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Design, synthesis and biological evaluation of novel pyrimidine, 3-cyanopyridine and *m*-amino-*N*-phenylbenzamide based monocyclic EGFR tyrosine kinase inhibitors

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ABSTRACT

36 new compounds with the typical skeleton of 4-anilino-5-vinyl/ethynyl pyrimidine, 4-anilino-3-cyano-5-vinyl/ethynyl/phenyl pyridine, and *m*-amino-*N*-phenylbenzamide, are designed, synthesized and selectively tested on EGFR, ErbB-2 kinases, and A-549, HL60 cells growth inhibition. Results from the bioactivity and chemical structures yield preliminary structure–activity relationships (SARs). The most potent 5-ethynylpyrimidine derivative **20a** has an IC_{50} value of 45 nM to EGFR kinase. Several compounds of other series also show IC_{50} values <1 μM for EGFR and <5 μM for A-549 and HL60 cells growth inhibition.

1. Introduction

The epidermal growth factor receptor (EGFR/ErbB-1) and the related human epidermal growth factor receptor-2 (ErbB-2/Her-2) belong to the family of trans-membrane growth factor receptor protein tyrosine kinases (PTKs), which play critical roles in many of the signal transduction processes that control cell growth, differentiation, mitosis and apoptosis.[1] The ErbB receptors can be activated through homo or heterodimerization with other receptors resulting in phosphorylation events and downstream signaling that produce excessive growth by inducing cell proliferation and inhibiting apoptotic pathways.[2] Overexpression of these receptors is found in a variety of cancers such as breast, ovarian, colon and non-small-cell lung cancers (NSCLC), and has been associated with poor prognosis in patients.[3] In addition, coexpression of EGFR and Her-2 has been found in various cancers such as breast, ovarian, colon and prostate cancers, and is associated with poor prognosis of the patients. Therefore, simultaneous inhibition of EGFR and Her-2 is expected to provide superior efficacy to single receptor targeting.[4]

Over the past decades, there are two clinically approved 4-anilinoquinazoline inhibitors, gefitinib and erlotinib, selective for EGFR inhibition.[5] The third one lapatinib is an equipotent inhibitor of both EGFR and ErbB-2, which has been expected to provide better efficacy than single receptor targeting.[6] The chemical structures are shown in Figure 1. Among the known kinase inhibitors, the 4-anilinoquinazoline scaffold is the most common template for inhibitors of the EGFR family.[7] In the last few years, a large structural variety of compounds, such as 4-anilino-3-cyanoquinolines (neratinib),[8] 4-anilinopyrazolo[3,4-*d*]pyrimidines,[9] 4-anilinopyrazolo and 4-anilinopyrroloquinazolines,[10] were reported as EGFR tyrosine kinase inhibitors.

In our effort to develop new non-quinazoline derivatives as effective EGFR kinase inhibitors, we focused on the monocyclic scaffolds. Several series of structures were designed, including 4-anilino-5-vinyl and 5-ethynylpyrimidines (**16**, **20**), 3-cyano-4-anilino-5-vinyl and 5-ethynylpyridines (**29**, **33**), 3-cyano-4-anilino-5-phenylpyridines (**35**), and *m*-amino-*N*-phenylbenzamides (**48**–**50**,**61**),asdepictedinTables1–4.Wehopethe6-methylpyrimidine scaffold, the 3-cyano-6-methylpyridine scaffold, or even the simple 5-amido-4-ethoxybenzamide structure can mimic the function of the classic quinazoline or 3-cyanoquinoline moiety and are expected to offer better physicochemical properties.[11]

Six designed compounds were selected and overlaid with lapatinib and neratinib respectively before the synthesis work, as depicted in Figures 2 and 3. PyMOL software was used to align the compounds. Lapatinib and compounds **16b**, **20a** and **33a** were

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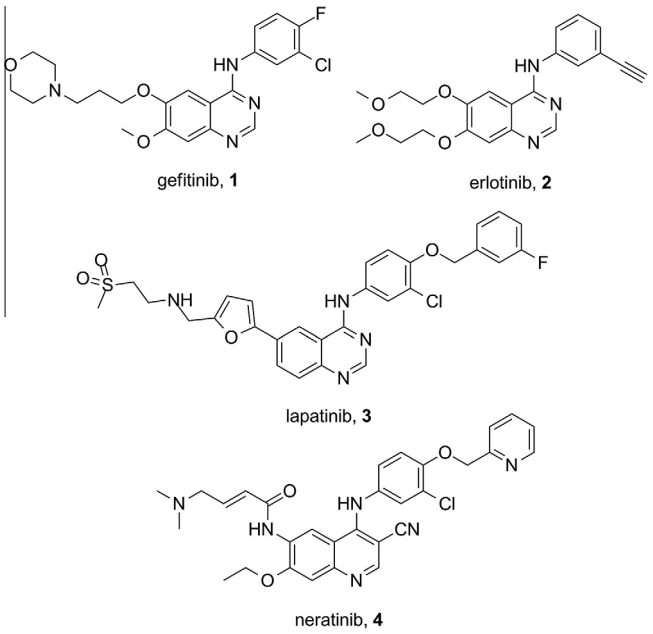


Figure 1. Chemical structure of compounds **1**–**4**.

imported into PyMOL, and optimized the dynamics conformation respectively. Then the 3 compounds were overlaid on lapatinib manually to give the image in Figure 2. The same process was used to give Figure 3. Except for **33a**, other five can give good to medium overlap results.

The 5-alkenyl and alkynyl substituents can be coplanar with the pyrimidine or 3-cyanopyridine ring, these scaffolds should be able to effectively mimic the well-known 4-anilinoquinazoline or 3-cyanoquizoline kinase inhibitor template. The phenyl ring as the C-5 substituent (**35a**) was introduced to the 3-cyanopyridine ring to mimic the 3-cyanoquizoline template. 2-Nitro-*N*-phenylbenzamides (**48a**) and 2-methyl-*N*-phenylbenzamides (**61a**) were also designed to mimic the 4-anilinoquinazoline and 3-cyanoquizoline template, as shown in Figure 4.[12]

In this paper, we will report the synthesis, bioactivity assay and a primary structure–activity relationships (SARs) study of the above monocyclic skeleton as inhibitors of EGFR kinase. The new 3-cyanopyridine and pyrimidine compounds were tested respectively on EGFR and ErbB-2 kinases inhibition activities, some compounds were also selected to test in a carcinoma cell growth inhibition assay on two cell lines, A-549 and HL60. The IC_{50} data of the tyrosine kinases inhibition or the cell viability assay were given. The SARs were provided based on the biological activity for these inhibitors of the EGFR signaling.

2. Results and discussion

2.1. Structure–activity relationships

On the basis of the existed structure−activity relationship (SAR) information,[11,12] we designed pyrimidine or 3-cyanopyridine based analogues with a 5-vinyl/ethynyl/phenyl moiety at the C5 position and bulky aniline moiety at the C4 position to secure irreversible EGFR inhibitory activity. *m*-Amino-*N*-phenylbenzamides were also designed to mimic the 4-anilinequinazoline core.

Our effort is to discover and develop non-quinazoline or non-3-cyanoquinoline derivatives for effective EGFR inhibitors as antitumor agents, we focused on the pyrimidine, 3-cyanopyridine, and *m*-amino-*N*-phenylbenzamide scaffolds which could mimic the function of the quinazoline and 3-cyanoquinoline moiety and could be expected to offer better physicochemical properties. We thought that the linker in our pyrimidine series should be in similar orientation as the phenyl ring of quinazoline, that is, a substituent at the C-5 position was required to be coplanar with the pyrimidine ring. Thus, we tried to find a linker that could maintain coplanarity with the pyrimidine nucleus. Similar idea was used for design the 5-substituted-3-cyanopyridine from 3-cyanoquinoline.

The eight compounds **16**, **20**, **29** and **33** were designed and synthesized, as depicted in Schemes 1–3, and tested on EGFR and ErbB-2 kinase inhibition activities. The IC_{50} values are shown in Table 1. In the same assay neratinib has an IC_{50} value of 2.2 nM.[13] The most potent one among the eight is **20a** (IC_{50} = 45 nM) which has the 5-ethynylpyrimidine skeleton with 3-chloro-4-(3-fluorobenzyloxy)anilino substituent at the 4-position. The 5-vinylpyrimidine **16b** also has an IC_{50} value <1 μM, which has the best overlay results with lapatinib, as shown in Figure 2. Other derivatives show no interesting bioactivities, especially to ErbB-2 kinase that most of them have poor inhibition value.

Another series of 3-cyano-4-anilino-5-phenylpyridines **35** were designed and synthesized (Scheme 4). The structures and bioassay data are depicted in Table 2. A 4-substituted phenyl ring was introduced to the 5-position to extend the conformation of the molecule. The 4-anilino chains of lapatinib and neratinib were introduced to the 4-position respectively. While this modification was not successful, only **35c** has an IC_{50} value of 0.6 μM on EGFR and **35d** has some inhibition activities on the cells growth, which indicated that the 3-cyano-5-phenylpyridine skeleton is not potent as EGFR kinase inhibitor.

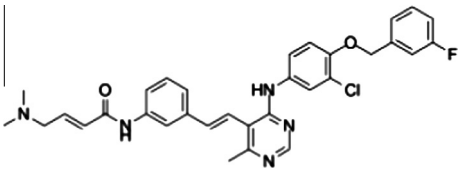
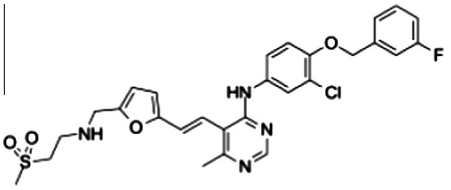
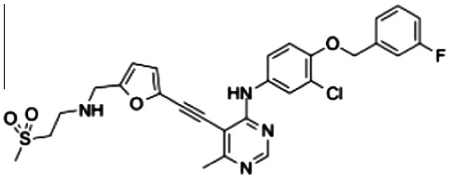
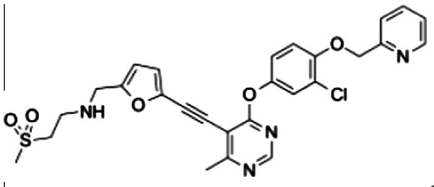
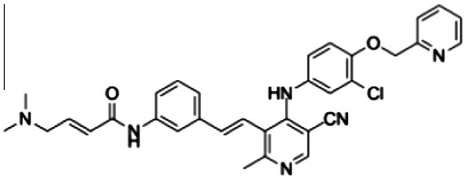
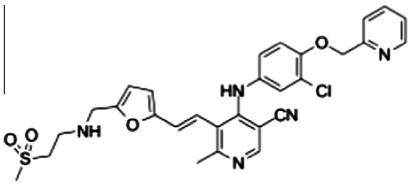
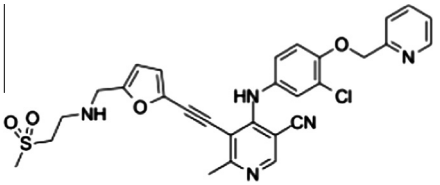
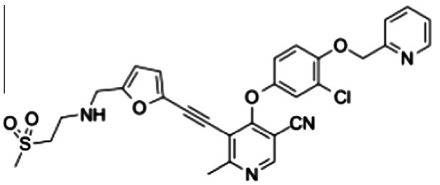
The other part of our work is design the 5-amido-4-ethoxybenzamide compounds **48**–**50** (Schemes 5 and 6) and the 2-methyl substituted analogues **61** (Scheme 7), with the purpose of unfolding the N-heterocyclic ring and keeping the phenyl ring of the classic quinazoline or 3-cyanoquinoline inhibitors. Therefore the side chains of lapatinib and neratinib, as well as the moiety developed by us previously, were introduced to the related positions in our new designed molecules. The structures and biological data are shown in Tables 3 and 4, while in the present situation, no exciting results were obtained. With regard to the EGFR kinase inhibition assay, several derivatives show <1 μM IC_{50} value, including compounds **48a, 48b**, **49a** and **50a**, which has the 3-chloro-4-(pyridin-2-ylmethoxy)anilino substituent in the corresponding position. The 2-nitro substituted compounds **48** can have better inhibition activity than the related 2-amino compounds **49** on the cells growth assay. Only two kinds of side chains were investigated at the 5-position, (dimethylamino)but-2-enamide and [(4-methylpiperazin-1-yl)methyl]benzamide. In general, the former analogues can possess better bioactivities than the latter.

Lastly four 2-methyl-5-amidobenzamides **61** were synthesized which have the similar bioassay results to compounds **48**–**50**. The 3-chloro-4-(3-fluorobenzyloxy)anilino substituted **61a** and **61b** have ~0.5 μM IC_{50} value, while most of these derivatives show no high bioactivities to inhibition of the cells growth.

2.2. Chemical synthesis

The 4-anilino-5-vinylpyrimidines **16** and 5-ethynylpyrimidines **20** were synthesized, as shown in Schemes 1 and 3. The side chain compounds **7** and **8** were prepared using the known method.[14] The key 4-chloropyrimidine intermediate **12** was prepared from ethyl acetoacetate and thiourea based on the simple route,[15] which coupled with 3-chloro-4-(3-fluorobenzyloxy)aniline (**13**) to produce the compound **14** in good yield. Using the Stille coupling,[16] intermediate **15** was obtained, which reacted with **7** and **8** respectively under the Heck reaction condition[17] to give the final 5-vinyl products **16**. Base on the similar method, 5-ethynyl compounds **20** were produced. A different 3-chloro-4-(pyridin-2-ylmethoxy)phenoxy substituent was introduced to the 4-position.

Table 1
Biological activity data of compounds **16**, **20**, **29** and **33**

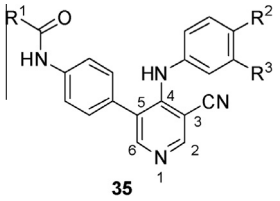
Compd	Structure	IC_{50}[a] (μM)	
		EGFR	ErbB2
16a		>5	>10
16b		0.46	>10
20a		0.045	>10
20b		>5	–
29a		>5	>10
29b		>5	>10
33a		1.5	–
33b		>5	–

[a] The IC_{50} values are the average of three separate experiments. Standard deviations were below ±30%.

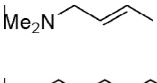
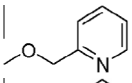
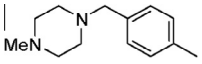
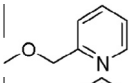
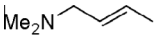
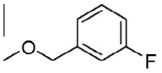
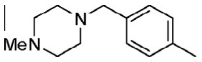
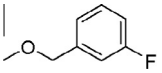
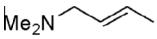
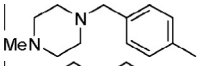
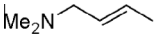
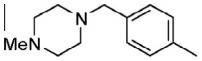
The 3-cyanopyridine derivatives **29** and **33** were synthesized using the similar method (Schemes 2 and 3). The key 4-chloro-3-cyanopyridine intermediate **25** was prepared from the 4-hydroxy nicotinic acid **22** through the amidation, iodosubstitution and chloration. Adopted the same method to preparation of **16** and **20**, **29** and **33** were obtained.

The synthetic route of **35** is shown in Scheme 4. 2-(4-Nitrophenyl)acetic acid was converted to the 3-oxobutanenitrile **37** through three simple steps, which then reacted in turn with DMF–DMA and veratrylamine to give the intermediate **39** based on the similar reported method.[18] The key 4-chloroquinoline **40** was obtained after the chloration. Compounds **35** were produced

Table 2
Chemical structure and biological activity data of compounds **35**



35

Compd	R^1	R^2	R^3	IC_{50}[a] (μM)		
					Cell growth inhibition	
				EGFR	A-549	HL60
35a			–Cl	>5	>10	>10
35b			–Cl	2.3	>10	6.0
35c			–Cl	0.66	7.0	>10
35d			–Cl	2.5	6.0	1.8
35e		–F	–Cl	3.5	>10	6.0
35f		–F	–Cl	3.0	>10	5.0
35g		-COMe	–H	>5	>10	>10
35h		-COMe	–H	>5	>10	5.0

[a] The IC_{50} values are the average of three separate experiments. Standard deviations were below ±30%.

by another two steps using the similar method described previously.

The synthetic method of **48** and **49** are depicted in Scheme 5. The materials **51** and **52** were prepared by our improved method,[19] which reacted with the anilines **13**, **26** or **41**, then went by reduction and amidation, compounds **48** and **49** was obtained. Using another material **55**, derivatives **50** were produced in the similar way (Scheme 6). Compounds **61** were also prepared by the similar method (Scheme 7).

3. Conclusion

In summary, 36 new compounds derived from four series of the monocyclic scaffolds, 5-vinyl/ethynyl pyrimidine (**16**, **20**), 3-cyano-5-vinyl/ethynyl pyridine (**29**, **33**), 3-cyano-5-phenylpyridine (**35**), and *m*-amino-*N*-phenylbenzamide (**48**–**50**, **61**), were designed, synthesized and tested respectively on EGFR and ErbB-2 kinases inhibition activities, most of the derivatives were also tested in a carcinoma cell growth inhibition assay on A-549 and HL60 cell lines. The biological activity results, the chemical structures, as well as the overlay processing of the representative compounds with lapatinib or neratinib, identified preliminary SARs. (1) 4-Anilino-5-vinyl/ethynyl pyrimidines have better activities to EGFR kinases than the similar 3-cyanopyridines. 5-Ethynyl substituted compounds are more potent than the 5-vinyl compounds, probably because the former have a similar molecular conformation to the typical 4-anilinoquinazoline inhibitors. The novel 5-[(furan-2-yl-)ethynyl]pyrimidine compound **20a** has a moderate inhibitory activity against EGFR kinases (IC_{50} = 45 nM). (2) The 3-cyano-5-phenylpyridines have poor inhibitory activities to EGFR kinases and cells growth, probably because the bulky 5-phenyl substituent transfers the molecular conformation too much to lose the bioactivities, except for the [(*N*-methyl piperazin-1-yl)methyl]benzamide substituted compounds (**35d** and **35f**) can have moderate inhibition to HL60 proliferation. (3) The *m*-aminobenzamides also have no interesting bioactivities to EGFR or to cells, which indicates that the pyrimidine core in the quinazoline, and the 3-cyanopyridine ring in the 3-cyanoquinaline are critical to keep the bioactivities.

4. Experimental

4.1. Materials and methods

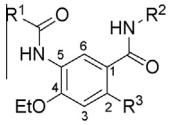
All commercially available materials and solvents were used as received without any further purification. ^1H and ^{13}C NMR spectra were recorded with a Gemini-300 spectrometer using TMS as an internal standard. The mass spectra were obtained from a Finnigan MAT-95/711 spectrometer. Melting points were measured on a Buchi-510 melting point apparatus and was uncorrected. The HPLC results were generated using a Waters 2487 UV/Visible Detector and Waters 515 Binary HPLC Pump.

4.2. Chemistry

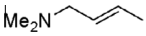
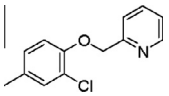
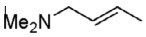
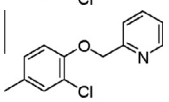
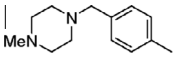
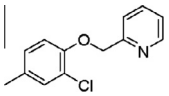
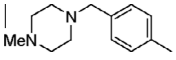
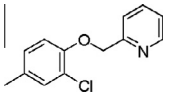
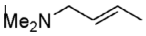
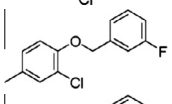
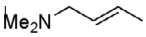
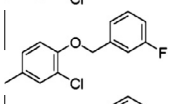
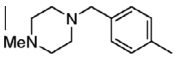
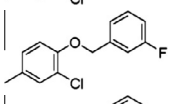
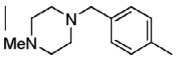
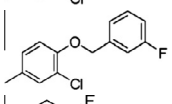
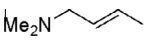
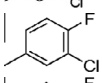
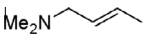
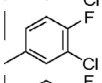
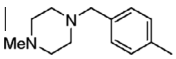
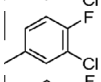
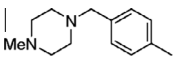
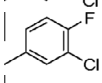
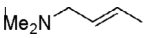
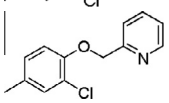
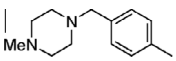
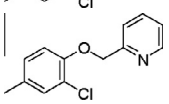
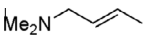
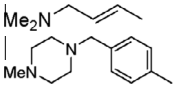
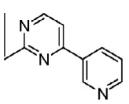
4.2.1. *N*-(3-Bromophenyl)-4-(dimethylamino)but-2-enamide (7)

4-(Dimethylamino)but-2-enoic acid hydrobromide **6** (0.5 g, 2.4 mmol) and DMF (9 μL, 5 mol %) were added into THF (8 mL) and the suspension was cooled in an ice-water bath under N_2

Table 3
Chemical structure and biological activity data of compounds **48–50**



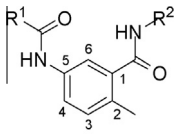
48 - 50

Compd	R^1	R^2	R^3	IC_{50}[a] (μM)		
					Cell growth inhibition	
				EGFR	A-549	HL60
48a			$-NO_2$	0.85	2.5	4.0
49a			$-NH_2$	0.80	>10	>10
48b			$-NO_2$	0.90	3.5	5.0
49b			$-NH_2$	1.0	6.0	8.0
48c			$-NO_2$	2.0	3.5	4.0
49c			$-NH_2$	1.5	5.5	6.0
48d			$-NO_2$	5.5	6.0	4.5
49d			$-NH_2$	5.0	6.0	7.0
48e			$-NO_2$	6.0	>10	6.0
49e			$-NH_2$	4.5	>10	>10
48f			$-NO_2$	5.0	6.0	5.5
49f			$-NH_2$	0.80	>10	>10
50a			–H	1.2	>10	>10
50b			–H	3.5	6.0	>10
50c			–H	6.0	>10	>10
50d			–H		>10	>10

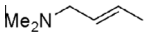
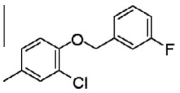
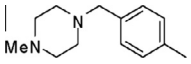
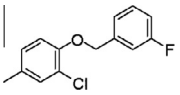
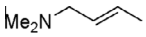
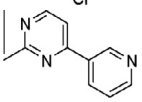
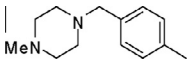
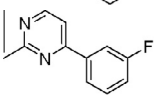
[a] The IC_{50} values are the average of three separate experiments. Standard deviations were below ±30%.

atmosphere. Oxalyl chloride (200 μL, 2.3 mmol) was added dropwise and the reaction suspension was stirred at 26–30 °C for 1.5 h, then cooled again in ice-water bath. 3-Bromoaniline (175 μL, 1.6 mmol) was added dropwise and the reaction suspension was stirred at 0 °C for another 4 h. The reaction solution was diluted with water and adjusted to pH ~9 with 10% NaOH solution, extracted with EtOAc. The organic layer was washed with water and brine, and dried over anhydrous Na_2SO_4. The solvent was

Table 4
Chemical structure and biological activity data of compounds **61**



61

Compd	R^1	R^2	IC_{50}[a] (μM)		
				Cell growth inhibition	
			EGFR	A-549	HL60
61a			0.45	7.0	5.5
61b			0.60	5.5	6.0
61c			5.0	>10	–
61d			6.0	>10	–

[a] The IC_{50} values are the average of three separate experiments. Standard deviations were below ±30%.

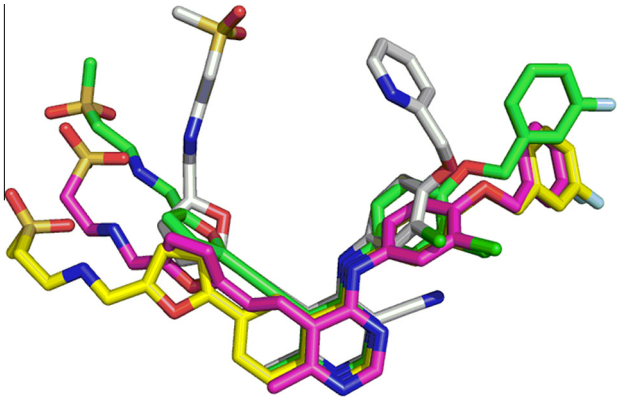


Figure 2. Overlay of lapatinib with compounds **16b**, **20a** and **33a**. Lapatinib is colored yellow. Compound **16b** is shown in pink, **20a** is green, and **33a** is grey. Atoms are colored by element with nitrogen in blue, oxygen red, chlorine dark green, sulfur yellow and fluorine cyan.

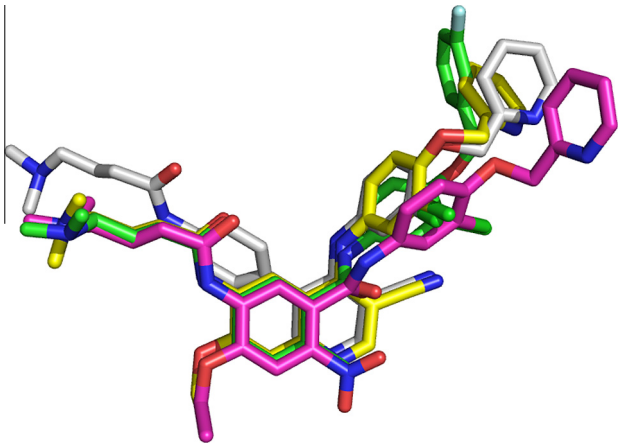


Figure 3. Overlay of neratinib with compounds **35a**, **48a** and **61a**. Neratinib is colored yellow. Compound **35a** is shown in grey, **48a** is pink, and **61a** is green. Atoms are colored by element with nitrogen in blue, oxygen red, chlorine dark green and fluorine cyan.

removed under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (eluent: PE/CH_2Cl_2 = 5/1–1/1) to give **7** (0.33 g, 75%) as a grey solid. ^{1}H NMR ($CDCl_3$, δ): 2.26(s, 6H), 3.12(d, 2H), 6.11(d, 1H), 6.91–7.01(m, 1H), 7.13–7.23(m, 1H), 7.46–7.54(m, 2H), 7.72(m, 1H), 7.82(s, 1H).

4.2.2. *N*-((5-Bromofuran-2-yl)methyl)-2-(methylsulfonyl) ethanamine (8)

2-(Methylsulfonyl)ethanamine hydrochloride (0.5 g, 3.1 mmol) was added to a NaOMe solution, which was prepared from Na (0.11 g, 4.78 mmol) and anhydrous MeOH (9 mL). The reaction mixture was stirred at room temperature for 15 min. The methanol was removed under reduced pressure and the residual was suspended into CH_2Cl_2 (15 mL) and filtered to removed the excess salt. The filtrate was concentrated to give 2-(methylsulfonyl)ethanamine as a colorless oil, which was dissolved into anhydrous MeOH (10 mL). 5-Bromo-2-furaldehyde (0.45 g, 2.6 mmol) was added and the resulting solution was stirred at room temperature for 2 h. $NaBH_4$ (0.48 g, 12.8 mmol) was added portion-wise and the solution was stirred at room temperature for another 4 h. The reaction solution was quenched with saturated Na_2CO_3, extracted with CH_2Cl_2. The organic layer was washed with water and brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give **8** (0.68 g, 94%) as a light-yellow oil. ^{1}H NMR ($CDCl_3$, δ): 2.99(s, 3H), 3.13(br, 4H), 3.76(s, 2H), 6.18(d, 1H), 6.22(s, 1H).

4.2.3. 4-Chloro-5-iodo-6-methylpyrimidine (12)

Na (30 g, 1.3 mol) was added to anhydrous MeOH (500 mL) which was cooled in an ice-water bath. After the sodium dissolved, thiourea (40 g, 0.52 mol) and ethyl acetoacetate (80 mL, 0.6 mol) were added and the reaction solution was stirred and heated to reflux for 8 h. The methanol was removed under reduced pressure and water (600 mL) was added to dissolve the residual solid. AcOH

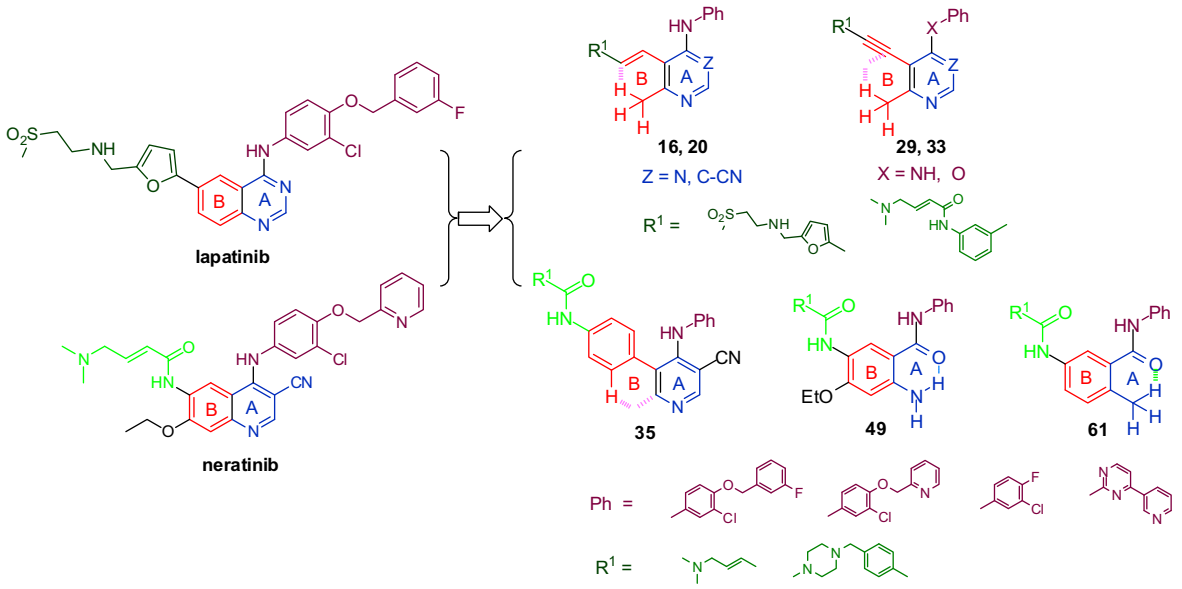
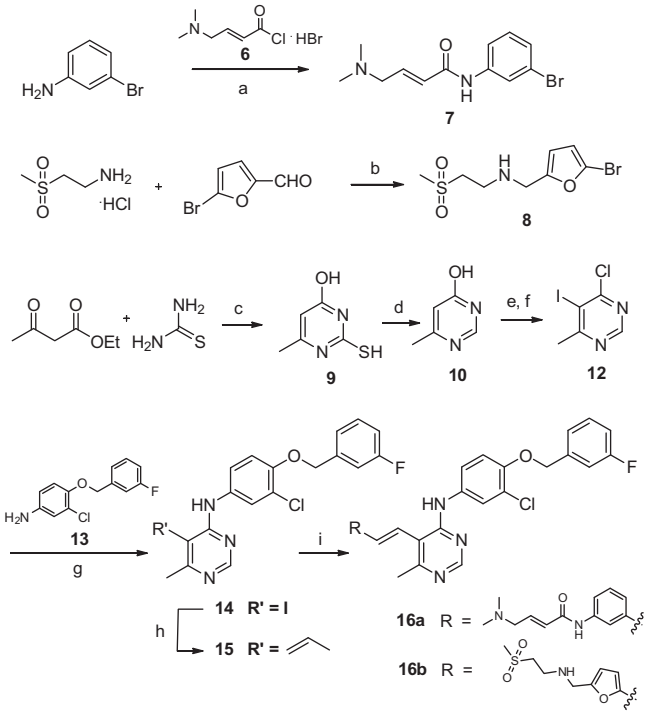
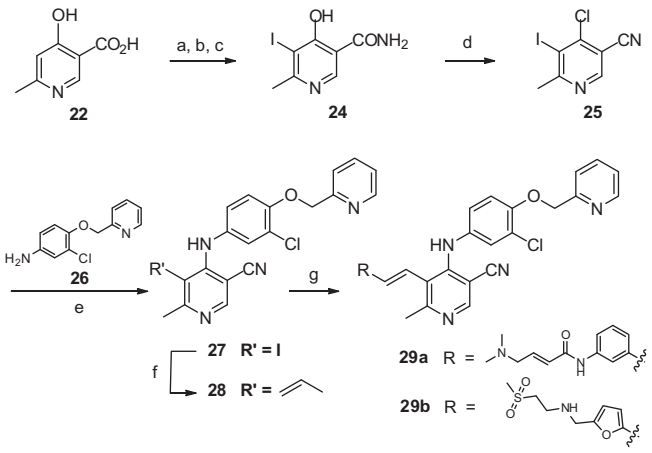


Figure 4. Design idea of the new compounds. (1) Open the B ring of lapatinib or neratinib to give the pyrimidine or 3-cyanopyridine compounds **16, 20, 29, 33** or **35**. (2) Open the A ring of lapatinib or neratinib to give the *m*-amino-*N*-phenylbenzamide compounds such as **49** and **61**. (3) The similar substituents of lapatinib, neratinib or other typical EGFR inhibitors are introduced to the related position on the new compounds.



Scheme 1. Reagents and conditions: (a) $(COCl)_2$, THF, 0–28 °C, 6 h, 75%; (b) $NaBH_4$, MeOH, rt, 94%; (c) NaOMe, MeOH, reflux, 89%; (d) H_2O_2, 80 °C, 58%; (e) I_2, NaOH, H_2O, reflux, 49%; (f) $POCl_3$, diglyme, 90 °C, 79%; (g) CH_3SO_3H (cat.), EtOH, 70 °C, 94%; (h) tributyl(vinyl)tin, $(Ph_3P)_2PdCl_2$, CuI, toluene, 110 °C, 76%; (i) **7** or **8**, NaOAc, $(Ph_3P)_2PdCl_2$, NMP, 110 °C, 45–47%.



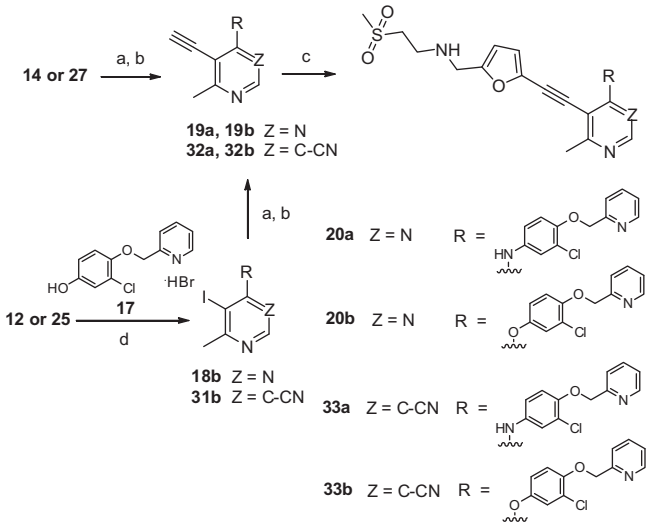
Scheme 2. Reagents and conditions: (a) $SOCl_2$, MeOH, reflux; (b) $NH_3{\cdot}H_2O$, 79%; (c) ICl, AcOH, 47%; (d) $POCl_3$, diglyme, 100 °C, 86%; (e) CH_3SO_3H (cat.), EtOH, 70 °C, 89%; (f) tributylethynylstannane, $(Ph_3P)_2PdCl_2$, CuI, toluene, reflux, 79%; (g) **7** or **8**, NaOAc, $(Ph_3P)_2PdCl_2$, NMP, 110 °C, 32–34%.

(90 mL, 0.15 mol) was added and the resulting white solid was collected by suction filtration, washed by water (150 mL × 2), dried at 50 °C to give 2-mercapto-6-methylpyrimidin-4-ol **9** (66 g, 89%).

Compound **9** (66 g, 0.46 mol) was added portion-wise over 1 h to a solution of 30% H_2O_2 (280 mL) and H_2O (400 mL) at 70 °C and stirred at 80–90 °C for another 1 h. After cooled down, Na_2CO_3 (~100 g) was added to the solution then stirred overnight to quench the excess H_2O_2. Water was removed under reduced pressure and the residual was dried at 50 °C to give a white solid, which was suspended in acetone (300 mL) and stirred for 20 min. The solid was filtered and washed by acetone (50 mL × 3). The combined filtrate was concentrated to give the 6-methylpyrimidin-4-ol **10** (30 g, 58%) as a white solid. MS-ESI (*m*/*z*): 109.2(M−H), 133.0 (M+Na); ^{1}H NMR ($CDCl_3$, δ): 2.34(s, 3H), 6.32(s, 1H), 8.09(s, 1H).

Compound **10** (3.5 g, 0.0318 mol) was dissolved in a solution of NaOH (1.5 g, 0.0375 mol) in H_2O (50 mL). I_2 (8.7 g, 0.0343 mol) was added and the reaction solution was heated to reflux for 3 h. After cooled to room temperature, the resulting solid was filtered and washed by water, dried to give 5-iodo-6-methylpyrimidin-4-ol **11** (3.6 g, 49%) as a grey solid. ^{1}H NMR (DMSO-d_6, δ): 2.45(s, 3H), 8.04(s, 1H).

$POCl_3$ (5.3 mL, 0.057 mol) was added to the suspension of **11** (3.4 g, 0.0144 mol) in diglyme (60 mL) and the reaction mixture was stirred at 90 °C for 2 h. After cooled to room temperature, the reaction mixture was poured into 10% K_2CO_3 solution (200 mL) and stirred for 1 h. The resulting solid was filtered and washed by water, dried to give 4-chloro-5-iodo-6-methylpyrimidine **12** (2.9 g, 79%).



Scheme 3. Reagents and conditions: (a) trimethylsilylacetylene, $(Ph_3P)_2PdCl_2$, CuI, THF, TEA; (b) TBAF, THF, 56–85%; (c) **8**, $(Ph_3P)_2PdCl_2$, CuI, THF, TEA, 40 °C, 23–58%; (d) K_2CO_3, DMF, 60 °C, 76–83%.

4.2.4. *N*-(3-(-2-(4-((3-Chloro-4-((3-fluorobenzyl)oxy)phenyl) amino)-6-methylpyrimidin-5-yl)vinyl)phenyl)-4-(dimethylamino) but-2-enamide (16a)

Compound **12** (1.6 g, 6.28 mmol), commercially available 3-chloro-4-(3-fluorobenzyloxy)aniline **13** (1.6 g, 6.3 mmol) and CH_3SO_3H (20 μL, 5 mol %) were added into anhydrous EtOH (40 mL) and the reaction mixture was heated at 70 °C for 2 h. After cooled down, the alcohol solution was diluted with water (150 mL) and the resulting solid was filtered, washed with water and dried to give *N*-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-5-iodo-6-methylpyrimidin-4-amine **14** (2.77 g, 94%) as a white solid. MS-ESI (*m*/*z*): 467.8(M−H), 470.0(M+H); ^{1}H NMR ($CDCl_3$, δ): 2.65(s, 3H), 5.15(s, 2H), 6.94(d, 1H), 7.02(t, 1H), 7.16(d, 1H), 7.22(d, 1H), 7.35(d, 2H), 7.67(s, 1H), 8.39(s, 1H).

Compound **14** (0.4 g, 0.85 mmol), tributyl(vinyl)tin (0.37 mL, 1.27 mmol), CuI (16 mg, 10 mol %) and $(Ph_3P)_2PdCl_2$ (30 mg, 5 mol %) were suspended in toluene (12 mL) under N_2 atmosphere and heated at 110 °C for 4 h. After cooled down to room temperature, the solution was diluted with CH_2Cl_2 (60 mL). The organic layer was washed with saturated $NaHCO_3$ and brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (eluent: PE/CH_2Cl_2 = 10/1–3/1) to give *N*-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-methyl-5-vinylpyrimidin-4-amine **15** (0.24 g, 76%) as a light-yellow solid. MS-EI (*m*/*z*): 260, 277, 369(M^+); ^{1}H NMR ($CDCl_3$, δ): 2.40(s, 3H), 5.12(s, 2H), 5.63(d, 1H), 5.80(d, 1H), 6.61(dd, 1H), 6.81(s, 1H), 6.91(d, 1H), 7.01(t, 1H), 7.17–7.23(m, 2H), 7.31–7.39(m, 2H), 7.66(d, 1H), 8.51(br, 1H).

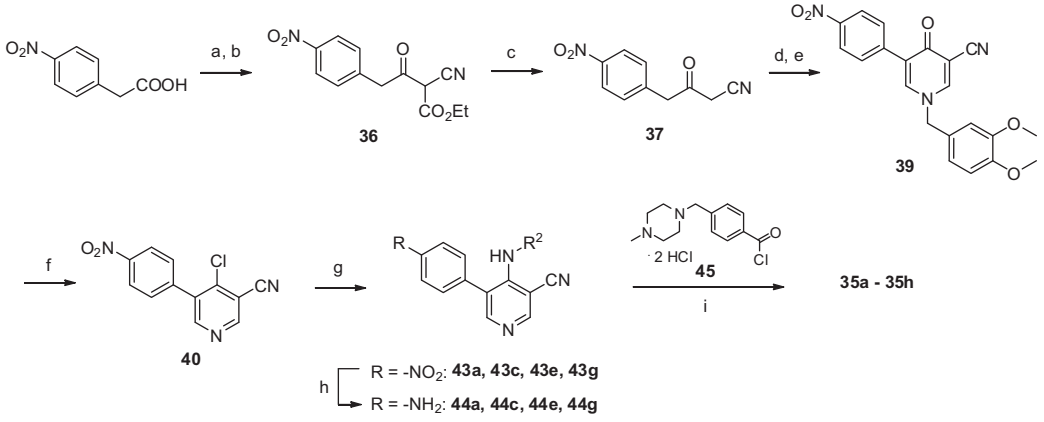
Compound **15** (40 mg, 0.108 mmol), **7** (45 mg, 0.16 mmol), NaOAc (18 mg, 0.22 mmol) and $(Ph_3P)_2PdCl_2$ (8 mg, 10 mol %) were added into NMP (1.5 mL) under N_2 atmosphere and heated at 110 °C for 3 h. After cooled down to room temperature, the solution was diluted with CH_2Cl_2 (40 mL). The organic layer was washed with saturated $NaHCO_3$ and brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (eluent: CH_2Cl_2–CH_2Cl_2/MeOH/TEA = 100/5/0.5) to give **16a** (29 mg, 46%) as a light-yellow solid. MS-ESI (*m*/*z*): 570.2(M−H), 606.1(M+Cl), 572.2(M+H); ^{1}H NMR ($CDCl_3$, δ): 2.35(s, 6H), 3.41(d, 2H), 5.05(s, 2H), 6.69(d, 1H), 6.77–6.90(m, 2H), 6.98(t, 1H), 7.10–7.21(m, 2H), 7.28–7.40(m, 1H), 7.65–7.73(m, 2H), 8.03(s, 1H), 8.40(s, 1H).

4.2.5. *N*-(3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-methyl-5-(2-(5-(((2-(methylsulfonyl)ethyl)amino)methyl)furan-2-yl)vinyl)pyrimidin-4-amine (16b)

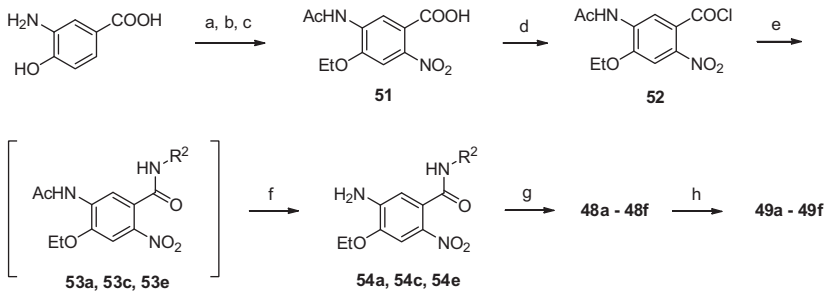
Prepared in a similar manner as for **16a**. Starting with **15** (80 mg, 0.216 mmol) and **8** (100 mg, 0.35 mmol) to produce **16b** (55 mg, 45%) as a beige solid. MS-ESI (*m*/*z*): 569.0(M−H), 604.9(M+Cl), 571.1(M+H), 593.1(M+Na); ^{1}H NMR ($CDCl_3$, δ): 2.46(s, 3H), 2.94(s, 3H), 3.20(br, 4H), 3.88(s, 2H), 5.12(s, 2H), 6.28(d, 1H), 6.33(d, 1H), 6.88–6.95(m, 2H), 6.96–7.05(m, 3H), 7.18–7.22(m, 4H), 7.30–7.35(m, 1H), 7.39(dd, 1H), 7.63(d, 1H), 8.48(s, 1H).

4.2.6. *N*-(3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-methyl-5-((5-(((2-(methylsulfonyl)ethyl)amino)methyl)furan-2-yl)ethynyl)pyrimidin-4-amine (20a)

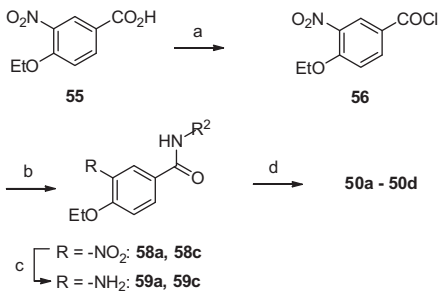
Compound **14** (0.21 g, 0.44 mmol), trimethylsilylacetylene (85 μL, 0.6 mmol), CuI (8 mg, 10 mol %) and $(Ph_3P)_2PdCl_2$ (15 mg, 5 mol %) were suspended in anhydrous THF (4 mL) under N_2 atmosphere and heated at 50 °C for 2 h. The solution was diluted with CH_2Cl_2 (60 mL). The organic layer was washed twice with saturated $NaHCO_3$, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give a brown flake, which was dissolved into CH_2Cl_2 (4 mL) at 0 °C and treated dropwise with a solution of TBAF (0.112 g, 0.44 mmol) in THF (2 mL). After stirred at 0 °C for another 30 min, the reaction solution was diluted with CH_2Cl_2 (60 mL). The organic layer was washed twice with brine,



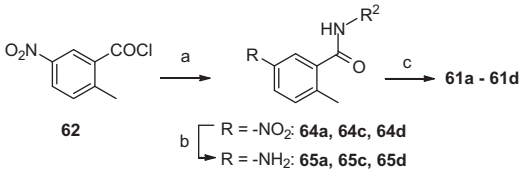
Scheme 4. Reagents and conditions: (a) $SOCl_2$, reflux; (b) ethyl cyanoacetate, NaOEt, 76%; (c) 95% DMSO/H_2O, 110 °C, 81%; (d) DMF–DMA, toluene, 110 °C; (e) veratrylamine, toluene, 110 °C, 85%; (f) $POCl_3$, 110 °C, 71%; (g) **13**, **26**, 3-chloro-4-fluoroaniline (**41**) or 4-aminoacetophenone (**42**), DME, Py·HCl, reflux, 75–90%; (h) Fe, NH_4Cl, HCl, EtOH, 70–80 °C, 80–90%; (i) **6**, $(COCl)_2$, THF, NMP, 0 °C to rt; or **45**, pyridine, 0 °C to rt, 24–54%.



Scheme 5. Reagents and conditions: (a) Ac_2O, AcOH, 60 °C, 92%; (b) C_2H_5Br, K_2CO_3, DMF, 60 °C, 96%; (c) fuming HNO_3, AcOH, 25–35 °C, 85%; (d) $SOCl_2$, reflux; (e) **13**, **26** or **41**, CH_2Cl_2, 0 °C to rt; (f) K_2CO_3, THF–MeOH, rt, 33–69%; (g) **6**, $(COCl)_2$, THF, NMP, 0 °C to rt; or **45**, pyridine, 0 °C to rt, 23–76%; (h) Zn, $CaCl_2$, EtOH–H_2O, reflux, 48–95%.



Scheme 6. Reagents and conditions: (a) $SOCl_2$, reflux; (b) **26**, CH_2Cl_2, 0 °C to rt; or 4-(pyridin-3-yl) pyrimidin-2-amine (**57**), pyridine, 0 °C to rt, 51–82%; (c) Fe, HCl, DME–MeOH, 60 °C, 84–98%; (d) **6**, $(COCl)_2$, THF, NMP, 0 °C to rt; or **45**, pyridine, 0 °C to rt, 19–49%.



Scheme 7. Reagents and conditions: (a) **13**, CH_2Cl_2, 0 °C to rt; **57** or 4-(3-fluorophenyl)pyrimidin-2-amine (**63**), pyridine, 0 °C to rt, 47–80%; (b) Fe, HCl, DME–MeOH, 60 °C, 91–98%; (c) **6**, $(COCl)_2$, THF, NMP, 0 °C to rt; or **45**, pyridine, 0 °C to rt, 21–54%.

and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (eluent: PE/CH_2Cl_2 = 10/1–3/1) to give *N*-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-5-ethynyl-6-methylpyrimidin-4-amine **19a** (0.124 g, 77%). MS-ESI (*m*/*z*): 368.2(M+H); ^{1}H NMR ($CDCl_3$, δ): 2.56(s, 3H), 3.86(s, 1H), 5.13(s, 2H), 6.93(d, 1H), 7.01(t, 1H), 7.01–7.25(m, 2H), 7.30–7.38(m, 1H), 7.39–7.47(m, 2H), 7.20(d, 1H), 8.54(s, 1H).

Compound **19a** (35 mg, 0.095 mmol), **8** (40 mg, 0.14 mmol), CuI (3 mg, 10 mol %) and $(Ph_3P)_2PdCl_2$ (5 mg, 5 mol %)) were suspended in anhydrous THF (2 mL) and Et_3N (0.3 mL) under N_2 atmosphere and heated at 45 °C for 12 h. The solvent was removed under reduced pressure and the residual was diluted with CH_2Cl_2 (40 mL). The organic layer was washed with saturated $NaHCO_3$ and brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (eluent: PE/CH_2Cl_2 = 10/1–1/1) to give **20a** (26 mg, 58%) as a beige solid. MS-ESI (*m*/*z*): 566.8(M−H), 602.9(M+Cl), 569.1(M+H); ^{1}H NMR ($CDCl_3$, δ): 2.56(s, 3H), 2.98(s, 3H), 3.18(s, 4H), 3.87(s, 2H), 5.14(s, 2H), 6.31(d, 1H), 6.69(d, 1H), 6.93(d, 1H), 7.01(dt, 1H), 7.18–7.24(m, 3H), 7.33(m, 1H), 7.42(dd, 1H), 7.72(m, 1H), 8.54(s, 1H).

4.2.7. 3-Chloro-4-(pyridin-2-ylmethoxy)phenol hydrobromide (17)

SO_2Cl_2 (8.3 mL, 0.103 mol) was added dropwise to a solution of 4-methoxyphenol (12.4 g, 0.1 mol) in $CHCl_3$ (70 mL) over 2 h and stirred for another 20 h at room temperature. The volatiles were removed to give 2-chloro-4-methoxyphenol as a light-yellow oil.

The above 2-chloro-4-methoxyphenol (16.0 g, 0.1 mol), 2-(chloromethyl)pyridine hydrochloride (16.4 g, 0.1 mol), K_2CO_3 (27.6 g, 0.2 mol) and KI (0.83 g, 5 mol %) were suspended in DMF (100 mL) and stirred at 60 °C for 12 h. The reaction suspension was diluted with water (400 mL) and stirred, the resulting solid was filtered, washed with water and dried to give 2-((2-chloro-4-methoxyphenoxy)methyl)pyridine (21.5 g, 86%) as a grey solid. ^{1}H NMR ($CDCl_3$, δ): 3.74(s, 3H), 5.20(s, 2H), 6.74(m, 1H), 6.89–6.98(m, 2H), 7.20(m, 1H), 7.65–7.73(m, 2H), 8.59(s, 1H).

The above product (11 g, 0.044 mol) was added to 40% HBr (90 mL) and the mixture was heated to reflux for 2 h. After cooled to 0 °C, the resulting solid was filtered, washed by water, dried to give **17** (8.4 g, 61%) as an off-white solid. ^{1}H NMR ($CDCl_3$, δ): 5.16(s, 2H), 6.61–6.92(m, 3H), 7.28–7.30(m, 1H), 7.68–7.79(m, 2H), 8.59(d, 1H).

4.2.8. *N*-((5-((4-(3-Chloro-4-(pyridin-2-ylmethoxy)phenoxy)-6-methylpyrimidin-5-yl)ethynyl)furan-2-yl)methyl)-2-(methylsulfonyl)ethanamine (20b)

Compound **17** (0.8 g, 2.46 mmol) and K_2CO_3 (0.68 g, 4.9 mmol) were suspended in DMF (8 mL) and stirred at room temperature for 20 min. **12** (0.61 g, 2.4 mmol) and KI (18 mg, 5 mol %) were added to the reaction mixture and stirred at 60 °C for 2 h. The reaction solution was diluted with water (60 mL) and the resulting solid was filtered, washed by water and dried to give 4-(3-chloro-4-(pyridin-2-ylmethoxy)phenoxy)-5-iodo-6-methylpyrimidine **18b** (0.89 g, 76%) as a beige solid. MS-ESI (*m*/*z*): 453.9(M+H), 475.9(M+Na); ^{1}H NMR (DMSO-d_6, δ): 2.66(s, 3H), 5.31(s, 2H), 7.18(dd, 1H), 7.29(d, 1H), 7.38(dd, 1H), 7.44(d, 1H), 7.58(d, 1H), 7.89(dt, 1H), 8.45(s, 1H), 8.60(d, 1H).

In a similar manner as for **19a**, started with **18b** (0.5 g, 1.1 mmol) to produce 4-(3-chloro-4-(pyridin-2-ylmethoxy)phenoxy)-5-ethynyl-6-methylpyrimidine **19b** (0.215 g, 56%) as a light-yellow solid. ^{1}H NMR ($CDCl_3$, δ): 2.68(s, 3H), 3.69(s, 1H), 5.28(s, 2H), 7.03(d, 2H), 7.27(t, 1H), 7.66(d, 1H), 7.77(dt, 1H), 8.56(s, 1H), 8.59(d, 1H).

In a similar manner as for **20a**, started with **19b** (70 mg, 0.2 mmol) and **8** (60 mg, 0.2 mmol) to produce **20b** (42 mg, 38%) as a beige solid. MS-ESI (*m*/*z*): 552.9(M+H); ^{1}H NMR ($CDCl_3$, δ): 2.70(s, 3H), 2.99(s, 3H), 3.17(br, 4H), 3.85(s, 2H), 5.31(s, 2H), 6.28(d, 1H), 6.69(d, 1H), 7.04(d, 2H), 7.28(s, 1H), 7.52(dd, 1H), 7.65–7.80(m, 2H), 8.55(s, 1H), 8.60(br, 1H).

4.2.9. 4-Chloro-5-iodo-6-methylnicotinonitrile (25)

$SOCl_2$ (5 mL, 0.066 mol) was added dropwise to a suspension of 4-hydroxy-6-methylnicotinic acid **22** (5 g, 0.033 mol) in anhydrous MeOH (100 mL). The reaction solution was heated to reflux for 5 h.

The volatiles were removed to give a white solid, which was added to 28% $NH_3 \cdot H_2O$ (50 mL) and the solution was stirred at room temperature overnight. The reaction mixture was cooled in an ice bath and the resulting solid was filtered, washed with water and dried to give 4-hydroxy-6-methylnicotinamide **23** (4 g, 79%) as a white solid. MS-ESI (*m*/*z*): 152.1(M−H), 153.0(M+H); ^{1}H NMR (DMSO-d_6, δ): 2.24(s, 3H), 6.22(s, 1H), 7.37(br, 1H), 8.29(s, 1H), 9.58(br, 1H), 12.03(br, 1H).

ICl (1 mL, 20 mmol) was added dropwise over 20 min to a solution of **23** (2 g, 13.1 mmol) in AcOH (20 mL) and the solution was stirred at room temperature overnight. The resulting solid was filtered, washed with water and dried to give 4-hydroxy-5-iodo-6-methylnicotinamide **24** (1.7 g, 47%) as a white solid.

In a similar manner as for **12**, started with **24** (1.7 g, 6.1 mmol) to produce **25** (1.5 g, 86%) as a faint-red solid. MS-ESI (*m*/*z*): 277.1(M−H); ^{1}H NMR (DMSO-d_6, δ): 2.81(s, 3H), 8.88(s, 1H).

4.2.10. 3-Chloro-4-(pyridin-2-ylmethoxy)aniline (26)

2-(Chloromethyl)pyridine hydrochloride (16.4 g, 0.1 mol) and K_2CO_3 (27.6 g, 0.2 mol) were suspended in DMF (100 mL) and stirred at room temperature for 30 min. 2-Chloro-4-nitrophenol (17.4 g, 0.1 mol) and KI (0.83 g, 5 mol %) were added in and the reaction mixture was stirred at 60 °C for 12 h. The reaction suspension was diluted with water (400 mL) and the resulting solid was filtered, washed with water and dried to give 2-((2-chloro-4-nitrophenoxy)methyl)pyridine (26 g, 98%) as a white solid. Mp 149.2–149.9 °C; MS-EI (*m*/*z*): 92, 229, 263(M$^+$).

The above product (13.2 g, 0.05 mol), iron powder (11.2 g, 0.2 mol) and 12 M HCl (4 mL, 0.05 mol) were added into 90% EtOH/H_2O (200 mL) and the reaction mixture was stirred at 70 °C for 1 h. The dark solution was filtered through a Celite pad. The filtrate was concentrated and the residual was dissolved in CH_2Cl_2 (200 mL). The organic layer was washed twice with water, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give **26** (10.9 g, 93%) as a light-yellow solid. Mp 90.9–91.8 °C; MS-EI (*m*/*z*): 93, 142, 199, 234(M$^+$); ^{1}H NMR (DMSO-d_6, δ): 4.95(s, 2H), 5.07(s, 2H), 6.45(dd, 1H), 6.65(d, 1H), 6.90(d, 1H), 7.35(t, 1H), 7.55(d, 1H), 7.85(t, 1H), 8.55(d, 1H).

4.2.11. *N*-(3-(2-(4-((3-Chloro-4-(pyridin-2-ylmethoxy)phenyl)amino)-5-cyano-2-methylpyridin-3-yl)vinyl)phenyl)-4-(dimethylamino)but-2-enamide (29a)

In a similar manner as for **14**, started with **25** (1.4 g, 5 mmol) and **26** (1.3 g, 5.5 mmol) to produce 4-((3-chloro-4-(pyridin-2-ylmethoxy)phenyl)amino)-5-iodo-6-methylnicotinonitrile **27** (2.1 g, 89%) as a off-white solid. ^{1}H NMR (CD_3OD, δ): 2.82(s, 3H), 5.34(s, 2H), 7.22(s, 2H), 7.42(s, 1H), 7.46(d, 1H), 7.75(d, 1H), 7.95(t, 1H), 8.29(s, 1H), 8.61(d, 1H).

In a similar manner as for **15**, started with **27** (0.6 g, 1.16 mmol) to produce 4-((3-chloro-4-(pyridin-2-ylmethoxy)phenyl)amino)-6-methyl-5-vinylnicotinonitrile **28** (0.34 g, 79%) as a yellow solid. MS-EI (*m*/*z*): 284, 341, 376(M$^+$); ^{1}H NMR ($CDCl_3$, δ): 2.48(s, 3H), 5.26(s, 2H), 5.53(d, 1H), 5.77(d, 1H), 6.44–6.56(m, 2H), 6.93–7.03(m, 2H), 7.22(d, 1H), 7.62(d, 1H), 7.75(t, 1H), 8.36(s, 1H), 8.58(d, 1H).

In a similar manner as for **16a**, started with **28** (45 mg, 0.12 mmol) and **7** (50 mg, 0.176 mmol) to produce **29a** (25 mg, 32%) as a beige solid. MS-ESI (*m*/*z*): 579.2(M+H); ^{1}H NMR (DMSO-d_6, δ): 2.48(s, 3H), 2.68(s, 6H), 3.49(d, 2H), 5.20(s, 2H), 6.63(d, 1H), 6.75–7.10(m, 3H), 7.21(d, 2H), 7.60(s, 1H), 7.72(t, 1H), 7.92(s, 1H), 8.31(s, 1H), 8.56(d, 1H), 9.76(br, 1H).

4.2.12. 4-((3-Chloro-4-(pyridin-2-ylmethoxy)phenyl)amino)-6-methyl-5-(2-(5-(((2-(methylsulfonyl)ethyl)amino)methyl)furan-2-yl)vinyl)nicotinonitrile (29b)

In a similar manner as for **16a**, started with **28** (60 mg, 0.16 mmol) and **8** (82 mg, 0.29 mmol) to produce **29b** (31 mg, 34%) as a beige solid. MS-ESI (*m*/*z*): 576.0(M−H), 611.8(M+Cl), 578.0(M+H); ^{1}H NMR ($CDCl_3$, δ): 2.54(s, 3H), 2.88(s, 3H), 3.15(s, 4H), 3.83(s, 2H), 5.25(s, 2H), 6.27(d, 1H), 6.49(m, 1H), 6.68(m, 1H), 6.90–7.10(m, 2H), 7.52(dd, 2H), 7.60(d, 1H), 7.69–7.75(m, 3H), 8.35(s, 1H), 8.56(d, 1H).

4.2.13. 4-((3-Chloro-4-(pyridin-2-ylmethoxy)phenyl)amino)-6-methyl-5-((5-(((2-(methylsulfonyl)ethyl)amino)methyl)furan-2-yl)ethynyl)nicotinonitrile (33a)

In a similar manner as for **19a**, started with **27** (0.5 g, 1.05 mmol) to produce 4-((3-chloro-4-(pyridin-2-ylmethoxy)phenyl)amino)-5-ethynyl-6-methylnicotinonitrile **32a** (0.335 g, 85%) as a faint-yellow solid. MS-EI (*m*/*z*): 282, 339, 374(M$^+$); ^{1}H NMR ($CDCl_3$, δ): 2.69(s, 3H), 3.85(s, 1H), 5.42(s, 2H), 7.05(d, 1H), 7.15(m, 1H), 7.36(d, 1H), 7.75(d, 1H), 7.89(dt, 1H), 8.35(s, 1H), 8.61(d, 1H).

In a similar manner as for **20a**, started with **32a** (140 mg, 0.375 mmol) and **8** (110 mg, 0.375 mmol) to produce **33a** (48 mg, 23%) as a brown solid. MS-ESI (*m*/*z*): 576.2(M+H), 598.1(M+Na); ^{1}H NMR ($CDCl_3$, δ): 2.51(s, 3H), 2.89(s, 3H), 3.15(br, 4H), 3.87(s, 2H), 5.21(s, 2H), 6.35(d, 1H), 6.93(s, 1H), 7.01(dt, 1H), 7.14–7.24(m, 3H), 7.31(m, 1H), 7.39(dd, 1H), 7.70(m, 1H), 8.58(s, 1H).

4.2.14. 4-(3-Chloro-4-(pyridin-2-ylmethoxy)phenoxy)-6-methyl-5-((5-(((2-(methylsulfonyl)ethyl)amino)methyl)furan-2-yl)ethynyl)nicotinonitrile (33b)

In a similar manner as for **18b**, started with **25** (0.58 g, 2.1 mmol) and **17** (0.71 g, 2.2 mmol) to produce 4-(3-chloro-4-(pyridin-2-ylmethoxy)phenoxy)-5-iodo-6-methylnicotinonitrile **31b** (0.83 g, 83%) as a brown solid. MS-ESI (*m*/*z*): 478.0(M+H); ^{1}H NMR (DMSO-d_6, δ): 2.82(s, 3H), 5.27(s, 2H), 6.96(dd, 1H), 7.24(d, 1H), 7.32(d, 1H), 7.37(t, 1H), 7.57(d, 1H), 7.87(dt, 1H), 8.58(d, 1H), 8.83(s, 1H).

In a similar manner as for **19a**, started with **31b** (0.5 g, 1.05 mmol) to produce 4-(3-chloro-4-(pyridin-2-ylmethoxy)phenoxy)-5-ethynyl-6-methylnicotinonitrile **32b** (0.32 g, 81%) as a faint-yellow solid. ^{1}H NMR ($CDCl_3$, δ): 2.75(s, 3H), 3.53(s, 1H), 5.26(s, 2H), 6.86(d, 1H), 6.94(s, 1H), 7.12(d, 1H), 7.25(t, 1H), 7.62(d, 1H), 7.76(dt, 1H), 8.57(d, 1H), 8.63(s, 1H).

In a similar manner as for **20a**, started with **32b** (80 mg, 0.213 mmol) and **8** (62 mg, 0.213 mmol) to produce **33b** (33 mg, 27%) as a brown solid. MS-ESI (*m*/*z*): 577.4(M+H), 599.3(M+Na); ^{1}H NMR (DMSO-d_6, δ): 2.68(s, 3H), 2.99(s, 3H), 3.32(br, 4H), 3.77(s, 2H), 5.25(s, 2H), 6.41(s, 1H), 6.66(d, 1H), 7.15(d, 1H), 7.24(s, 1H), 7.30–7.39(m, 2H), 7.49–7.56(m, 2H), 7.83(d, 1H), 8.57(s, 1H), 8.89(s, 1H).

4.2.15. 4-Chloro-5-(4-nitrophenyl)nicotinonitrile (40)

Ethyl cyanoacetate (24.3 mL, 0.227 mol) was added to a NaOEt solution prepared from Na (4.76 g, 0.207 mol) and anhydrous EtOH (200 mL) and the reaction solution was stirred at 50 °C for 1 h before cooled in an ice-water bath.

2-(4-Nitrophenyl)acetic acid (15 g, 0.083 mol) was mixed with $SOCl_2$ (150 mL) and heated to reflux for 1 h. The volatiles were removed under reduced pressure to give a dark-brown oil, which was dissolved into THF (100 mL) and added dropwise to the above EtOH solution at 0 °C and stirred for another 1 h. The reaction mixture was diluted with water (700 mL) and adjusted to pH ~3 by 10% H_2SO_4. The resulting solid was filtered, washed with water

and dried to give ethyl 2-cyano-4-(4-nitrophenyl)-3-oxobutanoate **36** (18 g, 76%).

Compound **36** (18 g, 0.065 mol) was added to 95% DMSO/H_2O (100 mL). The resulting mixture was heated at 110 °C for 1 h. The reaction mixture was diluted with water (500 mL) and adjusted to pH ~2 by 10% H_2SO_4. The resulting solid was filtered, washed with water and dried to give 4-(4-nitrophenyl)-3-oxobutanenitrile **37** (10 g, 81%) as a tan solid. MS-ESI (*m*/*z*): 203.1(M−H); ^{1}H NMR (DMSO-d_6, δ): 3.92(s, 2H), 4.01(s, 2H), 7.32(d, 2H), 8.05(d, 2H).

Compound **37** (6.1 g, 0.03 mol) and DMF–DMA (10 mL, 0.075 mol) were added to toluene (60 mL) and heated to reflux for 6 h. Then veratrylamine (7 g, 0.036 mol) was added to the solution and the mixture was heated to reflux for another 2 h. After cooled down to room temperature, the resulting solid was filtered, washed with toluene and dried to give 1-(3,4-dimethoxybenzyl)-5-(4-nitrophenyl)-4-oxo-1,4-dihydropyridine-3-carbonitrile **39** (10 g, 85%) as a tan solid. MS-ESI (*m*/*z*): 390.2(M−H), 392.2(M+H); ^{1}H NMR ($CDCl_3$, δ): 3.87(s, 3H), 3.89(s, 3H), 5.04(s, 2H), 6.78(s, 1H), 6.83–6.93(m, 2H), 7.63(d, 1H), 7.02(d, 2H), 7.91(d, 1H), 8.00(s, 1H), 8.18(d, 2H).

Compound **39** (5 g, 0.0118 mol) and LiCl (4.2 g, 0.1 mol) were added to $POCl_3$ (50 mL) and the solution was heated to reflux for 3 h. The volatiles were removed under reduced pressure and the residual was treated with water, adjusted pH ~7 by 10% aqueous NaOH. The resulting solid was filtered, washed with water and dried to give **40** (2.2 g, 71%) as a tan solid. ^{1}H NMR (DMSO-d_6, δ): 7.86(d, 2H), 8.39(d, 2H), 8.92(s, 1H), 9.19(s, 1H).

4.2.16. *N*-(4-(4-((3-Chloro-4-(pyridin-2-ylmethoxy)phenyl)amino)-5-cyanopyridin-3-yl)phenyl)-4-(dimethylamino)but-2-enamide (35a)

Compound **40** (0.66 g, 2.56 mmol), **26** (0.6 g, 2.56 mmol) and pyridine hydrochloride (0.3 g, 2.56 mmol) were added to DME (15 mL) and the solution was heated to reflux for 5 h. The reaction mixture was diluted with water (100 mL) and the resulting solid was filtered, washed with water and dried to give 4-((3-chloro-4-(pyridin-2-ylmethoxy)phenyl)amino)-5-(4-nitrophenyl)nicotinonitrile **43a** (0.88 g, 75%) as a faint-yellow solid. MS-ESI (*m*/*z*): 458.4(M+H), 480.3(M+Na); ^{1}H NMR (DMSO-d_6, δ): 5.19(d, 2H), 6.99–7.08(m, 2H), 7.18(s, 1H), 7.37(m, 1H), 7.47(d, 1H), 7.67(d, 2H), 7.85(t, 1H), 8.21(d, 2H), 8.34(s, 1H), 8.57(d, 1H), 8.66(s, 1H), 8.72(s, 1H).

Compound **43a** (0.86 g, 1.92 mmol), iron powder (0.65 g, 11.5 mmol), NH_4Cl (0.1 g, 1.9 mmol) and 2 M HCl (2 mL) were added into EtOH (20 mL) and the reaction solution were stirred at 70 °C for 1 h. The dark solution was filtered through a Celite pad. The filtrate was concentrated to ~10 mL and the residual was diluted with water (20 mL). The resulting solid was filtered, washed with water and dried to give 5-(4-aminophenyl)-4-((3-chloro-4-(pyridin-2-ylmethoxy)phenyl)amino)nicotinonitrile **44a** (0.77 g, 94%) as a tan solid. MS-ESI (*m*/*z*): 428.2(M+H), 450.2(M+Na).

Compound **6** (0.15 g, 0.7 mmol) and DMF (2 μL, 5 mol %) were added into THF (4 mL) and the suspension was cooled in an ice-water bath under N_2 atmosphere. Oxalyl chloride (62 μL, 0.7 mmol) was added and the reaction suspension was stirred at 26–30 °C for 1.5 h, then cooled again in ice-water bath. A solution of **44a** (0.2 g, 0.467 mmol) in NMP (3 mL) was added into the reaction suspension and the mixture was stirred at 0 °C for 3 h. The reaction solution was diluted with water (30 mL) and adjusted to pH ~9 with 10% NaOH solution, extracted with CH_2Cl_2. The organic layer was washed with water and brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (eluent: CH_2Cl_2–CH_2Cl_2/MeOH/TEA = 100/5/0.5) to give **35a** (72 mg, 29%) as a faint-yellow solid. MS-ESI (*m*/*z*): 539.5(M+H), 561.4(M+Na). ^{1}H NMR ($CDCl_3$, δ): 2.25(s, 6H), 3.08(d, 2H), 5.20(s, 2H), 6.19(d, 1H), 6.55(s, 1H), 6.86–6.98(m, 3H), 7.12(d, 1H), 7.20–7.25(m, 2H), 7.58(d, 1H), 7.65–7.76(m, 3H), 8.23(s, 1H), 8.33(s, 1H), 8.47(s, 1H), 8.55(d, 1H).

4.2.17. *N*-(4-(4-((3-Chloro-4-(pyridin-2-ylmethoxy)phenyl)amino)-5-cyanopyridin-3-yl)phenyl)-4-((4-methylpiperazin-1-yl)methyl)benzamide (35b)

Commercially available 4-((4-methylpiperazin-1-yl)methyl) benzoic acid dihydrochloride (5 g, 0.016 mol) was added into $SOCl_2$ (40 mL)andthemixturewasheatedtorefluxfor24 h.Thevolatileswere removed under reduced pressure to give 4-((4-methylpiperazin-1-yl)methyl)benzoyl chloride dihydrochloride (**45**) as a white solid.

Compound **45** (0.25 g, 0.767 mmol) and **44a** (0.2 g, 0.467 mmol) were suspended in pyridine (4 mL) and stirred at 0 °C for 4 h. The reaction solution was diluted with water (30 mL) and adjusted to pH ~9 with 10% NaOH solution, extracted with CH_2Cl_2. The organic layer was washed with water and brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (eluent: CH_2Cl_2–CH_2Cl_2/MeOH/TEA = 100/5/0.5) to give **35b** (0.164 g, 54%) as a faint-brown solid. MS-ESI (*m*/*z*): 644.6(M+H), 666.5(M+Na). ^{1}H NMR (DMSO-d_6, δ): 2.21(s, 3H), 2.05(br, 8H), 3.53(s, 2H), 5.20(s, 2H), 7.02(d, 1H), 7.10(d, 1H), 7.20(d, 1H), 7.32–7.44(m, 4H), 7.51(d, 1H), 7.70–7.92(m, 4H), 8.29(s, 1H), 8.48(s, 1H), 8.56(s, 2H), 10.36(s, 1H).

4.2.18. *N*-(4-(4-((3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)-5-cyanopyridin-3-yl)phenyl)-4-(dimethylamino)but-2-enamide (35c)

In a similar manner as for **43a**, started with **40** (0.6 g, 2.31 mmol) and **13** (0.58 g, 2.31 mmol) to produce 4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)-5-(4-nitrophenyl)nicotinonitrile **43c** (0.89 g, 82%) as a grey solid. MS-ESI (*m*/*z*): 475.3(M+H); ^{1}H NMR (DMSO-d_6, δ): 5.15(d, 2H), 7.04–7.06(m, 2H), 7.15–7.26(m, 4H), 7.45(m, 1H), 7.67(d, 2H), 8.23(d, 2H), 8.35(s, 1H), 8.67(s, 1H).

In a similar manner as for **44a**, started with **43c** (0.8 g, 1.68 mmol) to produce 5-(4-aminophenyl)-4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)nicotinonitrile **44c** (0.67 g, 90%) as a yellow solid. MS-ESI (*m*/*z*): 445.4(M+H).

In a similar manner as for **35a**, started with **44c** (0.2 g, 0.45 mmol) and **6** (0.15 g, 0.7 mmol) to produce **35c** (63 mg, 25%) as a white solid. MS-ESI (*m*/*z*): 554.4(M−H), 590.5(M+Cl), 556.4(M+H); ^{1}H NMR ($CDCl_3$, δ): 2.24(s, 6H), 3.07(d, 2H), 5.07(s, 2H), 6.20(d, 1H), 6.53(s, 1H), 6.83(d, 1H), 6.92–7.00(m, 2H), 7.12(d, 1H), 7.18(d, 2H), 7.27(m, 2H), 7.70(d, 2H), 8.23(s, 1H), 8.32(s, 1H), 8.47(s, 1H).

4.2.19. *N*-(4-(4-((3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)-5-cyanopyridin-3-yl)phenyl)-4-((4-methylpiperazin-1-yl)methyl)benzamide (35d)

In a similar manner as for **35b**, started with **44c** (0.2 g, 0.45 mmol) and **45** (0.25 g, 0.76 mmol) to produce **35d** (116 mg, 39%) as a white solid. MS-ESI (*m*/*z*): 661.5(M+H); ^{1}H NMR (DMSO-d_6, δ): 2.19(s, 3H), 2.39(br, 8H), 3.53(s, 2H), 5.16(s, 2H), 7.03–7.28(m, 6H), 7.36–7.44(m, 5H), 7.83–7.91(m, 4H), 8.29(s, 1H), 8.47(s, 1H), 8.56(s, 1H), 10.32(s, 1H).

4.2.20. *N*-(4-(4-((3-Chloro-4-fluorophenyl)amino)-5-cyanopyridin-3-yl)phenyl)-4-(dimethylamino)but-2-enamide (35e)

In a similar manner as for **43a**, started with **40** (0.8 g, 3.08 mmol) and 3-chloro-4-fluoroaniline **41** (0.45 g, 3.08 mmol) to produce 4-((3-chloro-4-fluorophenyl)amino)-5-(4-nitrophenyl)nicotinonit-

rile **43e** (0.95 g, 85%) as a faint-brown solid. MS-ESI (*m*/*z*): 369.2(M+H); ^{1}H NMR (DMSO-d_6, δ): 7.06(s, 1H), 7.25(d, 2H), 7.68(m, 2H), 8.24(d, 2H), 8.44(s, 1H), 8.73(s, 1H), 8.91(s, 1H).

In a similar manner as for **44a**, started with **43e** (0.9 g, 2.4 mmol) to produce 5-(4-aminophenyl)-4-((3-chloro-4-fluorophenyl)amino)nicotinonitrile **44e** (0.73 g, 89%) as a yellow solid. MS-ESI (*m*/*z*): 339.3(M+H).

In a similar manner as for **35a**, started with **44e** (0.2 g, 0.59 mmol) and **6** (0.19 g, 0.88 mmol) to produce **35e** (78 mg, 29%) as a white solid. MS-ESI (*m*/*z*): 450.4(M+H); ^{1}H NMR (DMSO-d_6, δ): 2.17(s, 6H), 3.06(d, 2H), 6.28(d, 1H), 6.68–6.78(m, 1H), 7.01(m, 1H), 7.18–7.26(m, 2H), 7.33(d, 2H), 7.69(d, 2H), 8.37(s, 1H), 8.64(s, 1H), 8.69(s, 1H), 10.19(s, 1H).

4.2.21. *N*-(4-(4-((3-Chloro-4-fluorophenyl)amino)-5-cyanopyridin-3-yl)phenyl)-4-((4-methylpiperazin-1-yl)methyl)benzamide (35f)

In a similar manner as for **35b**, started with **44e** (0.2 g, 0.59 mmol) and **45** (0.29 g, 0.88 mmol) to produce **35f** (140 mg, 43%) as a white solid. MS-ESI (*m*/*z*): 555.3(M+H); ^{1}H NMR ($CDCl_3$, δ): 2.29(s, 3H), 2.44(br, 8H), 3.54(s, 2H), 6.65(s, 1H), 6.95–7.12(m, 2H), 7.29(d, 2H), 7.41(d, 2H), 7.72(d, 2H), 7.81(d, 2H), 8.26(d, 1H), 8.41(s, 1H), 8.47(d, 1H).

4.2.22. *N*-(4-(4-((4-Acetylphenyl)amino)-5-cyanopyridin-3-yl)phenyl)-4-(dimethylamino)but-2-enamide (35g)

In a similar manner as for **43a**, started with **40** (0.8 g, 3.08 mmol) and 4-aminoacetophenone **42** (0.42 g, 3.08 mmol) to produce 4-((4-acetylphenyl)amino)-5-(4-nitrophenyl)nicotinonitrile **43g** (0.96 g, 88%) as a faint-brown solid. MS-ESI (*m*/*z*): 359.2(M+H); ^{1}H NMR (DMSO-d_6, δ): 2.44(s, 3H), 6.96(d, 2H), 7.65(d, 2H), 7.75(d, 2H), 7.96(d, 2H), 8.18–8.27(m, 5H), 8.55(s, 1H), 8.64(s, 1H), 8.90(s, 1H).

In a similar manner as for **44a**, started with **43g** (0.9 g, 2.5 mmol) to produce 4-((4-acetylphenyl)amino)-5-(4-aminophenyl)nicotinonitrile **44g** (0.74 g, 90%) as a yellow solid. MS-ESI (*m*/*z*): 329.4(M+H).

In a similar manner as for **35a**, started with **44g** (0.2 g, 0.61 mmol) and **6** (0.19 g, 0.88 mmol) to produce **35g** (68 mg, 24%) as a white solid. MS-ESI (*m*/*z*): 440.5(M+H), 462.5(M+Na); ^{1}H NMR (DMSO-d_6, δ): 2.17(s, 6H), 2.45(s, 3H), 3.05(d, 2H), 6.91(d, 2H), 7.32(d, 2H), 7.66(d, 2H), 7.74(d, 2H), 8.60(s, 1H), 8.83(s, 1H), 9.11(br, 1H), 10.17(br, 1H).

4.2.23. *N*-(4-(4-((4-Acetylphenyl)amino)-5-cyanopyridin-3-yl)phenyl)-4-((4-methylpiperazin-1-yl)methyl)benzamide (35h)

In a similar manner as for **35b**, started with **44g** (0.2 g, 0.61 mmol) and **45** (0.29 g, 0.88 mmol) to produce **35h** (136 mg, 41%) as a white solid. MS-ESI (*m*/*z*): 545.5(M+H), 567.5(M+Na); ^{1}H NMR ($CDCl_3$, δ): 2.25(s, 3H), 2.43(br, 8H), 2.48(s, 3H), 3.52(s, 2H), 6.97(d, 1H), 7.25(d, 1H), 7.38(d, 2H), 7.67(d, 1H), 7.81(d, 3H), 8.41(s, 1H), 8.48(s, 1H), 8.58(d, 1H).

4.2.24. 5-Acetamido-4-ethoxy-2-nitrobenzoyl chloride (52)

Ac_2O (150 mL, 1.5 mol) was added dropwise over 1 h to a solution of 3-amino-4-hydroxybenzoic acid (155 g, 1 mol) in AcOH (1 L) at 60 °C. The reaction solution was poured into water (3 L) and stirred, the resulting solid was filtered, washed with water (500 mL × 3) and dried at 50 °C to give 3-acetamido-4-hydroxybenzoic acid (180 g, 92%) as a white solid, which was suspended with K_2CO_3 (190 g, 1.4 mol) in DMF (1 L) and heated to 60 °C. C_2H_5Br (105 mL, 1.4 mol) was added dropwise to the suspension over 1 h. The reaction solution was poured into water (3 L) and stirred, the resulting solid was filtered, washed with water (500 mL × 3) and dried at 50 °C to give 3-acetamido-4-ethoxybenzoic acid (200 g, 96%) as a white solid.

Fuming HNO_3 (120 mL, 3 mol) was added dropwise over 1 h to a solution of 3-acetamido-4-ethoxybenzoic acid (220 g, 1 mol) in AcOH (1.5 L) to keep the inner temperature below 35 °C. The reaction solution was stirred at 25–35 °C for another 6 h. The reaction solution was poured into water (4 L) and stirred, the resulting solid was filtered, washed with water (500 mL × 3) and dried at 50 °C to give 5-acetamido-4-ethoxy-2-nitrobenzoic acid **51** (230 g, 85%) as a faint-brown solid. Mp 234–236 °C (dec). ESI-MS (*m*/*z*) 267(M−H). ^{1}H NMR (DMSO-d_6, δ): 1.40 (t, 3H, *J* = 6.9 Hz), 2.17 (s, 3H), 4.26 (q, 2H, *J* = 6.9 Hz), 7.61 (s, 1H), 8.56 (s, 1H), 9.46 (s, 1H), 13.55 (br s, 1H). ^{13}C NMR (DMSO-d_6, δ): 14.14, 24.12, 65.29, 107.35, 119.04, 120.87, 131.10, 144.23, 149.64, 165.65, 169.49. Anal. Calcd for $C_{11}H_{12}N_2O_6$: C, 49.26; H, 4.51; N, 10.44. Found: C, 49.25; H, 4.58; N, 10.54.

Compound **51** (14 g, 0.052 mol) and $SOCl_2$ (15 mL, 0.2 mol) were suspended in CH_2Cl_2 (150 mL) and the reaction mixture was heated to reflux for 1 h to give a clear solution. The volatiles were removed under reduced pressure to give **52** as a white solid.

4.2.25. *N*-(3-Chloro-4-(pyridin-2-ylmethoxy)phenyl)-5-(4-(dimethylamino)but-2-enamido)-4-ethoxy-2-nitrobenzamide (48a) and 2-amino-*N*-(3-chloro-4-(pyridin-2-ylmethoxy)phenyl)-5-(4-(dimethylamino)but-2-enamido)-4-ethoxybenzamide (49a)

A solution of **26** (2.3 g, 0.01 mol) in CH_2Cl_2 (20 mL) was added dropwise to the solution of **52** (2.9 g, 0.01 mol) in CH_2Cl_2 (30 mL) at 0 °C and the reaction suspension was stirred for another 30 min. The resulting solid was filtered and dried to give a grey solid, which was suspended with K_2CO_3 (1.38 g, 0.01 mol) into MeOH (100 mL) and THF (40 mL) and stirred at room temperature for 3 h. The reaction suspension was treated with 12 M HCl to pH ~7. The solvent were removed under reduced pressure and the residual was added into water (40 mL) and stirred, the resulting solid was filtered, washed with water, and dried to give 5-amino-*N*-(3-chloro-4-(pyridin-2-ylmethoxy)phenyl)-4-ethoxy-2-nitrobenzamide **54a** (3.1 g, 69%) at a bright-yellow solid.

In a similar manner as for **35a**, started with **54a** (0.44 g, 1 mmol) and **6** (0.42 g, 2 mmol) to produce **48a** (135 mg, 25%) as a light-yellow solid. MS-ESI (*m*/*z*): 552.1(M−H), 587.8(M+Cl), 554.0(M+H); ^{1}H NMR ($CDCl_3$, δ): 1.54(t, 3H), 2.28(s, 6H), 3.12(d, 2H), 4.24(q, 2H), 5.25(s, 2H), 6.18(d, 1H), 6.91–7.05(m, 2H), 7.22(t, 1H), 7.39(dd, 1H), 7.58(s, 1H), 7.62(d, 1H), 7.72(d, 1H), 7.78(d, 1H), 8.03(d, 2H), 8.57(d, 1H), 8.75(s, 1H).

Compound **48a** (80 mg, 0.153 mmol), zinc powder (0.2 g, 3 mmol) and $CaCl_2$ (85 mg, 0.76 mmol) were added into 80% EtOH/H_2O (10 mL) and the reaction mixture was stirred at reflux for 10 h. The solvent was removed under reduced pressure and the residual was dissolved in CH_2Cl_2 (30 mL). The organic layer was washed twice with brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (eluent: CH2Cl2 – CH2Cl2/MeOH/TEA CH_2Cl_2–CH_2Cl_2/MeOH/TEA = 100/5/0.5) to give **49a** (55 mg, 48%) as a white solid. MS-ESI (*m*/*z*): 524.1(M+H), 546.1(M+Na); ^{1}H NMR ($CDCl_3$, δ): 1.45(t, 3H), 2.28(s, 6H), 3.01(d, 2H), 4.07(q, 2H), 5.25(s, 2H), 5.75(br, 2H), 6.12(d, 1H), 6.14(s, 1H), 6.88–6.97(m, 2H), 7.22(t, 1H), 7.35(dd, 1H), 7.62(s, 2H), 7.71–7.75(m, 2H), 8.39(s, 1H), 8.58(s, 2H)

4.2.26. *N*-(3-Chloro-4-(pyridin-2-ylmethoxy)phenyl)-4-ethoxy-5-(4-((4-methylpiperazin-1-yl)methyl)benzamido)-2-nitrobenzamide (48b) and 2-amino-*N*-(3-chloro-4-(pyridin-2-ylmethoxy)phenyl)-4-ethoxy-5-(4-((4-methylpiperazin-1-yl)methyl)benzamido)benzamide (49b)

In a similar manner as for **35b**, started with **54a** (0.48 g, 1.1 mmol) and **45** (0.6 g, 1.35 mmol) to produce **48b** (610 mg, 87%)

as a grey solid. MS-ESI (*m*/*z*): 657.2(M−H), 692.8(M+Cl), 659.2(M+H); ^{1}H NMR ($CDCl_3$, δ): 1.57(t, 3H), 2.28(s, 3H), 2.48(br, 8H), 3.57(s, 2H), 4.27(q, 2H), 5.25(s, 2H), 6.93(d, 1H), 7.22(t, 1H), 7.41(dd, 1H), 7.49(d, 2H), 7.62(d, 2H), 7.72(d, 1H), 7.78(s, 1H), 7.80(d, 2H), 8.13(s, 1H), 8.57(d, 1H), 8.74(s, 1H), 8.83(s, 1H).

In a similar manner as for **49a**, started with **48b** (0.12 g, 0.18 mmol) to produce **49b** (0.11 g, 95%) as a grey solid. MS-ESI (*m*/*z*): 629.2(M+H); ^{1}H NMR ($CDCl_3$, δ): 1.47(t, 3H), 2.32(s, 3H), 2.52(br, 8H), 3.56(s, 2H), 4.08(q, 2H), 5.24(s, 2H), 5.77(br, 2H), 6.17(s, 1H), 6.90(d, 1H), 7.21(t, 1H), 7.36(d, 1H), 7.43(d, 2H), 7.62(d, 2H), 7.72(s, 1H), 7.78(d, 2H), 8.30(d, 2H), 8.56(d, 1H), 8.66(s, 1H).

4.2.27. *N*-(3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)-5-(4-(dimethylamino)but-2-enamido)-4-ethoxy-2-nitrobenzamide (48c) and 2-amino-*N*-(3-chloro-4-((3-fluorobenzyl)oxy) phenyl)-5-(4-(dimethylamino)but-2-enamido)-4-ethoxybenzamide (49c)

In a similar manner as for **54a**, started with **52** (1 g, 3.7 mmol) and **13** (0.94 g, 3.7 mmol) to give 5-amino-*N*-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-4-ethoxy-2-nitrobenzamide **54c** (0.59 g, 33%) as a tan solid. ^{1}H NMR (DMSO-d_6, δ): 1.39(t, 3H), 4.17(q, 2H), 5.21(s, 2H), 6.51(br, 2H), 6.65(s, 1H), 7.15–7.31(m, 4H), 7.47(d, 2H), 7.55(s, 1H), 7.83(d, 1H), 10.38(s, 1H).

In a similar manner as for **35a**, started with **54c** (0.31 g, 0.674 mmol) and **6** (0.21 g, 1 mmol) to produce **48c** (89 mg, 23%) as a light-yellow solid. MS-ESI (*m*/*z*): 571.5(M+H), 593.5(M+Na); ^{1}H NMR ($CDCl_3$, δ): 1.52(t, 3H), 2.27(s, 6H), 3.13(d, 2H), 4.22(q, 2H), 5.12(s, 2H), 6.17(d, 1H), 6.89(d, 1H), 6.95–7.04(m, 2H), 7.17–7.24(m, 2H), 7.30–7.37(m, 1H), 7.43(dd, 1H), 7.56(s, 1H), 7.72(d, 1H), 8.02(s, 1H), 8.23(s, 1H), 8.67(s, 1H).

In a similar manner as for **49a**, started with **48c** (35 mg, 0.061 mmol) to produce **49c** (29 mg, 87%) as a grey solid. MS-ESI (*m*/*z*): 541.3(M+H), 563.6(M+Na); ^{1}H NMR ($CDCl_3$, δ): 1.44(t, 3H), 2.29(s, 6H), 3.12(d, 2H), 4.04(q, 2H), 5.10(s, 2H), 6.12(s, 1H), 6.13(d, 1H), 6.22(br, 2H), 6.85–7.02(m, 3H), 7.17–7.22(m, 2H), 7.30–7.40(m, 2H), 7.62(s, 1H), 7.71(d, 1H), 8.50(s, 1H), 8.59(s, 1H).

4.2.28. *N*-(3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)-4-ethoxy-5-(4-((4-methylpiperazin-1-yl)methyl)benzamido)-2-nitrobenzamide (48d) and 2-amino-*N*-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-4-ethoxy-5-(4-((4-methylpiperazin-1-yl)methyl)benzamido)benzamide (49d)

In a similar manner as for **35b**, started with **54c** (120 mg, 0.182 mmol) and **45** (90 mg, 0.27 mmol) to produce **48d** (230 mg, 76%) as a yellow solid. MS-ESI (*m*/*z*): 676.6(M+H); ^{1}H NMR ($CDCl_3$, δ): 1.54(t, 3H), 2.28(s, 3H), 2.49(br, 8H), 3.54(s, 2H), 4.23(q, 2H), 5.07(s, 2H), 6.86(d, 1H), 6.99(t, 1H), 7.16–7.21(m, 2H), 7.32(t, 1H), 7.40(d, 1H), 7.46(d, 2H), 7.53(s, 1H), 7.71–7.78(m, 3H), 8.41(s, 1H), 8.69(s, 1H), 8.75(s, 1H).

In a similar manner as for **49a**, started with **48d** (140 mg, 0.207 mmol) to produce **49d** (110 mg, 82%) as a tan solid. MS-ESI (*m*/*z*): 646.7(M+H); ^{1}H NMR ($CDCl_3$, δ): 1.46(t, 3H), 2.28(s, 3H), 2.48(br, 8H), 3.56(s, 2H), 4.07(q, 2H), 5.08(s, 2H), 5.75(br, 2H), 6.16(s, 1H), 6.86(d, 1H), 6.98(t, 1H), 7.16–7.22(m, 2H), 7.30–7.45(m, 4H), 7.74(d, 1H), 7.77(d, 2H), 8.31(s, 1H), 8.56(s, 1H), 8.67(s, 1H).

4.2.29. *N*-(3-Chloro-4-fluorophenyl)-5-(4-(dimethylamino)but-2-enamido)-4-ethoxy-2-nitrobenzamide (48e) and 2-amino-*N*-(3-chloro-4-fluorophenyl)-5-(4-(dimethylamino)but-2-enamido)-4-ethoxybenzamide (49e)

In a similar manner as for **54a**, started with **52** (1 g, 3.7 mmol) and **41** (0.54 g, 3.7 mmol) to give 5-amino-*N*-(3-chloro-4-fluorophenyl)-4-ethoxy-2-nitrobenzamide **54e** (0.45 g, 35%) as a yellow solid. ^{1}H NMR (DMSO-d_6, δ): 1.39(t, 3H), 4.17(q, 2H), 6.54(br, 2H), 6.66(s, 1H), 7.40(t, 1H), 7.50–7.56(m, 2H), 7.96(d, 1H), 10.56(s, 1H).

In a similar manner as for **35a**, started with **54e** (0.24 g, 0.678 mmol) and **6** (0.21 g, 1 mmol) to produce **48e** (110 mg, 35%) as a light-yellow solid. MS-ESI (*m*/*z*): 465.4(M+H), 487.4(M+Na); ^{1}H NMR ($CDCl_3$, δ): 1.52(t, 3H), 2.28(s, 6H), 3.12(d, 2H), 4.22(q, 2H), 6.15(d, 1H), 6.92–7.02(m, 1H), 7.08(t, 1H), 7.42(m, 1H), 7.54(s, 1H), 7.80(dd, 1H), 8.00(s, 1H), 8.60(s, 1H), 8.66(s, 1H).

In a similar manner as for **49a**, started with **48e** (66 mg, 0.142 mmol) to produce **49e** (32 mg, 52%) as a grey solid. MS-ESI (*m*/*z*): 435.4(M+H), 457.5(M+Na); ^{1}H NMR ($CDCl_3$, δ): 1.45(t, 3H), 2.27(s, 6H), 3.12(d, 2H), 4.03(q, 2H), 5.75(br, 2H), 6.09(s, 1H), 6.86–6.98(m, 1H), 7.03(t, 1H), 7.42(m, 1H), 7.61(s, 1H), 7.77(dd, 1H), 8.44(s, 1H), 8.84(s, 1H).

4.2.30. *N*-(3-Chloro-4-fluorophenyl)-4-ethoxy-5-(4-((4-methylpiperazin-1-yl)methyl)benzamido)-2-nitrobenzamide (48f) and 2-amino-*N*-(3-chloro-4-fluorophenyl)-4-ethoxy-5-(4-((4-methylpiperazin-1-yl)methyl)benzamido)benzamide (49f)

In a similar manner as for **35b**, started with **54e** (160 mg, 0.45 mmol) and **45** (220 mg, 0.68 mmol) to produce **48f** (100 mg, 39%) as a yellow solid. MS-ESI (*m*/*z*): 570.4(M+H), 592.4(M+H); ^{1}H NMR ($CDCl_3$, δ): 1.57(t, 3H), 2.30(s, 3H), 2.49(br, 8H), 3.58(s, 2H), 4.28(q, 2H), 7.10(t, 1H), 7.42–7.51(m, 3H), 7.61(s, 1H), 7.78–7.81(m, 3H), 8.25(s, 1H), 8.73(s, 1H), 8.79(s, 1H).

In a similar manner as for **49a**, started with **48f** (50 mg, 0.088 mmol) to produce **49f** (40 mg, 85%) as a tan solid. MS-ESI (*m*/*z*): 540.5(M+H); ^{1}H NMR ($CDCl_3$, δ): 1.48(t, 3H), 2.27(s, 3H), 2.45(br, 8H), 3.56(s, 2H), 4.07(q, 2H), 5.74(br, 2H), 6.14(s, 1H), 7.03(t, 1H), 7.40–7.48(m, 3H), 7.77–7.81(m, 3H), 8.29(s, 1H), 8.53(s, 1H), 8.86(s, 1H).

4.2.31. *N*-(3-Chloro-4-(pyridin-2-ylmethoxy)phenyl)-3-(4-(dimethylamino)but-2-enamido)-4-ethoxybenzamide (50a)

4-Ethoxy-3-nitrobenzoic acid **55** (0.21 g, 1.1 mmol) was suspended in $SOCl_2$ (5 mL) and the reaction mixture was heated to reflux for 2 h to give a clear solution. The volatiles were removed under reduced pressure to give 4-ethoxy-3-nitrobenzoyl chloride **56** as a light-yellow solid.

A solution of **26** (0.23 g, 1 mmol) in CH_2Cl_2 (3 mL) was added dropwise to the solution of **56** (1.1 mmol) in CH_2Cl_2 (4 mL) at 0 °C and the reaction suspension was stirred for another 30 min. The resulting solid was filtered and dried to give *N*-(3-chloro-4-(pyridin-2-ylmethoxy)phenyl)-4-ethoxy-3-nitrobenzamide **58a** (0.35 g, 82%) as a yellow solid. ^{1}H NMR (DMSO-d_6, δ): 1.37(t, 3H), 4.31(q, 2H), 5.26(s, 2H), 7.24(d, 1H), 7.36(dd, 1H), 7.50(d, 1H), 7.56(d, 1H), 7.62(dd, 1H), 7.87(dt, 1H), 7.95(d, 1H), 8.25(dd, 1H), 8.50(d, 1H), 8.59(d, 1H), 10.37(s, 1H).

Compound **58a** (0.3 g, 0.71 mmol), iron powder (0.25 g, 4.2 mmol) and 2 M HCl (0.7 mL, 1.4 mmol) were added into DME (40 mL) and MeOH (40 mL) and the reaction solution were stirred at 60 °C for 5 h. The dark solution was filtered through a Celite pad. The filtrate was concentrated under reduced pressure and the residual was added into 5% K_2CO_3 solution and stirred to give 3-amino-*N*-(3-chloro-4-(pyridin-2-ylmethoxy)phenyl)-4-ethoxybenzamide **59a** (0.29 g, 98%) as a brown solid.

In a similar manner as for **35a**, started with **59a** (100 mg, 0.234 mmol) and **6** (100 mg, 0.467 mmol) to produce **50a** (49 mg, 41%) as a white solid. MS-ESI (*m*/*z*): 509.1(M+H); ^{1}H NMR ($CDCl_3$, δ): 1.46(t, 3H), 2.27(s, 6H), 3.11(d, 2H), 4.14(q, 2H), 5.23(s, 2H), 6.17(d, 1H), 6.87–6.92(m, 2H), 7.20(m, 1H), 7.42(dd, 1H), 7.60(d, 1H), 7.71(m, 2H), 7.80(d, 1H), 7.89(s, 1H), 8.84(s, 1H), 8.56(d, 1H), 8.87(s, 1H).

4.2.32. *N*-(3-Chloro-4-(pyridin-2-ylmethoxy)phenyl)-4-ethoxy-3-(4-((4-methylpiperazin-1-yl)methyl)benzamido)benzamide (50b)

In a similar manner as for **35b**, started with **59a** (120 mg, 0.3 mmol) and **45** (150 mg, 0.47 mmol) to produce **50b** (90 mg, 49%) as an off-white solid. MS-ESI (*m*/*z*): 612.1(M−H), 647.9(M+Cl), 614.2[M+H]; ^{1}H NMR ($CDCl_3$, δ): 1.52(t, 3H), 2.30(s, 3H), 2.50(br, 8H), 3.58(s, 2H), 4.22(q, 2H), 5.27(s, 2H), 6.93(d, 1H), 7.00(d, 1H), 7.23(t, 1H), 7.45–7.51(m, 3H), 7.63(d, 1H), 7.71–7.79(m, 2H), 7.83(d, 3H), 8.34(s, 1H), 8.58(d, 1H), 8.64(s, 1H), 8.96(d, 1H).

4.2.33. 3-(4-(Dimethylamino)but-2-enamido)-4-ethoxy-*N*-(4-(pyridin-3-yl)pyrimidin-2-yl)benzamide (50c)

Commercially available 4-(pyridin-3-yl) pyrimidin-2-amine **57** (0.3 g, 1.74 mmol) was added to the solution of **56** (0.38 g, 2 mmol) in pyridine (8 mL) at 0 °C and stirred at room temperature for 3 h. The reaction solution was diluted with water (50 mL) and the resulting solid was filtered, washed with water, and dried to give 4-ethoxy-3-nitro-*N*-(4-(pyridin-3-yl)pyrimidin-2-yl)benzamide **58c** (0.31 g, 51%) as a light-brown solid. ^{1}H NMR (DMSO-d_6, δ): 1.37(t, 3H), 4.32(q, 2H), 7.50(d, 1H), 7.75(dd, 1H), 7.99(d, 1H), 8.29(dd, 1H), 8.54(d, 1H), 8.74(d, 1H), 8.83(d, 1H), 8.88(d, 1H), 9.44(d, 1H), 11.33(br, 1H).

In a similar manner as for **59a**, started with **58c** (0.3 g, 0.89 mmol) to give 3-amino-4-ethoxy-*N*-(4-(pyridin-3-yl)pyrimidin-2-yl)benzamide **59c** (0.23 g, 84%) as a tan solid.

In a similar manner as for **35a**, started with **59c** (220 mg, 0.65 mmol) and **6** (210 mg, 1 mmol) to produce **50c** (65 mg, 22%) as a white solid. MS-ESI (*m*/*z*): 447.3(M+H); ^{1}H NMR (DMSO-d_6, δ): 1.38(t, 3H), 2.77(s, 6H), 3.92(d, 2H), 4.11(q, 2H), 6.18(d, 1H), 6.70–6.81(m, 1H), 6.90(d, 1H), 7.34(s, 1H), 7.60(m, 1H), 7.89(d, 1H), 8.56(d, 1H), 8.74(d, 1H), 8.81(d, 1H), 9.39(s, 1H), 10.72(s, 1H).

4.2.34. 4-Ethoxy-3-(4-((4-methylpiperazin-1-yl)methyl)benzamido)-*N*-(4-(pyridin-3-yl)pyrimidin-2-yl)benzamide (50d)

In a similar manner as for **35b**, started with **59c** (210 mg, 0.63 mmol) and **45** (310 mg, 1 mmol) to produce **50d** (60 mg, 19%) as a tan solid. MS-ESI (*m*/*z*): 552.2(M+H); ^{1}H NMR (CD_3COCD_3, δ): 1.51(t, 3H), 2.32(s, 3H), 2.55(br, 8H), 3.60(s, 2H), 4.31(q, 2H), 7.20(d, 1H), 7.50–7.55(m, 3H), 7.81(d, 1H), 7.89(d, 1H), 7.95(d, 2H), 8.59(d, 1H), 8.75(dd, 2H), 9.05(s, 1H), 9.40(s, 1H).

4.2.35. *N*-(3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)-5-(4-(dimethylamino)but-2-enamido)-2-methylbenzamide (61a)

In a similar manner as for **58a**, started with 2-methyl-5-nitrobenzoic acid (0.2 g, 1.1 mmol) and **13** (0.25 g, 1 mmol) to produce *N*-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-2-methyl-5-nitrobenzamide **64a** (0.33 g, 80%) as a yellow solid.

In a similar manner as for **59a**, started with **64a** (0.3 g, 0.723 mmol) to produce 5-amino-*N*-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-2-methylbenzamide **65a** (0.28 g, 98%) as a brown solid.

In a similar manner as for **35a**, started with **65a** (100 mg, 0.259 mmol) and **6** (82 mg, 0.39 mmol) to produce **61a** (33 mg, 25%) as a white solid. MS-ESI (*m*/*z*): 494.0(M−H), 529.9(M+Cl), 496.1(M+H); ^{1}H NMR ($CDCl_3$, δ): 2.21(s, 6H), 2.35(s, 3H), 3.03(d, 2H), 5.09(s, 2H), 6.17(dd, 1H), 6.82–6.91(m, 2H), 6.95–7.07(m, 1H), 7.15–7.23(m, 2H), 7.32(d, 1H), 7.47(t, 2H), 7.67(d, 1H), 7.81(s, 1H), 8.30(s, 1H), 8.93(s, 1H), 8.98(s, 1H).

4.2.36. *N*-(3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)-2-methyl-5-(4-((4-methylpiperazin-1-yl)methyl)benzamido)benzamide (61b)

In a similar manner as for **35b**, started with **65a** (110 mg, 0.286 mmol) and **45** (140 mg, 0.43 mmol) to produce **61b** (93 mg, 54%) as a tan solid. MS-ESI (*m*/*z*): 599.2(M−H), 635.0(M+Cl), 601.2(M+H); ^{1}H NMR (DMSO-d_6, δ): 2.21(s, 3H), 2.33(s, 3H), 2.41(br, 8H), 3.54(s, 2H), 5.22(s, 2H), 7.17(dt, 1H), 7.21(s, 1H), 7.24(s, 1H), 7.26–7.33(m, 2H), 7.42–7.47(m, 3H), 7.65(dd, 1H), 7.88–7.92(m, 2H), 7.93–7.99(m, 1H), 10.25(d, 2H).

4.2.37. 5-(4-(Dimethylamino)but-2-enamido)-2-methyl-*N*-(4-(pyridin-3-yl)pyrimidin-2-yl)benzamide (61c)

In a similar manner as for **58c**, started with 2-methyl-5-nitrobenzoyl chloride **62** (0.35 g, 2 mmol) and **57** (0.3 g, 1.74 mmol) to produce 2-methyl-5-nitro-*N*-(4-(pyridin-3-yl)pyrimidin-2-yl)benzamide **64c** (0.31 g, 51%) as a yellow solid.

In a similar manner as for **59a**, started with **64c** (0.3 g, 0.895 mmol) to produce 5-amino-2-methyl-*N*-(4-(pyridin-3-yl)pyrimidin-2-yl)benzamide **65c** (0.26 g, 92%) as a brown solid.

In a similar manner as for **35a**, started with **65c** (230 mg, 0.753 mmol) and **6** (230 mg, 1.1 mmol) to produce **61c** (64 mg, 21%) as a white solid. MS-ESI (*m*/*z*): 415.2(M−H), 451.0(M+Cl), 417.1(M+H); ^{1}H NMR ($CDCl_3$, δ): 2.20(s, 6H), 2.40(s, 3H), 3.05(d, 2H), 6.41(d, 1H), 6.90(m, 1H), 7.15(d, 1H), 7.38(dd, 1H), 7.43(d, 1H), 7.82(d, 1H), 7.97(s, 1H), 8.23(d, 1H), 8.62–8.68(m, 2H), 9.12(s, 1H), 9.70(s, 1H).

4.2.38. 4-(3-Fluorophenyl)pyrimidin-2-amine (63)

1-(3-Fluorophenyl)ethanone (8 mL, 0.065 mol) and DMF–DMA (25 mL, 0.2 mol) were heated to reflux in toluene (40 mL) for 24 h. Petroleum ether (30 mL) was added to the reaction solution and let it cool to room temperature. The resulting solid was filtered, washed with petroleum ether and dried to give 3-(dimethylamino)-1-(3-fluorophenyl)prop-2-en-1-one (9.8 g, 78%).

The above product (5 g, 0.026 mol), guanidine nitrate (3.16 g, 0.026 mol) and NaOH (1.04 g, 0.026 mol) were added into ^{i}PrOH (40 mL) and heated to reflux for 2 h. After cooled in an ice-water bath, the resulting solid was filtered, washed with ^{i}PrOH and dried to give **63** (3.1 g, 63%). ^{1}H NMR ($CDCl_3$, δ): 5.18(br, 2H), 7.02(d, 1H), 7.17(dt, 1H), 7.39–7.47(m, 1H), 7.71–7.77(m, 2H), 8.37(d, 1H).

4.2.39. *N*-(4-(3-Fluorophenyl)pyrimidin-2-yl)-2-methyl-5-(4-((4-methylpiperazin-1-yl)methyl)benzamido)benzamide (61d)

In a similar manner as for **58c**, started with **62** (0.43 g, 2.3 mmol) and **63** (0.36 g, 1.9 mmol) to produce *N*-(4-(3-fluorophenyl)pyrimidin-2-yl)-2-methyl-5-nitrobenzamide **64d** (0.31 g, 47%) as a yellow solid.

In a similar manner as for **59a**, started with **64d** (0.3 g, 0.88 mmol) to produce 5-amino-*N*-(4-(3-fluorophenyl)pyrimidin-2-yl)-2-methylbenzamide **65d** (0.25 g, 91%) as a brown solid.

In a similar manner as for **35b**, started with **65d** (160 mg, 0.47 mmol) and **45** (250 mg, 0.76 mmol) to produce **61d** (95 mg, 39%) as a tan solid. MS-ESI (*m*/*z*): 539.2(M+H); ^{1}H NMR ($CDCl_3$, δ): 2.28(s, 3H), 2.44(br, 11H), 3.52(s, 2H), 5.29(s, 2H), 7.15(t, 1H), 7.22(d, 1H), 7.35–7.42(m, 3H), 7.63(d, 1H), 7.72(d, 2H), 7.84(d, 2H), 7.88(s, 1H), 8.44(s, 1H), 8.56(d, 1H), 9.10(br, 1H).

4.3. Biology

4.3.1. EGFR and ErbB-2 kinase assays by DELFIA/time-resolved fluorometry

Compounds were dissolved in DMSO and diluted to the appropriate concentrations with 25 mM HEPES at pH 7.4. In each well, 10 μL of compound was incubated with 10 μL (5 ng for EGFR or 12.5 ng for ErbB-2) of recombinant enzyme (1:80 dilution in 100 mM HEPES) for 10 min at room temperature. Then 10 μL of 5× buffer (containing 20 mM HEPES, 2 mM $MnCl_2$, 100 μM Na_3VO_4, and 1 mM DTT) and 20 μL of 0.1 mM ATP–50 mM $MgCl_2$ was added for 1 h. Positive and negative controls were included in each plate by incubation of enzyme with or without ATP–$MgCl_2$.

At the end of incubation, liquid was aspirated, and plates were washed three times with wash buffer. A 75 μL (400 ng) sample of europiumlabeled anti-phosphotyrosine antibody was added to each well for another 1 h of incubation. After washing, enhancement solution was added and the signal was detected with excitation at 340 nm and emission at 615 nm. The IC_{50} was obtained from curves of percentage inhibition.

4.3.2. Cell proliferation assay

The cell proliferation assays were done with two human carcinoma cell lines: A-549 and HL60. Cells were plated in 96-well plates at densities of 5×10^4/mL in RPMI-1640 medium supplemented with 5% fetal bovine serum. On the next day, compounds were dosed at 0.5, 5, 50, 500, and 5000 ng/mL concentrations and the cells were cultured for 2 days. At the end of incubation, cell survival was determined by the sulforhodamine B assay. The IC_{50} values were obtained from the growth curves.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.03.053. These data include MOL files and InChiKeys of the most important compounds described in this article.

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