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Discovery and evaluation of 3-phenyl-1*H*-5-pyrazolylamine-based derivatives as potent, selective and efficacious inhibitors of FMS-like tyrosine kinase-3 (FLT3)

Wen-Hsing Lin [†], Shu-Yi Hsieh [†], Shih-Chieh Yen [†], Chiung-Tong Chen, Teng-Kuang Yeh, Tsu Hsu, Cheng-Tai Lu, Ching-Ping Chen, Chun-Wha Chen, Ling-Hui Chou, Yu-Lin Huang, An-Huei Cheng, Yun-I Chang, Ya-Ju Tseng, Kuei-Rong Yen, Yu-Sheng Chao, John T.-A. Hsu *, Weir-Torn Jiaang *

Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, No. 35, Keyan Rd., Zhunan Town, Miaoli Country 350, Taiwan, ROC

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ABSTRACT

Preclinical investigations and early clinical trial studies suggest that FLT3 inhibitors offer a viable therapy for acute myeloid leukemia. However, early clinical data for direct FLT3 inhibitors provided only modest results because of the failure to fully inhibit FLT3. We have designed and synthesized a novel class of 3-phenyl-1*H*-5-pyrazolylamine-derived compounds as FLT3 inhibitors which exhibit potent FLT3 inhibition and high selectivity toward different receptor tyrosine kinases. The structure–activity relationships led to the discovery of two series of FLT3 inhibitors, and some potent compounds within these two series exhibited comparable potency to FLT3 inhibitors sorafenib (**3**) and ABT-869 (**4**) in both wt-FLT3 enzyme inhibition and FLT3-ITD inhibition on cell growth (MOLM-13 and MV4;11 cells). In particular, the selected compound **12a** exhibited the ability to regress tumors in mouse xenograft models using MOLM-13 and MV4;11 cells.

1. Introduction

The FMS-like tyrosine kinase-3 (FLT3) is a member of the class III membrane-bound receptor tyrosine kinase (RTK) family together with cKIT, FMS, and PDGFR.[1,2] Inhibition of RTK signaling pathways has become an important area of new cancer drug discovery.[3–5] Acute myeloid leukemia (AML) is the most common type of leukemia in adults. AML is an aggressive disease with rapid growth of abnormal leukemic cells in bone marrow that results in failure of production of normal blood cells. In spite of the success of initial conventional chemotherapy, there is a relatively high relapse rate for patients with AML. The failure of initial chemotherapy or the refractory nature of AML has been associated with the acquisition of mutations that is involved in the signaling pathway of proliferation and survival in leukemia cells (constitutively activate kinases essential for AML cell survival).[6,7] Activating mutations in the FLT3 receptor tyrosine kinase are present in ~30% of AML patients.[2,8] These mutations involve internal tandem duplications (ITDs) in the juxtamembrane domain of the receptor and missense point or short-length mutations in the activation loop of the tyrosine kinase domain (TKD).[9–11] In addition to FLT3 mutation, wt-FLT3 has been found to be highly expressed in most acute leukemia and FLT3 overexpression is an unfavorable prognostic factor for overall survival in AML; moreover, it has been revealed that overexpressed FLT3 had the same sensitivity to the FLT3 inhibitor as FLT3/ITD.[12,13]

Over the past decade, due to the incidence and poor prognosis associated with FLT3 and the refractory nature of this disease associated with the mutant forms, numerous agents have been developed to directly inhibit the activity of wild type and mutated FLT3, and several of them, such as lestaurtinib (CEP-701, **1**), sunitinib (SU-11248, **2**), sorafenib (BAY-43–9006, **3**) and ABT-869 (**4**) shown in Figure 1, have been investigated as potential agents for treating AML.[14] However, early clinical data for direct FLT3 inhibitors provided modest results because of limited reduction in bone marrow blasts and short-term responses. Many suggest that the reason for the minimal success of these agents may be mainly due to lack of sufficient potency and/or adequate tolerability at efficacious doses that result in the failure to afford complete and sustained inhibition of FLT3 in patients' leukemic blast cells. Therefore, second-generation FLT3 inhibitors may be required. These agents have been optimized to inhibit FLT3 with very high potency and are highly selective for both wt-FLT3 and mutants to offer less toxicity due to less 'other or off-target' inhibition.[14–16]

We initially developed, through a structure-based design, a novel class of 3-phenyl-1*H*-5-pyrazolylamine-based derivatives (Fig. 2) as

* Corresponding authors. Tel.: +886 37 246166x35712; fax: +886 37 586456.
E-mail address: wtjiaang@nhri.org.tw (W.-T. Jiaang).
† Wen-Hsing Lin, Shu-Yi Hsieh and Shih-Chieh Yen contributed equally.

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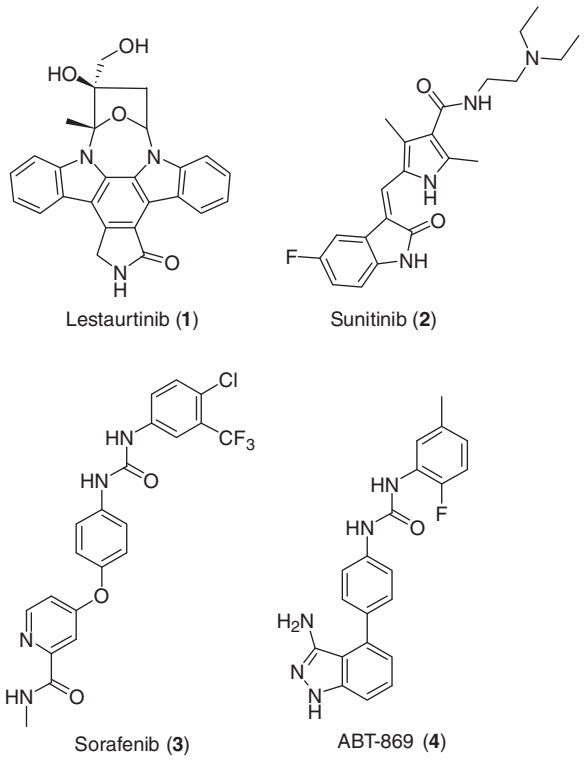


Figure 1. FLT3 inhibitors.

potential inhibitors for Aurora kinase A.[17] A number of compounds from this class showed moderate inhibition (IC_{50} >100 nM) against Aurora kinase A in enzyme-based assay, however none of them had effect on the growth of HCT-116 cells (GI_{50} >10 μM). Fortunately, a counterscreening against in-house kinases identified a sulfonamide series of benzamide compounds (Structure **I**) exhibiting both FLT3 enzymatic and cellular inhibitory activity. Utilizing a rational design strategy, we were able to identify a second sulfonamide series of pyrimidine compounds (Structure **J**) as potent inhibitors of FLT3 (Fig. 2). In this paper, the structure–activity relationships of two series of structure **I** and **J**-based FLT3 inhibitors as well as their in vitro properties are reported. This study has led to the discovery of novel and selective inhibitors of FLT3 with comparable in vitro potency to other FLT3 inhibitors **3** and **4** in clinical development. In addition, in vivo efficacy of the selected compound **12a** is also presented.

2. Chemistry

The general synthetic route for the preparation of 3-phenyl-1*H*-5-pyrazolylamine benzamide derivatives **7–10** is illustrated in Scheme 1 and these derivatives are listed in Tables 1 and 2. The synthesis began with commercially available or prepared 3 or 4-nitro substituted 3-phenyl-1*H*-5-pyrazolylamine **5** as a starting material. Compound **5** was prepared according to the literature procedures.[18,19] Benzoyl chlorides **6** with different water-solubilizing groups were prepared from their corresponding acid which are commercially available or prepared according to the literature procedures with some modifications.[16] Substituted benzoyl chlorides **6** acylate amines **5** in pyridine to form amides **7**, which were subjected to the next reaction involving reduction of the nitro groups using a Pd/C catalyst under H_2 to yield corresponding amines **7**. Amines **7** were coupled with phenyl or alkyl sulfonyl chlorides to afford the corresponding sulfonamides **8**, coupled with benzoyl chloride to yield the desired amide **9** or coupled with phenyl isocyanate to generate the desired urea **10**.

The general synthetic route for the preparation of 3-phenyl-1*H*-5-pyrazolylamine pyrimidines **12** is shown in Scheme 1 and these derivatives are listed in Table 3. Treatment of 3-phenyl-1*H*-5-pyrazolylamine **5** with 4,6-dichloropyrimidine in the presence of 4 N HCl/1,4-dioxane and DMF at 80 °C provided 4-monosubstituted pyrimidine derivatives **11**. Reaction of **11** with the appropriate 1° or 2° amines in pyridine at 80 °C gave 4,6-disubstituted pyrimidine derivatives. The crude products were subjected to reduction by the treatment with Pd/C under H_2 to yield the corresponding amines, which were subsequently coupled with phenyl or alkyl sulfonyl chlorides to obtain the final pyrimidine sulfonamides **12**.

3. Biological evaluation

3.1. In vitro kinase and cellular activity

A brief SAR study to identify potent structure **I**-based FLT3 inhibitors which have sulfonamide group at the 3-position of phenyl ring connecting with pyrazolylamine at the 3-position is shown in Table 1. The compounds were first tested in the FLT3 assay, and then potent compounds were selected to further evaluate the inhibition of proliferation of the human leukemia cell line with FLT3 mutation (MOLM-13) in a cellular assay. Compound **8a** without substituent at R^1 position was found to show modest inhibition against FLT3 (54% inhibition in the presence of 1.0 μM of compound **8a**). Introduction of an electron-withdrawing substituent ($R^1 = CF_3$ or CO_2H) at *meta*- or *para*-position of benzamide (**8b–e**) did not improve potency. The FLT3 potency improved remarkably when R^1 is water-solubilizing group. 4-Methylpiperazine benzamide **8f** was a potent FLT3 inhibitor with an IC_{50} value of 13 nM and had potent growth inhibitory activity against MOLM-13 cells with GI_{50} value of 12 nM. The crucial result indicated that the difference in solubilizing group between **8f** and **8d** affects the binding to the active site of FLT3 enzyme and provide a direction to optimize the skeleton (Structure **I**) further. Next, the position of sulfonamide group at the phenyl ring was investigated. A significant drop in both enzymatic and cellular potency was observed when the sulfonamide group of **8f** was moved from the *meta*-position to *para*-position (**8g**). The importance of sulfonamide group was further demonstrated by the significant loss of cellular and/or enzymatic

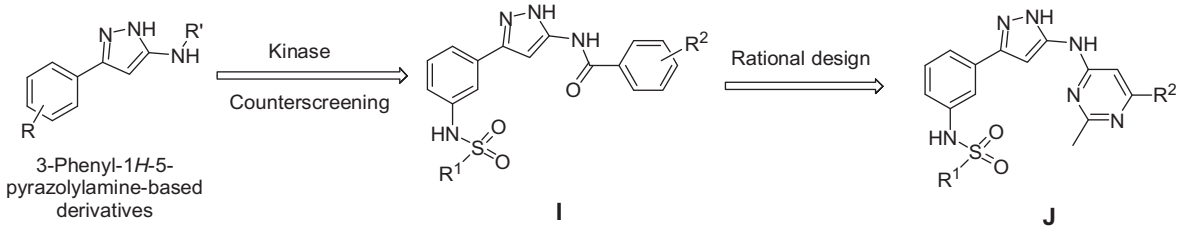
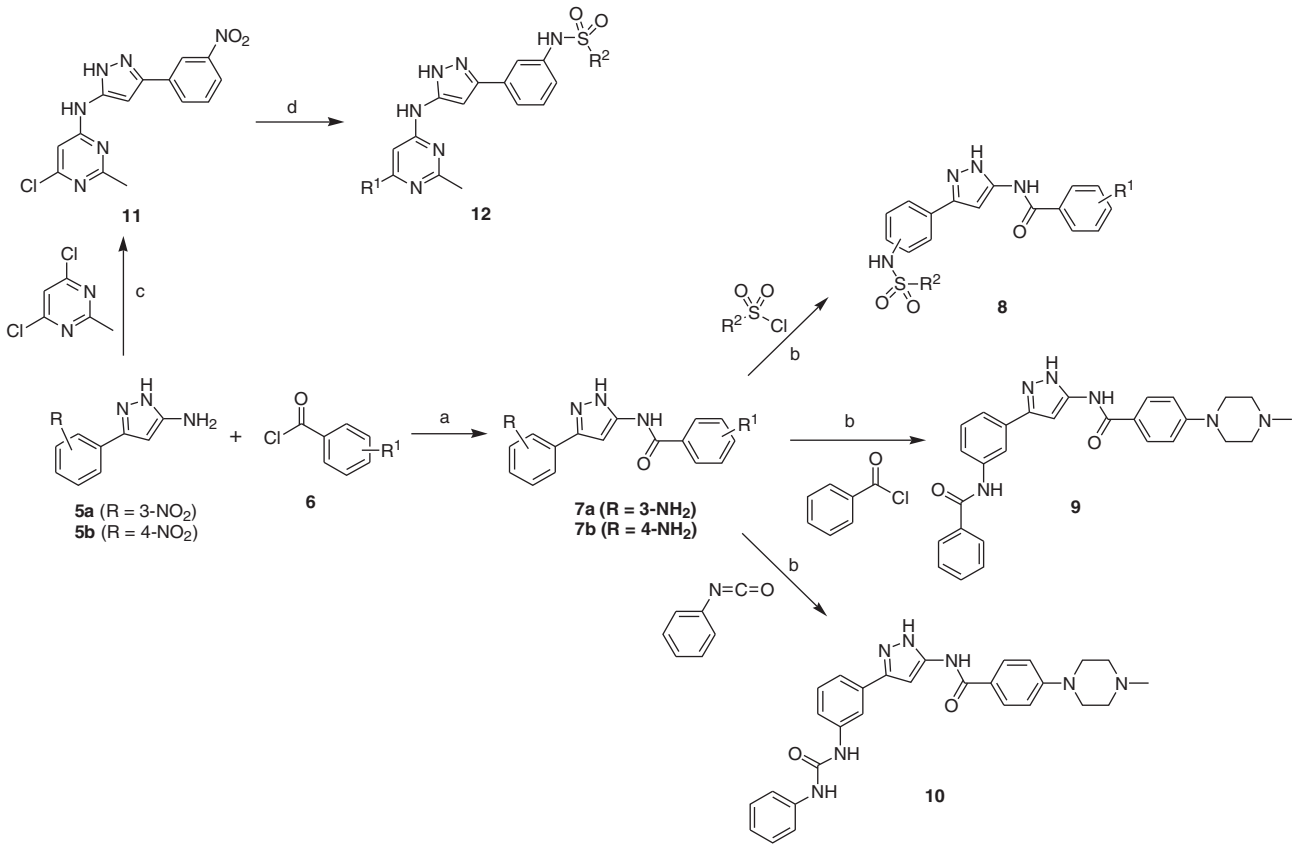


Figure 2. Identification of 3-phenyl-1*H*-5-pyrazolylamine-based derivatives as FLT3 inhibitors.

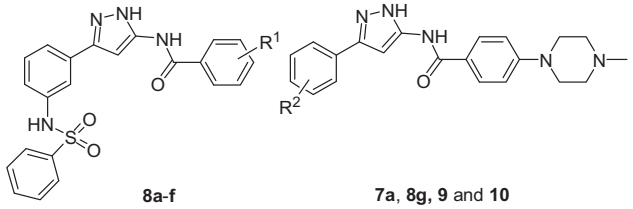


Scheme 1. Reagents and conditions: (a) (i) Pyridine, rt; (ii) H_2, Pd/C; (b) pyridine, rt; (c) 4 N HCl/1,4-dioxane, DMF, 80 °C; (d) (i) 1° or 2° amines, pyridine, 80 °C; (ii) H_2, Pd/C; (iii) R^2SO_2Cl, pyridine, rt.

potency suffered by the amide analogue **9**, urea analogue **10** and unsubstituted phenylamine **7a**.

Having demonstrated the importance of the water-solubilizing group and sulfonamide moiety, we then focused on investigating the effects on the change of terminal phenyl ring and solubilizing group of compound **8f**. In addition to FLT3, further screening of the 3-phenyl-1*H*-5-pyrazolylamine sulfonamides against vascular endothelial growth factor receptor (VEGFR) and Aurora kinase A is also present in Table 2. Firstly, we investigated the effects on the substitution of sulfonamide moiety. As shown in Table 2, chloro substitution at *meta*-position (**8h**) and methyl substitution at *meta*- and *para*-position (**8j**–**k**) provided very similar inhibitory potency against FLT3 (IC_{50} = 7–15 nM) when compared with the unsubstituted **8f**. Incorporation of chloro and methoxy group at the *para*-position resulted in significant loss of FLT3 potency (*p*-Cl **8i**: FLT3 IC_{50} = 120 nM and *p*-OCH_3 **8l**: FLT3 IC_{50} = 90 nM). Only **8h** is potent VEGFR1 inhibitor (IC_{50} = 40 nM), **8f** and **8i**–**l** were not potent against VEGFR and Aurora kinase A (IC_{50} >120 nM). In AML cell line MOLM-13, *meta*-methyl analogue **8j** and *para*-methyl analogue **8k** displayed a similar cellular activity to that shown by **8f**. When the terminal phenyl ring of sulfonamide was replaced by alkyl groups such as ethyl group (**8m**), *n*-propyl group (**8n**) and isopropyl group (**8o**), the compounds **8m**–**o** were found to be more potent in the cellular inhibition assay (MOLM-13) than in the enzymatic inhibition assay (FLT3). The likely reasons for this behavior include changes in enzyme conformation between the in vitro system and cells and the difference in amount of protein present in the two assays.[16] These alkyl substituted sulfonamides (**8m**–**o**) exhibited weak to modest inhibition against VEGFR1–2 and Aurora A (IC_{50} >1 μM). Next, we investigated the effects on the change of solubilizing group on the

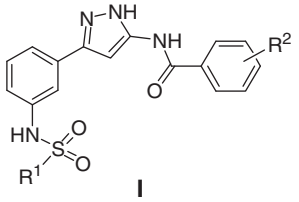
Table 1
Identification of sulfonamide series of inhibitors



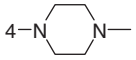
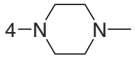
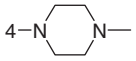
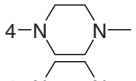
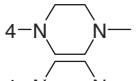
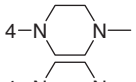
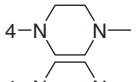
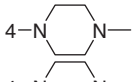
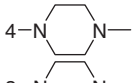
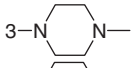
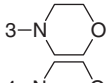
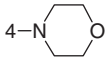
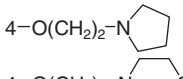
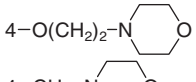
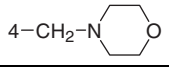
Compd	R^1	R^2	IC_{50} (μM) wt-FLT3	GI_{50}[b] (μM) MOLM-13
8a	H		54%[a]	ND
8b	*p*-CF_3		>1.0	ND
8c	*m*-CF_3		>1.0	ND
8d	*p*-CO_2H		61%[a]	ND
8e	*m*-CO_2H		56%[a]	ND
8f			0.013	0.012
8g			0.59	0.050
9			0.27	0.21
10			0.020	0.15
7a		3-NH_2	0.014	0.077

[a] Inhibition at 1 μM.
[b] ND: not determined.

Table 2
Inhibitory activity of structure-**I** sulfonamide analogues



I

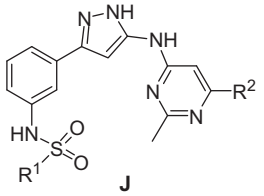
Compd	R^1	R^2	IC_{50} (μM)				GI_{50} (μM)
			wt-FLT3	VEGFR1	VEGFR2	Aurora A	MOLM-13 Cell
8f	C_6H_5		0.013	0.21	0.13	0.34	0.012
8h	*m*-Cl–C_6H_5		0.008	0.04	0.25	1.71	0.039
8i	*p*-Cl–C_6H_5		0.12	0.57	>1.0	4.99	0.074
8j	*m*-CH_3–C_6H_5		0.007	0.14	0.07	0.43	0.016
8k	*p*-CH_3–C_6H_5		0.015	>1.0	>1.0	50%[a]	0.024
8l	*p*-CH_3O–C_6H_5		0.09	0.42	0.16	>1.0	0.05
8m	Ethyl		0.36	>1.0	>1.0	>1.0	0.033
8n	*n*-Propyl		0.10	2.61	2.72	1.64	0.056
8o	Isopropyl		0.16	>1.0	>1.0	>1.0	0.044
8p	C_6H_5		0.065	>1.0	0.41	0.85	0.19
8q	C_6H_5		>1.0	>1.0	>1.0	>1.0	0.26
8r	C_6H_5		0.029	>1.0	0.45	0.39	0.10
8s	C_6H_5		0.007	0.27	0.20	1.17	0.025
8t	C_6H_5		0.07	>1.0	>1.0	50%[a]	0.072
8u	C_6H_5		0.35	52%[a]	0.56	0.22	0.067

[a] Inhibition at 1 μM.

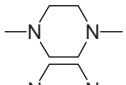
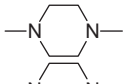
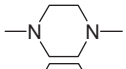
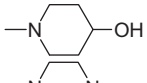
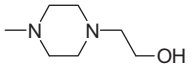
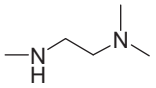
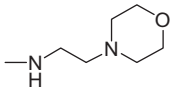
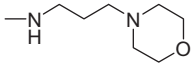
benzamide. Moving the *N*-methylpiperazine group of **8f** to the 3-position (**8p**) had a significant loss in both FLT3 enzymatic and cellular potency. Replacing the *N*-methylpiperazine ring of **8p** with morpholine (**8q**) had a profound negative effect on inhibition against FLT3, but it was approximately equipotent with **8p** in the MOLM-13 cell proliferation assay. Due to no improvement in installation of the water-solubilizing group at 3-position, we turned our attention to study the effect on varying the solubilizing group at 4-position. By keeping the phenyl ring of sulfonamide moiety, compound **8r** with a morpholine group is detrimental to cell potency compared to **8f**. Installation of the water solubilizing pyrrolidine group linked by a two-carbon ether tether yielded compound **8s** with equal FLT3 enzymatic and cellular potency. Morpholinoethoxy analogue **8t** was almost sixfold less active than **8f** in both the enzyme and cell proliferation assay. In comparison with **8t**, shortening by one carbon (**8u**) decreased the inhibitory potency of FLT3 by fivefold, had no effect on cell potency. As we can see for the selectivity profile of *N*-methylpiperazines **8f** and **8i**–**o**, these analogues **8p**–**u** also exhibited weak to modest inhibition against VEGFR1–2 and Aurora A kinases.

According to literature reports, the 3-aminopyrazole moiety is stereochemically well suited to form hydrogen bonding interactions with the kinase hinge region of the ATP pocket[17] and 2-methylpyrimidine is a known scaffold for kinase inhibitors.[20] Based on these facts, we replaced the benzamide with 2-methyl-pyrimidine which had a solubilizing group at 4-position to create structure-**J** sulfonamide analogues. As shown in Table 3, pyrimidine **12a** maintained potency in both enzymatic (FLT3, VEGFR1, VEGFR2 and Aurora A) and cellular (MOLM-13) assays compared to the related benzamide **8f**. Incorporation of *m*-methyl substitution (**12b**) at the sulfonamide terminal phenyl ring of **12a** and replacement of terminal phenyl ring of **12a** with *n*-butyl group (**12c**) had detrimental effects in potency. Next, we investigated the effects of water solubilizing groups on the 4-position of pyrimidine ring and compared the potency of these compounds (**12d**-**h**) with that of *N*-methylpipera-

Table 3
Inhibitory activity of structure-**J** sulfonamide analogues



J

Compd	R^1	R^2	IC_{50} (μM)				GI_{50} (μM)
			wt-FLT3	VEGFR1	VEGFR2	Aurora A	MOLM-13 Cell
12a	C_6H_5		0.006	0.31	0.29	0.29	0.021
12b	*m*-CH_3–C_6H_5		0.12	>1000	>1000	>1000	0.078
12c	*n*-Butyl		0.11	>1000	>1000	>1000	0.16
12d	C_6H_5		0.01	>1000	>1000	0.54	0.12
12e	C_6H_5		0.05	>1000	0.14	0.17	0.024
12f	C_6H_5		0.071	>1000	55%[a]	>1000	0.18
12g	C_6H_5		0.06	>1000	>1000	>1000	0.46
12h	C_6H_5		0.01	>1000	55%[a]	0.68	0.38
3			0.054	0.018	0.003	3.8	0.056
4			0.02	0.037	0.005	0.76	0.037

[a] Inhibition at 1 μM.

Table 4
Comparison of growth inhibitory profile of **12a** with ureas **3** and **4** on leukemia cell lines

Cell lines	Cell proliferation, GI_{50} (μM)		
	12a	**3**	**4**
MOLM-13	0.021	0.056	0.037
MV4;11	0.027	0.030	0.040
RS4;11	10	9.4	9.2
K562	>10	6.2	>20
U937	>20	3.4	19
MOLT-4	7.4	9.0	6.7

zine (**12a**). 4-Hydroxypiperidine (**12d**) was approximately equipopotent in the FLT3 assay, but it was about sixfold weaker in the MOLM-13 cell proliferation assay. On the countrary, *N*-(2-hydroxyethyl)piperazine (**12e**) was eightfold weaker in enzymatic assay, but maintained potency in cellular assay. Furthermore, we brought the nitrogen out of the ring system and linked water solubilizing group with a two-carbon tether, such as *N*,*N*-dimethylethylenediamine (**12f**) or 4-(2-aminoethyl)morpholine (**12g**), leading to a dramatic decrease in both inhibitory activity and anti-proliferative activity against FLT3 enzyme and MOLM-13 cells, respectively. As for three-carbon tether, *N*-(3-aminopropyl)morpholine (**12h**) had a minimal effect on enzymatic potency but resulted in a 19-fold loss in cellular potency compared to **12a**.

In comparison with urea FLT3 inhibitors **3** and **4** currently in clinical development (Table 3), some potent structure-**I** and -**J** sulfonamide compounds shown in Tables 2 and 3 are more selective

Table 5
Pharmacokinetic parameters for compounds **8f**, **8j**, **8s** and **12a**[a]

Parameter[b]	Unit	Compound			
		8f	**8j**	**8s**	**12a**
$AUC_{0\text{-inf}}$	ng/mL h	551	1202	3937	1402
CL	mL/min/kg	102.4	67.4	22.3	57.4
Vd_{ss}	L/kg	15.5	2.6	10.7	6.6
$t_{1/2}$	h	4.5	1.7	9.9	4.2
F	%	ND[c]	ND	ND	ND

[a] Sprague–Dawley rat PK. Animals were dosed at 5/20 mg/kg iv/po.
[b] PK parameters derived from iv dosing.
[c] ND: not detected.

for FLT3 over VEGFR1 and VEGFR2. In addition, representative compound **12a** was assayed against a panel of selected eight protein tyrosine kinases at 0.1 μM concentration. This result indicates that **12a** shows strong inhibition against TRKA (96%) and RET (71%), modest to weak inhibition against SRC (44%), VEGFR3 (31%), c-Kit (27%), PDGFRα (24%), PDGFRβ (18%) and CSF1R (FMS, 16%). Furthermore, we evaluated the effects of **12a** on cell growth of AML cell lines with or without FLT3 mutations (Table 4). In addition to MOLM-13, compound **12a** showed growth inhibitory activity against FLT3/ITD harboring human AML cell line MV4;11 with a GI_{50} value of 27 nM. Compound **12a** was equipotent or slightly more potent than **3** and **4** in MOLM-13 and MV4;11 cells. In wt-FLT3 cell, RS4;11, the anti-proliferative effect of **12a** was not significant (GI_{50} = 10 μM). Compound **12a** did not showed growth inhibitory activities against K562 cells which had wt-BCR/ABL[13]

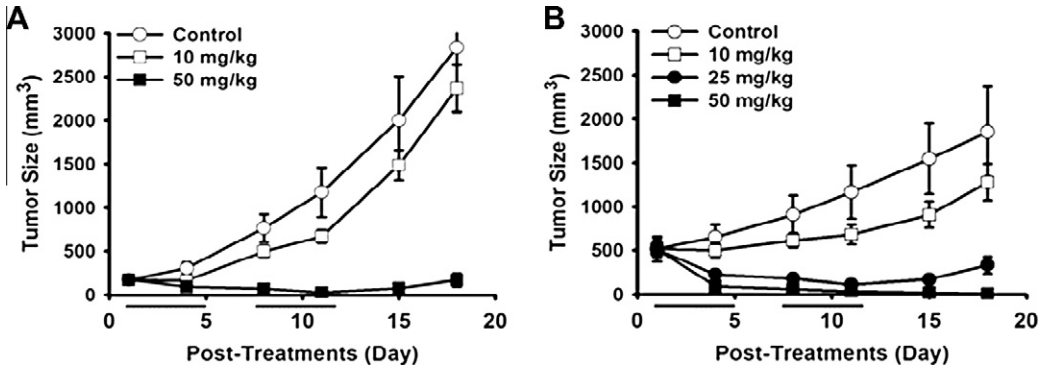


Figure 3. Compound **12a** demonstrates antitumor efficacy after intravenous (iv) treatment in the MOLM-13 (A) and MV4;11 (B) xenograft models (qd for 1–5 and 8–12 days). Tumor sizes are expressed as mean ± SEM (n = 7–8/group).

and U937 cells which did not express FLT3[21] (GI_{50} >10 μM). Compound **12a** had weak effect on MOLT-4 cell proliferation (GI_{50} = 7.4 μM). The growth inhibitory profile of **12a** in leukemia cell lines is similar to that of ureas **3** and **4**.

3.2. In vivo efficacy in tumor models

Compounds (**8f**, **8j**, **8s** and **12a**) with promising in vitro enzymatic and cellular inhibitory activity were further evaluated for their toxicities in mice. The pharmacokinetic studies in rats indicate that these four compounds are not orally available (Table 5). Therefore, compounds were administered via the intravenous route in the toxicity studies. The results indicated that compound **12a** was the safest of these potent compounds; it was well tolerated with no body weight loss at the highest intravenous dose of 50 mg/kg tested for continuous 5 days in normal mice. On the contrary, compound **8f**, **8j** or **8s** caused body weight loss and/or lethality at the same intravenous dose. Based on this reason, pyrimidine **12a** was chosen for further evaluation of its in vivo efficacy in the MOLM-13 and MV4;11 tumor xenograft models. Figure 3A illustrates the dose-dependent tumor growth inhibition in the MOLM-13 xenograft model. Compound **12a** was dosed at 10 and 50 mg/kg given iv qd for 1–5 and 8–12 days. At 10 mg/kg of **12a**, tumor growth was inhibited during the first 5 days of dosing, after which they started regrowing during the dosing period. At the dose of 50 mg/kg, **12a** was able to cause the complete regression of established tumors during the dosing period. In the MV4;11 tumor xenograft model (Fig. 3B), iv treatment of **12a** at doses of 10, 25 and 50 mg/kg once a day was initiated when the MV4;11 tumors reached an average of 500 mm^3 in volume. The 50 mg/kg dose of **12a** resulted in rapid and complete regression of tumors and the tumor volume stayed suppressed after dosing was halted, while tumors appeared to regrow after 20 days. No significant body weight loss (<3%) was observed in animals treated with **12a** at all doses of both xenograft models. These results demonstrate that **12a** can effectively reduce tumor size within 2 weeks of treatment and is well tolerated in mice at efficacious dose.

4. Conclusion

Through counterscreening and rational design approaches, we identified a novel class of 3-phenyl-1*H*-5-pyrazolylamine-based sulfonamide derivatives which contains benzamide series (structure-**I**) and pyrimidine series (structure-**J**) as FLT3 inhibitors. This report describes the SAR studies of these two series of compounds to develop some potent and selective FLT3 inhibitors. These potent compounds exhibited equipotent or slightly more potent than other FLT3 inhibitors **3** and **4** in both enzymatic and cellular assays. The extensive kinase profiling has proven that selected compound **12a** is highly selective for FLT3 over the closely related kinases, VEGFR1–3, SRC, c-Kit, PDGFRα, PDGFRβ and CSF1R. In addition, compound **12a** showed highly antitumor efficacy and tolerability in the MOLM-13 and MV4;11 human AML xenograft models.

5. Experimental

5.1. Chemistry

All commercial chemicals and solvents are reagent grade and were used without further treatment unless otherwise noted. ^{1}H NMR spectra were obtained with a Varian Mercury-300 or a Varian Mercury-400 spectrometer. Chemical shifts were recorded in parts per million (ppm, δ) and were reported relative to the solvent peak or TMS. LC/MS data were measured on an Agilent MSD-1100 ESI-MS/MS System. High-resolution mass spectra (HRMS) were measured with a Finnigan (MAT-95XL) electron impact (EI) mass spectrometer. Flash column chromatography was done using silica gel (Merck Kieselgel 60, No. 9385, 230–400 mesh ASTM). Reactions were monitored by TLC using Merck 60 F_{254} silica gel glass backed plates (5 × 10 cm); zones were detected visually under ultraviolet irradiation (254 nm) or by spraying with phosphomolybdic acid reagent (Aldrich) followed by heating at 80 °C. All starting materials and amines were commercially available unless otherwise indicated.

5.2. General procedure for the preparation of compounds 8, 9 and 10

The starting materials **5** were synthesized according to the literature report.[18,19] Pyrazolylamine **5** (1.0 equiv) in pyridine at room temperature was added acyl chloride **6** (1.2 equiv). After 1 h of stirring at room temperature, the reaction mixture was evaporated to dryness and the residue suspended in 1 N $NaHCO_3$. The suspension was vigorously stirred at room temperature for 1 h, and the resultant solid was collected by filtration and dried in vacuo. The crude amides were used for the next reaction without further purification. To a solution of crude amides prepared from last step in MeOH was added 12 N HCl (2.0 equiv) and a catalytic amount of 10% Pd/C under argon at room temperature. The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 12 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The crude products were purified by flash column chromatography (eluted with CH_2Cl_2/CH_3OH) yielded the desired amines **7a** and **7b**. Only one of **7a** and one of **7b** are selected to show its NMR spectrum and Mass.

5.2.1. *N*1-[3-(3-Aminophenyl)-1*H*-5-pyrazolyl]-4-(4-methylpiperazino)benzamide (7a)

Mp 272–274 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.69 (s, 1H), 10.48 (s, 1H), 7.91 (d, J = 8.8 Hz, 2H), 7.07 (t, 1H) 6.97 (d, J = 8.8 Hz, 2H), 6.87 (d, J = 8.0 Hz, 3H), 6.53 (d, J = 7.6 Hz, 1H), 5.18 (s, 2H), 3.27 (t, J = 4.8 Hz, 4H), 2.43 (t, J = 5.0 Hz, 4H), 2.21 (s, 3H); MS (ES$^+$) m/z calcd for $C_{21}H_{24}N_6O$: 376.20; found: 377.2 (M+H$^+$).

5.2.2. *N*1-[3-(4-Aminophenyl)-1*H*-5-pyrazolyl]-4-(4-methylpiperazino)benzamide (7b)

Mp 251–252 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.41 (s, 1H), 10.43 (s, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 7.6 Hz, 2H), 6.96 (d, J = 8.8 Hz, 2H), 6.71 (s, 1H), 6.59 (d, J = 8.0 Hz, 2H), 5.30 (s, 2H), 3.26 (t, J = 4.2 Hz, 4H), 2.42 (t, J = 4.4 Hz, 4H), 2.21(s, 3H); MS (ES$^+$) m/z calcd for $C_{21}H_{24}N_6O$: 376.20; found: 377.2 (M+H$^+$), 399.2 (M+Na$^+$).

To a solution of **7a** or **7b** (1.0 equiv) and pyridine (5.0 equiv) in DMSO at room temperature was added sulfonyl chlorides, benzoyl chloride or phenyl isocyanate (1.2 equiv). After 1 h of stirring at room temperature, the reaction mixture was evaporated to dryness and the residue suspended in 1 N $NaHCO_3$. The suspension was vigorously stirred at room temperature for 1 h before the resultant solid was collected by filtration and dried in vacuo. The crude products were purified by flash column chromatography (eluted with CH_2Cl_2/CH_3OH) yielded the desired sulfonamides **8**, amide **9** or urea **10**.

5.2.3. *N*1-(3-3-[(Phenylsulfonyl)amino]phenyl-1*H*-5-pyrazolyl) benzamide (8a)

Mp 106–109 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.92 (br s, 1H), 10.40 (s, 1H), 8.02 (d, J = 7.2 Hz, 2H), 7.80 (dt, J = 1.8, 6.3 Hz, 2H), 7.61–7.41 (m, 9H), 7.30 (t, J = 7.8 Hz, 1H), 7.07–7.04 (m, 1H); MS (ES$^+$) m/z Calcd for $C_{22}H_{18}N_4O_3S$: 418.1; found: 419.1 (M+H$^+$).

5.2.4. *N*1-(3-3-[(Phenylsulfonyl)amino]phenyl-1*H*-5-pyrazolyl)-4-(trifluoromethyl)benzamide (8b)

Mp 237–240 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.00 (s, 1H), 11.18 (s, 1H), 10.43 (br s, 1H), 8.20 (d, J = 8.0 Hz, 2H), 7.89 (d, J = 7.6 Hz, 2H), 7.80 (d, J = 8.0 Hz, 2H), 7.64–7.52 (m, 3H), 7.43 (d, J = 8.4 Hz, 2H), 7.31 (t, J = 8.0 Hz, 1H), 7.06 (d, J = 7.6 Hz, 1H), 6.93 (s, 1H); MS (ES$^+$) m/z Calcd for $C_{23}H_{17}F_3N_4O_3S$: 486.48; found: 487.1 (M+H$^+$).

5.2.5. *N*1-(3-3-[(Phenylsulfonyl)amino]phenyl-1*H*-5-pyrazolyl)-3-(trifluoromethyl) benzamide (8c)

Mp 117–120 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.05 (s, 1H), 11.25 (s, 1H), 10.44 (s, 1H), 8.40–8.21 (m, 2H), 7.99–7.94 (m, 1H), 7.82–7.75 (m, 3H), 7.63–7.54 (m, 4H), 7.51–7.37 (m, 2H), 7.32 (t, J = 7.6 Hz, 1H), 7.08 (d, J = 7.6 Hz, 1H), 6.95 (s, 1H); MS (ES$^+$) m/z calcd for $C_{23}H_{17}F_3N_4O_3S$: 486.47; found: 487.0 (M+H$^+$)

5.2.6. 4-[(3-3-[Amino(phenylsulfonyl)methyl]phenyl-1*H*-5-pyrazolyl)amino]carbonylbenzoic acid (8d)

Mp 338–340 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.12 (s, 1H), 10.42 (s, 1H), 8.08 (ABq, $\Delta\nu_{AB}$ = 13.2 Hz, J_{AB} = 8.7 Hz, 4H), 7.80 (td, J = 1.6 Hz, J = 6.0 Hz, 2H), 7.61–7.42 (m, 5H), 7.31 (t, J = 7.9 Hz, 1H), 7.06 (d, J = 9.32 Hz, 1H), 6.83 (s, 1H), 5.76 (br s, 2H); MS (ES$^+$) m/z calcd for $C_{23}H_{18}N_4O_5S$: 462.49; found: 463.1 (M+H$^+$).

5.2.7. 3-[(3-3-[(Phenylsulfonyl)amino]phenyl-1*H*-5-pyrazolyl) amino]carbonylbenzoic acid (8e)

^{1}H NMR (300 MHz, DMSO-d_6): δ 11.57 (s, 1H), 10.84 (s, 1H), 8.99 (s, 1H), 8.67 (d, J = 8.1 Hz, 1H), 8.55 (d, J = 7.8 Hz, 1H), 8.26–8.18 (m, 2H), 8.12–7.92 (m, 4H), 7.92–7.82 (m, 2H), 7.72 (t, J = 7.8 Hz, 1H), 7.48 (d, J = 7.2 Hz, 1H), 7.24 (s, 1H); MS (ES$^+$) m/z calcd for $C_{23}H_{18}N_4O_5S$: 462.49; found: 463.1 (M+H$^+$).

5.2.8. *N*1-(3-3-[(Phenylsulfonyl)amino]phenyl-1*H*-5-pyrazolyl)-4-(4-methylpiperazino) benzamide (8f)

Mp 247–248 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.88 (s, 1H), 10.55 (s, 1H), 10.41(s, 1H), 7.92 (d, J = 8.0 Hz, 2H), 7.79 (d, J = 7.2 Hz, 2H), 7.62–7.53 (m, 3H), 7.42–7.29 (m, 3H), 7.05–6.98 (m, 4H), 3.29 (t, J = 4.8 Hz, 4H), 2.45 (t, J = 4.8 Hz, 4H), 2.23 (s, 3H); MS (ES$^+$) m/z calcd for $C_{27}H_{28}N_6O_3S$: 516.62; found: 517.3 (M+H$^+$); HRMS (FAB) calcd for $C_{27}H_{28}N_6O_3S$: 516.1944; found: 516.1937.

5.2.9. *N*1-(3-4-[(Phenylsulfonyl)amino]phenyl-1*H*-5-pyrazolyl)-4-(4-methylpiperazino) benzamide (8g)

Mp 274–276 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.73 (s, 1H), 10.48 (s, 1H), 10.44 (s, 1H), 7.91 (d, J = 8.7 Hz, 2H), 7.79 (d, J = 6.6 Hz, 2H), 7.64–7.53 (m, 5H), 7.15 (d, J = 8.4 Hz, 2H), 6.97 (d, J = 8.4 Hz, 1H), 6.91 (s, 1H), 3.28 (t, J = 4.8 Hz, 4H), 2.44 (t, J = 4.8 Hz, 4H), 2.22 (s, 3H); MS (ES$^+$) m/z calcd for $C_{27}H_{28}N_6O_3S$: 516.62; found: 517.2 (M+H$^+$); HRMS (FAB) calcd for $C_{27}H_{28}N_6O_3S$: 516.1944; found: 516.1951.

5.2.10. *N*1-[3-(3-[(3-Chlorophenyl)sulfonyl]aminophenyl)-1*H*-5-pyrazolyl]-4-(4-methylpiperazino)benzamide (8h)

Mp 204–207 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.63 (s, 1H), 7.93 (d, J = 11.0 Hz, 2H), 7.79–7.68 (m, 3H), 7.61–7.55 (m, 3H), 7.50–7.29 (m, 4H), 7.06–6.97 (m, 3H), 6.80 (br s, 1H), 3.28 (br s, 4H), 2.44 (br s, 4H), 2.22(s, 3H); MS (ES$^+$) m/z calcd for $C_{27}H_{28}N_6O_3S$: 550.06; found: 551.0 (M+H$^+$); HRMS (FAB) calcd for $C_{27}H_{27}ClN_6O_3S$: 550.1554; found: 550.1545.

5.2.11. *N*1-[3-(3-[(4-Chlorophenyl)sulfonyl]aminophenyl)-1*H*-5-pyrazolyl]-4-(4-methylpiperazino)benzamide (8i)

Mp 205–207 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.89 (s, 1H), 12.61 (s, 1H), 10.59 (s, 1H), 7.92 (d, J = 8.1 Hz, 2H), 7.67 (d, J = 8.7 Hz, 2H), 7.66–7.58 (m, 3H), 7.45–7.31 (m, 3H), 7.05–6.98 (m, 2H), 6.91 (s, 1H), 3.29 (t, J = 4.8 Hz, 4H), 2.45 (t, J = 4.8 Hz, 4H), 2.23 (s, 3H); MS (ES$^+$) m/z calcd for $C_{27}H_{27}ClN_6O_3S$: 551.06; found: 551.1 (M+H$^+$).

5.2.12. *N*1-[3-(3-[(3-Methylphenyl)sulfonyl]aminophenyl)-1*H*-5-pyrazolyl]-4-(4-methylpiperazino)benzamide (8j)

Mp 200–202 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.89 (s, 1H), 10.55 (s, 1H), 10.36 (s, 1H), 7.93 (d, J = 8.8 Hz, 2H), 7.63–7.58 (m, 2H), 7.46–7.40 (m, 4H), 7.31 (t, J = 8.0 Hz, 1H), 7.05–6.97 (m, 3H), 6.89 (s, 1H), 3.28 (t, J = 4.8 Hz, 4H), 2.44 (t, J = 4.8 Hz, 4H), 2.34 (s, 3H), 2.22 (s, 3H); MS (ES$^+$) m/z calcd for $C_{28}H_{30}N_6O_3S$: 530.64; found: 531.1 (M+H$^+$); HRMS (FAB) calcd for $C_{28}H_{30}N_6O_3S$: 530.2100; found: 530.2108.

5.2.13. *N*1-[3-(3-[(4-Methylphenyl)sulfonyl]aminophenyl)-1*H*-5-pyrazolyl]-4-(4-methylpiperazino)benzamide (8k)

Mp 169–170 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.59 (s, 1H), 7.93 (d, J = 8.7 Hz, 2H), 7.69 (d, J = 8.4 Hz, 2H), 7.51–7.27 (m, 5H), 7.07–6.99 (m, 3H), 6.88 (s, 1H), 3.30 (t, J = 4.8 Hz, 4H), 2.46 (t, J = 4.8 Hz, 4H), 2.33 (s, 3H), 2.24 (s, 3H); MS (ES$^+$) m/z calcd for $C_{28}H_{30}N_6O_3S$: 530.64; found: 531.1 (M+H$^+$).

5.2.14. *N*1-[3-(3-[(4-Methoxyphenyl)sulfonyl]aminophenyl)-1*H*-5-pyrazolyl]-4-(4-methylpiperazino)benzamide (8l)

Mp 155–158 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.89 (s, 1H), 10.56 (s, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.73 (dd, J = 7.5, 1.5 Hz, 2H), 7.40 (d, J = 7.2 Hz, 2H), 7.29 (t, J = 7.8 Hz, 1H), 7.09–6.97 (m, 5H), 6.89 (s, 1H), 3.77 (s, 3H), 3.28 (t, J = 4.8 Hz, 4H), 2.44 (t, J = 4.8 Hz, 4H), 2.22 (s, 3H); MS (ES$^+$) m/z calcd for $C_{28}H_{30}N_6O_4S$: 546.64;

found: 547.1 ($M+H^+$); HRMS (FAB) calcd for $C_{28}H_{30}N_6O_4S$: 546.2049; found: 546.2056.

5.2.15. *N*1-(3-3-[(Ethylsulfonyl)amino]phenyl-1*H*-5-pyrazolyl)-4-(4-methylpiperazino) benzamide (8m)

Mp 256–258 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 12.61 (s, 1H), 10.56 (s, 1H), 9.90 (s, 1H), 7.92 (d, J = 8.1 Hz, 2H), 7.47 (d, J = 7.8 Hz, 1H), 7.52–7.39 (m, 2H), 7.17 (d, J = 6.9 Hz, 1H), 6.98 (d, J = 7.8 Hz, 1H), 3.29 (d, J = 4.5 Hz, 4H), 3.16–3.14 (m, 2H), 2.44 (t, J = 4.5 Hz, 4H), 2.22 (s, 3H), 1.22 (t, J = 7.2 Hz, 3H); MS (ES^+) m/z calcd for $C_{23}H_{28}N_6O_3S$: 468.6; found: 491.9 (M+Na), 469.1 ($M+H^+$).

5.2.16. *N*1-(3-3-[(Propylsulfonyl)amino]phenyl-1*H*-5-pyrazolyl)-4-(4-methylpiperazino) benzamide (8n)

Mp 228–230 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 12.92 (s, 1H), 10.57 (s, 1H), 9.90 (s, 1H), 7.93 (d, J = 8.4 Hz, 2H), 7.52–7.40 (m, 3H), 7.17 (d, J = 7.5 Hz, 1H), 7.00–6.97 (m, 3H), 3.29 (t, J = 4.8 Hz, 4H), 3.12 (t, J = 7.5 Hz, 2H), 2.44 (t, J = 4.8 Hz, 4H), 2.22 (s, 3H), 1.71 (qt, J = 7.5, 7.5 Hz, 2H), 0.95 (t, J = 7.5 Hz, 3H); MS (ES^+) m/z calcd for $C_{24}H_{30}N_6O_3S$: 482.59; found: 483.1 ($M+H^+$); HRMS (FAB) calcd for $C_{24}H_{30}N_6O_3S$: 482.2100; found: 482.2103.

5.2.17. *N*1-(3-3-[(Isopropylsulfonyl)amino]phenyl-1*H*-5-pyrazolyl)-4-(4-methylpiperazino) benzamide (8o)

Mp 170–172 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 10.56 (s, 1H), 9.87 (s, 1H), 7.92 (d, J = 7.8 Hz, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.54–7.37 (m, 2H), 7.19 (d, J = 7.8 Hz, 1H), 6.99 (d, J = 8.4 Hz, 1H), 3.29 (t, J = 4.8 Hz, 4H), 2.44 (t, J = 4.8 Hz, 4H), 2.22 (s, 3H), 1.81 (s, 1H), 1.27 (s, 3H), 1.25 (s, 3H); MS (ES^+) m/z calcd for $C_{24}H_{30}N_6O_3S$: 482.6; found: 505.1 (M+Na), 483.1 ($M+H^+$).

5.2.18. *N*1-(3-3-[(Phenylsulfonyl)amino]phenyl-1*H*-5-pyrazolyl)-3-(4-methylpiperazino) benzamide (8p)

Mp 104–105 °C. ^1H NMR (400 MHz, DMSO-d_6): δ 12.96 (s, 1H), 10.86 (s, 1H), 10.43 (s, 1H), 7.80 (d, J = 7.2 Hz, 2H), 7.62- 7.53 (m, 4H), 7.43–7.39 (m, 3H), 7.34–7.28 (m, 2H), 7.12 (d, J = 6.4 Hz, 1H), 7.05 (d, J = 8.0 Hz, 1H), 6.91 (s, 1H), 3.23 (t, J = 4.8 Hz, 4H), 2.48 (t, J = 4.8 Hz, 4H), 2.24 (s, 3H); MS (ES^+) m/z calcd for $C_{27}H_{28}N_6O_3S$: 516.62; found: 517.1 ($M+H^+$); HRMS (FAB) calcd for $C_{27}H_{28}N_6O_3S$: 516.1944; found: 516.1952.

5.2.19. *N*1-(3-3-[(Phenylsulfonyl)amino]phenyl-1*H*-5-pyrazolyl)-3-morpholino benzamide (8q)

^1H NMR (400 MHz, DMSO-d_6): δ 12.96 (s, 1H), 10.87 (s, 1H), 10.42 (s, 1H), 7.80 (d, J = 8.4 Hz, 2H), 7.63- 7.53 (m, 4H), 7.46–7.41 (m, 3H), 7.35 (t, J = 8.0 Hz, 1H), 7.30 (t, J = 8.0 Hz, 1H), 7.15 (d, J = 8.4 Hz, 1H), 7.05 (d, J = 8.0 Hz, 1H), 6.92 (br s, 1H), 3.77 (t, J = 4.8 Hz, 4H), 3.20 (t, J = 4.8 Hz, 4H); MS (ES^+) m/z calcd for $C_{26}H_{25}N_5O_4S$: 503.57; found: 504.0 ($M+H^+$).

5.2.20. *N*1-(3-3-[(Phenylsulfonyl)amino]phenyl-1*H*-5-pyrazolyl)-4-morpholino benzamide (8r)

Mp 257–260 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 12.86 (s, 1H), 10.59 (s, 1H), 10.39 (s, 1H), 7.94 (d, J = 10.8 Hz, 2H), 7.79 (d, J = 11.2 Hz, 2H), 7.63–7.52 (m, 3H), 7.45–7.40 (m, 2H), 7.29 (t, J = 10.0 Hz, 1H), 7.02 (t, J = 12.0 Hz, 3H), 6.87 (br s, 1H), 3.74 (t, J = 6.0 Hz, 4H), 3.26 (t, J = 6.0 Hz, 4H); MS (ES^+) m/z calcd for $C_{26}H_{25}N_5O_4S$: 503.57; found: 504.0 ($M+H^+$).

5.2.21. *N*1-(3-3-[(Phenylsulfonyl)amino]phenyl-1*H*-5-pyrazolyl)-4-(2-tetrahydro-1*H*-1-pyrrolylethoxy)benzamide (8s)

Mp 238–240 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 10.87 (s, 1H), 10.46 (s, 1H), 8.05 (d, J = 8.7 Hz, 2H), 7.79 (d, J = 6.9 Hz, 2H), 7.61-7.41 (m, 4H), 7.31–7.26 (m, 2H), 7.12–7.03 (m, 3H), 6.78 (s, 1H), 4.43 (t, J = 4.8 Hz, 2H), 3.59 (br, 4H), 3.14–3.04 (m, 2H), 2.00–1.86 (m, 4H); MS (ES^+) m/z calcd for $C_{28}H_{29}N_5O_4S$: 531.63; found: 532.1 ($M+H^+$); HRMS (FAB) calcd for $C_{28}H_{29}N_5O_4S$: 531.1940; found: 531.1944.

5.2.22. *N*1-(3-3-[(Phenylsulfonyl)amino]phenyl-1*H*-5-pyrazolyl)-4-(2-morpholinoethoxy)benzamide (8t)

Mp 106–107 °C. ^1H NMR (400 MHz, DMSO-d_6): δ 12.92 (s, 1H), 10.73 (s, 1H), 10.41 (s, 1H), 8.00 (d, J = 8.4 Hz, 2H), 7.79 (d, J = 6.8 Hz, 2H), 7.63–7.55 (m, 3H), 7.43–7.37 (m, 2H), 7.30 (t, J = 8.0 Hz, 1H), 7.05 (d, J = 7.6 Hz, 1H), 6.89 (s, 1H), 4.17 (t, J = 5.6 Hz, 2H), 3.58 (t, J = 4.8 Hz, 4H), 2.71 (t, J = 5.6 Hz, 2H), 2.48 (t, J = 4.8 Hz, 4H); MS (ES^+) m/z calcd for $C_{28}H_{29}N_5O_5S$: 547.63; found: 548.2 ($M+H^+$).

5.2.23. *N*1-(3-3-[(Phenylsulfonyl)amino]phenyl-1*H*-5-pyrazolyl)-4-(morpholinomethyl)benzamide (8u)

Mp 06–108 °C. ^1H NMR (400 MHz, DMSO-d_6): δ 12.99 (s, 1H), 10.91 (s, 1H), 10.45 (s, 1H), 8.02 (d, J = 5.6 Hz, 2H), 7.80 (d, J = 6.8 Hz, 2H), 7.63–7.53 (m, 4H), 7.43 (d, J = 8.0 Hz, 1H), 7.35–7.28 (m, 3H), 7.06 (d, J = 8.0 Hz, 1H), 6.92 (s, 1H), 3.63 (br s, 6H), 2.41 (br s, 4H); MS (ES^+) m/z calcd for $C_{27}H_{27}N_5O_4S$: 517.60; found: 518.1 ($M+H^+$); HRMS (FAB) calcd for $C_{27}H_{27}N_5O_4S$: 517.1784; found: 517.1793.

5.2.24. *N*1-3-[3-(Benzoylamino)phenyl]-1*H*-5-pyrazolyl-4-(4-methylpiperazino)benzamide (9)

Mp 197–200 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 12.88 (s, 1H), 10.62(s, 1H) 10.34 (s, 1H), 8.20 (s, 1H), 7.99 (d, J = 8.1 Hz, 2H), 7.93 (d, J = 8.7 Hz, 2H), 7.76 (d, J = 8.1 Hz, 1H), 7.63–7.40 (m, 5H), 6.98 (d, J = 8.7 Hz, 1H), 3.28 (t, J = 4.8 Hz, 4H), 2.43 (t, J = 4.8 Hz, 4H), 2.21 (s, 3H); MS (ES^+) m/z calcd for $C_{28}H_{28}N_6O_2$: 480.22; found: 481.3 ($M+H^+$).

5.2.25. *N1*-(3-{3-[(Anilinocarbonyl)amino]phenyl}-1*H*-5-pyrazolyl)-4-(4-methylpiperazino) benzamide (10)

^1H NMR (300 MHz, DMSO-d_6): δ 12.85 (s, 1H), 10.57 (s, 1H), 8.81 (d, J = 10.8 Hz, 2H), 7.93 (d, J = 8.7 Hz, 2H), 7.89 (s, 1H), 7.48 (d, J = 7.8 Hz, 2H), 7.35–7.24 (m, 5H), 6.99–6.94 (m, 3H), 3.28 (t, J = 4.8 Hz, 4H), 2.44 (t, J = 4.8 Hz, 4H), 2.22 (s, 3H); MS (ES^+) m/z calcd for $C_{28}H_{29}N_7O_2$: 495.24; found: 496.3 ($M+H^+$).

5.3. General procedure for the preparation of compound 12

To a solution of **5a** (1.0 equiv) and 4,6-dichloro-2-methylpyrimidine (1.5 equiv) in DMF at room temperature was added hydrogen chloride solution (4.0 M in dioxane, 1.6 equiv) and the resultant mixture was stirred at 80 °C for 4 h. After cooling to room temperature, the solvent was evaporated in vacuo to give a viscous residue, which was partitioned between EtOAc and 1 N $NaHCO_3$. The organic layer was separated, dried over $MgSO_4$ and concentrated in vacuo. Purification by flash column chromatography (eluted with CH_2Cl_2/CH_3OH) yielded compounds **11**. Only one of **11** is selected to show its NMR spectrum and Mass.

5.3.1. *N*-(6-Chloro-2-methyl-4-pyrimidinyl)-*N*-[5-(3-nitrophenyl)-1*H*-3-pyrazolyl]amine (11)

Mp 238–240 °C. ^1H NMR (400 MHz, DMSO-d_6): δ 13.21(s, 1H), 10.34(s, 1H) 10.34 (s, 1H), 8.58 (s, 1H), 8.18 (d, J = 7.2 Hz, 2H), 7.74 (t, J = 8.0 Hz, 1H), 6.85 (br s, 1H), 2.46 (s, 3H); MS (ES^+) m/z calcd for $C_{14}H_{11}ClN_6O_2$: 330.06; found: 331.1 ($M+H^+$).

To a solution of **11** (1.0 equiv) in pyridine at room temperature was added 1° or 2° amines (2.0 equiv) and the resultant mixture was stirred at 80 °C for 2 h. After cooling to room temperature, the reaction mixture was evaporated to dryness and the residue suspended in 1 N $NaHCO_3$. The suspension was vigorously stirred at room temperature for 1 h, and the resultant solid was collected by filtration and dried in vacuo to yield crude products which were

used without further purification. To a solution of the crude product prepared from last step in MeOH was added 12 N HCl (2.0 equiv) and a catalytic amount of 10% Pd/C under argon at room temperature. The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 12 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The crude amines were used for the next reaction without further purification. Starting with the crude amines, the same procedures were followed as described in the synthesis of sulfonamides **8** to yield compounds **12**.

5.3.2. *N*1-[3-(5-[2-Methyl-6-(4-methylpiperazino)-4-pyrimidinyl]amino-1*H*-3-pyrazolyl)phenyl]-1-benzenesulfonamide (12a)

Mp 113–115 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.72 (s, 1H), 10.41 (s, 1H), 9.35 (s, 1H), 7.81–7.78 (m, 2H), 7.63–7.53 (m, 3H), 7.38–7.26 (m, 3H), 7.04 (d, J = 7.6 Hz, 1H), 6.66 (s, 1H), 6.36 (s, 1H), 3.49 (t, J = 4.4 Hz, 4H), 2.38 (t, J = 4.4 Hz, 4H), 2.30 (s, 3H), 2.22 (s, 3H); MS (ES$^+$) m/z calcd for $C_{25}H_{28}N_8O_2S$: 504.61; found: 505.1 (M+H$^+$); HRMS (FAB) calcd for $C_{25}H_{28}N_8O_2S$: 504.2056; found: 504.2066.

5.3.3. *N*1-[3-(5-[2-Methyl-6-(4-methylpiperazino)-4-pyrimidinyl]amino-1*H*-3-pyrazolyl)phenyl]-3-methyl-1-benzenesulfonamide (12b)

Mp 252–254 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.70 (s, 1H), 10.31 (s, 1H), 9.38 (s, 1H), 7.59 (d, J = 6.8 Hz, 1H), 7.44–7.36 (m, 3H), 7.28 (t, J = 7.6 Hz, 1H), 7.21 (t, J = 7.6 Hz, 1H), 7.12 (d, J = 7.6 Hz, 1H), 7.04 (d, J = 8.0 Hz, 1H), 6.63 (s, 1H), 6.35 (s, 1H), 3.49 (br, 4H), 2.40 (br, 4H), 2.33 (s, 3H), 2.30 (s, 3H), 2.23 (s, 3H); MS (ES$^+$) m/z calcd for $C_{26}H_{30}N_8O_2S$: 518.64; found: 519.1 (M+H$^+$).

5.3.4. *N*1-[3-(5-[2-Methyl-6-(4-methylpiperazino)-4-pyrimidinyl]amino-1*H*-3-pyrazolyl)phenyl]-1-butanesulfonamide (12c)

Mp 121–123 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.72 (s, 1H), 9.84 (s, 1H), 9.30 (s, 1H), 7.47–7.36 (m, 3H), 7.16 (d, J = 6.8 Hz, 1H), 6.86 (s, 1H), 6.69 (s, 1H), 3.43 (br, 4H), 3.08 (t, J = 7.6 Hz, 2H), 2.32 (br, 4H), 2.24 (s, 3H), 2.16 (s, 3H), 1.61 (tt, J = 7.6, 7.6 Hz, 2H), 1.30 (qt, J = 7.2, 7.6 Hz, 2H), 0.78 (t, J = 7.2 Hz, 3H); MS (ES$^+$) m/z calcd for $C_{23}H_{32}N_8O_2S$: 484.62; found: 485.1 (M+H$^+$).

5.3.5. *N*1-[3-(5-[6-(4-Hydroxypiperidino)-2-methyl-4-pyrimidinyl]amino-1*H*-3-pyrazolyl)phenyl]-1-benzenesulfonamide (12d)

Mp 105–106 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.72 (s, 1H), 10.43 (s, 1H), 9.32 (s, 1H), 7.80 (d, J = 7.2 Hz, 2H), 7.63–7.53 (m, 3H), 7.39–7.28 (m, 3H), 7.04 (d, J = 7.6 Hz, 1H), 6.34 (s, 1H), 4.78 (s, 1H), 3.96 (d, J = 12.8 Hz, 2H), 3.75–3.69 (m, 1H), 3.17 (t, J = 10.4 Hz, 2H), 2.34 (s, 3H), 1.78 (d, J = 9.6 Hz, 2H), 1.39–1.23 (m, 2H); MS (ES$^+$) m/z calcd for $C_{25}H_{27}N_7O_3S$: 505.59; found: 506.1 (M+H$^+$).

5.3.6. *N*1-3-[5-(6-[4-(2-Hydroxyethyl)piperazino]-2-methyl-4-pyrimidinylamino)-1*H*-3-pyrazolyl]phenyl-1-benzenesulfonamide (12e)

Mp 98–100 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.83 (s, 1H), 10.44 (s, 1H), 9.56 (s, 1H), 7.79 (d, J = 6.8 Hz, 2H), 7.63–7.53 (m, 3H), 7.37–7.28 (m, 3H), 7.03 (d, J = 8.0 Hz, 1H), 6.59 (s, 1H), 6.37 (s, 1H), 3.56–3.49 (m, 8H), 2.50–2.45 (m, 4H), 2.30 (s, 3H); MS (ES$^+$) m/z calcd for $C_{26}H_{30}N_8O_3S$: 534.63; found: 535.1 (M+H$^+$); HRMS (FAB) calcd for $C_{26}H_{30}N_8O_3S$: 534.2162; found: 534.2166.

5.3.7. *N*1-(3-5-[(6-[2-(Dimethylamino)ethyl]amino-2-methyl-4-pyrimidinyl)amino]-1*H*-3-pyrazolylphenyl)-1-benzenesulfonamide (12f)

Mp 109–111 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.59 (s, 1H), 9.13 (s, 1H), 7.79 (d, J = 7.2 Hz, 2H), 7.63–7.53 (m, 4H), 7.37–7.27 (m, 3H), 7.02 (d, J = 8.0 Hz, 1H), 6.59 (s, 1H), 6.37–6.29 (m, 1H), 3.42–3.19 (m, 1H, overlapping with H_2O peak), 2.39 (t, J = 6.8 Hz, 2H), 2.25 (s, 2H), 2.18 (s, 9H); MS(ES$^+$) m/z calcd, for $C_{24}H_{28}N_8O_2S$: 492.60; found: 493.2 (M+H$^+$).

5.3.8. *N*1-3-[5-(2-Methyl-6-[(2-morpholinoethyl)amino]-4-pyrimidinylamino)-1*H*-3-pyrazolyl]phenyl-1-benzenesulfonamide (12g)

Mp 252–254 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.59 (s, 1H), 10.41 (s, 1H), 9.13 (s, 1H), 7.79 (d, J = 7.2 Hz, 2H), 7.63–7.53 (m, 3H), 7.39–7.27 (m, 3H), 7.04 (d, J = 8.0 Hz, 1H), 6.64 (s, 1H), 6.38 (s, 1H), 3.33 (br s, 4H), 2.40 (t, J = 6.4 Hz, 2H), 2.25 (s, 3H), 2.18 (br s, 6H); MS (ES$^+$) m/z calcd for $C_{26}H_{30}N_8O_3S$: 534.63; found: 535.1 (M+H$^+$).

5.3.9. *N*1-3-[5-(2-Methyl-6-[(3-morpholinopropyl)amino]-4-pyrimidinylamino)-1*H*-3-pyrazolyl]phenyl-1-benzenesulfonamide (12h)

Mp 109–110 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.62 (s, 1H), 10.42 (s, 1H), 9.23 (s, 1H), 7.79 (d, J = 7.2 Hz, 2H), 7.63–7.53 (m, 3H), 7.39–7.26 (m, 3H), 7.03 (d, J = 7.6 Hz, 1H), 6.95 (s, 1H), 6.33 (s, 1H), 3.69 (br s, 4H), 3.24 (br s, 2H), 2.99–2.67 (m, 6H), 2.28 (s, 3H), 1.79 (br s, 2H); MS (ES$^+$) m/z calcd for $C_{27}H_{32}N_8O_3S$: 548.66; found: 549.1 (M+H$^+$); HRMS (FAB) calcd for $C_{27}H_{32}N_8O_3S$: 548.2318; found: 548.2321.

5.4. Biochemical characterization of compounds

5.4.1. Kinase assays

FLT3. GST-FLT3-KD^{WT} containing the FLT3 kinase catalytic domain (residues Y567∼S993) were expressed in Sf9 insect cells transfected the baculovirus containing pBac-PAK8-GST-FLT3-KD plasmid. The FLT3^{WT} Kinase-Glo assays were carried out in 96-well plates at 30 °C for 4 h and tested compound in a final volume of 50 μl including the following components: 75 ng GST-FLT3-KD^{WT} proteins, 25 mM HEPES, pH 7.4, 4 mM $MnCl_2$, 10 mM $MgCl_2$, 2 mM DTT, 0.02% Triton X-100, 0.1 mg/ml bovine serum albumin, 25 μM Her2 peptide substrate, 0.5 mM Na_3VO_4, and 1 μM ATP.

VEGFR1/2. The recombinant GST-VEGFR1 (residues R781–I1338) or GST-VEGFR2 (residues V789–V1356) containing kinase domain were expressed in Sf9 insect cells. The kinase assay were carried out in 96-well plates with tested compound in a final volume of 50 μl reaction at 30 °C for 120 min with following components: 25 mM HEPES pH 7.4, 10 mM $MgCl_2$, 4 mM $MnCl_2$, 0.5 mM Na3VO4, 2 mM DTT, 0.02% Triton X-100, 0.01% BSA, 1 μM ATP, 2 μM polyGlu4:Tyr peptide, 50–100 ng recombinant VEGFR1 or VEGFR2.

Aurora kinase A. The recombinant GST-Aurora A (residues S123–S401) containing kinase domain was expressed in Sf9 insect cells. The kinase assay was carried out in 96-well plates with tested compound in a final volume of 50 μl reaction at 37 °C for 90 min with following components: 50 mM Tris–HCl pH 7.4, 10 mM NaCl, 10 mM $MgCl_2$, 0.01% BSA, 5 μM ATP, 1 mM DTT and 15 μM tetra(-LRRASLG) peptide, and 150 ng recombinant Aurora A.

Following incubation, 50 μl Kinase-Glo Plus Reagent (Promega, Madison, WI, USA) was added and the mixture was incubated at 25 °C for 20 min. A 70-μL aliquot of each reaction mixture was transferred to a black microliter plate and the luminescence was measured on Wallac Vector 1420 multilabel counter (PerkinElmer, Shelton, CT, USA).

5.4.2. Cell lines and MTS cell viability assay

The leukemias cell lines MOLM-13, MV4:11, RSV4;11, MOLT-4, U937, and K562 were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). All leukemias cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 10 U/ml penicillin, and 10 g/ml streptomycin at

37 °C and 5% CO_2. To determine cell viability after drug treatment, assays were performed by seeding 10,000 cells (leukemias cell lines) per well in a 96-well culture plate. After 16 h, cells were then treated with vehicle or various concentrations of compound in medium for 72 h. Viable cells were quantitated using the MTS method (Promega, Madison, WI, USA) according to the manufacturer's recommended protocol. The results were determined by measuring the absorbance at 490 nm using a plate reader (Victor2; PerkinElmer, Shelton, CT, USA). The IC_{50} value was defined as the amount of compound that caused 50% reduction in cell viability in comparison with DMSO-treated (vehicle) control and was calculated using Prism version 4 software (GraphPad, San Diego, CA, USA).

5.5. Pharmacological studies (in vivo assessment of 12a)

Nine weeks old athymic nu/nu nude mice were obtained from BioLASCO, Taiwan Co., Ltd (Ilan, Taiwan). The mice were housed in sterilized cages equipped with an air filter and sterile bedding materials at the Laboratory Animal Center. All mice were fed with sterilized water and chow under 12-hour light/dark schedule. 100 μl suspensions of MOLM-13 or MV4;11 tumor cells (final concentration: 1×107 cells/ml; 1×106/flank) were transplanted s.c. into the left flank of nu/nu nude mice. Tumor diameters are measured with digital calipers, and the tumor volume in mm^3 is calculated by the formula: Volume = (width $\times$ length^2)/2. In Figure 3A, tumor-bearing mice were randomized when the mean tumor volume was $\sim$200 mm^3. The mice were randomly divided into three groups of 7–8 animals each, and the treatment was initiated. Compound **12a** was dissolved in vehicle formulation (10% DMSO/20% Cremophor/70% Saline, v/v/v) and intravenously administrated at 10 and 50 mg/kg once a day for two continuous weeks (on days 1–5 and 8–12). A vehicle control group received intravenously in the same manner. In Figure 3B, tumor-bearing mice were randomized when the mean tumor volume was $\sim$500 mm^3. Compound **12a** was intravenously administrated at 10, 25 and 50 mg/kg once a day for two continuous weeks (on days 1–5 and 8–12, $n = 8$ per group) and the vehicle control was dosed in the same manner. (Animal Use Protocol for this project has been approved by National Health Research Institutes, the protocol No: NHRI-IACUC-099088-A).

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