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Novel and orally active 5-(1,3,4-oxadiazol-2-yl)pyrimidine derivatives as selective FLT3 inhibitors

Hiroshi Ishida [a,*], Shoichi Isami [a], Tsutomu Matsumura [a], Hiroshi Umehara [a], Yoshinori Yamashita [a], Jiro Kajita [a], Eiichi Fuse [a], Hitoshi Kiyoi [b], Tomoki Naoe [c], Shiro Akinaga [a], Yukimasa Shiotsu [a], Hitoshi Arai [a]

[a] Pharmaceutical Research Center, Kyowa Hakko Kogyo Co., Ltd, 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8731, Japan
[b] Department of Infectious Diseases, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8560, Japan
[c] Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8560, Japan

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ABSTRACT

5-(1,3,4-Oxadiazol-2-yl)pyrimidine derivative **1** was identified as a new class of FLT3 inhibitor from our compound library. With the aim of enhancement of antitumor activity of **2** prepared by minor modification of **1**, structure optimization of side chains at the 2-, 4-, and 5-positions of the pyrimidine ring of **2** was performed to improve the metabolic stability. Introduction of polar substituents on the 1,3,4-oxadiazolyl group contributed to a significant increase in the metabolic stability. As a result, a series of compounds showed increased efficacy against MOLM-13 xenograft model in mice by oral administration.

FLT3 (FMS-like receptor tyrosine kinase 3) also referred to as fetal liver kinase-2 (FLK-2) or stem cell kinase 1 (STK-1) is a class III receptor tyrosine kinase together with KIT, FMS, and platelet-derived growth factor receptor.[1,2] In 1996, FLT3/ITD (Internal Tandem Duplication) mutation was found in AML (Acute Myeloid Leukemia) cells. This mutation is formed by the duplication of juxtamembrane domain-coding sequence in a direct head-to-tail orientation.[3] The ITD mutation occurs in 15–35% of AML patients and promotes ligand-independent dimerization, auto-phosphorylation, and constitutive activation of the FLT3 receptor.[4] Patients carrying the ITD mutations are found to have an increased incidence of leukocytosis and a decreased overall survival when compared with patients without ITD mutations.[5] Therefore, inhibition of activated and mutated FLT3 kinase is effective strategy for the treatment of AML. To date, several FLT3 inhibitors have been subjected to clinical trials in several hematopoietic malignancies including AML.[6–9]

In the course of our discovery study for FLT3 inhibitors, the 5-(1,3,4-oxadiazol-2-yl)pyrimidine derivative **1** was identified as a primary hit compound via high-throughput screening of our compound library. In our preliminary SAR investigations, we found the compound **2** which showed about 70-fold more potent FLT3 inhibitory activity than **1** (Fig. 1).

Compound **2** showed not only FLT3 inhibitory activity but antiproliferative activity against MOLM-13 cells, a human AML cell line expressing ITD activating mutation.[10] This compound also showed

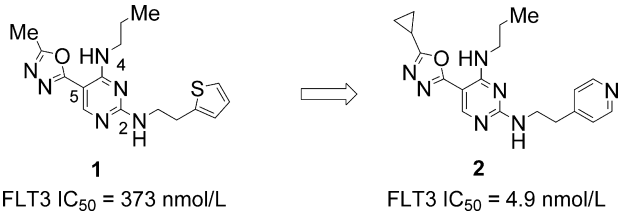

Figure 1. FLT3 inhibitors identified via high-throughput screening (**1**) and subsequent analog screening (**2**).

modest antitumor activity against MOLM-13 xenograft model in SCID mice. However, **2** was found metabolically unstable in both human and mice liver microsomes, and oxidative reaction of the *n*-propylamino group at the 4-position and the 4-(2-aminoethyl)pyridyl group at the 2-position were shown to be major metabolic pathways from the analysis of metabolite profiles. Thus, further structure optimization to acquire metabolic stability was required to enhance in vivo activity, and we started the structure optimization study of **2**. We herein report the synthesis and evaluation of novel 5-(1,3,4-oxadiazol-2-yl)pyrimidine derivatives as FLT3 inhibitors.

The synthesis of derivatives with modified side chains at the 2- and 4-positions is outlined in Scheme 1. Functionalization of the 4-position of a commercially available ethyl 4-chloro-2-methylthiopyrimidine-5-carboxylate **3** followed by hydrolysis of the ester group and condensation with hydrazine monohydrate gave the pyrimidinecarboxylic acid hydrazide **4**. Cyclization reaction[11] of **5**

* Corresponding author. Tel.: +81 55 989 2025; fax: +81 55 986 7430.
E-mail address: hiroshi.ishida@kyowa.co.jp (H. Ishida).

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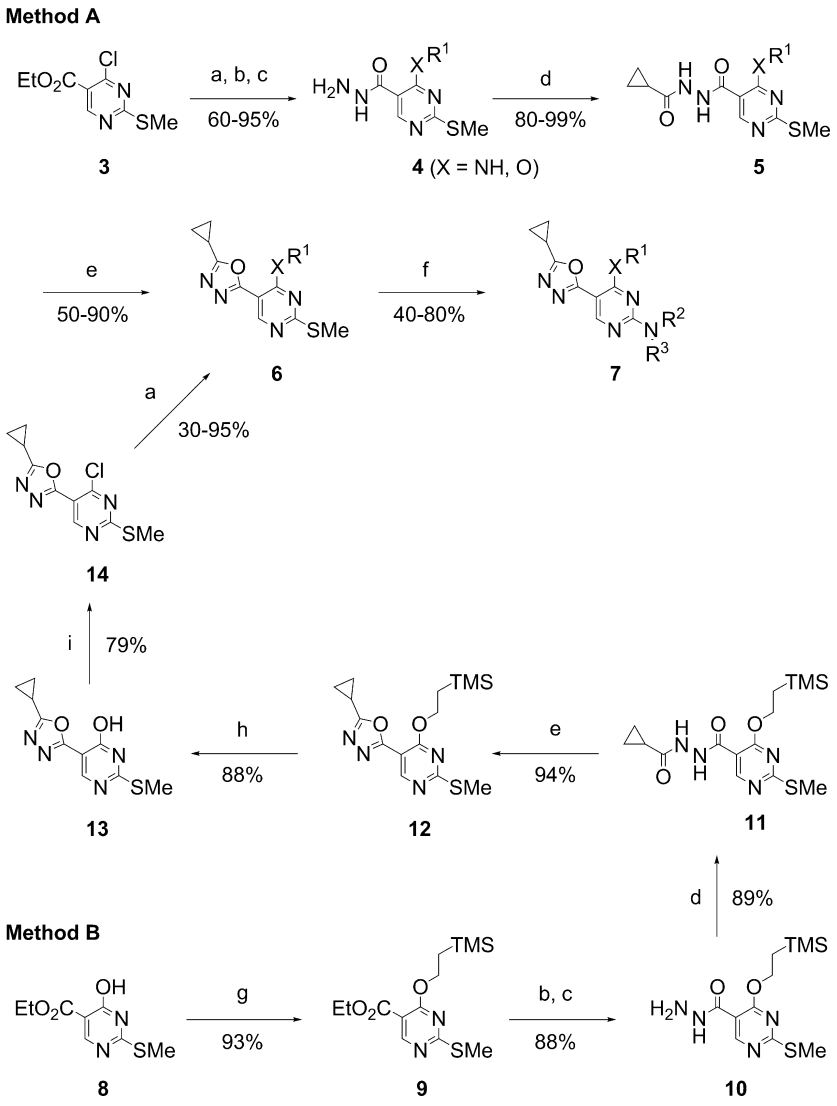


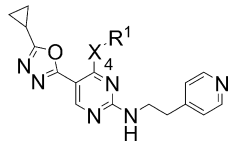
Scheme 1. Reagents and conditions: (a) R^1ONa (for X = O) or R^1NH_2 (for X = NH), Et_3N, THF; (b) 2.0 mol/L NaOH aq, EtOH; (c) i—CDI, THF; ii—$NH_2NH_2{\cdot}H_2O$, THF; (d) cyclopropanecarbonyl chloride, $CHCl_3$, satd $NaHCO_3$ aq, 0 °C; (e) PPh_3, CCl_4, Et_3N, CH_3CN; (f) i—mCPBA, CH_2Cl_2; ii—R^2R^3NH, Et_3N, THF; (g) PPh_3, DEAD, 2-(trimethylsilyl)ethanol, toluene; (h) 1.0 mol/L TBAF in THF; (i) $POCl_3$, 60 °C.

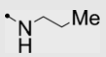
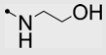
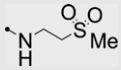
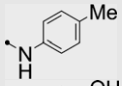
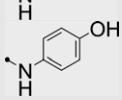
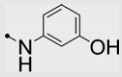
obtained by acylation of the hydrazide moiety in **4** provided the 5-(1,3,4-oxadiazol-2-yl)pyrimidine derivatives **6**. Subsequent substitution reaction at the 2-position of **6** afforded the desired compounds **7** (Method A). Alternatively, to aim for more efficient optimization at the 4-position by introducing the side chain in the latter stage of the reaction route, another method was examined (Method B). Introduction of a (2-trimethylsilyl)ethoxy group[12] to the 4-position of **8** prepared by the reported procedure[13] was performed using Mitsunobu reaction, followed by conversion to **12** according to Method A. Then, removal[14] of the (2-trimethylsilyl)ethyl moiety and treatment with $POCl_3$ yielded **14**, which was converted to **7** via formation of **6**.

Our SAR study was started on the 4-position. These results are summarized in Table 1. Shortening the carbon chain (**7a**) or introduction of a polar group (**7b**) resulted in a significant increase in the metabolic stability. However, their FLT3 kinase inhibitory activity and anti-proliferative activity against MOLM-13 were less potent compared to that of **2**. A similar tendency was also observed in the ethoxy analog **7i**. Anti-proliferative activity of the anilino analogs (**7d**–**7h**) was maintained. Among them, a 4-hydroxyanilino analog **7g** showed the most potent FLT3 inhibitory activity as well as anti-proliferative activity against MOLM-13 and good stability in human microsomes. However, compound **7g** was not expected to show in vivo activity, since **7g** was not detected in plasma after oral administration in mice (data not shown). From these results, we chose *n*-propylamino, anilino, and methylamino groups as desirable substituents for further optimization study because **2**, **7a**, and **7d** show either potent anti-proliferative activity against MOLM-13 (**2**, **7d**) or good metabolic stability (**7a**).

With the results in mind, SAR investigations on the 2-position were also examined for structural tolerability of the pyridyl moiety with the anilino group at the 4-position, which was identified as potent anti-proliferative activity in Table 1. These results are summarized in Table 2. The compounds were evaluated for anti-proliferative activity against MOLM-13 cells preferentially to identify more active compound against tumor cells. In contrast to the 4-position, structure tolerance for anti-proliferative activity in the 2-position was quite limited. Replacement with pyridyl *N*-oxide (**7j**), pyrimidyl (**7k**), and 4-aminophenyl (**7q**) analogs improved metabolic stability, but significantly lowered anti-proliferative activity. As mentioned above, oxidative reaction of the 4-(2-aminoethyl)pyridyl moiety at the 2-position of **2** was observed in the metabolite analysis. Introduction of a methyl group to aim for blocking the oxidative reaction of the nitrogen atom, however,

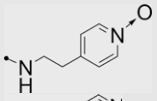
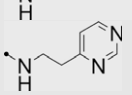
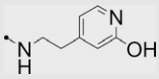
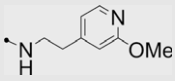
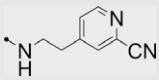
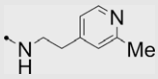
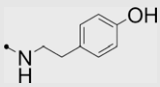
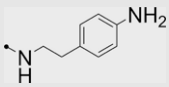
Table 1
SAR for the C-4 modified analogs



Compound	XR^1	FLT3IC$_{50}$[a] (nmol/L)	MOLM-13 GI$_{50}$[b] (nmol/L)	HLM CL[c] (h/L/kg)
2		4.9 ± 1.0	36 ± 3.4	13
7a		35 ± 10	139 ± 14	1.2
7b		63 ± 10	123 ± 7.7	1.2
7c		515 ± 146	588 ± 235	n.t.[d]
7d		47 ± 7.4	40 ± 6.3	19
7e		134 ± 31	40 ± 8 7	13
7f		106 ± 30	23 ± 1.8	11
7g		4.8 ± 1.2	3.2 ± 0.34.8	
7h		34 ± 17	10 ± 1.5	16
7i		80 ± 14	263 ± 139	1.4

IC_{50} and GI_{50} values are ±SEM means of three experiments.
[a] See Ref. 15 for assay details.
[b] See Ref. 16 for assay details.
[c] See Ref. 17 for assay details.
[d] n.t., not tested.

Table 2
SAR for the C-2 and C-4 modified analogs

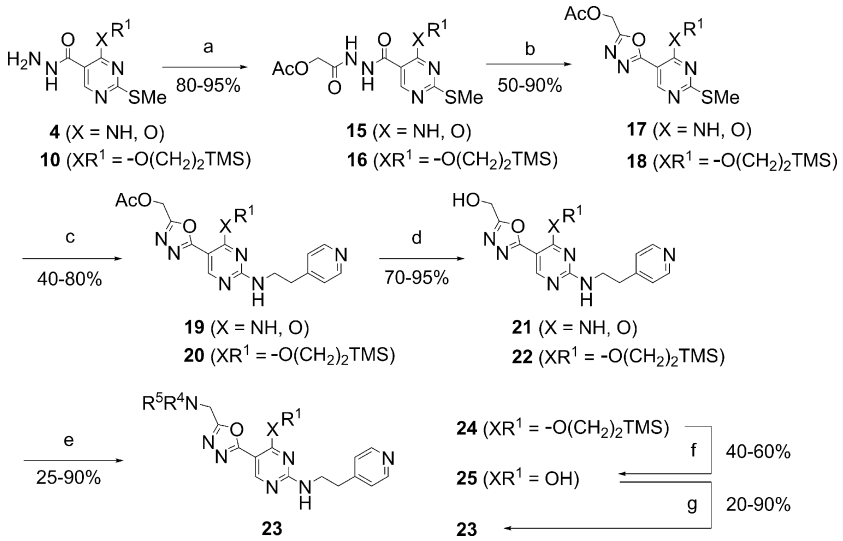
Compound	NR^2R^3	MOLM-13 GI$_{50}$[a] (nmol/L)	HLM CL[b] (h/L/kg)
7j		3610 ± 657	0.2
7k		775 ± 205	4.4
7l		113 ± 6.6	2.5
7m		999 ± 254	n.t.[c]
7n		>10,000	n.t.
7o		137 ± 10	22
7p		44 ± 6.7	5.1
7q		287 ± 33	4.1

GI_{50} values are ±SEM means of three experiments.
[a] See Ref. 16 for assay details.
[b] See Ref. 17 for assay details.
[c] n.t., not tested.

did not improve metabolic stability (**7o**). 2-Methoxypyridyl (**7m**) and 2-cyanopyridyl (**7n**) analogs also showed diminished activity. Only 2-hydroxypyridyl (**7l**) and 4-hydroxyphenyl (**7p**) analogs satisfied both criteria of anti-proliferative activity and microsomal stability. However, compounds **7l** and **7p** were not detected in plasma after oral administration in mice likewise **7g** (data not shown). From the results, we estimated the 4-(2-aminoethyl)pyridyl group was quite important to show in vivo activity.

Through these unsatisfied results, we decided to examine modification of the side chain on the 1,3,4-oxadiazolyl group at the 5-position, since SAR investigations on the position were carried out to a lesser extent. Thus, we planned to reduce their lipophilicity through introduction of polar substituents at the end of the 5-position to improve metabolic stability. To incorporate the above design into the structure, we developed new synthetic routes as shown in Scheme 2. Treatment of compounds **4** used as starting materials with acetoxyacetyl chloride afforded the corresponding **15** followed by the cyclization reaction to give **17**. Compounds **19** were obtained by introduction of the side chain at the 2-position of **17**. Hydrolysis of the acetyl group of **19** gave **21** followed by conversion to mesylates and subsequent substitution reaction with various amines provided **23**. Synthesis of **23** with XR^1 = OH could be alternatively achieved. Compounds **24** were prepared from **10** according to the above method, which underwent further deprotection of the hydroxy group to give **25**. Conversion of a 4-hydroxy group of the pyrimidine ring to mesylates and substitution reaction[18] with amines provided the desired compounds **23** successfully.

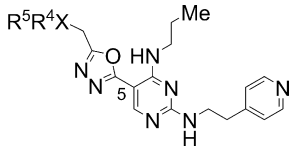
These compounds were evaluated for anti-proliferative activity against MOLM-13 cells. Table 3 summarizes the SAR studies for the substituted 1,3,4-oxadiazolyl group. We identified the 5-position with extensive structural tolerability. Polar substituents on the side chain of the 1,3,4-oxadiazolyl group were well tolerated for anti-proliferative activity against MOLM-13 cells. It is also noteworthy that the microsomal intrinsic clearance values of these compounds varied depending on the side chains. The distinct improvement of metabolic stability by the introduction of dimethylamino (**23a**), morpholino (**23b**), and 4-acetylpiperazinyl (**23d**) groups was not observed. Meanwhile, the introduction of hydroxy (**21a**), 4-methylpiperazinyl (**23c**), piperazinyl (**23f**), and 2-(dimethylamino)ethylamino (**23i**) groups increased metabolic stability. Above all, compound**23f** gave a best result and substituted or unsubstituted piperazinyl groups were shown to be suitable for the improvement of metabolic stability. We therefore tried to explore a combination of the side chains at the 4- and 5-positions which showed both potent activity and good metabolic stability. Table 4 summarizes the SAR studies for the 4-position in combination with the favorable piperazinyl groups on the side chain of the 5-position, which were identified in Table 3. Reduction of lipophil-

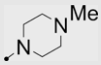
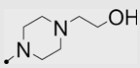
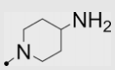
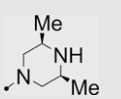
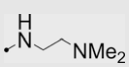


Scheme 2. Reagents and conditions: (a) $AcOCH_2COCl$, $CHCl_3$, satd $NaHCO_3$ aq, 0 °C; (b) PPh_3, CCl_4, Et_3N, CH_3CN; (c) i—mCPBA, CH_2Cl_2; ii—4-(2-aminoethyl)pyridine, THF; (d) 2.0 mol/L NaOH aq, MeOH; (e) i—Ms_2O, Et_3N, CH_2Cl_2; ii—R^4R^5NH, THF; (f) 1.0 mol/L TBAF in THF; (g) i—Ms_2O, Et_3N, CH_2Cl_2; ii—R^1NH_2 (for X = NH), Et_3N, CH_2Cl_2.

icity at the 4-position (e.g., **23c** vs **23j** or **23k**, **23h** vs **23r**) was found also effective for metabolic stabilization, which was already shown in Table 1. Incorporation of F atom to aim for blocking

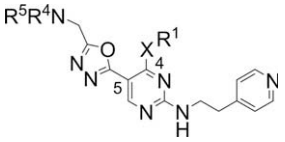
Table 3
SAR for the C-5 modified analogs

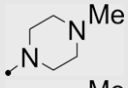
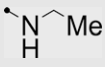
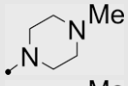
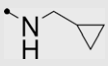
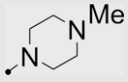
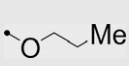
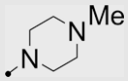
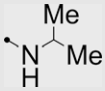
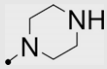
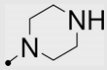
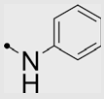
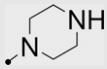
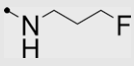
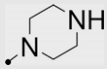
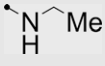
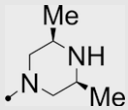


Compound	XR^4R^5	MOLM-13 GI_{50}[a] (nmol/L)	HLM CL[b] (h/L/kg)	*c*log*p*[c]
21a		58 ± 8.6	2.1	0.74
23a		73 ± 8.1	11	1.61
23b		43 ±4.2	10	1.52
23c		19 ± 3.6	4.9	1.97
23d		66 ± 4.6	8.2	1.11
23e		57 ± 4.3	3.0	1.43
23f		29 ± 1.9	1.1	1.51
23g		74 ± 5.8	3.0	0.82
23h		43 ± 8.1	6.9	2.55
23i		78 ± 4.1	3.0	1.53

GI_{50} values are ±SEM means of three experiments.
[a] See Ref. 16 for assay details.
[b] See Ref. 17 for assay details.
[c] Calculated by Daylight.[19]

Table 4
SAR for the C-4 and C-5 modified analogs



Compound	XR^1	NR^4R^5	MOLM-13 GI_{50}[a] (nmol/L)	HLM CL[b] (h/L/kg)	*c*log*p*[c]
23j			262 ± 51	<0.1	0.91
23k			102 ± 5.1	2.7	1.44
23l			52 ± 4.4	5.2	2.0
23m			308 ± 55	0.7	1.51
23n			49 ± 4.0	1.5	1.29
23o			50 ± 7.7	6.3	1.37
23p			74 ± 5.8	2.3	2.43
23q			99 ± 4.9	<0.1	0.93
23r			94 ± 10	0.8	2.02

GI_{50} values are ±SEM means of three experiments.
[a] See Ref. 16 for assay details.
[b] See Ref. 17 for assay details.
[c] Calculated by Daylight.[19]

Table 5
Anti-proliferative activity of 5-(1,3,4-oxadiazol-2-yl)pyrimidine derivatives against several cell lines

Compound	MOLM-13 GI_{50} (nmol/L)	ML-1 GI_{50} (nmol/L)	FLT3-Δ599GI_{50} (nmol/L)	FLT-D835Y GI_{50} (nmol/L)
2	36 ± 3.4	4318 ± 905	36 ± 9.2	52 ± 13
21a	56 ± 8.6	5958 ± 1552	76 ± 22	116 ± 38
23c	19 ± 3.6	>10,000	37 ± 11	31 ± 5.9
23f	29 ± 1.9	9108 ± 1045	74 ± 14	65 ± 21
23k	102 ± 5.1	>10,000	114 ± 13	63 ± 10
23r	94 ± 10	>10,000	74 ± 14	48 ± 19

GI_{50} values are ±SEM means of three experiments. See Ref. 16 for assay details.

Table 6
Antitumor activity of 5-(1,3,4-oxadiazol-2-yl)pyrimidine derivatives

Compound	Dose[a] (mg/kg)	T/C_{min}[b] (on day)	Regression V/V_0 min[c] (on day)	Mortality
Control		1.00	—	0/5
2	50	0.47 (7)	—	0/5
	100	0.34 (7)	—	1/5
23a	50	0.45 (7)	—	0/5
23c	50	0.27 (7)	—	0/5
23f	50	0.25 (10)	—	0/5
	100	0.10 (7)	0.571 (4)	0/5
	200	0.006 (10)	0.098 (10)	0/5
23k	50	0.24 (7)	—	0/5
23r	50	0.20 (7)	1.39 (7)	0/5
	100	0.014 (7)	0.12 (7)	0/5

[a] Compounds were orally administrated at b.i.d. intervals for 5 days.
[b] Averages of tumor volume ratio in the treated to control mice.
[c] Averages of the relative tumor volume to the initial tumor volume on day 0. See Ref. 10 for assay details.

metabolism (**23f** vs **23q**) at the 4-position was also effective; however, cycloalkyl moieties (**23l** and **23o**) exhibited relatively low metabolic stability. Introduction of an alkoxy unit (**23m**) resulted in a substantial reduction in activity likewise for **7i**.

Among them, several compounds were subjected to evaluation for anti-proliferative activity against human leukemia cell lines (Table 5).[20,21] These selected compounds showed significant inhibition for mutant FLT3-expressing cells in addition to MOLM-13 cells, while weak or no inhibition for ML1 cells expressing wild-type FLT3 was observed. Compound **23f** also exhibited selective kinase inhibitory profile against FLT3 kinase as previously described.[10]

Finally, these antitumor effects were evaluated by oral administration to sc MOLM-13 tumor xenograft model in SCID mice (Table 6). All compounds except for **23a** revealed more potent activity than that of **2** without mortality nor body weight loss. Above all, **23f** and **23r** showed significant antitumor activity including tumor regression in a dose dependent manner.

In summary, we have demonstrated a novel class of 5-(1,3,4-oxadiazol-2-yl)pyrimidine derivatives showed anti-proliferative activity against MOLM-13 cells by SAR investigations to aim for metabolic stability. Decreased lipophilicity by incorporating polar group into the 1,3,4-oxadiazolyl group at the 5-position of the pyrimidine ring was effective to improve metabolic stability. Interestingly, our compounds showed selective anti-proliferative activity against multiple leukemia cell lines having FLT3/ITD mutation without inhibition of wild-type FLT3 expressing cells. As a result, some of them showed potent in vivo antitumor activity with tumor regression against sc tumor xenograft model in SCID mice. Further SAR studies of this class of FLT3 inhibitors will be reported elsewhere together with detailed biological data.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.09.031.

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15. *FLT3 kinase assays.* To evaluate the kinase inhibitory activities against FLT3 by ELISA, GST-tagged FLT3 cytoplasmic domain and biotinylated poly-(Glu/Tyr 4:1) substrate (CIS bio International) bound to the surface of 96-well assay plates were used. Phosphorylated substrate was bound to anti-phosphotyrosine antibody conjugated to europium. The bound antibody was measured using ARVO (Perkin-Elmer) at excitation/emission of 340/615 nm.
16. *Growth inhibition assays.* MOLM-13, ML-1, FLT3/Δ599, and FLT3/D835Y were maintained in RPMI1640 with 10% heat-inactivated FBS, 1% penicillin, and 1% streptomycin. HMC-1 cells were maintained in IMEM with 10% heat-inactivated FBS, 0.01% α-thioglycerol, 1% penicillin, and 1% streptomycin. These cell lines were seeded in 96-well culture plates in appropriate media and incubated for 72 h in the presence of test compounds. Cell viability was measured using the cell proliferation reagent WST-1 (Roche) according to the instructions of the manufacture; see also Ref. 19 and 20.
17. *Human liver microsomes (HLM) stability procedure.* Human pooled liver microsomes were purchased from Human and Animal Bridging Research Organization. Reaction mixtures ($n = 1$ or 2) containing test compound (0.1 or 1 μmol/L), liver microsomes (0.2 mg protein/mL), nicotinamide adenine dinucleotide phosphate, reduced form (5 mmol/L), phosphate buffer (100 mmol/L, pH 7.4), $MgCl_2$ (6 mmol/L), EDTA (0.1 mmol/L), and DMSO (0.01 or 0.001 vol%) or methanol (1 vol%) were incubated at 37 °C for constant time. Just before and after incubation, aliquots of the reaction mixtures were added to twofold volume of CH_3CN containing internal standard to stop the reaction (samples for quantitative analysis). After centrifugation, the supernatant was

analyzed by LC–MS or LC–MS/MS. The values of intrinsic clearance were calculated from percent remaining of the test compound.
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