m/z*: 375 (M^++H); HR-MS (ESI) *m/z*: 375.14180 (Calcd for $C_{17}H_{23}N_6S_2$: 375.14256); *Anal.* Calcd for $C_{17}H_{22}N_6S_2\cdot 2.5HCl\cdot 1.25H_2O$: C, 41.82; H, 5.44; Cl, 18.29; N, 17.02; S, 12.95. Found: C, 41.68; H, 5.44; Cl, 18.29; N, 17.02; S, 12.95.

***tert*-Butyl[(2,2-dimethylpent-4-en-1-yl)oxy]diphenylsilane (27)** To a mixture of isobutyraldehyde **25** (25 ml, 0.275 mol) and allyl alcohol **26** (12.5 ml, 0.183 mol) in *p*-cymene (40 ml) was added *p*-TsOH (60 mg) and stirred under reflux for 24 h. Distillation gave 2,2-dimethyl-4-pentenal (14.8 g, 72%) as a colorless oil. To a suspension of LAH (0.8 g, 21 mmol) in Et_2O (60 ml) was added dropwise 2,2-dimethyl-4-pentenal (8.00 g, 71 mmol) in Et_2O (40 ml) at room temperature, and stirred under reflux for 3 h. After cooling to 0 °C, MeOH (1.8 ml), water (0.8 ml), 15% NaOH aq. (0.8 ml), and water (2.4 ml) were added to the reaction mixture successively. The precipitate was filtered through Celite. The filtrate was concentrated under reduced pressure and the residue was purified by vacuum distillation to afford 2,2-dimethyl-4-pentenol (5.9 g, 73%) as a colorless oil. To a solution of pentenol (2.28 g, 20 mmol) in THF (50 ml) were added imidazole (1.50 g, 22 mmol) and TBDPSCl (5.7 ml, 22 mmol). The reaction mixture was stirred at room temperature for 20 h. After EtOAc and water were added, the two layers were separated. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel eluted with *n*-hexane/EtOAc (50 : 1) to afford title compound **27** (5.05 g, 72%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 0.85 (6H, s), 1.05 (9H, s), 2.05 (2H, d, J=8.0 Hz), 3.32 (2H, s), 4.95—5.02 (2H, m), 5.71—5.82 (1H, m), 7.35—7.77 (10H, m).

Methyl 2-Amino-5-(2-{[*tert*-butyl(diphenyl)silyl]oxy}-1,1-dimethylethyl)thiophene-3-carboxylate (23g) To a solution of **27** (5.00 g, 14.2 mmol) in a mixture of THF (140 ml) and H_2O (70 ml) were slowly added OsO_4 (180 mg, 5 mol%) and $NaIO_4$ (6.10 g, 28.5 mmol), and the mixture was stirred at 50 °C for 2 h. Et_2O and sat. $Na_2S_2O_3$ aq. were added to the reaction mixture, and the two layers were separated. The aqueous layer was extracted with Et_2O and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel eluted with *n*-hexane/EtOAc (20 : 1) to afford 4-{[*tert*-butyl(diphenyl)silyl]oxy}-3,3-dimethylbutanal (2.50 g, 50%) as a colorless oil. The resulting aldehyde was added to a solution of methyl cyanoacetate (0.65 ml, 7.5 mmol), Et_3N (1.05 ml, 7.5 mmol), and DMF (1.1 ml, 14 mmol) under N_2 atmosphere. After 3 min of stirring, S_8 (225 mg, 7.0 mmol) was added and stirred at room temperature for 72 h. The reaction mixture was poured into water and extracted with EtOAc. The aqueous layer was extracted with EtOAc and the combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel eluted with *n*-hexane/EtOAc (9 : 1) to afford title compound **23g** (2.50 g, 76%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.05 (9H, s), 1.29 (6H, s), 3.48 (2H, s), 3.78 (3H, s), 5.77 (2H, br), 6.68 (1H, s), 7.33—7.61 (10H, m).

6-(2-{[*tert*-Butyl(diphenyl)silyl]oxy}-1,1-dimethylethyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (28) A solution of **23g** (2.50 g, 5.34 mmol) in formamide (12.5 ml) was heated at 210 °C for 2 h. After cooling, precipitate was formed, which was collected and washed with water. The crude solid was chromatographed on silica gel eluted with *n*-hexane/EtOAc (1 : 1) to afford title compound **28** (1.43 g, 58%) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (9H, s), 1.42 (6H, s), 3.61 (2H, s), 7.26 (1H, s), 7.32—7.59 (10H, m), 8.01 (1H, br); IR (KBr) cm^{-1}: 3571, 2854, 1689, 1577; MS (FAB) *m/z*: 463 (M^++H); HR-MS (ESI) *m/z*: 463.18572 (Calcd for $C_{26}H_{31}N_2O_2SSi$: 463.18755); *Anal.* Calcd for $C_{26}H_{30}N_2O_2SSi$: C, 67.49; H, 6.54; N, 6.05; S, 6.93. Found: C, 67.54; H, 6.82; N, 6.22; S, 6.67.

6-(2-{[*tert*-Butyl(diphenyl)silyl]oxy}-1,1-dimethylethyl)-4-hydrazinothieno[2,3-*d*]pyrimidine (24g) Compound **24g** was obtained from **28** as a colorless solid (76%) by following the chlorination and hydrazinolysis procedure described for **24a**. ^{1}H-NMR ($CDCl_3$) δ: 1.02 (9H, s), 1.42 (6H, s), 3.63 (2H, s), 6.35 (1H, br), 6.90 (1H, s), 7.32—7.55 (10H, m), 8.51 (1H, s); IR (KBr) cm^{-1}: 3301, 3268, 2929, 1589, 1548; MS (FAB) *m/z*: 477 (M^++H); HR-MS (ESI) *m/z*: 477.21018 (Calcd for $C_{26}H_{33}N_4OSSi$: 477.21443).

4-[(Methylamino)methyl]-1,3-thiazole-2-carbaldehyde [6-(2-Hydroxy-1,1-dimethylethyl)thieno[2,3-*d*]pyrimidin-4-yl]hydrazone (36) A mixture of **24g** (885.6 mg, 1.86 mmol) and **3d** (500 mg, 1.95 mmol) in benzene (17 ml) was stirred under reflux for 3 h and cooled to room temperature. The reaction mixture was concentrated under reduced pressure. The residue was chromatographed on silica gel eluted with *n*-hexane/EtOAc (2 : 1) to afford crude hydrazone (777 mg). To a solution of the hydrazone in THF (8 ml) was added TBAF (1 M THF solution, 1.3 ml, 1.31 mmol) at 0 °C, and stirred at 75 °C for 15 h. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel eluted with *n*-hexane/EtOAc (1 : 3) to afford crude hydroxide (35.8 mg). To a solution of the hydroxide in 1,4-dioxane (1.5 ml) was added 4 N HCl in 1,4-dioxane (1.5 ml) at 0 °C. The mixture was stirred at room temperature for 16 h and concentrated under reduced pressure. The residue was recrystallized from EtOH, EtOAc, and *n*-hexane to afford title compound **36** (9.3 mg, 2%) as a yellow solid. ^{1}H-NMR (DMSO-d_6) δ: 1.40 (6H, s), 2.45 (3H, s), 3.49 (2H, s), 4.00 (2H, s), 5.06 (1H, br), 7.70 (1H, s), 7.83 (1H, s), 8.42 (1H, s), 8.50 (1H, s); IR (ATR) cm^{-1}: 1558, 1508, 1348; MS (FAB) *m/z*: 377 (M^++H); HR-MS (ESI) *m/z*: 377.11975 (Calcd for $C_{16}H_{21}N_6OS_2$: 377.12183); *Anal.* Calcd for $C_{16}H_{20}N_6OS_2\cdot 1.5HCl\cdot 0.25H_2O$: C, 44.11; H, 5.09; N, 19.29; S, 14.72. Found: C, 44.46; H, 4.89; N, 18.98; S, 14.57.

6-(2-Fluoro-1,1-dimethylethyl)-4-hydrazinothieno[2,3-*d*]pyrimidine (24h) To a solution of **28** (8.60 g, 18.4 mmol) in THF (100 ml) was added TBAF (1 M THF solution, 36.7 ml, 36.7 mmol) at 0 °C, and stirred overnight at 70 °C. After cooling, $CHCl_3$ and brine were added, and the two layers were separated. The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel eluted with $CHCl_3$/MeOH (100 : 3). The eluate was concentrated under reduced pressure, and the residue was recrystallized from $CHCl_3$ and EtOAc to afford 6-(1,1-dimethyl-2-hydroxyethyl)thieno[2,3-*d*]pyrimidin-4-one (2.01 g, 49%) as a brownish solid. To a solution of afforded compound (1.60 g, 7.14 mmol) in CH_2Cl_2 (240 ml) was added (dimethylamino)sulfur trifluoride (3.77 ml, 28.6 mmol) at −78 °C and warmed to room temperature over 2 h with stirring. The reaction mixture was neutralized with a solution of sat $NaHCO_3$, and the two layers were separated. The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel eluted with $CHCl_3$/MeOH (100 : 1) to afford 6-(2-fluoro-1,1-dimethylethyl)thieno[2,3-*d*]pyrimidin-4-one as a colorless solid. Compound **24h** was obtained from the resulting compound as a colorless solid (1.16 g, 68%) by following the procedure described for **24g**. ^{1}H-NMR (DMSO-d_6) δ: 1.33 (3H, s), 1.38 (3H, s), 3.17 (2H, d, J=22.2 Hz), 4.57 (2H, br), 7.39 (1H, br), 8.31 (1H, s), 9.07 (1H, s); IR (ATR) cm^{-1}: 335, 2985, 1580, 1513, 1317; MS (FAB) *m/z*: 241 (M^++H); HR-MS (ESI) *m/z*: 241.09405 (Calcd for $C_{10}H_{14}FN_4S$: 241.09232); *Anal.* Calcd for $C_{10}H_1FN_4S$: C, 49.98; H, 5.45; F, 7.91; N, 23.32; S, 13.34. Found:

C, 49.99; H, 5.46; F, 7.91; N, 23.12; S, 13.38.

4-[(Methylamino)methyl]-1,3-thiazole-2-carbaldehyde [6-(2-Fluoro-1,1-dimethylethyl)thieno[2,3-*d*]pyrimidin-4-yl]hydrazone (37) Compound **37** was obtained from **3d** and **24h** as a yellow solid (97%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 1.39 (3H, s), 1.44 (3H, s), 2.59 (3H, t, J=5.4 Hz), 3.30 (2H, d, J=22.0 Hz), 4.28 (2H, t, J=5.4 Hz), 7.89 (1H, s), 7.98 (1H, s), 8.56 (1H, s), 8.65 (1H, br), 9.40 (2H, br); MS (FAB) *m*/*z*: 379 (M^++H); IR (ATR) cm^{-1}: 3350, 2696, 1631, 1589, 1372; HR-MS (ESI) *m*/*z*: 379.10884 (Calcd for $C_{16}H_{20}FN_6S_2$: 379.11749); *Anal.* Calcd for $C_{16}H_{19}N_6FS_2\cdot 2HCl$: C, 42.57; H, 4.69; Cl, 15.71; F, 4.21; N, 18.62; S, 14.21. Found: C, 42.52; H, 4.74; Cl, 15.80; F, 4.27; N, 18.68; S, 14.15.

4-(1-Aminoethyl)-1,3-thiazole-2-carbaldehyde [6-(2-Fluoro-1,1-dimethylethyl)thieno[2,3-*d*]pyrimidin-4-yl]hydrazone (38) Compound **38** was obtained from **3h** and **24h** as a yellow solid (88%) by following the procedure described for **4d**. ^{1}H-NMR (DMSO-d_6) δ: 1.39 (3H, s), 1.44 (3H, s), 1.59 (3H, d, J=6.8 Hz), 3.31 (2H, d, J=22.2 Hz), 4.52—4.64 (1H, m), 7.92 (1H, s), 7.94 (1H, s), 8.54—8.90 (6H, m); MS (FAB) *m*/*z*: 379 (M^++H); IR (ATR) cm^{-1}: 2872, 1633, 1581, 1552, 1424, 1211; HR-MS (ESI) *m*/*z*: 379.11307 (Calcd for $C_{16}H_{20}FN_6S_2$: 379.11749); *Anal.* Calcd for $C_{16}H_{19}FN_6S_2\cdot 2.4HCl\cdot 0.95H_2O$: C, 39.78; H, 4.86; Cl, 17.61; F, 3.93; N, 17.40; S, 13.27. Found: C, 40.15; H, 4.80; Cl, 17.66; F, 3.61; N, 17.00; S, 13.01.

Kinase Inhibition Assay CDK4, CDK2, cyclin D, and cyclin E proteins were purified from baculovirus-infected Sf-9 insect cells. Glutathione *S*-transferase Rb (GST-Rb) protein was expressed and purified from bacteria using glutathione-sepharose beads as per standard procedure. Enzyme assays to determine the concentration of compounds that cause 50% of kinase inhibition (IC_{50}) were performed in 96-well filter plates (Whatmann, GF/C filter). The assay mixture (30 μl of 10 mM ATP contained 0.2 mCi [^{33}P]ATP, 30 μl of enzymes (Cdk4/D1 or Cdk2/E), 30 μl of 25% GST-Rb with glutathione sepharose beads in kinase assay buffer (50 mM Hepes pH 7.4, 10 mM $MgCl_2$, 1 mM DTT, 2.5 mM EGTA, 5 mg/ml AMSF, 5 mg/ml aprotinine, 0.1 mM NaF, 10 ml β-glycerophosphate, and 0.1 mM sodium-*o*-vanadate) and 10 μl of the tested compound in a final volume of 100 μl) was incubated on the plate for 30 min at 30 °C. The plate was washed four times and the kinase activities were measured. The IC_{50} values were determined by analyzing the dose–response inhibition curves.

Cytotoxicity Assay An 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay was performed against HCT116 and PC-6 cell lines to examine the growth inhibitory effects of our compounds. HCT116 cell line was purchased from American Type Culture Collection (ATCC, U.S.A.). PC-6 cell line was obtained from Immuno-Biological Laboratories (Gunma, Japan). The cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum. The cells were plated in 96-well micro plates on Day 0, and stock solutions serially diluted in DMSO were added to each well on Day 1. After 3 d of culture (Day 4), the number of viable cells was determined by the MTT assay. The concentration of compounds that cause 50% of growth inhibition was calculated by the following equation: $100\times[(T-T_0)/(C-T_0)]=50$ (C: control optical density; T: test optical density; T_0: optical density at time zero).

Cell Cycle Distribution Analysis HCT116 cells were treated with test compounds for 16 h and were stained with propidium iodide using a Cycle Test Kit (Becton Dickinson). Then the cells were analyzed using a FACscan flow cytometer with CellQuest software (Becton Dickinson) in accordance with the manufacturer's recommendations. The percentage of cells in the G0/G1, S, and G2/M phases of the cell cycle were quantified using Modifit software (Verity Software House, Inc.).

Antitumor Activity Assay For *in vivo* studies, HCT116 tumor cells were subcutaneously transplanted into the right hind limb of male BALB/c-nu/nu mice (Japan SLC, Inc.), aged 5 or 6 weeks. After the subcutaneous tumors averaged 80—100 mm^3 in size, the tested compounds dissolved in 20% Captisol® (β-cyclodextrin sulfobutylether sodium salt, CyDex, Inc.) solution were administered intravenously or orally once a day for 4—6 d at the doses. The tumor volume was measured with a caliper periodically.

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