Accepted Manuscript

Development of a potent and selective FLT3 kinase inhibitor by systematic expansion of a non-selective fragment-screening hit

Hirofumi Nakano, Tsukasa Hasegawa, Riyo Imamura, Nae Saito, Hirotatsu Kojima, Takayoshi Okabe, Tetsuo Nagano

PII: S0960-894X(16)30226-8
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.03.006
Reference: BMCL 23649

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 9 January 2016
Revised Date: 27 February 2016
Accepted Date: 3 March 2016

Please cite this article as: Nakano, H., Hasegawa, T., Imamura, R., Saito, N., Kojima, H., Okabe, T., Nagano, T., Development of a potent and selective FLT3 kinase inhibitor by systematic expansion of a non-selective fragment-screening hit, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.03.006

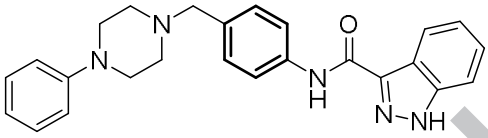
Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Development of a potent and selective FLT3 kinase inhibitor by systematic expansion of a non-selective fragment-screening hit

Hirofumi Nakano, Tsukasa Hasegawa, Riyo Imamura, Nae Saito, Hirotatsu Kojima, Takayoshi Okabe, Tetsuo Nagano

Leave this area blank for abstract info.



26

Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Development of a potent and selective FLT3 kinase inhibitor by systematic expansion of a non-selective fragment-screening hit

Hirofumi Nakano [a], Tsukasa Hasegawa [a], Riyo Imamura [a], Nae Saito [a], Hirotatsu Kojima [a], Takayoshi Okabe [a], and Tetsuo Nagano [a, *]

[a] *Drug Discovery Initiative, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan*

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
FLT3
Kinase inhibitor
Kinase selectivity
Fragment based drug discovery
Acute myeloid leukemia

ABSTRACT

A non-selective inhibitor (**1**) of FMS-like tyrosine kinase-3 (FLT3) was identified by fragment screening and systematically modified to afford a potent and selective inhibitor **26**. We confirmed that **26** inhibited the growth of FLT-3-activated human acute myeloid leukemia cell line MV4-11. Our design strategy enabled rapid development of a novel type of FLT3 inhibitor from the hit fragment in the absence of target-structural information.

* Corresponding author. Tel.: +81-3-5841-1989; fax: +81-3-5841-1959; e-mail:tlong@mol.f.u-tokyo.ac.jp

FMS-like tyrosine kinase 3 (FLT3), a class-III transmembrane receptor-type tyrosine kinase, is a validated therapeutic target for acute myeloid leukemia (AML).[1] It is expressed mainly in hematopoietic cells, and is activated by its extracellular ligand. FLT3 signal cascades regulate the survival and proliferation of hematopoietic cells, and uncontrolled over-activation causes leukemia. About 30% of AML patients possess FLT3-activating mutations, such as point mutation at D835 or internal tandem domain repeat (ITD) mutation in the juxtamembrane domain. These mutations have been reported to be associated with poor prognosis. Therefore, FLT3 kinase is an attractive therapeutic target for treatment of AML.

A number of FLT3 inhibitors have been reported, but none has yet been approved for clinical use.[1] Compounds such as lestaurtinib (CEP-701), midostaurin (PKC-412) and tandutinib (MLN-518) are representative first-generation FLT3 inhibitors, but they were not originally developed as FLT3 inhibitors, and show broad kinase inhibition profiles or insufficient potency against FLT3. Quizartinib (AC220) is a potent and selective second-generation FLT3 inhibitor, which is in clinical trials.[1,2] However, emergence of quizartinib-resistant FLT3-mutants has already been reported.[3]

Drug candidates with different profiles can contribute to cancer chemotherapy, since they can be used in a mutually complementary fashion to manage inter-individual differences in drug-metabolism and the emergence of resistance mutations. In the case of FLT3, previously reported FLT3 inhibitors generate non-overlapping drug-resistant mutants in vitro.[4] In cancer chemotherapy, it is a general strategy to treat patients with several inhibitors belonging to different chemical classes. BCR-ABL inhibitors, for example, are have different clinical spectra, and multiple drugs have been marketed to cope with drug-resistant mutants.[5] In the case of FLT3, crenolanib (CP-868,596) and gilteritinib (ASP2215) are in clinical trials to overcome quizartinib resistance.[6,7]

Fragment screening is a strategy that provides opportunities to search chemical space that has not been efficiently explored by other methods. Typically, fragments are defined as small organic molecules whose molecular weights (MWs) are in the range of 120-300 Da. It has been estimated that 10^7 fragments with up to 12 heavy atoms exist (average MW = 153, 87% of compounds have MW $\leq$ 160).[8] On the other hand, 10^{33} compounds can be generated with up to 36 heavy atoms (MW $\leq$ 500).[9] These numbers imply that screening on a scale sufficient to provide reasonable coverage of the chemical space is only practical for molecules containing quite small numbers of atoms. In other words, we may be more likely to pick up hit compounds with novel types of structures if we focus on screening fragment libraries.

To find fragments with FLT3-inhibitory activity, we screened an in-house collection of 9,000 fragments. As a result, we discovered **1** (MW = 237) as a potent FLT3 inhibitor (IC_{50} = 417 nM). Previously, **1** was reported as a CDK2-inhibiting fragment (CDK2 IC_{50} = 3 μM).[10] In a kinase selectivity study, we found that **1** inhibited several kinases, including CDK2, in addition to FLT3 (*vide infra*). Therefore, our next challenge was to improve especially the selectivity, as well as the potency, of **1**.

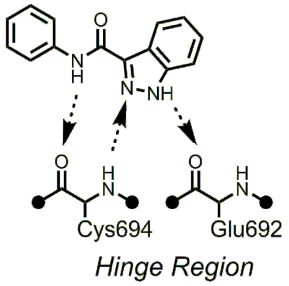
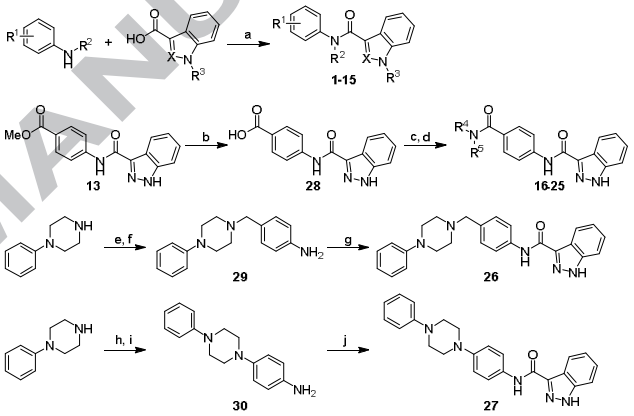


Figure 1. Hypothetical binding mode of **1**.

Compounds were synthesized as follows (Scheme 1). **1**-**15** were synthesized by amide coupling of aniline and carboxylic acid precursors, both of which are commercially available. **13** was hydrolyzed to yield a carboxylic acid derivative, and then coupled with secondary amines to furnish **16**, **17**, and **19**-**25**. To obtain **18**, the resultant amide was deprotected under acidic conditions. To obtain **26** and **27**, 4-phenylpiperazine was reacted with 4-nitrobenzyl bromide or 1-fluoro-4-nitrobenzene, and the nitro groups were reduced. The resulting 4-substituted anilines were coupled with indazole-3-carboxylic acid to obtain **26** and **27**.



Scheme 1. Reagents and conditions: (a) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, HOBt.H_2O, DMF, 100°C, 6-83%; (b) 4 *N* NaOH, THF-MeOH, rt, 30%; (c) secondary amine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, HOBt.H_2O, DMF, rt, 10-99%; (d) For preparation of **18**: TFA, CH_2Cl_2, rt, 48%; (e) 4-nitrobenzyl bromide, Et_3N, CH_2Cl_2, rt, 87%; (f) Fe powder, NH_4Cl, EtOH-H_2O, 80°C, 85%; (g) indazole-3-carboxylic acid, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, HOBt.H_2O, DMF, 100°C, 10%; (h) 1-fluoro-4-nitrobenzene, DMSO, rt, 85%; (i) $NiCl_2.6H_2O$, $NaBH_4$, MeOH, 0°C, then rt, 74%: (j) indazole-3-carboxylic acid, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, pyridine, rt, 28%.

We hypothesized that **1** binds to the hinge region of FLT3 in active form (Figure 1), as in the previously reported CDK2-**1** analogue co-crystal structure (PDB ID: 2VTI), which contains three hydrogen bonds in the hinge region.[10] To validate this hypothesis, we prepared derivatives of **1** that are unable to form these hydrogen bonds by masking the proposed donor NHs with methyl groups, or by replacing the putative acceptor N with CH (Table 1, compounds **2**-**4**). All three modified derivatives lost potency, clearly confirming the importance of the three hydrogen bonds for inhibitory potency, and supporting the idea that the binding mode of FLT3-**1** is similar to that reported for the CDK2-**1** analogue. The methylation of the amide nitrogen would not only mask the hydrogen donor but also change the amide conformation from *trans* (as shown in Figure 1) to *cis*. Such conformation change would also be related to the potency loss of

2 because the phenyl ring in the *cis*-amide would not occupy the hydrophobic pocket but clash with the protein.

Structure-guided optimization of **1** was not feasible, because the crystal structure of FLT3 in the active form has not been reported, so systematic modification was the only way to evolve **1** into potent and selective lead compound.[11] In order to rapidly evaluate the potential of **1** as a starting compound, we focused on modification of the aniline ring to obtain initial SAR, because various aniline building blocks are commercially available, in contrast to indazole-3-carboxylic acid derivatives.

We first set out to identify appropriate positions in the aniline ring to attach pendant functionalities to improve potency. By scanning with a methyl group, methoxy group, and methoxycarbonyl group, we found that the 4-position in the aniline tolerates these modifications (Table 1, compounds **5**-**13**). Then, we examined 4-amide derivatives, and found that tertiary amide **15** seemed promising for further modification.

Next, we synthesized various tertiary amide derivatives (Table 2). After comparing the potency of **15** (Table 1) and **16**-**19** (Table 2), we focused on piperazine amide derivatives. We attached several moieties to the lateral NH group of the piperazine, and found that a phenylpiperazine derivative, **24**, potently inhibits FLT3 with an IC_{50} value of 34 nM, which is a 10-fold improvement over **1**.

Further, we changed the linker moiety between the fragment core and the pendant phenylpiperazine group. Transformation of the linker from sp^2 carbonyl to sp^3 methylene improved the potency (Table 2, compound **26**), indicating that the flexible sp^3 methylene linker allows the phenylpiperazine ring to interact more efficiently with the protein. As expected, removal of the linker resulted in loss of potency (**27**).

In addition to potency, we monitored the Ligand Efficiency (LE) of the derivatives.[12] Evolution of **1** into **26** resulted in a change of the LE from 0.50 to 0.36, which is above the preferred minimum score of 0.3 for lead compounds.[12]

Selective inhibition of the targeted kinase is a major concern in the design of kinase inhibitors, because poor kinase selectivity may cause unwanted side effects, such as cardiotoxicity.[13] Kinase selectivity data for **1**, **24**, and **26** are summarized in Fig. 2 and Table S1 in Supporting Information. The compounds were tested against a panel of 20 tyrosine kinases and 30 serine/threonine kinases at a concentration 50-fold higher than the IC_{50} value in FLT3 inhibition assay (carried out at Carna Biosciences, Japan). To find suitable inhibitor concentrations for selectivity study, the FLT3 IC_{50} values of **1**, **24**, and **26** were determined at Carna Biosciences; they were found to be 350 nM, 23 nM, and 9 nM, respectively. Therefore, **1**, **24**, and **26** were used at concentrations of 17.5 μM, 1.15 μM, and 0.450 μM, respectively, in the kinase selectivity study.

The selectivity profile of **1** shows almost complete inhibition of two off-target kinases, TRKA and HGK, as well as FLT3 (Figure 2). In addition, **1** potently inhibited ITK, SYK and CDK2 by more than 70%. Other kinases that were inhibited by more than 50% were JAK3 (52%), CK1ε (51%), DYRK1B (54%), and GSK3β (51%). Overall selectivity was evaluated in terms of the Gini coefficient,[14] and the calculated score was 0.578.

The selectivity profile of **24** is cleaner than that of **1**, as shown in Fig. 2. Although TRKA and HGK, two major off-targets of **1**, were still potently inhibited by **24** (90% and 91%), the other off-targets were much less potently inhibited. Besides TRKA and HGK, ITK (51%) was the only kinase that was inhibited by more than 50%. Improvement of selectivity was reflected in an increase of the Gini coefficient to 0.678.

Compound **26** showed an even better selectivity profile. We found that converting the benzyl position from sp^2 carbon (**24**) to sp^3 carbon (**26**) improved not only the potency, but also the selectivity, presumably due to the three-dimensional character of the sp^3 carbon derivative.[15] Inhibition rates of TRKA and HGK were reduced to 81% and 63%, respectively. The IC_{50} value for TRKA was 137 nM (15-fold selectivity vs FLT3), and that for HGK was 410 nM (45-fold selectivity vs FLT3). The other 47 kinases were inhibited by less than 40%. The Gini coefficient was improved from 0.678 to 0.757. Thus, we successfully developed **26** as a potent and selective inhibitor of FLT3 by simple, systematic structural evolution of fragment **1**.

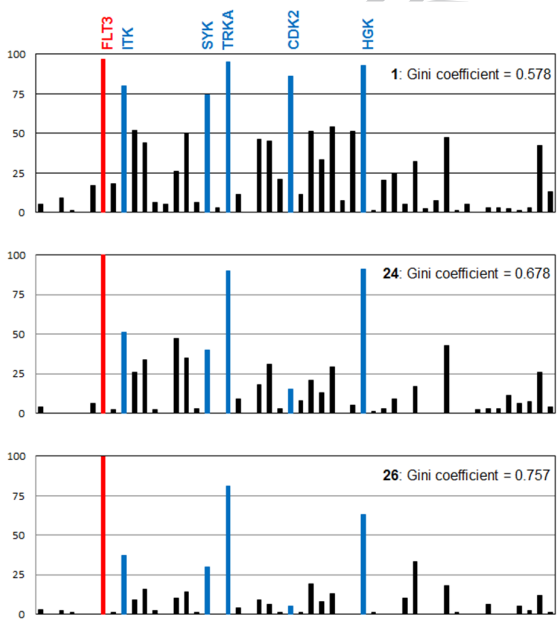


Figure 2. Kinase selectivity profiles of **1**, **24**, and **26**. Selectivity was evaluated against a panel of 50 kinases at a concentration 50 times higher than the IC_{50} value for FLT3 (**1**: 17.5 μM, **24**: 1.15 μM, **26**: 0.450 μM). The y axis shows % inhibition of each kinase.

To further evaluate the potential of **26**, we carried out cell-based experiments using the FLT3-ITD-harboring human leukemia cell line MV4-11. In growth inhibition assay, **26** potently inhibited the growth of MV4-11 (IC_{50} = 164 nM), but negligibly inhibited the growth of normal human diploid lung fibroblast cell line WI-38, which was used as a surrogate for general toxicity (Table 3). Next, we examined the phosphorylation-inhibitory activity of **26**. In MV4-11 cells, FLT3 is reported to auto-phosphorylate Tyr-596. MV4-11 cells were incubated with **26** for 1.5 h, then lysed, and the phosphorylation status of FLT3 was evaluated by western blotting. As shown in Figure 3A, **26** dose-dependently inhibited phosphorylation of FLT3. Finally, we examined whether or not **26** induces apoptosis of MV4-11 cells during 48 h incubation, by means of Annexin V staining (Figure 3B). As expected, **26** induced apoptosis, as has been reported for the representative FLT3 inhibitor quizartinib.[2c,d] These results indicate that **26** is a promising lead compound.

To confirm the potential of **26** for further development, the in vitro ADME properties were examined (Table 4).[16] Among five major CYP isozymes, CYP2C19 and CYP2D6 were inhibited about 60% by **26** at 10 μM, and the others were inhibited by less

than 50%. Metabolic stability in human liver microsomes was moderate, and a half of the compound was recovered after 1 h incubation. In Caco-2 permeability assay, **26** showed moderate permeability of 4 x 10^{-6} cm/s, indicating that the compound has potential for oral administration. Improvement of the poor aqueous solubility (3 μM in PBS, pH 7.4) is a major issue to be addressed in further development.

In this letter, we report the development of a potent, selective, and cell-active FLT3 inhibitor **26** by means of fragment-based screening followed by systematic modification of the non-selective inhibitor hit **1**. By careful selection of suitable substituents, we achieved significant improvement of both on-target potency and selectivity.

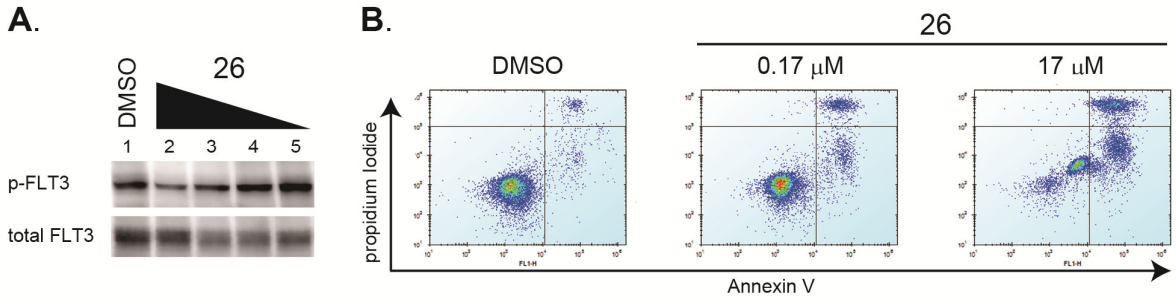
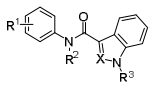


Figure 3. Cellular study of compound **26**. (A) Inhibition of auto-phosphorylation of FLT3 at Tyr-596 was evaluated by immunoblotting. MV4-11 was incubated with **26** for 1.5 h. Lane 1: DMSO control; Lane 2: 1 μM; Lane 3: 0.3 μM; Lane 4: 0.1 μM; Lane 5: 0.03 μM. (B) Induction of apoptosis was evaluated by means of Annexin V and PI staining. MV4-11 was incubated with compounds for 48 h.

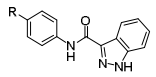
Table 1. Effects of various substituents on FLT3-inhibitory potency

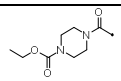
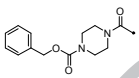
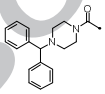
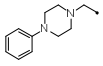


Compounds	R^1	R^2	R^3	X	FLT3 IC_{50} (nM)[a]	LE[b]
1	H	H	H	N	417	0.50
2	H	Me	H	N	>2000	ND[c]
3	H	H	Me	N	>2000	ND[c]
4	H	H	H	CH	>2000	ND[c]
5	2-Me	H	H	N	>2000	ND[c]
6	3-Me	H	H	N	443	0.47
7	4-Me	H	H	N	310	0.48
8	2-OMe	H	H	N	>2000	ND[c]
9	3-OMe	H	H	N	786	0.43
10	4-OMe	H	H	N	352	0.45
11	2-COOMe	H	H	N	>2000	ND[c]
12	3-COOMe	H	H	N	>2000	ND[c]
13	4-COOMe	H	H	N	552	0.40
14	4-CONHMe	H	H	N	281	0.42
15	4-$CONMe_2$	H	H	N	110	0.42

[a] IC_{50} values are shown as the average of two independent experiments (n = 4). [ATP] = 30 μM. [b] LE = –1.4•log(IC_{50})/HAC; HAC = heavy atom count. [c] Not determined.

Table 2. Effects of 4-substituents on the aniline moiety upon FLT3-inhibitory potency.



Compound	R	FLT3 IC_{50} (nM)[a]	LE[b]	Compound	R	FLT3 IC_{50} (nM)[a]	LE[b]
16		107	0.39	**22**		46	0.33
17		113	0.37	**23**		42	0.29
18		61	0.39	**24**		34	0.33
19		95	0.38	**25**		56	0.26
20		90	0.37	**26**		10	0.36
21		43	0.33	**27**		>2000	ND[c]

[a] IC_{50} values are shown as the average of two independent experiments (n = 4). [ATP] = 30 μM. [b] LE = –1.4•log(IC_{50})/HAC; HAC = heavy atom count. [c] Not determined.

Table 3. Cell growth-inhibitory potency of **1** and **26**. [a]

FLT3-ITD-positive leukemia cell line MV4-11 IC_{50} (nM)		Diploid lung fibroblast cell line WI-38 IC_{50} (nM)	
1	**26**	**1**	**26**
7,700	164	>10,000	>10,000

[a] Incubated for 48 h.

Table 4. In vitro ADME properties of **26**. [a]

CYP (%)[b]					Human liver microsomes (%)[c]	Caco-2 (cm/s)[d]	Aqueous solubility (μM)[e]
1A2	2C9	2C19	2D6	3A4			
36	36	57	62	48	50	4×10^{-6}	3

[a] Carried out at Cerep. [b] Compound concentration was 10 μM. [c] % Remaining after incubation of 1 μM compound at 37°C for 1 h. [d] A-B permeability at 10 μM. [e] Aqueous solubility in PBS, pH 7.4.

Acknowledgments

This work was supported by the Platform for Drug Discovery, Informatics and Structural Life Science from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References and notes

1. (a) Prescott, H.; Kantarjian, H.; Cortes, J.; Ravandi, F. *Expert Opin. Emerging Drugs* **2011**, *16*, 407. (b) Pemmaraju, N.; Kantarjian, H.; Ravandi, F.; Cortes, J. *Cancer*, **2011**, *117*, 3293. (c) Wander, S. A.; Levis, M. J.; Fathi, A. T. *Ther. Adv. Hematol.* **2014**, *5*, 65.
2. (a) Zarrinkar, P. P.; Gunawardane, R. N.; Cramer, M. D.; Gardner, M. F.; Brigham, D.; Belli, B.; Karaman, M. W.; Pratz, K. W.; Pallares, G.; Chao, Q.; Sprankle, K. G.; Patel, H. K.; Levis, M.; Armstrong, R. C.; James, J.; Bhagwat, S. S. *Blood* **2009**, *114*, 2984. (b) Chao, Q.; Sprankle, K. G.; Grotzfeld, R. M.; Lai, A. G.; Carter, T. A.; Velasco, A. M.; Gunawardane, R. N.; Cramer, M. D.; Gardner, M. F.; James, J.; Zarrinkar, P. P.; Patel, H. K.; Bhagwat, S. S. *J. Med. Chem.* **2009**, *52*, 7808. (c) Gunawardane, R. N.; Nepomuceno, R. R.; Rooks, A. M.; Hunt, J. P.; Ricono, J. M.; Belli, B.; Armstrong, R. C. *Mol. Cancer Ther.* **2013**, *12*, 438. (d) Kampa-Schittenhelm, K. M.; Heinrich, M. C.; Akmut, F.; Döhner, H.; Döhner, K.; Schittenhelm, M. M. *Molecular Cancer* **2013**, *12*, 19.
3. Smith, C. C.; Wang, Q.; Chin, C.-S.; Salerno, S.; Damon, L. E.; Levis, M. J.; Perl, A. E.; Travers, K. J.; Wang, S.; Hunt, J. P.; Zarrinkar, P. P.; Schadt, E. E.; Kasarskis, A.; Kuriyan, J.; Shah, N. P. *Nature*, **2012**, *485*, 260.
4. (a) von Bubnoff, N.; Engh, R. A.; Åberg, E.; Sänger, J.; Peschel, C.; Duyster. J. *Cancer Res.* **2009**, *69*, 3032. (b) Williams, A. B.; Nguyen, B.; Li, L.; Brown, P.; Levis, M.; Leahy, D.; Small, D. *Leukemia*, **2013**, *27*, 48. (c) Smith, C. C.; Lasater, E. A.; Zhu, X.; Lin, K. C.; Stewart, W. K.; Damon, L. E.; Salerno, S.; Shah, N. P. *Blood*, **2013**, *121*, 3165.
5. (a) Desogus, A.; Schenone, S.; Brullo, C.; Tintori, C.; Musumeci, F. *Expert Opin. Ther. Patents* **2015**, *25*, 397. (b) Zhang, J.; Adrián, F. J.; Jahnke, W.; Cowan-Jacob, S. W.; Li, A. G.; Iacob, R. E.;

Sim, T.; Powers, J.; Dierks, C.; Sun, F.; Guo, G.-R.; Ding, Q.; Okram, B.; Choi, Y.; Wojciechowski, A.; Deng, X.; Liu, G.; Fendrich, G.; Strauss, A.; Vajpai, N.; Grzesiek, S.; Tuntland, T.; Liu, Y.; Bursulaya, B.; Azam, M.; Manley, P. W.; Engen, J. R.; Daley, G. Q.; Warmuth, M.; Gray, N. S. *Nature*, **2010**, *463*, 501.

6. (a) Zimmerman, E. I.; Turner, D. C.; Buaboonnam, J.; Hu, S.; Orwick, S.; Roberts, M. S.; Janke, L. J.; Ramachandran, A.; Stewart, C. F.; Inaba, H.; Baker, S. D. *Blood*, **2013**. *122*, 3607. (b) Galanis, A.; Ma, H.; Rajkhowa, T.; Ramachandran, A.; Small, D.; Cortes, J.; Levis, M. *Blood*, **2014**, *123*, 94. (c) Smith, C. C.; Lasater, E. A.; Lin, K. C.; Wang, Q.; McCreery, M. Q.; Stewart, W. K.; Damon, L. E.; Perl, A. E.; Jeschke, G. R.; Sugita, M.; Carroll, M.; Kogan, S. C.; Kuriyan, J.; Shah, N. P. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 5319.
7. Myers, S. H.; Brunton, V. G.; Unciti-Broceta, A. *J. Med. Chem.* in press.
8. Fink T.; Bruggesser H.; Reymond J.-L. *Angew. Chem. Int. Ed.* **2005**, *44*, 1504.
9. Polishchuk, P. G.; Madzhidov, T. I.; Varnek, A. *J. Comput. Aided Mol. Des.* **2013**, *27*, 675.
10. Wyatt, P. G.; Woodhead, A. J.; Berdini, V.; Boulstridge, J. A.; Carr, M. G.; Cross, D. M.; Davis, D. J.; Devine, L. A.; Early, T. R.; Feltell, R. E.; Lewis E. J.; McMenamin, R. L.; Navarro, E. F.; O'Brien, M. A.; O'Reilly, M.; Reule, M.; Saxty, G.; Seavers, L. C. A.; Smith, D.-M.; Squires, M. S.; Trewartha, G.; Walker, M. T.; Woodford, A. J.-A. *J. Med. Chem.* **2008**, *51*, 4986.
11. Crystal structure of FLT3 in inactive form has been reported. (a) Griffith, J.; Black, J.; Faerman, C.; Swenson, L.; Wynn, M.; Lu, F.; Lippke, J.; Saxena, K. *Molecular Cell* **2004**, *13*, 169. (b) Zorn, J. A.; Wang, Q.; Fujimura, E.; Barros, T.; Kuriyan, J. *PLoS ONE*, **2015**, *10*, e0121177.
12. (a) Hopkins, A. L.; Groom, C. R.; Alex, A. *Drug Discov. Today* **2004**, *9*, 430. (b) Schultes, S.; de Graaf, C.; Haaksma, E. E. J.; de Esch, I. J. P.; Leurs, R.; Krämer, O. *Drug Discov. Today Technol.* **2010**, *7*, e157.
13. (a) Hasinoff, B. B. *Toxicol. Appl. Pharmacol.* **2010**, *244*, 190. (b) Hasinoff, B. B.; Patel, D. *Toxicol. Appl. Pharmacol.* **2010**, *249*, 132.
14. Graczyk, P. P. *J. Med. Chem.* **2007**, *50*, 5773.
15. Lovering, F. *Med. Chem. Commun.* **2013**, *4*, 515.
16. In vitro ADME assay was carried out at Cerep.

Supplementary Material

Details of chemistry, protocols of biological assay and raw data of the kinase selectivity studies (compounds **1**, **24** and **26**).