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Design and synthesis of novel dasatinib derivatives as inhibitors of leukemia stem cells

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ABSTRACT

We used the concept of bioisosteres to design and synthesize a novel series of dasatinib derivatives for the treatment of leukemia. Unfortunately, most of the dasatinib derivatives did not show appreciable inhibition against leukemia cell lines K562 and HL60. However, acrylamide compound **2c** had comparable inhibitory activity with dasatinib against K562 cells (IC_{50} = 0.039 nM vs. 0.069 nM). And amide compound **2a** and acrylamide compound **2c** also had comparable inhibitory activity with dasatinib against the leukemia cell line HL60 (IC_{50} = 0.25 nM and 0.26 nM vs. 0.11 nM). Against the leukemia progenitor cell line KG1a, triazole compounds **15a** and **15d**–**15f** and oxadiazole compounds **24a**–**24d** were more potent than dasatinib. In particular, the hydroxyl compounds **15a** and **24a** were about 64 and 180 fold more potent than dasatinib against KG1a cells (IC_{50} = 0.14 μM and 0.05 μM vs. 8.98 μM). Compounds **15a** and **24a** also inhibited colony formation in MCF-7 cells and inhibited cell migration in the cell wound scratch assay in B16BL6 cells. Moreover, hydroxyl compounds **15a** and **24a** had low toxicity *in vivo*.

Dasatinib is a second-generation, orally available, potent, and multi-targeted inhibitor of Bcr-Abl and Src family kinases.[1,2] Dasatinib is effective in imatinib-resistant wild type and mutant BCR-ABL cell lines, except in those carrying the T315I mutation.[3–7] Dasatinib also has high selectivity for normal hematopoietic cells and leukemia cells.[8] When dasatinib was used in conjunction with bone marrow transplantation, however, many mutations emerged, including T315I/A, F317L/I/C/V, V299L (IC_{50} > 15 nM), Y253F, E255 K/V and G250E.[9–14] Dasatinib also fails to eliminate the quiescent chronic myeloid leukemia (CML) stem cell compartment.[15]

Bioisosterism is a very important strategy for drug design in medicinal chemistry, and 1,3,4-oxadiazole and 1,2,3-triazole are considered to be bioisosteres of the amide group. 1,3,4-Oxadiazole and 1,2,3-triazole rings have special biological properties and these moieties have been successfully used in multi-targeted drugs, including the anti-cancer drug, zibotentan.[16]

Here, a series of dasatinib derivatives, containing oxadiazole and triazole rings, was synthesized and the activity of the compounds was evaluated in the leukemia cell lines K562 and HL60, and the leukemia progenitor cell line, KG1a. Lead compounds were further evaluated by the colony forming assay in MCF-7 cells and the cell wound scratch assay in B16BL6 cells. Preliminary toxicity was also discussed.

Dasatinib derivatives were prepared using the sequences shown in Schemes 1–5.

Firstly, acid amide compounds **2a**–**2d** were synthesized (Scheme 1). Dasatinib was treated with phthalimide to provide compound **1**, which was deprotected to provide amine compound **2a**. Amine compound **2a** was then converted to compounds **2b**–**2d**. Treatment with CS_2 under basic conditions afforded isothiocyanate compound **2b**, treatment with acryloyl chloride provided the allyl amide compound **2c** and treatment with chloroacetyl chloride gave the chloroethyl amide compound **2d**.

Triazole ring compounds **15a**–**15f** were synthesized as shown in Schemes 2 and 3. Boc protection of amine compound **3** provided compound **4**, which was converted to iodide compound **5** using *N*-iodosuccinimide (NIS). Iodide compound **5** was reacted with trimethylsilylacetylene to provide compound **6**, which was then deprotected to give alkyne compound **7**. Azide compound **9** and alkyne compound **7** were treated with Cu_2O nanoparticles to give the triazole ring compound **10** using click chemistry. The Boc

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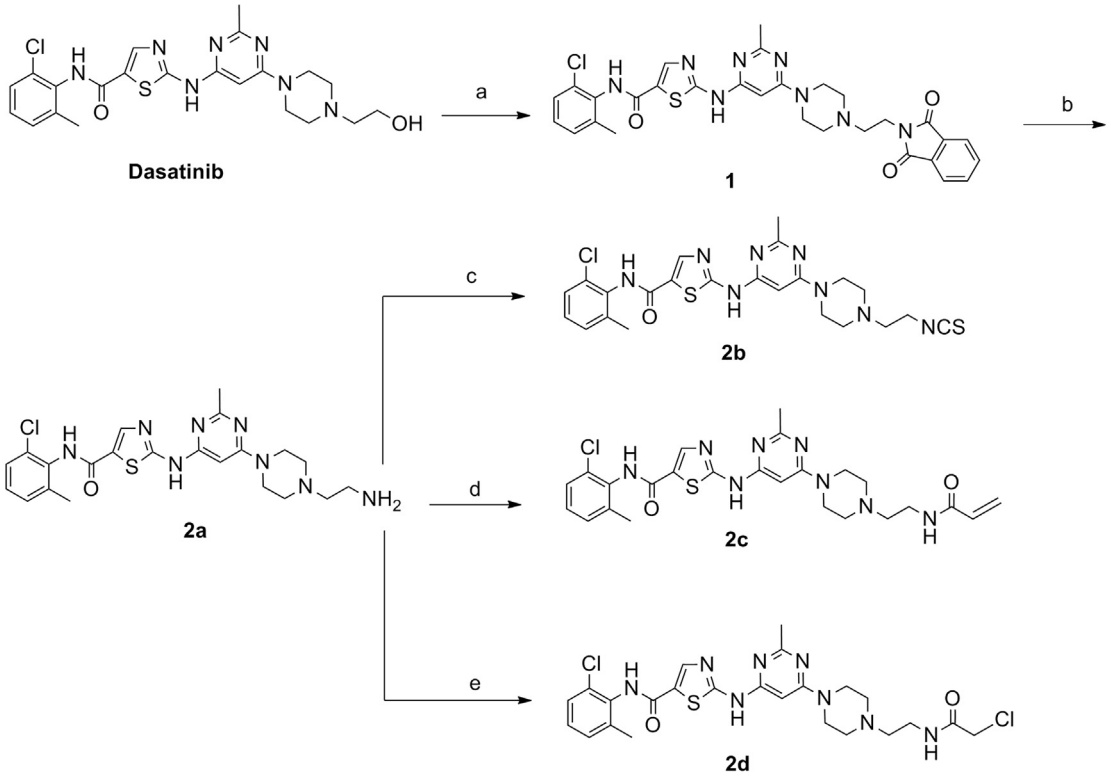


Scheme 1. Reagents and conditions: (a) PPh_3, phthalimide, DIAD, THF, r.t, 88%; (b) $N_2H_2{\cdot}H_2O$, EtOH, 78 °C, 72%; (c) DCC, CS_2, CH_2Cl_2, r.t, 52%; (d) acryloyl chloride, Et_3N, CH_2Cl_2, r.t, 49%; (e) chloroacetyl chloride, Et_3N, CH_2Cl_2, r.t, 55%;

protecting group was then removed from compound **10** to provide compound **11**. Condensation of compounds **11** and **12** gave compound **13**, which was treated with different amines to provide compounds **15a** and **15b**. Triazole amine compound **15c** was prepared from compound **15b** by removal of the Boc group. Compounds **15d**–**15f** were synthesized using the same procedures for synthesis of acid amide compounds **2b**–**2d**, respectively.

1,3,4-Oxadiazole compounds **24a**–**24e** were prepared as shown in Schemes 4 and 5. Amine compound **16** was treated with compound **12** to afford pyrimidine **17**, which was condensed with different amine by condensation reaction to provide compounds **19a** and **19b**. Compounds **19a** and **19b** were treated with hydrazine solution to provide carbohydrazide compounds **20a** and **20b**, which were treated with chloride **21** to give compounds **22a** and **22b**. Compounds **22a** and **22b** were then converted to compounds **23a** and **23b**. Deprotection of compounds **23a** and **23b** gave compounds **24a** and **24b**. Compound **24b** was then converted to compounds **24c**–**24e**, using the procedures described for the synthesis of compounds **2b**–**2d**.

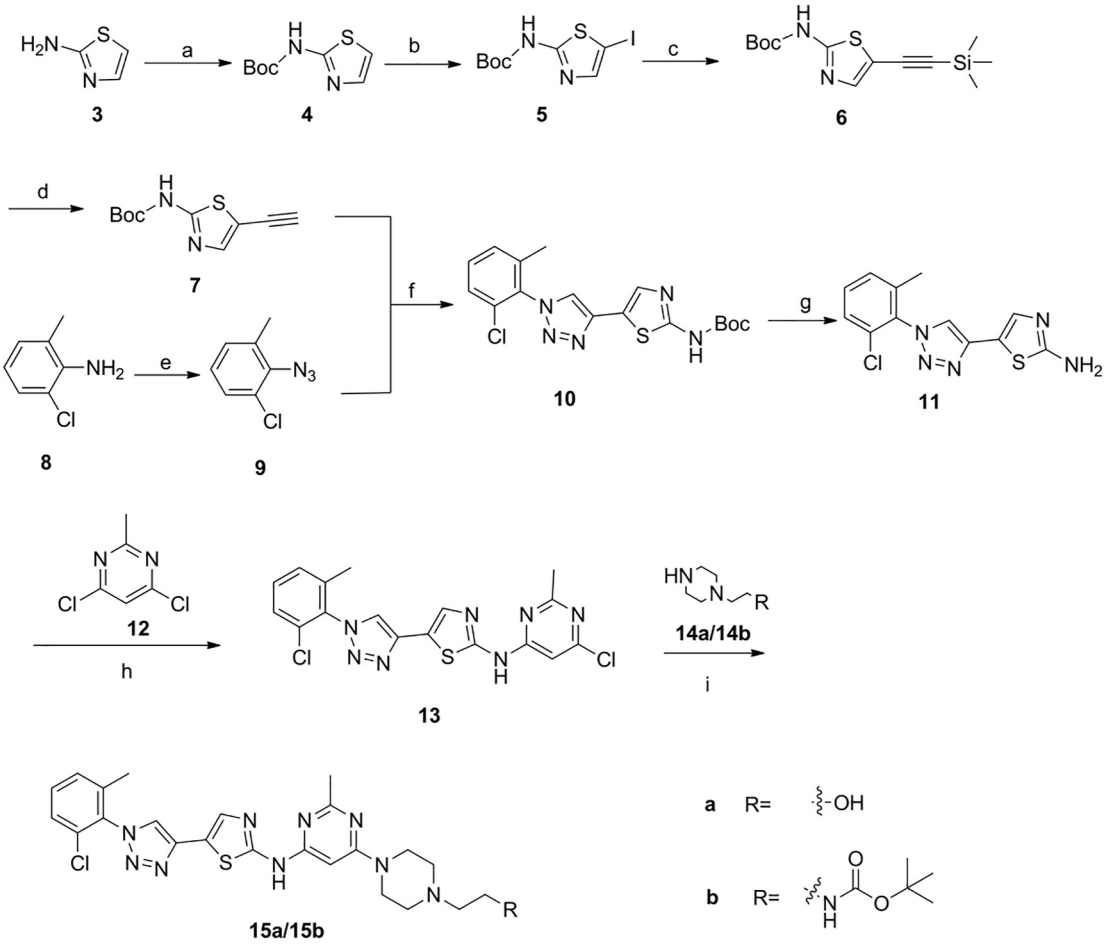
Most of the dasatinib derivatives did not noticeably inhibit the growth of K562 and HL60 cells, but both triazole and oxadiazole compounds were more potent than dasatinib against leukemia progenitor cell line KG1a (Table 1).

For compounds **2a**–**2d**, inhibitory activities did not increase. In K562 cells, the IC_{50} values of compounds **2a**–**2d** were 0.72, 3.77, 0.039 and 1.67 nM, respectively. The inhibitory activity of acrylamide compound **2c** was comparable to that of dasatinib (IC_{50} = 0.069 nM). In HL60 cells, the activities of compounds **2a** and **2c** were very similar to that of dasatinib (IC_{50} = 0.25 nM and 0.26 vs. 0.11 nM). Isothiocyanate compound **2b** (IC_{50} = 1.07 nM) had comparable inhibitory activity to that of compound **2d** (IC_{50} = 0.86 nM). In KG1a cells, isothiocyanate compound **2b** (IC_{50} = 1.96 μM) was more potent than dasatinib (IC_{50} = 8.98 μM) whereas compounds **2a**, **2c** and **2d** (IC_{50} = 25.39, 12.88, and 13.56 μM, respectively) were less potent than dasatinib.

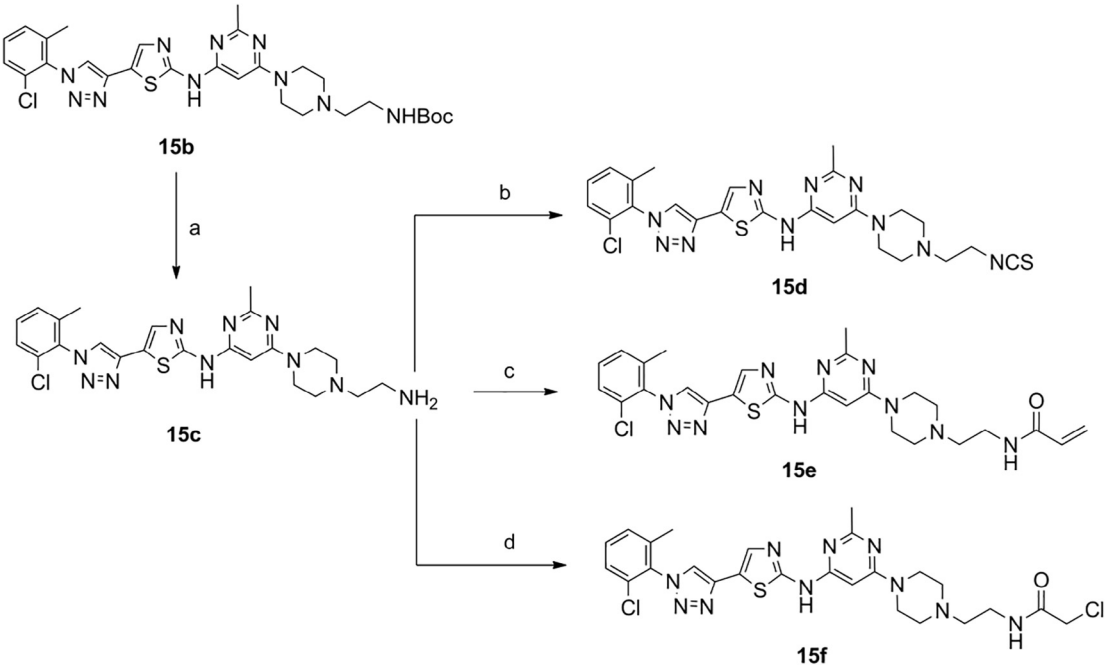
Inhibitory activities of triazoles **15a** and **15c**–**15f** were reduced 170–530-fold against K562 cells and 35–150-fold against HL60 cells whereas inhibitory activities were enhanced 4–64-fold against leukemia progenitor cells KG1a. Amongst the triazoles, isothiocyanate compound **15d** had the lowest inhibitory activity against K562 and HL60 cells (IC_{50} = 36.48 and 16.57 nM, respectively) and compounds **15a**, **15c**, **15e** and **15f** had comparable inhibitory activities (IC_{50} = 14.14, 11.73, 15.09 and 17.65 nM, respectively, against K562 cells and 4.19, 4.08, 5.46 and 9.82 nM, respectively, against HL60 cells). In KG1a cells, amine compound **15c** had the poorest inhibitory activity (IC_{50} = 2.06 μM) and compound **15a** had best inhibitory activity (IC_{50} = 0.14 μM). Compounds **15d**–**15f** had comparable inhibitory activities (IC_{50} = 0.32, 0.25 and 0.45 μM).

The IC_{50} values of the oxadiazole compounds against both K562 and HL60 cell lines were at least an order of magnitude higher than that of dasatinib. Against KG1a cells, compound **24e** (IC_{50} = 9.75 μM) had comparable inhibitory activity to dasatinib (IC_{50} = 8.98 μM). Compounds **24a**–**24d** (IC_{50} = 0.05, 0.68, 0.15, and 0.11 μM, respectively) were more potent than dasatinib. It is noteworthy that compound **24a** was about 180-fold more potent than dasatinib.

Dasatinib, and compounds **15a** and **24a**, significantly and dose-dependently decreased the formation of colonies in MCF-7 cells, compared with the control group (Fig. 1). The high dose of compound **15a** was more potent than dasatinib and the high dose of compound **24a** was comparable with dasatinib. Compounds **15a** and **24a** could thus inhibit the growth of cancer stem cells, and compound **15a** was more potent than compound **24a**.



Scheme 2. Reagents and conditions: (a) $(Boc)_2O$, Et_3N, DMAP, THF, r.t, 4 h, 64%; (b) NIS, THF, r.t, 2 h, 70%; (c) CuI, $PdCl_2(PPh_3)_2$, Trimethylsilylacetylene, Et_3N, THF, rt, 12 h, 60%; (d) K_2CO_3, MeOH, r.t, 0.5 h, 98%; (e) $NaNO_2$, HCl, NaN_3, H_2O, 3 h, 0 °C; (f) Cu_2O nanoparticles, CH_3CN:H_2O, r.t, overnight, 86%; (g) HCl, DCM, 4 h, 96%; (h) NaH, THF, −20 °C, 86%; (i) DIPEA, 1,4-dioxane, 5–18 h, for **15a**: 88% and for **15b**: 90%.



Scheme 3. Reagents and conditions: (a) TFA, CH_2Cl_2, r.t, 80%; (b) DCC, CS_2, CH_2Cl_2, r.t, 41%; (c) acryloyl chloride, Et_3N, CH_2Cl_2, r.t, 47%; (d) chloroacetyl chloride, Et_3N, CH_2Cl_2, r.t, 37%.

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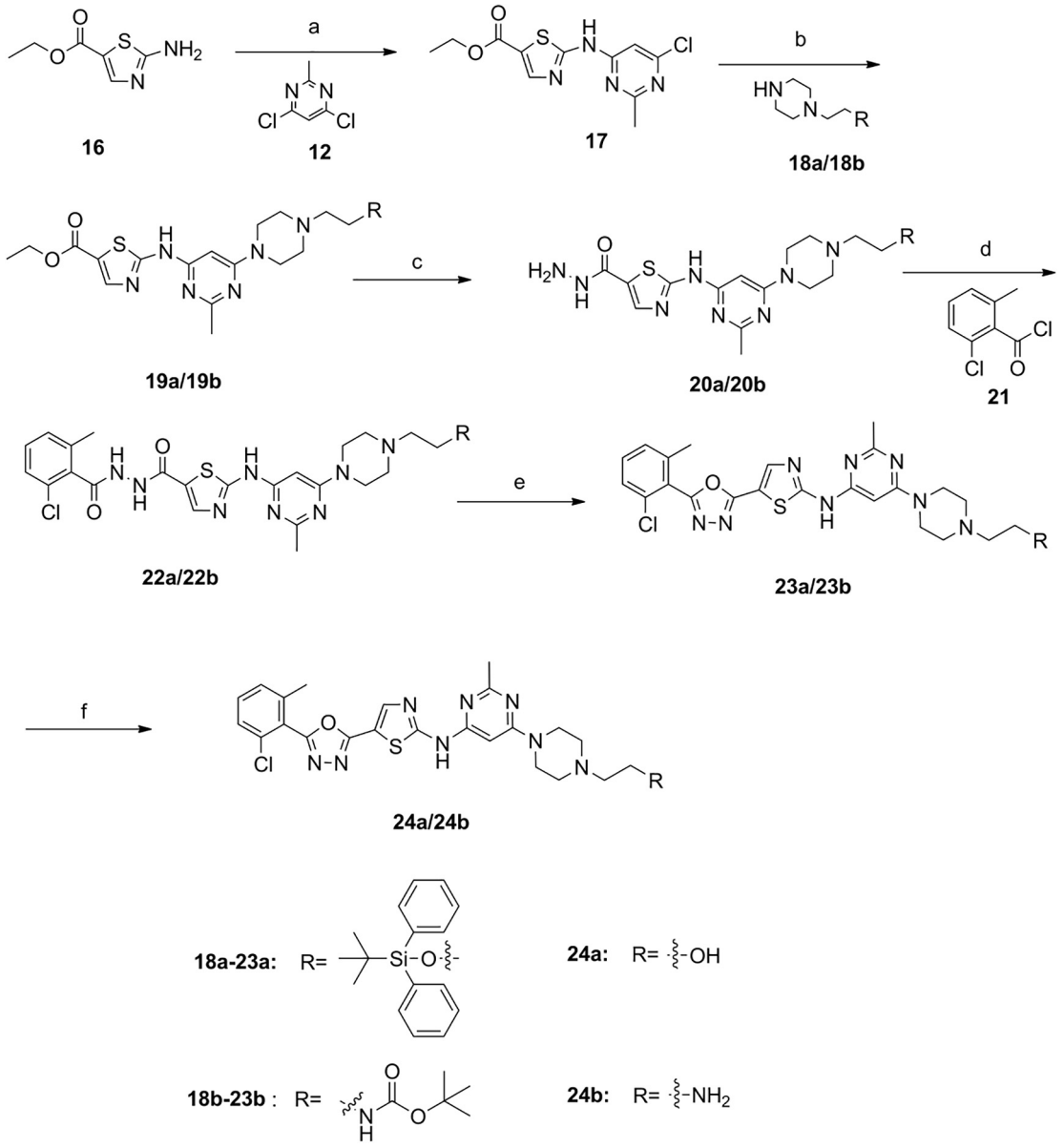

Scheme 4. Reagents and conditions: (a) Cs_2CO_3, DMF, 0 °C, 5 min, r.t, 8 h, 82%; (b) DIPEA, 1,4-dioxane, 103 °C, 5.5 h, for **19a**: 89% and for **19b**: 90%; (c) EtOH, $N_2H_4{\cdot}H_2O$, 78 °C, for **20b**: 59%; (d) Et_3N, r.t, 5 h, for **22a**: 73% and **for** 22b: 80%; (e) TsCl, K_2CO_3, r.t, for **23a**: 76% and for **23b**: 60%; (f) for: **9a**: TBAF, THF, 3 h, r.t, 96%; for: **9b**: TFA, CH_2Cl_2, 2 h, 83%.

Compared with control group, migration was inhibited in B16BL6 cells treated with dasatinib and compounds **15a** and **24a** (Fig. 2). The inhibitory effects of compounds **15a** and **24a** were comparable with the positive control dasatinib, and inhibition of **24a** was stronger than that of **15a**.

The survival rate of mice, dosed with dasatinib, and compounds **15a** and **24a** (all 200 mg/kg) once for 2 weeks, was 100% (Fig. 3). When the dose was increased, mice treated with compounds **15a** and **24a** also survived, demonstrating the low toxicity of compounds **15a** and **24a**.

In summary, we have designed and synthesized novel dasatinib derivatives and evaluated their *in vitro* activity against three different leukemia cell lines (K562, HL60 and KG1a). Compared with dasatinib, all of the newly synthesized triazole and oxadiazole derivatives showed good efficacy in the leukemia progenitor cell line KG1a. In particular, compounds **15a** and **24a** were about 64- and 180-fold more potent than dasatinib against KG1a cells. We speculate, therefore, that compounds **15a** and **24a** may be able to eradicate stem cells in CML. Compounds **15a** and **24a** inhibited colony formation in MCF-7 cells and also inhibited cell migration in the cell wound scratch assay in B16BL6 cells. Compounds **15a** and **24a** had low toxicity *in vivo* and provided a possibility for the treatment of CML.

Acknowledgments

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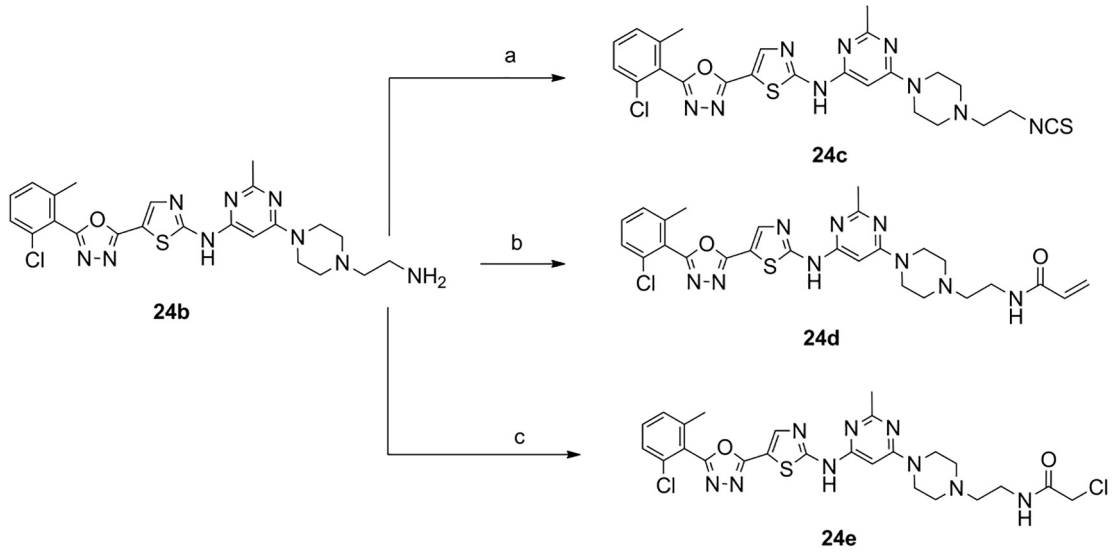
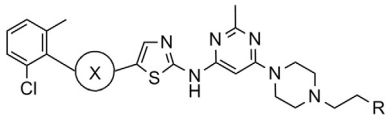
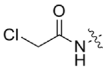
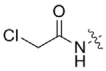
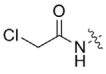


Scheme 5. Reagents and conditions: (a) DCC, CS_2, CH_2Cl_2, r.t, 37%; (b) acryloyl chloride, Et_3N, CH_2Cl_2, r.t, 39.7%; (c) chloroacetyl chloride, Et_3N, CH_2Cl_2, r.t, 35%.

Table 1
Inhibitory effects of dasatinib derivatives in K562, HL60 and KG1a cells.



Compound	X	R	K562 IC_{50} (nM)	HL60 IC_{50} (nM)	KG1a IC_{50} (μM)
Dasatinib		-OH	0.069 ± 0.004	0.11 ± 0.02	8.98 ± 0.03
2a		$-NH_2$	0.72 ± 0.35	0.25 ± 0.06	25.39 ± 0.35
2b		-NCS	3.77 ± 1.72	1.07 ± 0.55	1.96 ± 0.18
2c			0.039 ± 0.009	0.26 ± 0.13	12.88 ± 2.59
2d			1.67 ± 0.22	0.86 ± 0.08	13.56 ± 0.18
15a		-OH	14.14 ± 2.64	4.19 ± 0.78	0.14 ± 0.03
15c		$-NH_2$	11.73 ± 3.41	4.08 ± 0.61	2.06 ± 0.20
15d		-NCS	36.48 ± 10.69	16.57 ± 2.86	0.32 ± 0.10
15e			15.09 ± 2.99	5.46 ± 0.38	0.25 ± 0.06
15f			17.65 ± 1.62	9.82 ± 2.88	0.45 ± 0.07
24a		-OH	$(0.23 \pm 0.07) * 10^3$	$(0.15 \pm 0.08) * 10^3$	0.05 ± 0.008
24b		$-NH_2$	$(0.26 \pm 0.05) * 10^3$	$(0.11 \pm 0.04) * 10^3$	0.68 ± 0.06
24c		-NCS	$(1.81 \pm 0.63) * 10^3$	$(1.42 \pm 0.06) * 10^3$	0.15 ± 0.08
24d			$(0.15 \pm 0.04) * 10^3$	$(0.16 \pm 0.03) * 10^3$	0.11 ± 0.05
24e			$(3.06 \pm 0.23) * 10^3$	$(2.12 \pm 0.29) * 10^3$	9.75 ± 0.11

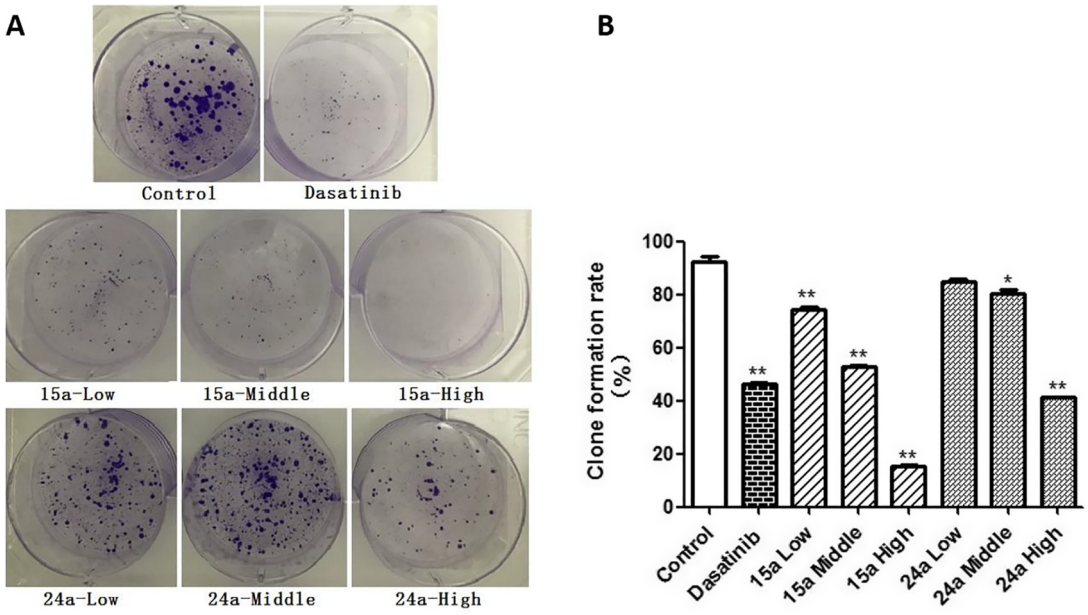


Fig. 1. The effects of **15a** and **24a** on the formation of clones in MCF-7 cell. Cells were administered with positive medicine Dasatinib (7.5 μM), compounds **15a** (Low: 0.96 μM, Middle: 1.90 μM, High: 3.80 μM) and **24a** (Low: 0.68 μM, Middle: 1.35 μM, High: 2.70 μM) for 10 days. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the control group.

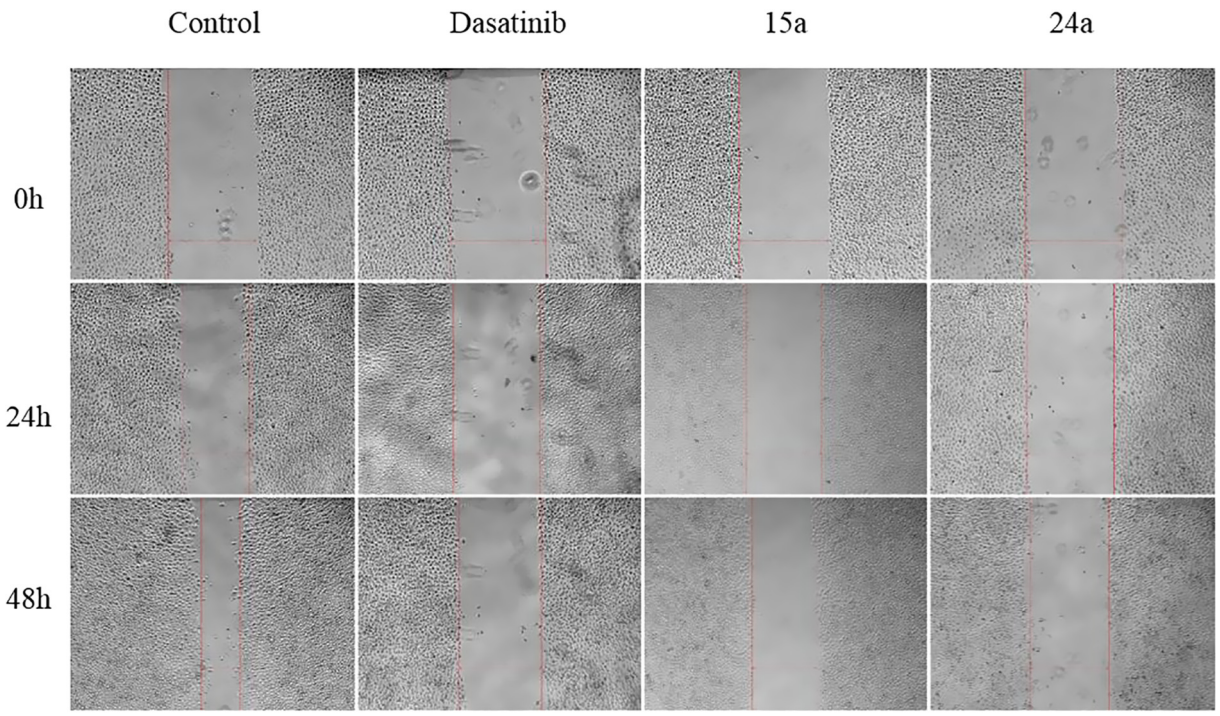


Fig. 2. The effects of compounds **15a** and **24a** on the migration of B16BL6 cells. Cells were administered with positive medicine Dasatinib (8.13 μM), compounds **15a** (0.82 μM) and **24a** (1.43 μM) for 2 days.

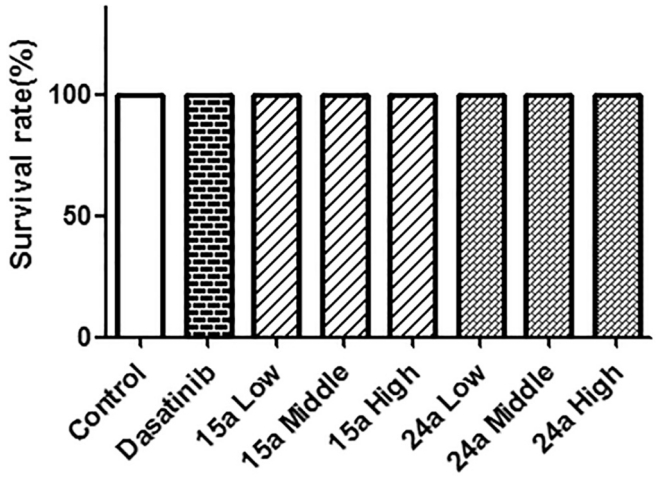


Fig. 3. The toxicity of compounds **15a** and **24a** on mice. Mice were orally administered with positive medicine Dasatinib (200 mg/kg), compounds **15a** (200 mg/kg, 500 mg/kg and 1000 mg/kg) and **24a** (200 mg/kg, 500 mg/kg and 1000 mg/kg) once and observed for 14 days.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2018.01.011.

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