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Synthesis and biological evaluation of diaryl urea derivatives as FLT3 inhibitors

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ABSTRACT

As a class III receptor tyrosine kinase (RTK), FMS-like tyrosine kinase 3 (FLT3) is always overexpressed in many cases of acute leukemia. This paper studies the structure-based synthesis and biological evaluation of diaryl urea derivatives as FLT3 inhibitors. Encouragingly, compounds **15b**, **16b**, **24a**, and **24c** showed excellent biological activities in a low nanomolar range. In particular, compound **16b** demonstrated significant inhibitory potency against FLT3-ITD (IC_{50} = 5.60 nM) and better antiproliferative activity than quizartinib against MV4-11 cell line (IC_{50} = 0.176 nM). It is indicated that compound **16b** for the treatment of acute myeloid leukemia could be very promising.

Acute myeloid leukemia (AML) is a malignant tumor of the bone marrow and blood caused by immature bone marrow progenitor cells.[1,2] In the United States, about 12,000 new cases of AML are reported each year. In China, the incidence of acute leukemia is significantly higher than that of chronic leukemia, with a ratio of 5.5–1.[3] Currently, conventional treatments include intensive chemotherapy and allogeneic hematopoietic stem cell transplantation (HSCT), but the efficacy is poor in elderly patients who are not able to withstand intensive chemotherapy.[4,5] Thus, there is a compelling medical need for developing more efficient targeted agents.

FMS-like tyrosine kinase 3 (FLT3) is an expression product of a proto-oncogene located on chromosome 13q12, which plays an important role in the regulation of survival, proliferation, and differentiation of hematopoietic progenitor cells.[6–8] FLT3 mutations are detected in approximately 30% of newly diagnosed AML cases and are associated with a high leukemic burden and poor prognosis.[9,10] The two most common FLT3 mutations are internal tandem duplication (ITD) in the juxtamembrane domain, which has been validated as the driving mutation, and point mutations with in the tyrosine kinase domain (TKD).[11,12] Despite the different activation pathways, both the ITD mutation and the TKD mutation could cause the abnormally activation of ligand-independent FLT3, thereby inducing the survival and proliferation of cytokine-independent AML cells.[13] Therefore, FLT3 is regarded as the most potential target for the treatment of AML.

At present, many small molecule FLT3 inhibitors have been developed,[14] and they are divided into type I FLT3 inhibitors and type II FLT3 inhibitors based on their mechanism of action. Type I inhibitors (e.g., midostaurin and gilteritinib) bind the FLT3 receptor in the active conformation near the activation loop or near the ATP binding pocket, type II (e.g., sorafenib and quizartinib (Fig. 1)) bind FLT3 receptor in the inactive conformation in a region adjacent to the ATP binding domain.[15,16] Diaryl ureas were identified as a scaffold that elicited potent inhibition of FLT3,[17] because it can form hydrogen bond interactions with Asp 829 and Glu 661 of the FLT3 receptor, and can form π-π stacks with Phe 691 and Phe 830, which has the effect of stabilizing the conformation of the compound.[18] These results indicate that diaryl ureas play a pivot role in maintaining the activities of the compounds. Based on the above analysis, our team retained the diaryl ureas and designed a series of diaryl urea compounds with imidazobenzothiazole as the basic skeleton. Here, the synthesis process and the relationship between structure and activity are reported.

The synthetic methods of these compounds are shown in Schemes 1–3. The commercially available 4-nitrophenylacetic acid (**1**) was esterified and reduced with Pd/C catalyst under H_2 to obtain compound **3**. Phenyl chloroformate reacted with the corresponding heterocyclic amine to produce **4a-4d**. Then **4a-4d** with compound **3** by amine transesterification obtained the corresponding ureas, and the esters were hydrolyzed with 0.5 mol/L NaOH aqueous solution to give the corresponding acids **6a-6d**. **6a-6d** were submitted to the coupling with commercially available 2-amino-6-methoxybenzothiazole (**7**) to afford compounds **8a-8d** (Scheme 1).

Alkylation of 4-nitrophenol (**9**) with 4-(2-chloroethy)morpholine

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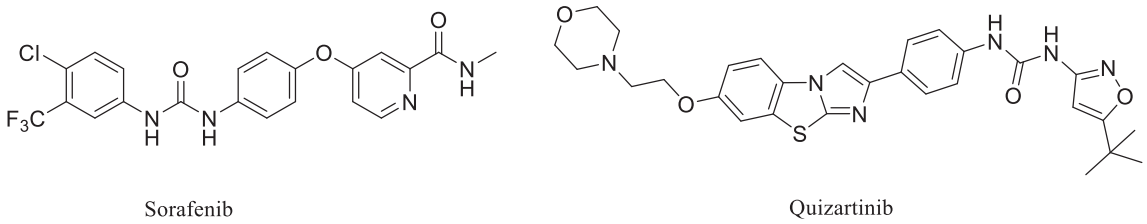
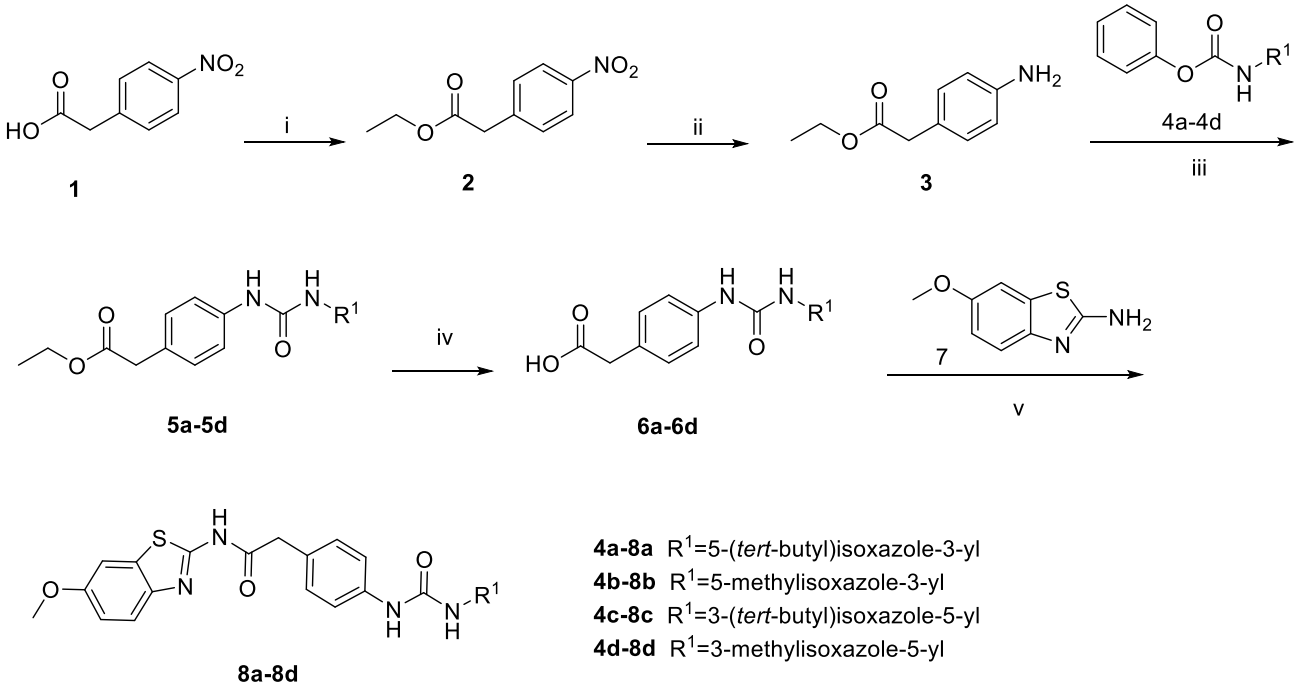


Fig. 1. Structures of some FLT3 inhibitors.



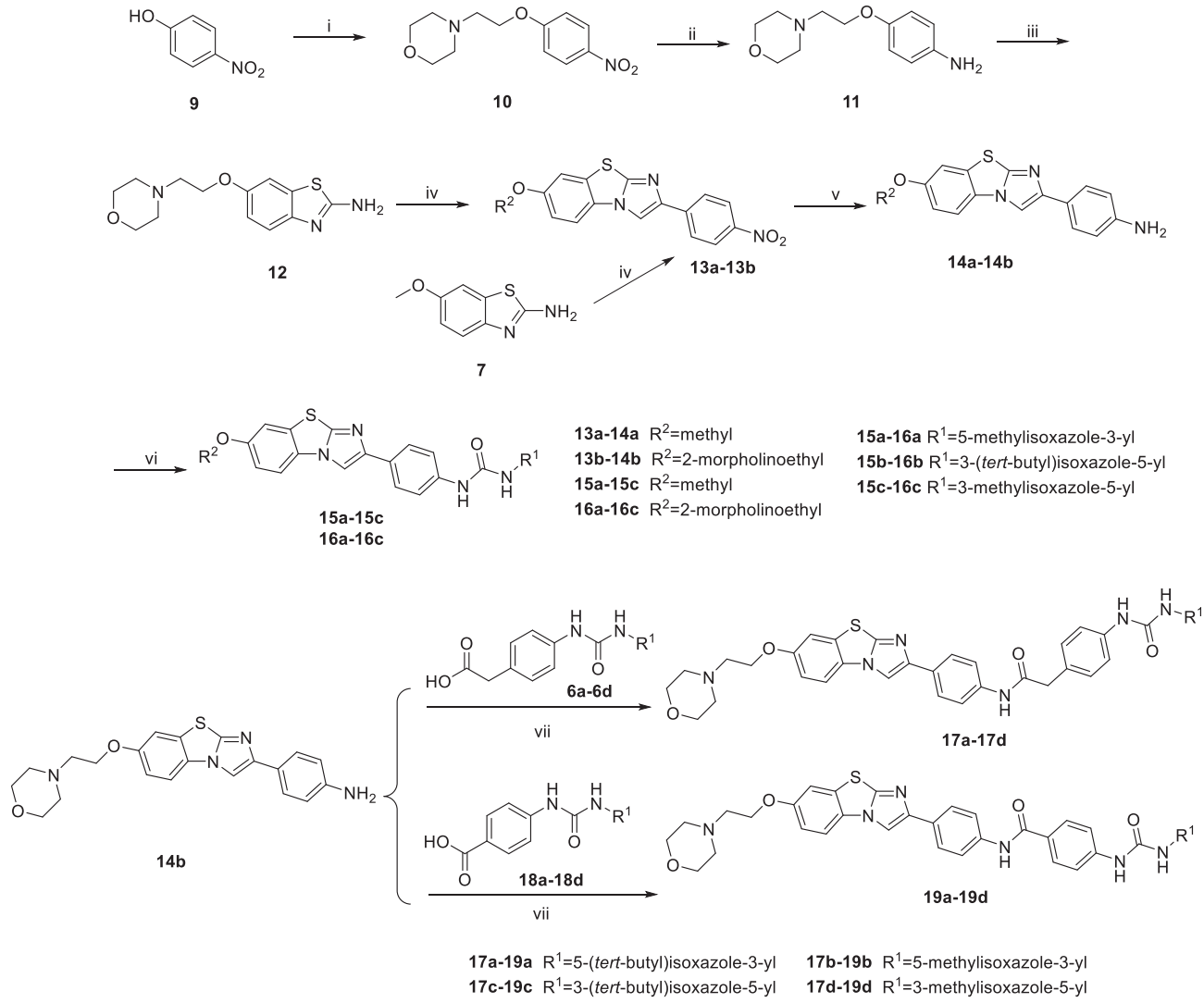
Scheme 1. Synthesis of compounds **8a-8d**. Reagents and conditions: (i) $SOCl_2$, EtOH, r.t., 2 h, 89.3%; (ii) 10% Pd/C, H_2, MeOH, r.t., 12 h, 92.5%; (iii) **4a-4d**, DMAP, TEA, $CHCl_3$, 50 °C, 12 h, 55.7–83.5%; (iv) 0.5 mol/L NaOH-wt, THF, 40 °C, overnight, 76.2–95.8%; (v) 2-amino-6-methoxybenzothiazole, HATU, DIPEA, DMF, r.t., 20 h, 15.2–48.3%.

hydrochloride followed by reduction of the nitro group with Pd/C catalyst under H_2 gave compound **11**. **11** and ammonium thiocyanate gave rise to compound **12** under the action of bromine and acetic acid. Then compound **12** or 2-amino-6-methoxybenzothiazole (**7**) was condensed with 2-bromo-4′-nitroacetophenone in refluxing isopropanol to yield **13a-13b**. Reduction of the nitro group using iron and ammonium chloride gave the corresponding amines **14a-14b**, which were reacted with the corresponding compound to yield the ureas **15a-16c**. **14b** reacted with the corresponding carboxylic acid to provide amides **17a-17d** and **19a-19d** (Scheme 2).

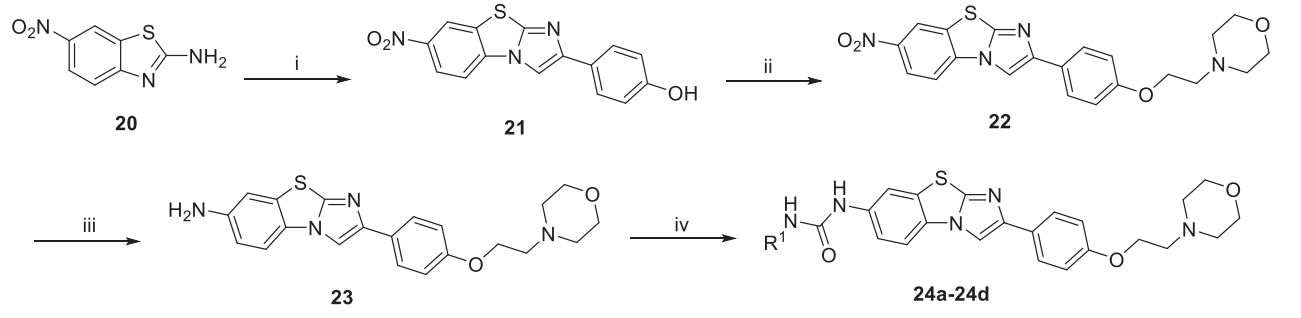
The synthetic route of the compounds **24a-24d** is shown in Scheme 3. Compound **21** was obtained by cyclization of 2-amino-6-nitrobenzothiazole (**20**) and 2-bromo-4′-hydroxyacetophenone. Mitsunobu reaction of **21** with 4-(2-chloroethy)morpholine hydrochloride gave compound **22** in basic conditions. After reduction of the nitro group, compound **23** and **4a-4d** reacted to generate **24a-24d**.

In order to have a preliminary screening, all synthesized compounds were tested for their inhibitory activities against FLT3-ITD at 500 nM with quizartinib as a positive control (Tables 1 and 2). Remarkably, many compounds showed good inhibitory activities against FLT3-ITD at 500 nM. We analyzed the preliminary structure–activity relationships (SARs) based on the results in Tables 1 and 2. Firstly, we tried to synthesize compounds **8a-8d** by ring-opening of the imidazole ring in the basic skeleton. Compared with compounds **15a-15c** (inhibition rates of 52.6–97.2%), ring-opening compounds **8a-8d** led to a significantly decreased inhibition rates against FLT3-ITD, and the inhibition rates were all less than 10%. This may be due to the change in the connection between the basic skeleton and the phenyl ring of the diaryl urea structure, resulting in it not being between the two phenylalanine residues of Phe 691 in the gatekeeper residue and Phe 830 in the DFG motif. It can only form a π-π interaction with Phe 691 in the gatekeeper residue, and cannot achieve the effect of stabilizing the active conformation of the compound.

Based on the above analysis, we fixed the imidazobenzothiazole ring as the basic skeleton and explored the effect of $\mathbf{R^2}$ group as methyl or 2-morpholinoethyl on inhibitory activities of compounds. 2-morpholinoethyl substituted compounds **16a-16c** showed higher FLT3 kinase inhibition rates compared to **15a-15c**. Overall, when the $\mathbf{R^2}$ group was 2-morpholinoethyl substituted, the kinase activities of the compounds were better than that of the methyl substituted compounds. Subsequently, we introduced *p*-substituted amide between the diaryl urea structure and the basic skeleton to obtain compounds **17a-17d** and **19a-19d**. As is showed in the results, this amide bonding pattern significantly reduced the kinase inhibitory activities of the compounds. Compared with compound **16b** (inhibition rate of 99.6%), the inhibition rates against FLT3-ITD of compounds **17c** and **19c** lowered to 38–41%. According to the above activity data, we try to exchange the urea structure and 2-morpholinoethoxy position in the structure of compounds **16a-16c** to obtain compounds **24a-24d**. We hypothesized that the position of urea structure and 2-morpholinoethoxy are exchanged, the benzene ring of the basic skeleton can play the same role as the benzene ring on the diaryl urea structure. So the benzene ring



Scheme 2. Synthesis of compounds **15a-17d** and **19a-19d**. Reagents and conditions: (i) 4-(2-chloroethy)morpholine hydrochloride, Cs_2CO_3, DMF, 100 °C, 2.5 h, 91.1%; (ii) 10% Pd/C, H_2, MeOH, reflux, 3.5 h, 94.0%; (iii) NH_4SCN, Br_2, AcOH, r.t., 4 h, 88.5%;(iv) 2-bromo-4′-nitroacetophenone, $NaHCO_3$, *i*-PrOH, reflux, 3 h, 67.4–89.3%; (v) Fe, NH_4Cl, EtOH, H_2O, reflux, 2.5 h, 84.7–90.8%; (vi) **4b-4d**, DMAP, TEA, $CHCl_3$, 50 °C, overnight, 50.2–85.4%. (vii) HATU, DIPEA, DMF, r.t, 20 h, 54.3–79.8%.



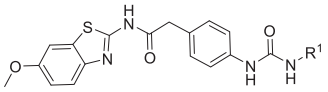
24a R^1=5-(*tert*-butyl)isoxazole-3-yl **24b** R^1=5-methylisoxazole-3-yl **24c** R^1=3-(*tert*-butyl)isoxazole-5-yl **24d** R^1=3-methylisoxazole-5-yl

Scheme 3. Synthesis of compounds **24a-24d**. Reagents and conditions: (i) 2-bromo-4′-hydroxyacetophenone, $NaHCO_3$, *n*-BuOH, reflux, 3 h, 32.4%; (ii) 4-(2-chloroethy)morpholine hydrochloride, Cs_2CO_3, DMF, 120 °C, 3 h, 93.4%; (iii) Fe, NH_4Cl, EtOH, H_2O, reflux, 2.5 h, 84.2%; (iv) **4a-4d**, DMAP, TEA, $CHCl_3$, 50 °C, overnight, 25.5–48.0%.

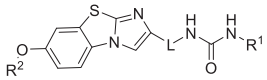
connected to the imidazole ring can be removed or replaced by other groups, which is significant for reducing the molecular weight and improving the physical and chemical properties of the compound. Encouragingly, compounds **24a-24d** displayed similar inhibition rates against FLT3-ITD to **16a-16c**.

Meanwhile, the inhibition rates of compounds **15b**, **16b**, **24a**, and **24c** at 500 nM were all more than 97.2%. Interestingly, we found that the $\mathbf{R^1}$ groups of these compounds were all *tert*-butyl substituted

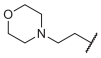
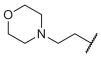
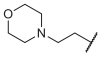
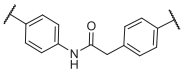
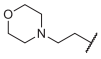
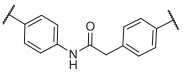
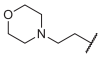
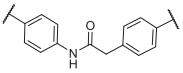
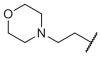
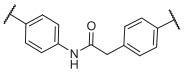
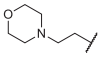
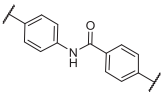
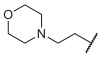
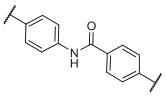
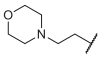
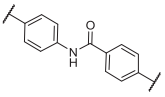
Table 1
Inhibition rates against FLT3-ITD at 500 nM by compounds **8a-8d**, **15a-15c**, **16a-16c**, **17a-17d**, **and 19a-19d**.



8a-8d



15a-17d and **19a-19d**

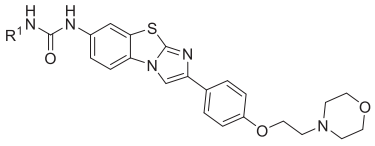
Compd.	L	R^1	R^2	Inhibit rate [a] (at 500 nM) against FLT3-ITD
8a	–		–	2.5% ± 0.4
8b	–		–	−2.0% ± 0.9
8c	–		–	6.4% ± 3.3
8d	–		–	4.5% ± 0.2
15a			$-CH_3$	67.3% ± 0.4
15b			$-CH_3$	97.2% ± 1.1
15c			$-CH_3$	52.6% ± 0.4
16a				79.9% ± 0.9
16b				99.6% ± 0.4
16c				66.7% ± 0.9
17a				36.0% ± 2.9
17b				35.8% ± 2.2
17c				38.5% ± 1.1
17d				45.9% ± 2.6
19a				35.0% ± 6.9
19b				29.0% ± 3.6
19c				41.9% ± 0.7
19d				28.1% ± 3.3
Quizartinib [b]	–	–	–	99.2% ± 0.9

[a] Each experiment was run in duplicate and the values shown are the average of the two.
[b] Used as a positive control.

isoxazole. The methyl-substituted isoxazole ring showed weaker inhibition activities. The results revealed that the inhibition effect against FLT3-ITD after methyl substitution was markedly reduced, compared with the *tert*-butyl substituted compounds.

On the basis of the above inhibition rate results, several potent compounds (**15b**, **16b**, **16c**, and **24a-24d**) were selected to evaluate for their IC_{50} values against FLT3-ITD and anti-proliferative activities against human AML cell line MV4-11 (Table 3). The results suggested that compound **16b** (FLT3-ITD IC_{50} = 5.60 nM, MV4-11 IC_{50} = 0.176 nM) showed better activity than quizartinib (FLT3-ITD IC_{50} = 6.51 nM, MV4-11 IC_{50} = 0.836 nM). In particular, the IC_{50} value against the MV4-11 cell line of compound **16b** was about 4.75-

Table 2
Inhibition rates against FLT3-ITD at 500 nM by compounds **24a-24d**.



Compd.	R^1	Inhibit rate [a] (at 500 nM) against FLT3-ITD
24a		98.8% ± 2.2
24b		82.5% ± 0.7
24c		99.3% ± 1.8
24d		76.9% ± 1.5
Quizartinib [b]	–	99.2% ± 0.9

[a] Each experiment was run in duplicate and the values shown are the average of the two.
[b] Used as a positive control.

Table 3
The IC_{50} values against FLT3-ITD kinase and MV4-11 cell line by compounds **15b**, **16b**, **16c**, and **24a-24d.**

Compd.	FLT3-ITD IC_{50} [a] (nM)	MV4-11 IC_{50} [b] (nM)
15b	20.52	1.967
16b	5.60	0.176
16c	157.05	33.09
24a	8.25	0.782
24b	82.56	39.94
24c	6.83	0.614
24d	109.21	88.72
Quizartinib [c]	6.51	0.836

[a] Values are means of at least two or more experiments.
[b] Values are means of at least three or more experiments.
[c] Used as a positive control.

fold lower than that of quizartinib. Compounds **24a** and **24c** showed similar biological activities to quizartinib, **24a** (FLT3-ITD IC_{50} = 8.25 nM, MV4-11 IC_{50} = 0.782 nM), **24c** (FLT3-ITD IC_{50} = 6.83 nM, MV4-11 IC_{50} = 0.614 nM). However, compounds **16c**, **24b**, and **24d** exhibited weak biological activities. Therefore, *tert*-butyl substitution is necessary to maintain the activity of the compound.

Many effective FLT3 inhibitors have been detected in the SAR studies of imidazobenzothiazole derivatives based on MV4-11 cell assays and FLT3-ITD kinase inhibition assays. Among them, compounds **15b**, **16b**, **24a,** and **24c** have displayed remarkable anti-proliferative activities in a low nanomolar range on the MV4-11 cell line, and the IC_{50} values were 1.967 nM, 0.176 nM, 0.782 nM and 0.614 nM, respectively. Especially, the inhibitory activity of compound **16b** was 4.75 times higher than that of quizartinib. Compounds **16b**, **24a** and **24c** also exhibited excellent biological activity against FLT3-ITD kinase (IC_{50} = 5.60–8.25 nM). Overall, the above statistics suggest the optimal compound **16b** with great promise for the treatment of AML.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127525.

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