Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of 6-phenylimidazo[2,1-*b*]thiazole derivatives as a new type of FLT3 inhibitors

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ARTICLE INFO

Article history:
Received 12 April 2015
Revised 23 August 2015
Accepted 26 August 2015
Available online xxxx

Keywords:
6-Phenylimidazo[2,1-*b*]thiazole
Acute myeloid leukemia (AML)
FLT3
FLT3-ITD
MV4-11
Anti-viability

ABSTRACT

In this investigation, a series of 6-phenylimidazo[2,1-*b*]thiazole derivatives were synthesized. Structure–activity relationship (SAR) analysis of these compounds based on cellular assays led to the discovery of a number of compounds that showed potent activity against FLT3-dependent human acute myeloid leukemia (AML) cell line MV4-11, but very weak or no activity against FLT3-independent human cervical cancer cell line Hela. FLT3 kinase inhibition assays were then performed on the three most active compounds. Among these compounds, 6-(4-(3-(5-(*tert*-butyl)isoxazol- 3-yl)ureido)phenyl)-*N*-(3-(dimethylamino)propyl)imidazo[2,1-*b*]thiazole-3-carboxamide (**19**) exhibited the highest potency in both cellular (MV4-11, IC_{50}: 0.002 μM) and enzymatic (FLT3, IC_{50}: 0.022 μM) assays. Further in-depth in vitro anti-AML activity and mechanism of action studies were carried out on compound **19**.

Acute myeloid leukemia (AML) is a malignant disease of the bone marrow and blood, characterized by the accumulation of rapidly growing abnormal white blood cells in the bone marrow that interferes with the production of normal blood cells.[1,2] Chemotherapies with cytotoxic agents such as cytarabine, daunorubicin, and mitoxantrone are still the main treatment strategy for AML.[3,4] Nevertheless, the toxicities of these conventional cytotoxic agents together with drug resistance and relapse force us to develop more efficient targeted drugs.[5,6] A number of studies have revealed that mutations and/or aberrant expression of specific protein tyrosine kinases (PTKs) are often responsible for the initiation and development of AML.[7,8] Of special note is the FMS-like tyrosine kinase 3 (FLT3). Activating mutations in FLT3 kinase are found in up to one-third of AML cases and the most prevalent activating mutation is 'internal tandem duplications' (ITDs) in the juxtamembrane domain that lead to constitutive, ligand-independent activation of the kinase.[9–11] Numerous studies have demonstrated that FLT3-ITD mutations represent a driving mutation for the development of AML and are associated with a poor prognosis for overall survival.[12–14] Therefore, FLT3 has been considered as a potential molecular target in the treatment of AML.

Discovery of FLT3 inhibitors has attracted much attention in recent years due to their potential therapeutic values in AML. A number of FLT3 inhibitors have been developed, and several of them have entered into clinical trials.[15–19] However the clinical efficacy of most of these FLT3 inhibitors in patients with AML seems unimpressive, mainly because of their potency and/or adverse events. Therefore, it is still necessary to discover more novel FLT3 inhibitors with high potency and low toxicity at present.

Currently, the 'enzyme-cell-animal' drug discovery mode faces some challenges. Firstly, the screening process is increasingly expensive, which restricts more academic institutes with limited fund support, like us, to participate in the drug screening campaign. Secondly, the screening mode sometimes does not work. For example, a compound that shows potent activity in enzyme may be not very effective in cell and animal. Cheap and reliable screening strategies are thus needed to overcome the shortcomings. In this investigation, a pure cell-based screening strategy was adopted to identify new FLT3 inhibitors, in which two cell lines MV4-11 and Hela were used.[20–24] MV4-11 is a typical FLT3-dependent human AML cell line, and Hela is an FLT3-independent human cervical carcinoma cell line. The use of Hela is for ruling out

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http://dx.doi.org/10.1016/j.bmcl.2015.08.068
0960-894X/

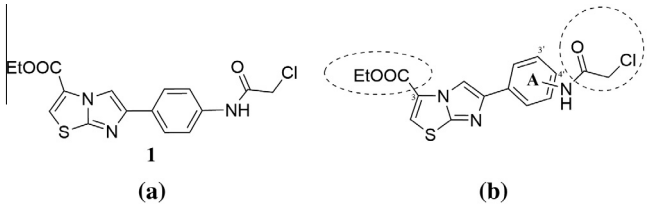


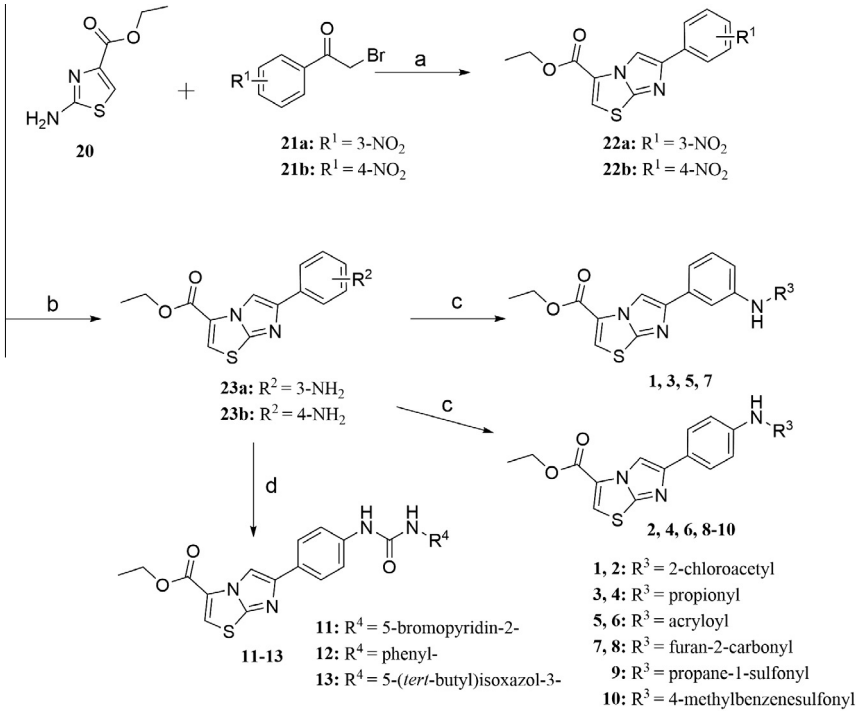
Figure 1. (a) The structure of compound **1**. (b) Schematic showing subgroups that are the focus of structural modifications.

activity due to non-FLT3 mediated effects ('off target' or cellular toxic effects). Compounds are likely better FLT3 inhibitors if they have high potency against MV4-11 cells but low or no activity against Hela. The enzymatic assays were also performed but just on compounds with higher potency at the cellular level.

With the cell-based screening strategy, we first performed a screening against an in-house chemical database containing about 200 compounds, which led to the discovery of a number of active compounds. Among these compounds, ethyl 6-(4-(2-chloroacetamido)phenyl)imidazo[2,1-*b*]thiazole-3-carboxylate (**1**, Fig. 1a) attracted our attention since it contains a new scaffold, namely 6-phenylimidazo[2,1-*b*]thiazole; 6-phenylimidazo[2,1-*b*]thiazole derivatives have not been reported as FLT3 inhibitors. Compound **1** displayed an IC_{50} (half maximal inhibitory concentration) value of 9.07 μM against MV4-11 cells and almost no activity against Hela cells. Obviously, the potency is not good. The purpose here is to further optimize the potency with the cell-based strategy. A series of 6-phenylimidazo[2,1-*b*]thiazole derivatives will be synthesized, and the structure–activity relationship (SAR) of these compounds will also be discussed.

The SAR analysis will focus on two regions: the 3′/4′-position substituent of phenyl ring (ring A) and the 3-position substituent of imidazo[2,1-*b*]thiazole (Fig. 1b). Firstly, we changed the substituent of phenyl ring with different groups and fixed the 3-position of imidazo[2,1-*b*]thiazole as its original ethyl ester. A total of 13 compounds were synthesized. The synthetic routes for these compounds are depicted in Scheme 1. Treatment of ethyl 2-aminothiazole-4-carboxylate **20** with 2-bromo-1-phenylethan-1-one **21a** or **21b** provided intermediate nitrobenzene **22a** or **22b**, respectively,[25] followed by reduction of the nitro group to give the corresponding aniline **23a** or **23b**. Then a series of amide compounds, **1**–**10**, were obtained by condensation of **23a**/**b** with a variety of acyl chlorides in a basic condition. Urea series of compounds **11**–**13** were synthesized from **23b** by condensation with diverse aromatic amines and triphosgene. Bioactivities of these compounds are shown in Table 1. Obviously, compounds with a *para*-substituent on the phenyl ring exhibited a higher anti-viability activity against MV-4-11 cells than corresponding ones with a *meta*-substituent. The most potent compound corresponds to **13**, which contains the substituent 1-(5-(*tert*-butyl)isoxazol-3-yl)urea at the *para*-position of phenyl ring. Compound **13** as well as others displayed a very weak or no activity against Hela, indicating that the anti-viability activities of these compounds against the MV4-11 cells were not due to cellular toxicity.

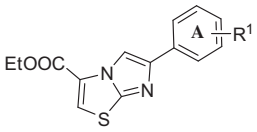
We next fixed the *para*-substituent of phenyl ring as the optimal 1-(5-(*tert*-butyl)isoxazol-3-yl)urea and varied the 3-position of imidazo[2,1-*b*]thiazole. A total of 6 compounds (**14**–**19**) were synthesized via the synthetic method outlined in Scheme 2. Compound **13** synthesized above was hydrolyzed to corresponding carboxylic acid **24** by sodium hydroxide. The amidation/esterification reaction of carboxylic acid **24** with diverse moderate size amines or alcohols was carried out to provide the desired compounds **14**–**19**. Table 2 summarizes the anti-viability activities of this set of compounds. Replacement of the ester group of compound **13** by a variety of amides led to a significantly increased bioactivity (**14**, **16**–**19** vs **13**) against MV4-11 cells, while the replacement of the ester group of compound **13** with another ester group showed a similar anti-viability activity (**15** vs **13**). These results imply that the existence of amide groups is critical for retaining potent cellular activity.

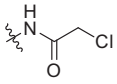
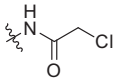
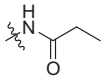
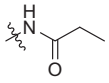
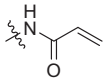
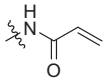
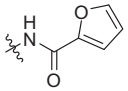
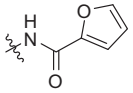
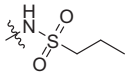
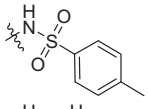
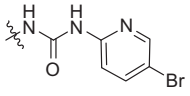
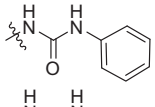
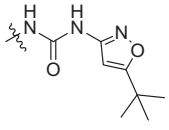


Scheme 1. Reagents and conditions: (a) dioxane, reflux, 10–12 h, 90%; (b) (i) $SnCl_2{\cdot}2H_2O$, EtOH, reflux; (ii) cond. HCl, reflux, 8 h, 60–65%; (c) acyl chloride, CH_2Cl_2, DIEA, 0 °C–rt, 1 h, 60–95%; (d) (i) aromatic amine, triphosgene, CH_2Cl_2, Et_3N, 0 °C–rt; (ii) toluene, reflux, 8–10 h, 50–85%.

Please cite this article in press as: Lin, X.-D.; et al. *Bioorg. Med. Chem. Lett.* (2015), http://dx.doi.org/10.1016/j.bmcl.2015.08.068

Table 1
Anti-viability activities of 6-phenylimidazo[2,1-*b*]thiazole derivatives with different *meta*- and *para*-position substituents against MV4-11 cells and HeLa cells



Compd	Attaching position to A	R^1	MV4-11[a] (IC_{50}, μM)	Hela[a] (IC_{50}, μM)
1	3		17.731 ± 1.88	~20
2	4		9.070 ± 1.04	>20
3	3		>20	>20
4	4		9.523 ± 1.44	>20
5	3		>20	>20
6	4		2.929 ± 0.27	>20
7	3		>20	>20
8	4		9.917 ± 1.63	>20
9	4		>20	>20
10	4		>20	>20
11	4		2.444 ± 0.23	>20
12	4		>20	>20
13	4		0.2741 ± 0.027	>20

[a] Each compound was tested in triplicate; the data are presented as the mean ± SD.

Collectively, the above SAR analysis led to the discovery of a number of compounds that exhibited high potency against FLT3-dependant MV4-11 cells. Among these compounds, **16**, **17** and **19** are the three most active ones, which all showed IC_{50} values less than 0.01 μM. Kinase inhibitory potencies against FLT3 were then measured for the three compounds. The results showed that the IC_{50} values of compounds **16**, **17** and **19** against FLT3 were 22 nM, 95 nM and 22 nM, respectively. Obviously, compound **16** and **19** showed the same highest potency in enzymatic assays. However, considering that compound **19** is the most active one in cellular assays, further studies including kinase inhibition profile, cancer cell selectivity, and signaling inhibition in intact cells were performed just on compound **19**.

Kinase inhibition profiles of compound **19** against a panel of selected recombinant human protein kinases are shown in Table 3. Compound **19** potently inhibited FLT3 and Fms kinases (IC_{50} = 0.022 μM for FLT3 and 0.043 μM for Fms). Compound **19** also exhibited considerable potency against several other kinases,

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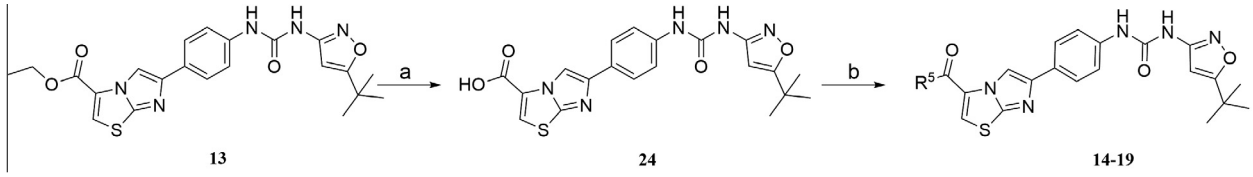
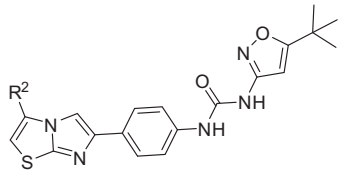
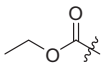
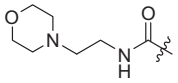
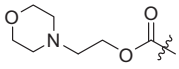
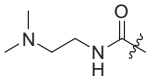
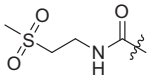
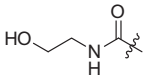
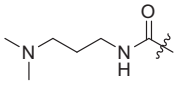


14: R^5 = 2-morpholinoethyl-1-NH-
15: R^5 = 2-morpholinoethyl-1-OH-
16: $R^5 = (CH_3)_2N(CH_2)_2NH$-
17: R^5 = 2-(methylsulfonyl)ethyl-1-NH-
18: $R^5 = HO(CH_2)_2NH$-
19: $R^5 = (CH_3)_2N(CH_2)_3NH$-

Scheme 2. Reagents and conditions: (a) NaOH, EtOH, reflux, overnight; (b) amines, HOBT, EDCI, DIEA, CH_2Cl_2, reflux, 10–12 h, 40–80%; or alcohols, DMAP, DCC, CH_2Cl_2, rt, 8 h, 38%.

Table 2
6-Phenylimidazo[2,1-*b*]thiazole derivatives with different substituents R^2, together with their anti-viability activities against MV4-11 cells and HeLa cells



Compd.	R^2	MV4-11[a] (IC_{50}, μM)	Hela[a] (IC_{50}, μM)
13		0.2741 ± 0.027	>20
14		0.01628 ± 0.003	>20
15		0.1456 ± 0.08	>20
16		0.0097 ± 0.002	~20
17		0.0049 ± 0.0018	>20
18		0.0156 ± 0.003	>20
19		0.002 ± 0.001	~20
Sorafinib		0.007 ± 0.0012	5.23 ± 0.38

[a] Each compound was tested in triplicate; the data are presented as the mean ± SD.

including cKit (IC_{50} = 0.294 μM), PDGFRα (IC_{50} = 0.37 μM), PDGFRβ (IC_{50} = 1.982 μM), Flt1 (IC_{50} = 2.826 μM), Flt4 (IC_{50} = 1.188 μM), KDR (IC_{50} = 2.458 μM), Pim-1 (IC_{50} = 4.037 μM), and Ret (IC_{50} = 0.986 μM). Compound **19** displayed almost no inhibitory activity against 40 other tested protein kinases (IC_{50} >10 μM). These data demonstrate that compound **19** is a potent FLT3 inhibitor with good kinase selectivity.

Table 4 shows the anti-viability activities of compound **19** against various cell lines, including leukemia and solid tumor cell lines. Except for potently inhibiting MV4-11 cells with an IC_{50} value of 0.002 μM (also see Fig. 2a), compound **19** just showed moderate activity against several cell lines including OCI-LY19 (lymphoma, IC_{50} = 3.3 μM), SH-SY5Y (neuroblastoma, IC_{50} = 3.3 μM),

Table 3
Kinase inhibition profiles of compound **19** against FLT3 and a panel of selected protein kinases

Kinase	IC_{50} (μM)	Kinase	IC_{50} (μM)
Flt3	0.022	EphB2	>10
cKit	0.294	ErbB2	>10
Fms	0.043	ErbB4	>10
PDGFRα	0.37	Fes	>10
PDGFRβ	1.982	FGFR2	>10
Flt1	2.826	Fyn	>10
Flt4	1.188	GSK3β	>10
KDR	2.458	IGF-1R	>10
Pim-1	4.037	IRE1	>10
Ret	0.986	JAK3	>10
Abl	>10	JNK3	>10
Arg	>10	Lck	>10
AMPKα1	>10	Lyn	>10
Aurora-A	>10	MEK1	>10
Aurora-B	>10	Met	>10
Axl	>10	MLK1	>10
Bmx	>10	PAK1	>10
BTK	>10	PAK2	>10
CaMKIV	>10	PAK4	>10
CDK2	>10	PKCμ	>10
CDK7	>10	PKD2	>10
CHK1	>10	Plk1	>10
c-RAF	>10	Rsk1	>10
DMPK	>10	Syk	>10
EGFR	>10	TrkB	>10

and negligible activity against the remaining 29 human cancer cell lines. These results indicate that compound **19** is relatively selective for the human AML cell line MV4-11.

The ability of compound **19** to inhibit the activation of FLT3 and downstream signaling proteins in intact cells was assessed by Western blot analysis.[26–28] As shown in Figure 2b, compound **19** inhibited FLT3 phosphorylation in a dose-dependent manner. Consistent with the downregulation of the phosphorylation of FLT3, the phosphorylation of the downstream signaling proteins STAT5 and ERK1/2 was also significantly inhibited at concentrations larger than 0.001 μM (Fig. 2b).

In conclusion, SAR studies of 6-phenylimidazo[2,1-*b*]thiazole derivatives based on cellular assays combined with kinase inhibition assays led to the discovery of a number of potent FLT3 inhibitors. Compound **19** is the most active one in both cellular and enzymatic levels. It also displayed very good selectivity for FLT3 against a panel of selected kinases and considerable cell selectivity for human AML cell line MV4-11. Western blot analysis showed that compound **19** significantly and dose-dependently down-regulated the phosphorylation of FLT3 and its downstream signal proteins STAT5 and ERK. Overall, compound **19** could be taken as a good lead compound for further lead optimization for the treatment of AML.

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Table 4
Anti-viability activities of compound **19** against various cancer cell lines

Cell line	Tumor type	IC_{50} (μM)	Cell line	Tumor type	IC_{50} (μM)
MV4-11	Leukemia, AML	0.002 ± 0.001	MDA-MB-157	Breast cancer	>10
Jurkat	Leukemia, ALL	10	MDA-MB-231	Breast cancer	10
Raji	Lymphoma	>10	MDA-MB-435	Breast cancer	>10
OCI-LY19	Lymphoma	3.3 ± 0.02	MDA-MB-453	Breast cancer	>10
SH-SY5Y	Neuroblastoma	3.3 ± 0.031	MCF7	Breast cancer	>10
Hela	Cervical cancer	>10	SKBr3	Breast cancer	10
UMUC3	Bladder cancer	10	ZR-75-1	Breast cancer	>10
HT29	Colon cancer	10	BT474	Breast cancer	10
HCT116	Colon cancer	10	H1975	Lung cancer	>10
DLD-1	Colon cancer	10	PC9	Lung cancer	>10
SW480	Colon cancer	10	Calu-6	Lung cancer	10
22Rv1	Prostatic cancer	>10	A549	Lung cancer	10
A875	Melanoma	10	KYSE150	Esophageal cancer	>10
A375	Melanoma	10	HepG2	Liver cancer	10
A2058	Melanoma	10	SK-Hep1	Liver cancer	10
U251	Spongiocytoma	>10			

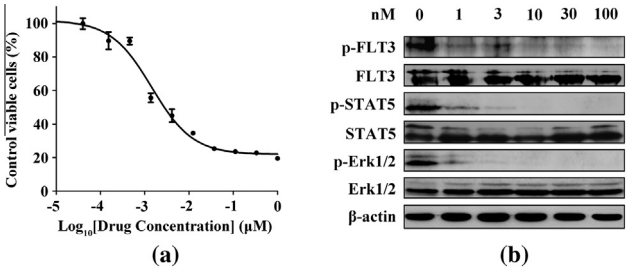

Figure 2. (a) Anti-viability profile of compound **19** against MV4-11 cells using the MTT assay method. (b) Western blot analysis showing the influence of compound **19** on the FLT3 signaling in intact MV4-11 cells.

Acknowledgement

This work was supported by the 973 Program (2013CB967204), and the National Natural Science Foundation of China (81172987, 81325021, 81473140), and partly by the National Science and Technology Major Project (2012ZX09501001-003, 2012ZX09102-101-002), and PCSIRT (IRT13031).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.08.068.

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