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Synthesis and biological evaluation of novel quinazoline-derived human Pin1 inhibitors

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ABSTRACT

A series of novel 2,4-disubstituted quinazoline derivatives were prepared and their inhibitory activities on hPin1 were evaluated. Of all the synthesized compounds, eight compounds displayed inhibitory activities with IC_{50} value at the level of 10^{-6} mol/L. Preliminary structure–activity relationships were analyzed in details and the binding mode of the titled compounds was predicted using FlexX algorithm. The design and optimization of novel small molecule Pin1 inhibitors will be guided by the results of this report.

1. Introduction

Pin1 (Protein interaction with NIMA1), a phosphorylation-dependent peptidyl-prolyl cis/trans isomerase (PPIase),[1,2] can catalyze the rotation of pSer-Pro/pThr-Pro amide bond in its substrate protein.[3] Thus, Pin1 works as a conformational switcher to regulate the function of many phosphoproteins. The over-expression of Pin1 could lead to oncogenesis by increasing cyclin D1 transcription and stability in multiple oncogenic signaling pathways.[4] It has been demonstrated that Pin1 is prevalently over-expressed in some commonly encountered cancers such as prostate, breast, brain, lung and colon cancer,[5] and its over-expression level correlates with tumor grades and clinical outcomes.[6] The depletion of Pin1 in various cancer cells may cause apoptosis and suppress the transformed phenotypes and tumorigenicity in nude mice.[7,8] Therefore, inhibition of Pin1 is becoming a potential therapeutic strategy for anticancer treatment and has attracted many efforts on the development of Pin1 inhibitors.

In this regard, a number of small molecules have been investigated as inhibitors of Pin1 by several groups.[9–11] Lately, the design of Pin1 inhibitors has been carried out using structure-based strategy and the enzymatic inhibition was achieved in nanomolar level with the aminophenylpropanol scaffold.[12–15] However, the reported highly potent Pin1 inhibitors have lacked the whole cell activity because of their poor permeability.[12] In view of the fact, the development of small molecule as Pin1 inhibitors is still highly desirable and challenging with respect to improving the binding affinity and physiochemical properties.

The crystal structure of Pin1 has shown that the active site of Pin1 consists of two hydrophobic domains and one basic cluster formed by the side chains of Lys63, Arg68 and Arg69 residues.[12–16] One of hydrophobic binding site defined as prolyl pocket formed by His59, His157, Met130 and Phe134 residues, the other one is a slightly shallow hydrophobic shelf and includes His59, Ala118 and Leu122 residues. As a part of our program to discover novel Pin1 inhibitors, quinazoline compound **6a** was identified as a hit with an IC_{50} of 4.87 μM by a random screening. In order to investigate the structure–activity relationships (SAR), a series of quinazoline derivatives were designed by diversifying the substituents at 2- and 4-position of the parent quinazolines. We envisioned that the introduced carboxyl group at 4-position of quinazoline allowed to bind with the charged side chains of the basic cluster. The 2-substituents on the quinazoline scaffold may produce a hydrophobic interaction with the shallow surface, and the prolyl pocket of Pin1 is able to be occupied by the present quinazoline fragment. In this work, the chemical synthesis of these new quinazoline derivatives is described in details. The inhibitory activities on Pin1 of these synthetic compounds are presented along with their SAR analysis as follows.

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2. Chemistry

The synthesis of a variety of 2,4-disubstituted quinazolines (**6a–6h**, **10a–10c** and **13a–13h**) was depicted in Schemes 1–3. Target compounds **6a–6h** were achieved in five steps using *o*-aminobenzoic acid derivatives as starting materials as shown in Scheme 1. Starting from 2-aminobenzoic acid or 5-nitro-2-aminobenzoic acid, the desired compounds **2a** and **2b** were obtained, respectively, (**2a**: 80% yield; **2b**: 65% yield) upon treatment with urea under microwave irradiation. Chlorination of compounds **2a** and **2b** was accomplished in 48% yield by employing $POCl_3$ or the mixture of $POCl_3$ and PCl_5. In the presence of DIEA, the regioselective substitution reaction at 4-position of compounds **3a** or **3b** was performed with an array of primary amines, thus furnishing compounds **4a–4f** in 63–96% yields. The subsequent substitution reaction of compounds **4a–4f** with several amines was carried out under the different reaction conditions. Under catalysis of concentrated HCl(aq), the reaction of compounds **4a–4f** with 4-phenoxyphenylamine or methyl 4-aminobenzate underwent readily, thus furnishing the desired products **5a**, **5c**, **5d**, **5g** and **5h** in moderate to high yields. In contrast, in the absence of any catalyst the amination reaction of **4c** and **4d** with 3,5-bis(trifluoromethyl)benzylamine proceeded smoothly to give compounds **5e** and **5f** in 75% and 67% yields, respectively. The conversion of compound **5a** to **5b** was successfully completed in quantitative yield under catalytic hydrogenation in hexafluoroisopropanol (HFIP) at room temperature. Upon treatment with basic reaction conditions, compounds **5a–5h** were hydrolyzed into the corresponding carboxylic acid derivatives **6a–6h** in a moderate to excellent yield.

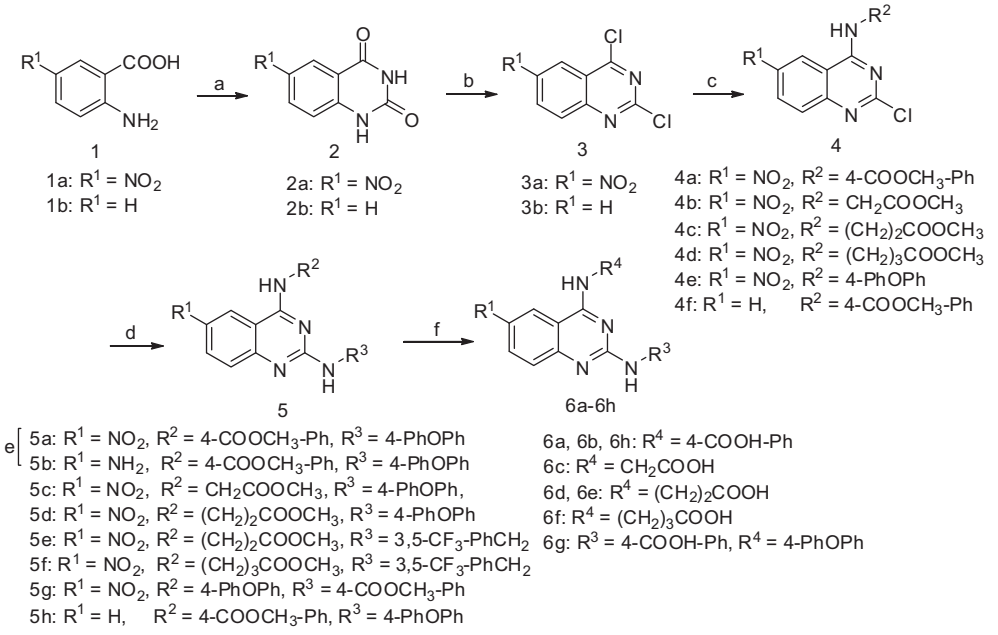
The synthesis of the phosphoric acid derivatives **10a–10c** was outlined in Scheme 2. The key intermediate **7** was obtained in 97% yield via the reaction of compound **3a** with 2-aminoethanol in the presence of hünig's base. The access to compounds **8a** and **8c** was achieved in 99% and 80% yields, respectively, via the reaction of compound **7** with 4-phenoxyphenylamine or 3-fluorophenylamine under catalysis of concentrated hydrochloric acid. Compound **8b** was easily accessed via the reaction of compound **7** with an excessive amount of benzylamine in isopropanol. In the presence of 1*H*-tetrazole and hydrogen peroxide, compounds **8a–8c** were reacted with dibenzyl diisopropylphosphoramidite to offer the phosphorylated products **9a–9c** in 74–90% yields, respectively. The target compounds **10a–10c** were provided in high yields when compounds **9a–9c** were deprotected with TFA.

Compounds **13a–13h** featuring a urea motif were synthesized according to Scheme 3. Under microwave irradiation, compounds **11a** and **11b** were obtained in 98% and 69% yields by the reaction of **4a** and **4c** with saturated NH_3 in ethanol, respectively. Compounds **11a** and **11b** were converted into the desired products **12a–12h** in moderate to good yields upon treatment with a variety of isocyanate derivatives under microwave irradiation. Finally, under basic condition the hydrolysis of compounds **12a–12h** furnished the corresponding target compounds **13a–13h**.

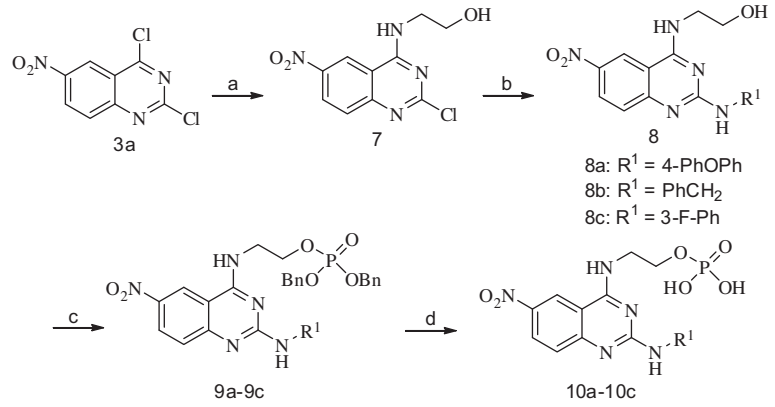
3. Biological results and discussion

The inhibitory activities on Pin1 of all target compounds (**6a–6h**, **10a–10c** and **13a–13h**) were evaluated by a protease-coupled enzyme assay with Suc-Ala-Glu-Pro-Phe-pNA as the substrate.[17,18] The corresponding results are expressed as IC_{50} values and presented in Tables 1–3.

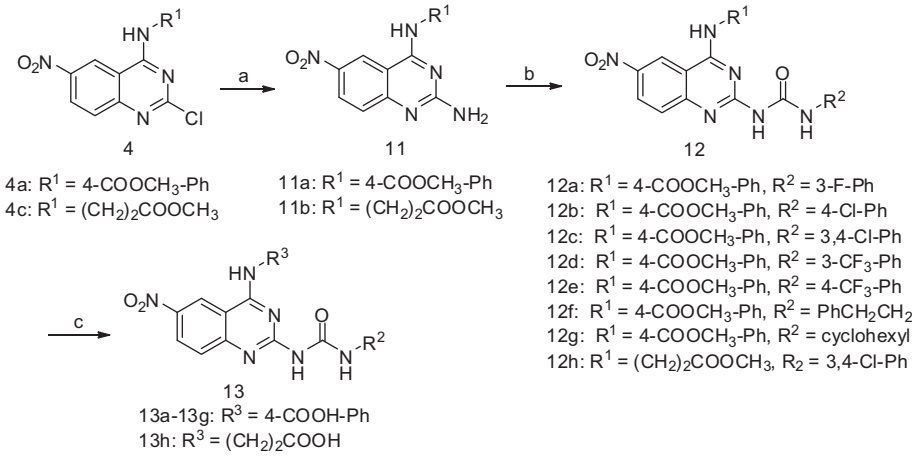
As shown in Table 1, the SAR was investigated initially on the series of compounds **6a–6h**. Compound **6a** with an aminobenzoic acid as R^4 group was found to have an IC_{50} value of 4.87 μM on the enzymatic inhibition. When the aminobenzoate substituent in compound **6a** was replaced by a carboxylmethylene (**6c**, IC_{50} 16.4 μM) or carboxylethylene (**6d**, IC_{50} 31.4 μM), the inhibitory activities were decreased to about 1/3 or 1/6, presumably due to the more flexible alkyl linker in compounds **6c** and **6d** resulting in additional entropy penalty. In comparison with compound **6d**, the inhibitory potency of compound **6e** (IC_{50} 16.4 μM) was increased to about twofold by changing the 4-phenoxyphenyl to 3,5-bis(trifluoromethyl)benzyl group. The potency of compound **6f** (IC_{50} 33.2 μM) is about half of that of compound **6e** when an additional CH_2 was introduced into the R^4 group. Based on the above the results, it is reasonable to hypothesize that the rigid linker can make a beneficial contribution to the binding affinity. In



Scheme 1. Reagents and conditions: (a) urea, microwave; (b) R^1 = H, $POCl_3$, *N*,*N*-dimethylaniline, reflux; R^1 = NO_2, $POCl_3/PCl_5$, reflux; (c) R^2NH_2, DIEA, *i*-PrOH, rt; (d) R^3 = aromatic amine, concd HCl(aq), acetone, reflux; R^3 = 3,5-CF_3-$PhCH_2$, *i*-PrOH, reflux/microwave; (e) Pd/C, HFIP, rt; (f) LiOH or KOH, THF/CH_3OH/H_2O, reflux.

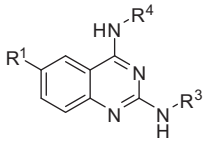


Scheme 2. Reagents and conditions: (a) 2-aminoethanol, DIEA, *i*-PrOH, rt; (b) R^1 = 4-phenoxylphenylamine or 3-fluorophenylamine, concd HCl, acetone/water, reflux; R^1 = benzyl amine, *i*-PrOH, reflux; (c) dibenzyl diisopropylphosphoramidite, 1*H*-tetrazole, H_2O_2, DMF/DCM, rt; (d) TFA, H_2O, rt.



Scheme 3. Reagents and conditions: (a) NH_3/ethanol, microwave; (b) R_2NCO, dioxane, microwave; (c) KOH, methanol/water, reflux.

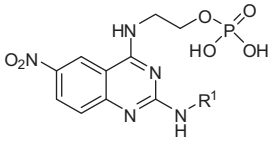
Table 1
The chemical structures and inhibitory activities on hPin1 of compounds **6a**–**6h**



Compound	R^1	R^3	R^4	IC_{50}[a] (μM)
6a	NO_2	4-PhOPh	4-COOH-Ph	4.87
6b	NH_2	4-PhOPh	4-COOH-Ph	23.05
6c	NO_2	4-PhOPh	CH_2COOH	16.4
6d	NO_2	4-PhOPh	$(CH_2)_2COOH$	31.4
6e	NO_2	3,5-CF_3-$PhCH_2$	$(CH_2)_2COOH$	16.4
6f	NO_2	3,5-CF_3-$PhCH_2$	$(CH_2)_3COOH$	33.2
6g	NO_2	4-COOH-Ph	4-PhOPh	9.46
6h	H	4-PhOPh	4-COOH-Ph	9.81

[a] Compound dose (μM) required to inhibit the Pin1 activity by 50%.

Table 2
The chemical structures and inhibitory activities on hPin1 of compounds **10a**–**10c**



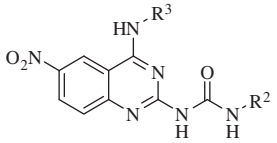
Compound	R^1	IC_{50}[a] (μM)
10a	4-PhOPh	9.75
10b	$PhCH_2$	46.5
10c	3-F-Ph	NA

[a] Compound dose (μM) required to inhibit the Pin1 activity by 50%.

addition, the effect of the substituents at C-6 position of quinazolines on the inhibition was investigated preliminarily. The inhibitory activity of compound **6b** with an amino substituent (IC_{50} = 23.05 μM) was decreased to about 1/5 in comparison with that of compound **6a**. Compound **6h** is devoid of a nitro group, and exhibited an IC_{50} of 9.81 μM. Therefore, we rationalized that the installment of hydrogen acceptor rather than hydrogen donor is advantageous to the interaction between the synthesized quinazoline analogues and Pin1. By comparison with compound **6a**, the inhibitory activity of compound **6g**, which has 4-carboxylphenyl as R^3 and 4-phenoxylphenyl as R^4 group, has shown an IC_{50} of 9.46 μM.

It has been disclosed that the charge–charge interactions between phosphate inhibitors and the active site is essential for Pin1 inhibition.[12,13] Since the initial modification of quinazoline scaffold did not bring about a strong Pin1 inhibitor, we attempted to integrate a phosphate into the quinazoline skeleton, thus providing compounds **10a**–**10c**. Indeed, compound **10a** (IC_{50} 9.75 μM) was a more potent Pin1 inhibitor than the corresponding carboxylate counterpart **6d** (IC_{50} 31.4 μM). However, the incorpo-

Table 3
The chemical structures and inhibitory activities on hPin1 of compounds **13a–13h**



Compound	R^2	R^3	IC_{50}[a] (μM)
13a	3-F-Ph	4-COOH-Ph	13.4
13b	4-Cl-Ph	4-COOH-Ph	9.0
13c	3,4-Cl-Ph	4-COOH-Ph	2.90
13d	3-CF_3-Ph	4-COOH-Ph	8.50
13e	4-CF_3-Ph	4-COOH-Ph	5.60
13f	$PhCH_2CH_2$	4-COOH-Ph	52.5
13g	Cyclohexyl	4-COOH-Ph	54.2
13h	3,4-Cl-Ph	$(CH_2)_2COOH$	>100

[a] Compound dose (μM) required to inhibit the Pin1 activity by 50%.

ration of phosphate into the quinazoline skeleton did not give rise to a significant enhance in potency as anticipated, presumably due to the longer linker between the phosphate and the quinazoline motif. Compound **10b** (IC_{50} 46.5 μM) was about 1/5 as active as compound **10a**, suggesting that the bulky hydrophobic group at 2-position of quinazolines would positively contribute to the binding.

After some modification on 4- and 6-substituents, 4-carboxylphenylamino and 6-nitro group were kept intact and a variety of hydrophobic moieties were introduced into 2-position of quinazoline via a urea linker, as shown in Table 3. All compounds **13a–13g** have shown the binding affinity to Pin1 with an IC_{50} value ranging from 2.9 to 54.2 μM. Compound **13c** exhibited the most potent inhibitory activity with an IC_{50} value of 2.9 μM. It has been demonstrated that aromatic hydrophobic substituents are more favorable for the binding than the alkyl hydrophobic groups since compounds **13a–13e** (IC_{50} 2.9–13.4 μM) with substituted phenyl moieties always presented higher inhibitory activity than that of 2-alkyl substituted quinazolines (compound **13f**: IC_{50} 52.5 μM and **13g**: IC_{50} 54.2 μM). The inhibitory activity was increased in the order of **13c** > **13e** > **13b** > **13a** further proved that the bulky hydrophobic fragments on 2-substituents are favorable for the interactions. Surprisingly, replacement of 4-carboxylphenylamino group in compound **13c** with carboxylethylene (compound **13h**, IC_{50} >100 μM) resulted in a loss of activity completely.

In order to get some insight for further structure-based modification of quinazolines Pin1 inhibitor, the binding mode was investigated using FlexX algorithm implemented in SYBYL 7.2.[19] The coordinates of X-ray co-crystal structure of a carboxylate inhibitor (reference molecule) with Pin1 (pdb code: 3JYJ) reported by Pfizer in 2010[13] was employed for docking the synthesized compounds. The binding modes of most of the evaluated target compounds are somewhat similar to that of the reference molecule and exemplified by the representative compound **6a** as shown in Figure 1. The carboxylate on 4-substituents anchored with the positive charged amino side chain of Arg69 and Lys63 via the crucial charge–charge interaction, the other two hydrophobic moieties are adjustable to fit into the hydrophobic pockets. The quinazoline scaffold was positioned in the hydrophobic prolyl pocket and the 6-nitro group formed hydrogen bonds with OH on the side chain of Ser154 and NH on both side chain and amide bond of GLN131. Nevertheless, in comparison with the disposition of the benzene ring of reference molecule, the quinazoline scaffold orientated slightly outward. The 2-substituents on quinazoline extended to the shallow hydrophobic region. However, the orientation of this hydrophobic contact is somewhat promiscuous within the whole series. Based on the analysis of the predicted binding mode and SAR studies of the titled compounds, we envisioned that disconnection of quinazoline scaffold might facilitate the phenyl fragment to extend into the prolyl pocket and therefore enhancing the hydrophobic contacts. In addition, incorporation of a hydrogen bond donor fragment on the appropriate location of the benzene ring might assist in the binding orientation and binding affinity via the hydrogen bond interactions with the side chain of GLN131 and Ser154.

4. Conclusion

In summary, a series of novel quinazoline-based chemical entities were synthesized as potent Pin1 inhibitors with IC_{50} value at micromolar level. The SAR and binding mode of the titled compounds were explored preliminarily, and that will shed light for the discovery of more potent novel small molecule Pin1 inhibitors.

5. Experimetal

5.1. Chemistry

5.1.1. General

Melting points were measured on a Yanaco micro melting point apparatus and are uncorrected. ^{1}H NMR (300 MHz) on a Varian Mercury 300 spectrometer was recorded in DMSO-d_6 or $CDCl_3$. Chemical shifts are reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). High resolution mass spectra (HRMS) were obtained on an Agilent Technologies LC/MSD TOF spectrometer. All chemicals and solvents used were of reagent grade without purified or dried before use. All the reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel G plates at 254 nm under a UV lamp. Column chromatography separations were performed with silica gel (200–300 mesh).

5.1.2. 4-(6-Nitro-2-(4-phenoxyphenylamino)quinazolin-4-ylamino)benzoic acid (6a)

5.1.2.1. 6-Nitroquinazoline-2,4-(1*H*, 3*H*)-dione (2a). The mixture of 2-amino-5-nitrobenzoic acid (10.0 g, 0.055 mol) and urea (32.2 g, 0.54 mol) was stirred at 150 °C for 10 h. The reaction mixture was cooled to 100 °C and then water (50 mL) was added to quench the reaction. The crude product was obtained by filtration, and then washed with water (50 mL × 3). After dried under vacuum condition, compound **2a** was obtained as yellow solid (9.1 g, 80%); mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 7.24 (d, J = 9.0 Hz, 1H, ArH), 8.36 (dd, J_1 = 9.0 Hz, J_2 = 2.7 Hz, 1H, ArH), 8.55 (d, J = 2.7 Hz, 1H, ArH).

5.1.2.2. 2,4-Dichloro-6-nitroquinazoline (3a). The reaction mixture of compound **2a** (0.78 g, 3.78 mmol), PCl_5 (4.11 g, 19.7 mmol) and $POCl_3$ (16 mL) was stirred at reflux for 6.5 h. The excess $POCl_3$ was removed by evaporation. The residue was dissolved in ice water, and then the solution pH was adjusted to pH 5–6 with saturated $NaHCO_3$. The water phase was extracted with EtOAc (60 mL × 5) and the organic layer was dried over anhydrous Na_2SO_4, concentrated to give the crude product which was purified by column chromatography on silica gel (petroleum ether/EtOAc = 40:1) to afford compound **3a** as white solid (0.58 g, 63%); mp 122–124 °C; ^{1}H NMR ($CDCl_3$) δ: 8.18 (d, J = 9.0 Hz, 1H), 8.76 (dd, J_1 = 9.3 Hz, J_2 = 2.1 Hz, 1H), 9.18 (d, J = 1.8 Hz, 1H).

5.1.2.3. Methyl 4-(2-chloro-6-nitroquinazolin-4-ylamino)benzoate (4a). To a stirred solution of compound **3a** (0.50 g, 2.05 mmol) in *i*-PrOH (8 mL) and CH_2Cl_2 (1.5 mL) was added methyl 4-aminobenzoate (0.34 g, 2.25 mmol) and DIEA (0.42 g,

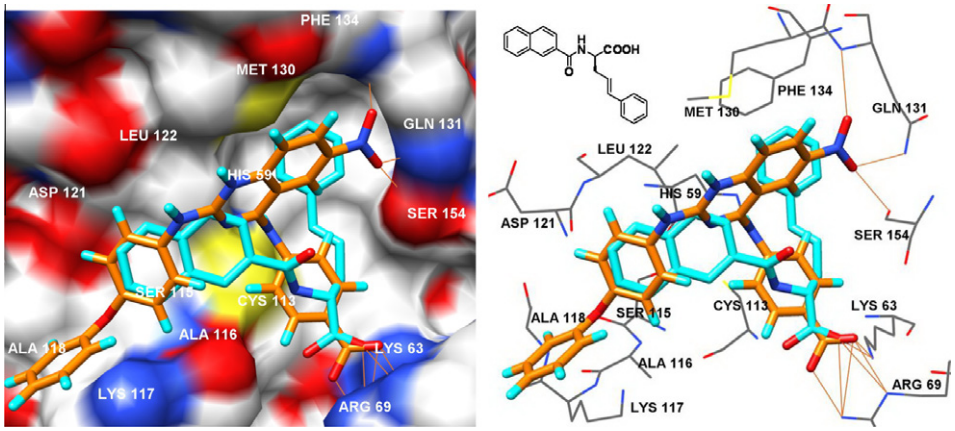


Figure 1. FlexX-modeled binding mode of compound **6a** (carbon atoms colored orange) in comparison with the crystal structure (3JYJ in PDB)[13] of a carboxylate Pin1 inhibitor (carbon atoms colored cyan). H-Bonding interactions are presented with yellow line. The chemical structure of reference molecule is presented. Molecular image was generated with UCSF Chimera.[20]

3.28 mmol). The reaction mixture was stirred at room temperature for 5 h. After filtration, compound **4a** was obtained as yellow solid (0.68 g, 93%); mp 279–282 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.98 (br s, 1H, NH), 9.71(d, J = 2.4 Hz, 1H, ArH), 8.60 (dd, J_1 = 9.3 Hz, J_2 = 2.4 Hz, 1H, ArH), 8.06 (d, J = 9.0 Hz, 2H, ArH), 7.99 (d, J = 9.0 Hz, 2H, ArH), 7.91 (d, J = 9.0 Hz, 1H, ArH), 3.86 (s, 3H, OCH_3).

5.1.2.4. Methyl 4-(6-nitro-2-(4-phenoxyphenylamino)quinazolin-4-ylamino)benzoate (5a). To a stirred suspension of compound **4a** (0.46 g, 1.27 mmol) in acetone (1.5 mL) and water (2.5 mL) was added 4-phenoxyphenylamine (0.13 g, 2.06 mmol) and co-NMR (DMSO-d_6) δ: 10.98 (s, 1H, NH), concd HCl (24 drops). The reaction mixture was refluxed for 10 h and then cooled to room temperature. The crude product was obtained by filtration and recrystalized with ethanol. Compound **5a** was obtained as yellow solid (0.56 g, 87%); mp 234–236 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.32 (br s, 1H, NH), 10.68 (br s, 1H, NH), 9.63 (s, 1H, ArH), 8.55 (d, J = 7.8 Hz, 1H, ArH), 7.55–8.01 (m, 7H, ArH), 7.40 (t, J = 8.4 Hz, 2H, ArH), 7.16 (t, J = 7.5 Hz, 1H, ArH), 7.00–7.04 (m, 4H, ArH), 3.82 (s, 3H, OCH_3); HRMS (ESI): m/z, calcd for $C_{28}H_{22}N_5O_5$ [M+H$^+$]: 508.1615, found 508.1621.

5.1.2.5. 4-(6-Nitro-2-(4-phenoxyphenylamino)quinazolin-4-ylamino)benzoic acid (6a). The reaction mixture of compound **5a** (0.15 g, 1.0 mmol) and LiOH·H_2O (0.11 g, 9.0 mmol) in water (12 mL) and THF (4.0 mL) was refluxed for 6.5 h. The solvent was removed by evaporation and the residue was dissolved in water (10 mL), which was cooled in ice-water bath and adjusted to pH 1.5 with diluted HCl. The resulting mixture was stirred for 5 h. After filtration, the title compound **6a** was obtained as brick red solid (0.13 g, 92%); mp >300 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.36 (br s, 1H, NH), 9.75 (s, 1H, NH), 9.54 (d, J = 2.1 Hz, 1H, ArH), 8.37 (dd, J_1 = 9.0 Hz, J_2 = 1.8 Hz, 1H, ArH), 7.94 (m, 6H, ArH), 7.52 (d, J = 9.3 Hz, 1H, ArH), 7.37 (t, J = 7.8 Hz, 2H, ArH), 7.08 (t, J = 7.2 Hz, 1H, ArH), 7.10 (d, J = 7.8 Hz, 4H, ArH); HRMS (ESI): m/z, calcd for $C_{27}H_{20}N_5O_5$ [M+H$^+$]: 494.1459, found 494.1457.

5.1.3. 4-(6-Amino-2-(4-phenoxyphenylamino)quinazolin-4-ylamino)benzoic acid (6b)

5.1.3.1. Methyl 4-(6-amino-2-(4-phenoxyphenylamino)quinazolin-4-ylamino)benzoate (5b). The mixture of compound **5a** (0.35 g, 0.69 mmol) and 10% Pd/C (0.14 g) in HFIP (15 mL) was hydrogenated at room temperature and 1 atm for 3.5 h. Pd/C was filtered off and the filtrate was concentrated to give compound **5b** as yellow solid (0.33 g, 100%); mp 232–234 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.73 (br s, 1H, NH), 10.11 (br s, 1H, NH), 7.85–7.93 (m, 4H, ArH), 7.57 (s, 1H, ArH), 7.37–7.47 (m, 5H, ArH), 7.27 (d, J = 8.7 Hz, 1H, ArH), 7.16 (t, J = 7.2 Hz, 1H, ArH), 7.01 (d, J = 8.4 Hz, 4H, ArH), 3.81 (s, 3H, OCH_3); HRMS (ESI): m/z, calcd for $C_{28}H_{24}N_5O_3$ [M+H$^+$]: 478.1874; found 478.1874.

5.1.3.2. 4-(6-Amino-2-(4-phenoxyphenylamino)quinazolin-4-ylamino)benzoic acid (6b). The reaction mixture of compound **5b** (0.072 g, 0.15 mmol) and LiOH·H_2O (0.063 g, 1.5 mmol) in water (6.0 mL) and THF (3.0 mL) was refluxed for 4.5 h. The solvent was removed by evaporation and the residue was dissolved in water (5.0 mL), which was cooled in ice-water bath and adjusted to pH 2.0 with diluted HCl solution. The resulting mixture stood for overnight. After filtration, the title compound **6b** was obtained as brick red solid (0.06 g, 85%); mp 208–210 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.15 (br s, 1H, NH), 9.56 (br s, 1H, NH), 7.97 (d, J = 8.7 Hz, 2H, ArH), 7.91 (d, J = 8.7 Hz, 2H, ArH), 7.65 (d, J = 7.8 Hz, 2H, ArH), 7.46 (s, 1H, ArH), 7.38 (t, J = 6.3 Hz, 3H, ArH), 7.21 (d, J = 8.7 Hz, 1H, ArH), 7.11 (t, J = 7.5 Hz, 1H, ArH), 6.97–7.01 (m, 4H, ArH); HRMS (ESI): m/z, calcd for $C_{27}H_{22}N_5O_3$ [M+H$^+$]: 464.1717, found 464.1712.

5.1.4. 2-(6-Nitro-2-(4-phenoxyphenylamino)quinazolin-4-ylamino) acetic acid (6c)

5.1.4.1. Methyl 2-(2-chloro-6-nitroquinazolin-4-ylamino) acetate (4b). To a suspension of 2,4-dichloro-6-nitroquinazoline **3a** (0.1 g, 0.41 mmol) in *i*-PrOH (3 mL) was added glycine methyl ester hydrochloride (0.061 g, 0.49 mmol) and DIEA (0.11 g, 0.82 mol). The resulting mixture was stirred at room temperature for 0.5 h and filtered to provide product **4b** as light yellow solid (0.077 g, 63%); mp 161–162 °C; ^{1}H NMR: ($CDCl_3$) δ (ppm): 8.81 (s, 1H, ArH), 8.50 (dd, J_1 = 9.3 Hz, J_2 = 2.4 Hz, 1H, ArH), 7.82 (d, J = 9.0 Hz, 1H, ArH), 4.49 (d, J = 4.8 Hz, 2H, CH_2), 3.91 (s, 3H, OCH_3); HRMS (ESI): m/z, calcd for $C_{11}H_{10}ClN_4O_4$ [M+H$^+$]: 297.0385, found 297.0392.

5.1.4.2. Methyl 2-(6-nitro-2-(4-phenoxyphenylamino)quinazolin-4-ylamino)acetate (5c). Following the preparation protocol of Section 5.1.2.4, the reaction mixture of compound **4b** (0.1 g, 0.34 mmol), 4-phenoxyphenylamine (0.10 g, 0.55 mmol) and concd HCl (24 drops) in acetone (4 mL) and water (5 mL) was refluxed for 3 h to give compound **5c** as brow solid (0.09 g, 61%); mp 203–205 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 9.36 (s, 1H, ArH), 8.55 (d, J = 9.3 Hz, 1H, ArH), 7.71 (d, J = 9.3 Hz, 1H, ArH), 7.56 (d, J = 6.6 Hz, 2H, ArH), 7.41 (t, J = 7.5 Hz, 2H, ArH), 7.15 (t, J = 7.5 Hz,

1H, ArH), 7.02–7.05 (m, 4H, ArH), 4.31 (d, J = 4.8 Hz, 2H, CH_2), 3.61 (s, 3H, OCH_3); HRMS (ESI): m/z, calcd for $C_{23}H_{20}N_5O_5$ [M+H$^+$]: 446.1459, found 446.1451.

5.1.4.3. 2-(6-Nitro-2-(4-phenoxyphenylamino)quinazolin-4-ylamino)acetic acid (6c). The reaction mixture of compound **5c** (0.05 g, 0.11 mmol) and NaOH (0.045 g, 1.12 mmol) in water (4 mL) and THF (2 mL) was stirred at room temperature for 2 h, compound **6c** was obtained as brow solid (0.043 g, 88%); mp >300 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 12.08 (br s, 1H, COOH), 9.69 (br s, 1H, NH), 9.19 (d, J = 2.4 Hz, 1H, ArH), 8.33 (dd, J_1 = 9.3 Hz, J_2 = 2.4 Hz, 1H, ArH), 7.80 (m, 2H, ArH), 7.45 (d, J = 9.0 Hz, 1H, ArH), 7.36 (t, J = 8.1 Hz, 2H, ArH), 7.09 (t, J = 7.5 Hz, 1H, ArH), 6.96 (d, J = 8.4 Hz, 4H, ArH), 4.21 (m, 2H, NCH_2); HRMS (ESI): m/z, calcd for $C_{22}H_{18}N_5O_5$ [M+H$^+$]: 432.1302, found 432.1292.

5.1.5. 3-(6-Nitro-2-(4-phenoxyphenylamino)quinazolin-4-ylamino)propanoic acid (6d)

5.1.5.1. Methyl 2-(2-chloro-6-nitroquinazolin-4-ylamino) propanoate (4c). To a suspension of 2,4-dichloro-6-nitroquinazoline **3a** (0.4 g, 1.64 mmol) in *i*-PrOH (10 mL) was added methyl 3-amino propanoate hydrochloride (0.27 g, 1.97 mmol) and DIEA (0.42 g, 3.28 mmol). The resulting mixture was stirred at room temperature for 0.5 h and filtered to provide compound **4c** as light yellow solid (0.48 g, 93%); mp 178–179 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 8.72 (d, J = 2.1 Hz, 1H, ArH), 8.50 (dd, J_1 = 9.0 Hz, J_2 = 2.4 Hz, 1H, ArH), 7.86 (d, J = 9.6 Hz, 1H, ArH), 4.02 (q, J = 5.4 Hz, 2H, CH_2), 3.76 (s, 3H, CH_3), 2.80 (t, J = 5.7 Hz, 2H, CH_2); HRMS (ESI): m/z, calcd for $C_{12}H_{12}ClN_4O_4$ [M+H$^+$]: 311.0542, found 311.0549.

5.1.5.2. Methyl 2-(6-nitro-2-(4-phenoxyphenylamino)quinazolin-4-ylamino)propanoate (5d). Following the preparation protocol of Section 5.1.2.4, the reaction mixture of compound **4c** (0.05 g, 0.17 mmol), 4-phenoxyphenylamine (0.51 g, 0.27 mmol) and concd HCl (4 drops) in acetone (2 mL) and water (3 mL) was refluxed for 2 h to give compound **5d** as yellow solid (0.07 g, 92%); mp 185–187 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.64 (br s, 1H, NH), 10.08 (br s, 1H, NH), 9.32 (d, J = 1.8 Hz, 1H, ArH), 8.53 (d, J = 8.7 Hz, 1H, ArH), 7.70 (d, J = 9.3 Hz, 1H, ArH), 7.63 (d, J = 9.0 Hz, 2H, ArH), 7.40 (t, J = 8.1 Hz, 2H, ArH), 7.17 (t, J = 7.5 Hz, 1H, ArH), 7.03–7.06 (m, 4H, ArH), 3.77 (m, 2H, CH_2), 3.58 (s, 3H, OCH_3), 2.78 (t, J = 6.9 Hz, 2H, CH_2); HRMS (ESI): m/z, calcd for $C_{24}H_{22}N_5O_5$ [M+H$^+$]: 460.1615, found 460.1614.

5.1.5.3. 3-(6-Nitro-2-(4-phenoxyphenylamino)quinazolin-4-ylamino)propanoic acid (6d). The reaction mixture of compound **5d** (0.2 g, 0.44 mmol) and NaOH (0.17 g, 4.34 mmol) in water (10 mL) and THF (8 mL) was stirred at room temperature for 30 min, compound **6d** was obtained as red solid (0.18 g, 96%); mp 266–268 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 12.27 (br s, 1H, COOH), 9.76 (br s, 1H, NH), 9.18 (d, J = 1.8 Hz, 1H, ArH), 8.94 (br s, 1H, NH), 8.32 (dd, J_1 = 9.6 Hz, J_2 = 2.1 Hz, 1H, ArH), 7.87 (d, J = 8.1 Hz, 2H, ArH), 7.44 (d, J = 9.3 Hz, 1H, ArH), 7.40 (t, J = 8.1 Hz, 2H, ArH), 7.36 (t, J = 8.4 Hz, 2H, ArH), 7.08 (t, J = 7.2 Hz, 1H, ArH), 6.95–7.00 (m, 4H, ArH), 3.74 (m, 2H, CH_2), 2.70 (t, J = 6.9 Hz, 2H, CH_2); HRMS (ESI): m/z, calcd for $C_{23}H_{20}N_5O_5$ [M+H$^+$]: 446.1459, found 446.1465.

5.1.6. 3-(2-(3,5-Bis(trifluoromethyl)benzylamino)-6-nitroquinazolin-4-ylamino)propanoic acid (6e)

5.1.6.1. Methyl 3-(2-(3,5-bis(trifluoromethyl)benzylamino)-6-nitroquinazolin-4-ylamino) propanoate (5e). The reaction mixture of compound **4c** (0.2 g, 0.64 mmol) and 3,5-bis(trifluoromethyl)benzylamine (0.78 g, 3.22 mmol) in isopropanol (4 mL) was heated by microwave (power 50 W, temperature 110 °C) for 10 min. The mixture was cooled to room temperature and the crude product was precipitated. After filtration and recrystallization with ethanol, compound **5e** was obtained as yellow crystals (0.25 g, 75%); mp 186–187 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 9.06 (br s, 1H, NH), 8.82 (br s, 0.5H, NH), 8.61 (br s, 0.5H, NH), 7.95–8.24 (m, 5H, ArH), 7.25 (d, J = 9.3 Hz, 1H, ArH), 4.71 (d, J = 5.7 Hz, 2H, $ArCH_2$), 3.53–3.69 (m, 5H, OCH_3 and CH_2), 2.73 (t, J = 6.6 Hz, 2H, CH_2); HRMS (ESI): m/z, calcd for $C_{21}H_{18}F_6N_5O_4$ [M+H$^+$]: 518.1257, found 518.1255.

5.1.6.2. 3-(2-(3,5-Bis(trifluoromethyl)benzylamino)-6-nitroquinazolin-4-ylamino)propanoic acid (6e). The reaction mixture of compound **5e** (0.05 g, 0.10 mmol) and NaOH (0.04 g, 0.96 mmol) in water (4 mL) and THF (2 mL) was stirred at room temperature for 1.5 h, compound **6e** was obtained as off-white solid (0.04 g, 84%); mp >300 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 12.24 (br s, 1H, OH), 9.54 (br s, 1H, NH), 9.24 (s, 0.6H, ArH), 9.12 (s, 0.4H, ArH), 8.76 (br s, 0.4H, NH), 8.62 (br s, 0.6H, NH), 8.39 (d, J = 9.0 Hz, 0.6H, ArH), 8.27 (d, J = 9.3 Hz, 0.4H, ArH), 8.09 (s, 2H, ArH), 7.98 (s, 1H, ArH), 7.48 (d, J = 8.7 Hz, 0.6H, ArH), 7.38 (d, J = 8.4 Hz, 0.4H, ArH), 4.78 (br s, 2H, $ArCH_2$), 3.64 (m, 2H, CH_2), 2.67 (m, 1H, CH_2), 2.49 (m, 1H, CH_2); HRMS (ESI): m/z, calcd for $C_{20}H_{16}F_6N_5O_4$ [M+H$^+$]: 504.1101, found 504.1110.

5.1.7. 4-(2-(3,5-Bis(trifluoromethyl)benzylamino)-6-nitroquinazolin-4-ylamino)butanoic acid (6f)

5.1.7.1. Ethyl 2-(2-chloro-6-nitroquinazolin-4-ylamino)butanoate (4d). To a suspension of 2,4-dichloro-6-nitroquinazoline **3a** (0.1 g, 0.41 mmol) in *i*-PrOH (3 mL) was added ethyl 4-amino butanoate hydrochloride (0.054 g, 0.49 mmol) and DIEA (0.11 g, 0.82 mmol). The resulting mixture was stirred at room temperature for 0.5 h and filtered to provide product **4d** as light yellow solid (0.11 g, 84%); mp 149–150 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 8.87 (s, 1H, ArH), 8.66 (d, J = 9.3 Hz, 1H, ArH), 8.01 (br s, 1H, NH), 7.83 (d, J = 9.0 Hz, 1H, ArH), 4.25 (q, J = 7.2 Hz, 2H, CH_2), 3.73 (m, 2H, CH_2), 2.62 (m, 2H, CH_2), 2.12 (m, 2H, CH_2), 1.30 (t, J = 7.5 Hz, 3H, CH_3); HRMS (ESI): m/z, calcd for $C_{14}H_{16}ClN_4O_4$ [M+H$^+$]: 339.0854, found 339.0855.

5.1.7.2. Ethyl 4-(2-(3,5-bis(trifluoromethyl)benzylamino)-6-nitroquinazolin-4-ylamino) butanoate (5f). The reaction mixture of compound **4d** (0.3 g, 0.89 mmol) and 3,5-bis(trifluoromethyl)benzylamine (1.08 g, 4.43 mmol) in isopropanol (5 mL) was heated by microwave (power 200 W, temperature 110 °C) for 45 min. The mixture was cooled to room temperature and the crude product was precipitated. After filtration and recrystallization with ethanol, compound **5f** was obtained as yellow crystals (0.32 g, 67%); mp 172–173 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 9.11 (br s, 0.5H, NH), 9.07 (br s, 0.5H, NH), 8.73 (br s, 0.5H, NH), 8.55 (br s, 0.5H, NH), 8.22 (d, J = 9.6 Hz, 1H, ArH), 7.95–8.12 (m, 4H, ArH), 7.26 (d, J = 9.3 Hz, 1H, ArH), 4.71 (d, J = 5.4 Hz, 2H, $ArCH_2$), 3.95–4.02 (m, 2H, CH_2), 3.51 (m, 1H, CH), 3.41 (m, 1H, CH), 2.41 (m, 1H, CH), 2.19 (m, 1H, CH), 1.92 (m, 1H, CH), 1.69 (m, 1H, CH), 1.11–1.13 (m, 3H, CH_3); HRMS (ESI): m/z, calcd for $C_{23}H_{22}F_6N_5O_4$ [M+H$^+$]: 546.1570, found 546.1561.

5.1.7.3. 4-(2-(3,5-Bis(trifluoromethyl)benzylamino)-6-nitroquinazolin-4-ylamino)butanoic acid (6f). The reaction mixture of compound **5f** (0.05 g, 0.09 mmol) and NaOH (0.367 g, 0.92 mmol) in water (3 mL) and THF (2 mL) was refluxed for 1.5 h, compound **6f** was obtained as light yellow solid (0.033 g, 69%); mp 266–268 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.04 (s, 1H, NH), 9.29 (s, 1H, ArH), 8.99 (t, J = 4.8 Hz, 1H, NH), 8.52 (d, J = 9.0 Hz, 1H, ArH), 8.14 (s, 2H, ArH), 7.99 (s, 1H, ArH), 7.68 (d, J = 9.3 Hz, 1H, ArH), 4.96 (d, J = 4.8 Hz, 2H, CH_2), 3.54 (m, 2H, CH_2), 2.20 (t, J = 6.6 Hz,

2H, CH_2), 1.73 (q, J = 6.6 Hz, 2H, CH_2); HRMS (ESI): m/z, calcd for $C_{21}H_{18}F_6N_5O_4$ [M+H$^+$]: 518.1257, found 518.1267.

5.1.8. 4-(6-Nitro-4-(4-phenoxyphenylamino)quinazolin-2-ylamino)benzoic acid (6g)

5.1.8.1. 2-Chloro-6-nitro-N-(4-phenoxyphenyl)quinazolin-4-amine (4e). To a suspension of 2,4-dichloro-6-nitroquinazoline **3a** (0.50 g, 2.03 mmol) in *i*-PrOH (30 mL) was added 4-phenoxyphenylamine (0.45 g, 2.43 mmol) and DIEA (0.32 g, 2.48 mmol). The resulting mixture was stirred at room temperature for 8 h and concentrated under vacuum. The residue was dissolved in EtOAc, washed with saturated $NaHCO_3$ aqueous solution and brine and dried over anhydrous $MgSO_4$. The crude product obtained after concentration was purified with column chromatography to afford product **4e** as red solid (0.64 g, 80%); mp 222–223 °C; ^{1}H NMR (acetone-d_6) δ (ppm): 9.13 (s, 1H, NH), 8.79 (d, J = 2.4 Hz, 1H, NH), 8.44 (dd, J_1 = 9.3 Hz, J_2=2.7 Hz, 1H, ArH), 8.03 (d, J = 8.4 Hz, 2H, ArH), 7.67 (d, J = 9.3 Hz, 1H, ArH), 7.37 (t, J = 8.1 Hz, 2H, ArH), 7.10 (t, J = 7.2 Hz, 1H, ArH), 7.03 (t, J = 8.7 Hz, 4H, ArH).

5.1.8.2. Methyl 4-(6-nitro-4-(4-phenoxyphenylamino)quinazolin-2-ylamino)benzoate (5g). Following the preparation protocol of Section 5.1.2.4, the reaction mixture of compound **4e** (0.2 g, 0.51 mmol), methyl 4-aminobenzoate (0.12 g, 0.76 mmol) and concd HCl (8 drops) in acetone and water was refluxed for 6.5 h to give the crude product, which was recrystallized from ethanol to afford compound **5g** as yellow solid (0.19 g, 74%); mp 260–261 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.29 (br s, 1H, NH), 10.78 (br s, 1H, NH), 9.58 (d, J = 2.1 Hz, 1H, ArH), 8.53 (dd, J_1 = 9.0 Hz, J_2 = 2.1 Hz, 1H, ArH), 7.67–7.82 (m, 7H, ArH), 7.44 (t, J = 8.1 Hz, 2H, ArH), 7.19 (t, J = 7.5 Hz, 1H, ArH), 7.05–7.12 (m, 4H, ArH), 3.81 (s, 3H, OCH_3); HRMS (ESI): m/z, calcd for $C_{28}H_{22}N_5O_5$ [M+H$^+$]: 508.1615, found 508.1619.

5.1.8.3. 4-(6-Nitro-4-(4-phenoxyphenylamino)quinazolin-2-ylamino)benzoic acid (6g). The reaction mixture of compound **5g** (0.05 g, 0.1 mmol) and KOH (0.112 g, 2.0 mmol) in water (12 mL) and CH_3OH (25 mL) was refluxed for 11 h, compound **6g** was obtained as yellow solid (0.044 g, 90%); mp >300 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.39 (s, 1H, NH), 10.01 (s, 1H, NH), 9.50 (d, J = 1.8 Hz, 1H, ArH), 8.41 (dd, J_1 = 9.3 Hz, J_2 = 2.1 Hz, 1H, ArH), 7.78–7.93 (m, 6H, ArH), 7.58 (d, J = 9.0 Hz, 1H, ArH), 7.44 (t, J = 7.8 Hz, 2H, ArH), 7.07–7.18 (m, 5H, ArH); HRMS (ESI): m/z, calcd for $C_{27}H_{20}N_5O_5$ [M+H$^+$]: 494.1459, found 494.1462.

5.1.9. 4-(2-(4-Phenoxyphenylamino)quinazolin-4-ylamino)benzoic acid (6h)

5.1.9.1. Quinazoline-2,4-(1*H*,3*H*)-dione (2b). The mixture of 2-aminobenzoic acid (0.5 g, 3.65 m mol) and urea (2.19 g, 0.36 mol) was stirred at 150 °C for 6 h. The reaction mixture was cooled to 100 °C and then water (5 mL) was added to quench the reaction. The crude product was obtained by filtration, and then washed with water (3 mL × 3) and methanol (3 mL × 3) to give the crude product 0.41 g, which was dissolved in heated NaOH aqueous solution, then cooled to 0 °C and adjusted pH 6 with diluted HCl aqueous solution. After stirred 20 min, the mixture was filtered and compound **2b** was obtained as white solid (0.37 g, 65%), which was used directly without further purification.

5.1.9.2. 2,4-Dichloroquinazoline (3b). The reaction mixture of compound **2b** (0.50 g, 3.09 mmol), $POCl_3$ (4.3 mL) and *N,N*-dimethylaniline (1.6 mL) was stirred at reflux for 7 h. The excess $POCl_3$ was removed by evaporation. The residue was dissolved in EtOAc which was washed with cold diluted HCl aqueous solution in order to remove the *N,N*-dimethylaniline. The organic phase was adjusted to pH 5–6 with saturated $NaHCO_3$. The water phase was extracted with EtOAc and the organic layer was dried over anhydrous $MgSO_4$, concentrated to give the crude product which was purified by column chromatography on silica gel (petroleum ether/EtOAc = 50:1) to afford compound **3b**, which was recrystalized with methanol as yellow solid (0.49 g, 80%); mp 116–117 °C; HRMS (ESI): m/z, calcd for $C_8H_5Cl_2N_2$ [M+H$^+$]: 198.9829 found 198.9832.

5.1.9.3. Methyl 4-(2-chloroquinazolin-4-ylamino)benzoate (4f). To a stirred solution of compound **3b** (0.10 g, 0.50 mmol) in *i*-PrOH (2.5 mL) and CH_2Cl_2 (0.5 mL) was added methyl 4-aminobenzoate (0.08 g, 0.55 mmol) and DIEA (0.14 mL). The reaction mixture was stirred at room temperature for 45 h. After filtration, compound **4f** was obtained as white solid (0.105 g, 67%); mp 217–219 °C; ^{1}H NMR (acetone-d_6, δ ppm) δ 9.65 (s, 1H, NH), 8.49 (d, J = 8.1 Hz, 1H), 8.10 (d, J = 9.0 Hz, 2H, ArH), 8.04 (d, J = 9.0 Hz, 2H, ArH), 7.91 (t, J = 7.2 Hz, 1H, ArH), 7.77 (d, J = 8.1 HZ, 1H, ArH), 7.65 (t, J = 7.2 Hz, 1H, ArH), 3.88 (s, 3H, OCH_3).

5.1.9.4. Methyl 4-(2-(4-phenoxyphenylamino)quinazolin-4-ylamino)benzoate (5h). To a stirred suspension of compound **4f** (0.05 g, 0.16 mmol) in acetone (1.5 mL) and water (2.5 mL) was added 4-phenoxyphenylamine (0.047 g, 0.25 mmol) and concd HCl (3 drops). The reaction mixture was refluxed for 7.5 h and then cooled to room temperature. The crude product was obtained by filtration and washed with acetone. Compound **5h** was obtained as yellow solid (0.069 g, 94%); mp 216–218 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.18 (s, 1H, NH), 10.55 (s, 1H, NH), 8.73 (d, J = 8.4 Hz, 1H, ArH), 7.90–7.93 (m, 5H, ArH), 7.67 (d, J = 8.4 Hz, 1H, ArH), 7.55 (t, J = 7.5 Hz, 1H, ArH), 7.39–7.47 (m, 4H, ArH), 7.18 (t, J = 7.2 Hz, 1H, ArH), 7.02–7.05 (m, 4H, ArH), 3.82 (s, 3H, OCH_3); HRMS (ESI): m/z, calcd for $C_{28}H_{23}N_4O_3$ [M+H$^+$]: 463.1765, found 463.1764.

5.1.9.5. 4-(2-(4-Phenoxyphenylamino)quinazolin-4-ylamino)benzoic acid (6h). The reaction mixture of compound **5h** (0.10 g, 0.22 mmol) and $LiOH{\cdot}H_2O$ (0.082 g, 1.95 mmol) in water (4 mL), methanol (18 mL) and THF (2.0 mL) was refluxed for 2 h. The solvent was removed by evaporation and the residue was dissolved in water, which was cooled in ice-water bath and adjusted to pH 2.0 with diluted HCl. The resulting mixture was stirred for 2 h. After filtration, the title compound **6h** was obtained as yellow solid (0.095 g, 98%); mp 224–226 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.10 (br s, 1H, COOH), 11.14 (s, 1H, NH), 10.56 (s, 1H, ArH), 8.73 (d, J = 8.1 Hz, 1H, ArH), 7.88–7.99 (m, 5H, ArH), 7.67 (d, J = 8.4 Hz, 1H, ArH), 7.54 (t, J = 7.8 Hz, 1H, ArH), 7.43 (q, J = 9.3 Hz, 4H, ArH), 7.16 (t, J = 7.2 Hz, 1H, ArH), 7.00–7.05 (m, 4H, ArH); HRMS (ESI): m/z, calcd for $C_{27}H_{21}N_4O_3$ [M+H$^+$]: 449.1608, found: 449.1610.

5.1.10. 2-(6-Nitro-2-(4-phenoxyphenylamino)quinazolin-4-ylamino)ethyl dihydrogen phosphate (10a)

5.1.10.1. 2-(2-Chloro-6-nitroquinazolin-4-ylamino)ethanol (7). To a stirred solution of compound **3a** (1.12 g, 5.0 mmol) in *i*-PrOH (11 mL) was added 2-aminoethanol (0.37 g, 6.0 mmol) and DIEA (0.97 g, 7.5 mmol). The reaction mixture was stirred at room temperature for 7 h. After filtration, compound **7** was obtained as yellow solid (1.30 g, 97%); mp 155–156 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 12.22 (s, 1H, ArNH), 9.95 (s, 1H, ArNH), 9.28 (t, 1H, NH), 9.19 (d, J = 1.8 Hz, 1H, ArH), 8.30 (dd, J_1 = 9.0 Hz, J_2 = 1.8 Hz, 1H, , ArH), 8.04 (s, 1H, ArH), 7.68 (d, J = 9.3 Hz, 1H, ArH), 7.54 (s, 2H, ArH), 3.68 (m, 2H, NCH_2), 2.65 (t, J = 6.6 Hz, 2H, CH_2CO).

5.1.10.2. 2-(6-Nitro-2-(4-phenoxyphenylamino)quinazolin-4-ylamino)ethanol (8a). To a stirred suspension of compound **7** (0.29 g, 1.07 mmol) in acetone (10 mL) and water (4.0 mL) was

added 4-phenoxyphenylamine (0.32 g, 1.72 mmol) and concd HCl (0.64 g, 6.42 mmol). The reaction mixture was refluxed for 1 h and then cooled to room temperature. Compound **8a** was obtained as yellow solid (0.44 g, 99%); mp 226–228 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.67 (br s, 1H, NH), 10.12 (br s, 1H, NH), 9.41 (d, J = 2.1 Hz, 1H, Ar), 8.53 (dd, J_1 = 9.0 Hz, J_2 = 1.8 Hz, 1H, ArH), 7.70 (d, J = 9.3 Hz, 1H, ArH), 7.62 (d, J = 8.4 Hz, 2H, ArH), 7.40 (t, J = 7.5 Hz, 2H, ArH), 7.15 (t, J = 7.2 Hz, 1H, Ar), 7.05 (m, 4H, ArH), 3.65 (m, 4H, CH_2CH_2); HRMS (ESI): m/z, calcd for $C_{22}H_{20}N_5O_4$ [M+H$^+$]: 418.1510, found 418.1514.

5.1.10.3. Dibenzyl 2-(6-nitro-2-(4-phenoxyphenylamino)quinazolin-4-ylamino)ethyl phosphate (9a). To a suspension of compound **8a** (0.104 g, 0.25 mmol) in dried DCM (3 mL) and DMF (0.5 mL) was added dibenzyl diisopropylphosphoramidite (0.13 g, 0.38 mmol) and 1H-tetrazole (0.028 g, 0.4 mmol), respectively. The resulting reaction mixture was stirred at room temperature for 1 h, and then 30% H_2O_2 (0.099 g, 0.88 mmol) was added. The reaction solution was stirred for another 35 min and then diluted with EtOAc (60 mL). The organic mixture was washed water (60 mL × 5) and saturated $NaHCO_3$ aqueous solution. The water phase was extracted with EtOAc (60 mL × 4), and the combined organic phase was dried over anhydrous Na_2SO_4, concentrated to give the crude product which was purified by column chromatography on silica gel (DCM/methanol = 100:1) to afford compound **9a**, which was recrystalized with petroleum ether and DCM to give the orange solid (0.15 g, 90%); mp 128–129 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 8.94 (s, 1H, ArH), 8.40 (d, J = 9.3 Hz, 1H, ArH), 8.17 (t, 1H, NH), 8.06 (d, J = 7.2 Hz, 1H, ArH), 7.64–7.68 (m, 3H, ArH), 7.50 (d, J = 8.1 Hz, 1H, ArH), 7.27–7.41 (m, 10H, ArH), 7.11 (t, J = 7.2 Hz, 1H, ArH), 7.02 (d, J = 8.7 Hz, 4H, ArH), 5.08 (d, J = 9.0 Hz, 2H, $ArCH_2$), 5.06 (d, J = 9.6 Hz, 2H, $ArCH_2$), 4.26 (m, 2H, CH_2), 3.80 (m, 2H, CH_2); HRMS (ESI): m/z, calcd for $C_{36}H_{33}N_5O_7P$ [M+H$^+$]: 678.2112, found 678.2118.

5.1.10.4. 2-(6-Nitro-2-(4-phenoxyphenylamino)quinazolin-4-ylamino)ethyl dihydrogen phosphate (10a). The reaction mixture of compound **9a** (0.24 g, 0.35 mmol), TFA (3.79 g, 33. 25 mmol) and water (0.032 g, 1.75 mmol) was stirred at room temperature for 4.5 h. The excess TFA was removed by evaporation, the residue was treated with EtOAc and the yellow solid precipitated. After filtration, the yellow solid was recrystalized with DMF and water to afford compound **10a** (0.16 g, 92%); mp 230–231 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 9.25 (br s, 1H), 9.19 (s, 1H, ArH), 8.25 (d, J = 8.4 Hz, 1H, ArH), 7.80 (br s, 3H), 6.91–7.40 (m, 10H, ArH), 4.16 (m, 2H, CH_2), 3.78 (m, 2H, CH_2); HRMS (ESI): m/z, calcd for $C_{22}H_{21}N_5O_7P$ [M+H$^+$]: 498.1173, found: 498.1175.

5.1.11. 2-(2-(Benzylamino)-6-nitroquinazolin-4-ylamino)ethyl dihydrogen phosphate (10b)

5.1.11.1. 2-(2-(Benzylamino)-6-nitroquinazolin-4-ylamino)ethanol (8b). To a stirred suspension of compound **7** (0.46 g, 1.7 mmol) in *i*-PrOH (10 mL) was added benzyl amine (0.64 g, 5.95 mmol). The reaction mixture was refluxed for 3 h. The crude product was obtained after filtration and recrystalized with ethanol to afford the desired product **8b** as light yellow solid (0.49 g, 85%); mp 194–196 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 9.11 (d, J = 2.4 Hz, 1H, ArH), 8.74 (br s, 0.5H), 8.49 (br s, 0.5H), 8.20 (d, J = 9.0 Hz, 1H, ArH), 8.01 (br s, 0.5H), 7.84 (br s, 0.5H), 7.42 (m, 1H, ArH), 7.17–7.35 (m, 5H, ArH), 4.74 (m, 1H, OH), 4.56 (s, 2H, $ArCH_2$), 3.62–3.64 (m, 4H, CH_2CH_2); HRMS (ESI): m/z, calcd for $C_{17}H_{18}N_5O_3$ [M+H$^+$]: 340.1404, found: 340.1406.

5.1.11.2. Dibenzyl 2-(2-(benzylamino)-6-nitroquinazolin-4-ylamino)ethyl phosphate (9b). Following the preparation protocol of Section 5.1.10.3, the mixture of compound **8b** (0.20 g, 0.6 mmol), dibenzyl diisopropylphosphoramidite (0.31 g, 0.9 mmol) and 1H-tetrazole (0.067 g, 0.96 mmol) was stirred for 40 min. Then 30% H_2O_2 (0.24 g, 2.1 mmol) was added and stirred for 50 min to provide compound **9b** (0.27 g, 74%); mp 113–115 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 8.75 (s, 1H, ArH), 8.29 (d, J = 7.8 Hz, 1H, ArH), 7.29 (m, 16H, ArH), 5.05 (d, J = 6.9 Hz, 4H, $2CH_2$), 4.70 (s, 2H, CH_2), 4.18 (s, 2H, CH_2), 3.73 (s, 2H, CH_2). HRMS (ESI): m/z, calcd for $C_{31}H_{31}N_5O_6P$ [M+H$^+$]: 600.2006, found: 600.2012.

5.1.11.3. 2-(2-(Benzylamino)-6-nitroquinazolin-4-ylamino) ethyl dihydrogen phosphate (10b). Following the preparation protocol of Section 5.1.10.4, the mixture of compound **9b** (0.12 g, 0.2 mmol), TFA (2.17 g, 19 mmol) and water (0.018 g, 1 mmol) was stirred at room temperature for 5 h to provide compound **10b** as yellow solid (0.08 g, 95%); mp 196–198 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 9.10 (br s, 1H), 8.21 (d, J = 7.5 Hz, 1H, ArH), 7.34 (d, J = 7.5 Hz, 2H, ArH), 7.29 (t, J = 7.5 Hz, 3H, ArH), 7.20 (d, J = 7.5 Hz, 2H, ArH), 4.56 (m, 2H, CH_2), 4.13 (m, 2H, CH_2), 3.63 (m, 2H, CH_2); HRMS (ESI): m/z, calcd for $C_{17}H_{19}N_5O_6P$ [M+H$^+$]: 420.1067, found: 420.1065.

5.1.12. 2-(2-(3-Fluorophenylamino)-6-nitroquinazolin-4-ylamino)ethyl dihydrogen phosphate (10c)

5.1.12.1. 2-(2-(3-Fluorophenylamino)-6-nitroquinazolin-4-ylamino)ethanol (8c). The reaction mixture of compound **7** (0.54 g, 2 mmol), 3-fluorophenylamine (0.36 g, 3.2 mmol) and concd HCl (1.2 g, 12 mmol) was refluxed for 30 min. After filtration, compound **8c** (0.55 g, 80%) was obtained as white solid; mp 241–243 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.85 (br s, 1H), 10.18 (br s, 1H), 9.43 (d, J = 2.1 Hz, 1H, ArH), 8.53 (dd, J_1 = 9.0 Hz, J_2 = 2.1 Hz, 1H, ArH), 7.62–7.71 (m, 2H, ArH), 7.38–7.48 (m, 2H, ArH), 6.99–7.04 (m, 1H, ArH), 3.66–3.71 (m, 4H, CH_2CH_2); HRMS (ESI): m/z, calcd for $C_{16}H_{15}FN_5O_3$ [M+H$^+$]: 344.1153, found 344.1157.

5.1.12.2. Dibenzyl 2-(2-(3-fluorophenylamino)-6-nitroquinazolin-4-ylamino)ethyl phosphate (9c). Following the preparation protocol of Section 5.1.10.3, the mixture of compound **8c** (0.12 g, 0.35 mmol), dibenzyl diisopropylphosphoramidite (0.18 g, 0.52 mmol) and 1H-tetrazole (0.039 g, 0.56 mmol) was stirred for 1 h. Then 30% H_2O_2 (0.14 g, 1.22 mmol) was added and stirred for 15 min to provide compound **9c** (0.18 g, 87%); mp 152–154 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 8.44 (d, J = 2.1 Hz, 1H, ArH), 8.35 (dd, J_1 = 9.0 Hz, J_2 = 2.1 Hz, 1H, ArH), 7.86 (d, J = 11.4 Hz, 1H, ArH), 7.66 (br s, 1H), 7.55 (d, J = 9.3 Hz, 1H, ArH), 7.19–7.36 (m, 12H, ArH), 6.75 (t, J = 7.5 Hz, 1H, ArH), 5.08 (d, J = 9.0 Hz, 2H, $ArCH_2$), 5.06 (d, J = 9.3 Hz, 2H, $ArCH_2$), 4.30 (m, 2H, CH_2), 3.81 (m, 2H, CH_2); HRMS (ESI): m/z, calcd for $C_{30}H_{28}FN_5O_6P$ [M+H$^+$]: 604.1756, found 604.1759.

5.1.12.3. 2-(2-(3-Fluorophenylamino)-6-nitroquinazolin-4-ylamino)ethyl dihydrogen phosphate (10c). Following the preparation protocol of Section 5.1.10.4, the mixture of compound **9c** (0.12 g, 0.2 mmol), TFA (2.17 g, 19 mmol) and water (0.018 g, 1 mmol) was stirred at room temperature for 5 h to provide the title compound **10c** as yellow solid (0.08 g, 100%); mp 237–239 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.09 (br s, 1H), 9.23 (s, 1H, ArH), 8.32 (d, J = 9.0 Hz, 1H, ArH), 7.85 (m, 1H, ArH), 7.57 (d, J = 7.5 Hz, 1H, ArH), 7.48 (d, J = 7.5 Hz, 1H, ArH), 7.27–7.34 (m, 1H, ArH), 6.79 (t, J = 7.8 Hz, 1H, ArH), 4.15 (m, 2H, CH_2), 3.79 (m, 2H, CH_2); HRMS (ESI): m/z, calcd for $C_{16}H_{16}FN_5O_6P$ [M+H$^+$]: 424.0817, found: 424.0815.

5.1.13. 4-(2-(3-(3-Fluorophenyl)ureido)-6-nitroquinazolin-4-ylamino)benzoic acid (13a)

5.1.13.1. Methyl 4-(2-amino-6-nitroquinazolin-4-ylamino)benzoate (11a). The reaction mixture of compound **4a** (0.54 g, 1.5 mmol) and the newly prepared saturated NH_3 ethanol solution

was irradiated by microwave (power 50 W, temperature 90 °C) for 135 min. The desired compound **11a** (0.5 g, 98%) was obtained as yellow solid after filtration and washed with ethanol. Mp 295–296 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.19 (s, 1H, NH), 9.45 (d, J = 2.1 Hz, 1H, ArH), 8.30 (dd, J_1 = 9.3 Hz, J_2 = 2.4 Hz, 1H, ArH), 8.18 (d, J = 8.7 Hz, 2H, ArH), 7.96 (d, J = 8.7 Hz, 2H, ArH), 7.34 (d, J = 9.6 Hz, 1H, ArH), 7.18 (s, 2H, NH_2), 3.84 (s, 3H, OCH_3); HRMS (ESI): m/z, calcd for $C_{16}H_{13}N_5O_4$ [M+H$^+$]: 340.1040, found 340.1044.

5.1.13.2. Methyl 4-(2-(3-(3-fluorophenyl)ureido)-6-nitroquinazolin-4-ylamino)benzoate (12a). The solution of compound **11a** (0.034 g, 0.1 mmol) and 3-fluorophenyl isocyanate (0.04 g, 0.3 mmol) in dioxane (1 mL) was heated by microwave (power 150 W, temperature 120 °C) for 15 min. After filtration, compound **12a** (0.026 g, 54%) was obtained as yellow solid; mp 242–243 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 12.05 (s, 1H, NH), 10.53 (s, 1H, NH), 10.39 (s, 1H, NH), 9.59 (d, J = 2.1 Hz, 1H, Ar), 8.44 (dd, J_1 = 9 Hz, J_2 = 1.8 Hz, 1H, ArH), 8.27 (d, J = 8.7 Hz, 2H, ArH), 7.89–7.94 (m, 3H, ArH), 7.53 (d, J = 11.4 Hz, 1H, ArH), 7.26–7.33 (m, 2H, ArH), 6.86 (t, J = 8.4 Hz, 1H, ArH), 3.84 (s, 3H, OCH_3); HRMS (ESI): m/z, calcd for $C_{23}H_{18}FN_6O_5$ [M+H$^+$]: 477.1317, found 477.1317.

5.1.13.3. 4-(2-(3-(3-Fluorophenyl)ureido)-6-nitroquinazolin-4-ylamino)benzoic acid (13a). The reaction mixture of compound **12a** (0.105 g, 0.22 mmol) and KOH (0.168 g, 2.99 mmol) in methanol (2 mL) and water (1.5 mL) was refluxed for 30 min, and then diluted with water (5 mL) and cooled with ice bath. The solution was adjusted to pH 2.0 with diluted HCl and stood for 2 h. The crude product was obtained after filtration and recrystalized with DMF/H_2O to give compound **13a** (0.075 g, 83%) as yellow solid; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 12.12 (s, 1H, NH), 10.64 (s, 1H, NH), 10.47 (s, 1H, NH), 9.68 (s, 1H, ArH), 8.52 (d, J = 9.0 Hz, 1H, ArH), 8.27 (d, J = 8.4 Hz, 2H, ArH), 8.02 (s, 1H, ArH), 7.97 (d, J = 8.7 Hz, 2H, ArH), 7.58 (d, J = 11.1 Hz, 1H, ArH), 7.32 (m, 2H, ArH), 6.89 (m, 1H, ArH); HRMS (ESI): m/z, calcd for $C_{22}H_{16}N_6O_5F$ [M+H$^+$]: 463.1160, found 463.1172.

5.1.14. 4-(2-(3-(4-Chlorophenyl)ureido)-6-nitroquinazolin-4-ylamino)benzoic acid (13b)

5.1.14.1. Methyl 4-(2-(3-(4-chlorophenyl)ureido)-6-nitroquinazolin-4-ylamino)benzoate (12b). The solution of compound **11a** (0.034 g, 0.1 mmol) and 4-chlorophenyl isocyanate (0.046 g, 0.3 mmol) in dioxane (1 mL) was heated by microwave (power 150 W, temperature 120 °C) for 15 min. After filtration, compound **12b** (0.036 g, 73%) was obtained as yellow solid; mp 251–253 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 12.04 (s, 1H, NH), 10.64 (s, 1H, NH), 10.45 (s, 1H, NH), 9.67 (s, 1H, ArH), 8.51 (d, J = 8.1 Hz, 1H, ArH), 8.32 (d, J = 8.1 Hz, 2H, ArH), 7.96 (d, J = 8.1 Hz, 3H, ArH), 7.63 (d, J = 8.7 Hz, 2H, ArH), 7.36 (d, J = 7.8 Hz, 2H, ArH), 3.86 (s, 3H, OCH_3); HRMS (ESI): m/z, calcd for $C_{23}H_{18}ClN_6O_5$ [M+H$^+$]: 493.1027, found 493.1022.

5.1.14.2. 4-(2-(3-(4-Chlorophenyl)ureido)-6-nitroquinazolin-4-ylamino)benzoic acid (13b). The reaction mixture of compound **12b** (0.108 g, 0.22 mmol) and KOH (0.168 g, 2.99 mmol) in methanol (2 mL) and water (1.5 mL) was refluxed for 4.5 h, and then diluted with water (5 mL) and cooled with ice bath. The solution was adjusted to pH 2.0 with diluted HCl and stood for 1 h. The crude product was obtained after filtration and recrystalized with DMF/H_2O to give compound **13b** (0.095 g, 93%) as yellow solid; mp >300 °C; ^{1}H NMR (CD_3OD-d_4) δ (ppm): 9.48 (d, J = 1.8 Hz, 1H, ArH), 8.60 (dd, J_1 = 6.6 Hz, J_2 = 1.8 Hz, 1H, ArH), 8.07 (d, J = 6.6 Hz, 2H, ArH), 7.94 (d, J = 6.6 Hz, 2H, ArH), 7.92 (s, 1H, ArH), 7.58 (d, J = 6.9 Hz, 1H, ArH), 7.36 (d, J = 6.6 Hz, 2H, ArH), 7.21 (d, J = 6.6 Hz, 2H, ArH); HRMS (ESI): m/z, calcd for $C_{22}H_{16}N_6O_4Cl$ [M+H$^+$]: 479.0865, found 479.0865.

5.1.15. 4-(2-(3-(3,4-Dichlorophenyl)ureido)-6-nitroquinazolin-4-ylamino)benzoic acid (13c)

5.1.15.1. Methyl 4-(2-(3-(3,4-dichlorophenyl)ureido)-6-nitroquinazolin-4-ylamino)benzoate (12c). The solution of compound **11a** (0.034 g, 0.1 mmol) and 3,4-dichlorophenyl isocyanate (0.056 g, 0.3 mmol) in dioxane (1 mL) was heated by microwave (power 150 W, temperature 120 °C) for 15 min. After filtration, compound **12c** (0.028 g, 53%) was obtained as yellow solid; mp 273–275 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 12.07 (s, 1H, NH), 10.57 (s, 1H, NH), 10.48 (s, 1H, NH), 9.60 (d, J = 2.1 Hz, 1H, ArH), 8.46 (dd, J_1 = 9 Hz, J_2 = 2.1 Hz, 1H, ArH), 8.25 (d, J = 8.7 Hz, 2H, ArH), 7.88–7.96 (m, 4H, ArH), 7.51 (s, 2H, ArH), 3.84 (s, 3H, OCH_3); HRMS (ESI): m/z, calcd for $C_{23}H_{17}Cl_2N_6O_5$ [M+H$^+$]: 527.0632, found 527.0625.

5.1.15.2. 4-(2-(3-(3,4-Dichlorophenyl)ureido)-6-nitroquinazolin-4-ylamino)benzoic acid (13c). The reaction mixture of compound **12c** (0.105 g, 0.2 mmol) and KOH (0.152 g, 2.72 mmol) in methanol (2 mL) and water (1.5 mL) was refluxed for 50 min, and then diluted with water (5 mL) and cooled with ice bath. The solution was adjusted to pH 2.0 with diluted HCl and stood for 1 h. The crude product was obtained after filtration and recrystalized with DMF/H_2O to give compound **13c** (0.095 g, 73%) as yellow solid; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 12.12 (s, 0.5H, NH), 11.07 (s, 0.5H, NH), 10.66 (s, 0.5H, NH), 10.54 (s, 0.5H, NH), 9.67 (s, 0.5H, ArH), 9.61 (s, 0.5H, ArH), 8.51–8.58 (m, 1H, ArH), 8.26 (d, J = 8.1 Hz, 1H, ArH), 7.95–8.05 (m, 4H, ArH), 7.64 (d, J = 9.0 Hz, 1H, ArH), 7.33 (s, 2H, ArH); HRMS (ESI): m/z, calcd for $C_{22}H_{15}Cl_2N_6O_5$ [M+H$^+$]: 513.0475, found 513.0466.

5.1.16. 4-(2-(3-(3-Trifluorophenyl)ureido)-6-nitroquinazolin-4-ylamino)benzoic acid (13d)

5.1.16.1. Methyl 4-(2-(3-(3-trifluorophenyl)ureido)-6-nitroquinazolin-4-ylamino)benzoate (12d). The solution of compound **11a** (0.034 g, 0.1 mmol) and 3-trifluorophenyl isocyanate (0.056 g, 0.3 mmol) in dioxane (1 mL) was heated by microwave (power 150 W, temperature 120 °C) for 10 min. After filtration, the title compound **12d** (0.037 g, 70%) was obtained as yellow solid; mp 275–276 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 12.14 (s, 1H, NH), 10.55 (s, 1H, NH), 10.45 (s, 1H, NH), 9.60 (d, J = 2.1 Hz, 1H, ArH), 8.46 (dd, J_1 = 9 Hz, J_2 = 2.1 Hz, 1H, ArH), 8.26 (d, J = 8.7 Hz, 2H, ArH), 8.10 (s, 1H, ArH), 7.92 (d, J = 8.7 Hz, 3H, ArH), 7.67 (d, J = 8.7 Hz, 1H, ArH), 7.53 (t, J = 7.5 Hz, 1H, ArH), 7.38 (d, J = 7.8 Hz, 1H, ArH), 3.84 (s, 3H, OCH_3); HRMS (ESI): m/z, calcd for $C_{24}H_{18}F_3N_6O_5$ [M+H$^+$]: 527.1285, found 527.1290.

5.1.16.2. 4-(2-(3-(3-Trifluorophenyl)ureido)-6-nitroquinazolin-4-ylamino)benzoic acid (13d). The reaction mixture of compound **12d** (0.132 g, 0.25 mmol) and KOH (0.19 g, 3.4 mmol) in methanol (2 mL) and water (1.5 mL) was refluxed for 30 min, and then diluted with water (15 mL) and cooled with ice bath. The solution was adjusted to pH 2.0 with diluted HCl and stood for 2.5 h. The crude product was obtained after filtration and recrystalized with DMF/H_2O to give compound **13d** (0.064 g, 50%) as yellow solid; mp >300 °C; ^{1}H NMR (pyridine-d_5) δ (ppm): 12.65 (br s, 1H, NH), 12.43 (s, 1H, NH), 11.65 (br s, 1H, NH), 9.53 (s, 1H, ArH), 8.57 (d, J = 8.7 Hz, 1H, ArH), 8.42 (d, J = 8.4 Hz, 2H, ArH), 8.05 (d, J = 7.5 Hz, 2H, ArH), 7.93 (d, J = 9.3 Hz, 1H, ArH), 7.77 (m, 1H, ArH), 7.48 (t, J = 7.5 Hz, 1H, ArH), 7.40 (d, J = 7.2 Hz, 1H, ArH); HRMS (ESI): m/z, calcd for $C_{23}H_{16}N_6O_5F_3$ [M+H$^+$]: 513.1128, found 513.1130.

5.1.17. 4-(2-(3-(4-Trifluorophenyl)ureido)-6-nitroquinazolin-4-ylamino)benzoic acid (13e)

5.1.17.1. Methyl 4-(2-(3-(4-trifluorophenyl)ureido)-6-nitroquinazolin-4-ylamino)benzoate (12e). The solution of compound **11a** (0.034 g, 0.1 mmol) and 4-trifluorophenyl isocyanate (0.056 g, 0.3 mmol) in dioxane (1 mL) was heated by microwave (power 150 W, temperature 120 °C) for 15 min. After filtration, compound **12e** (0.03 g, 57%) was obtained as yellow solid; mp 242–243 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 12.22 (s, 1H, NH), 10.60 (s, 1H, NH), 10.50 (s, 1H, NH), 9.62 (d, J = 2.1 Hz, 1H, ArH), 8.49 (dd, J_1 = 9 Hz, J_2 = 2.1 Hz, 1H, ArH), 8.28 (d, J = 9.0 Hz, 2H, ArH), 7.91–7.96 (m, 3H, ArH), 7.75 (d, J = 8.7 Hz, 2H, ArH), 7.30 (d, J = 8.7 Hz, 2H, ArH), 3.84 (s, 3H, OCH_3); HRMS (ESI): m/z, calcd for $C_{24}H_{18}F_3N_6O_5$ [M+H$^+$]: 527.1285, found 527.1290.

5.1.17.2. 4-(2-(3-(4-Trifluorophenyl)ureido)-6-nitroquinazolin-4-ylamino)benzoic acid (13e). The reaction mixture of compound **12e** (0.132 g, 0.25 mmol) and KOH (0.19 g, 3.4 mmol) in methanol (2.5 mL) and water (2.0 mL) was refluxed for 2 h, and then diluted with water (5 mL) and cooled with ice bath. The solution was adjusted to pH 2.0 with diluted HCl and stood for 2 h. The crude product was obtained after filtration and recrystalized with DMF/H_2O to give compound **13e** (0.107 g, 88%) as yellow solid; mp >250 °C; ^{1}H NMR (pyridine-d_5) δ (ppm): 12.63 (br s, 1H, NH), 12.41 (s, 1H, NH), 11.70 (br s, 1H, NH), 9.55 (s, 1H, ArH), 8.57 (dd, J = 8.7 Hz, 1H, ArH), 8.42 (d, J = 8.4 Hz, 2H, ArH), 8.05 (d, J = 7.5 Hz, 2H, ArH), 7.93 (d, J_1 = 6.6 Hz, J_2 = 1.5 Hz, 1H, ArH), 8.43 (d, J = 6.3 Hz, 2H, ArH), 8.04 (d, J = 6.3 Hz, 2H, ArH), 8.00 (m, 2H, ArH), 7.92 (d, J = 6.9 Hz, 1H, ArH), 7.73 (d, J = 6.3 Hz, 2H, ArH); HRMS (ESI): m/z, calcd for $C_{23}H_{16}N_6O_5F_3$ [M+H$^+$]: 513.1128, found 513.1122.

5.1.18. 4-(6-Nitro-2-(3-phenethylureido)quinazolin-4-ylamino) benzoic acid (13f)

5.1.18.1. Methyl 4-(6-nitro-2-(3-phenethylureido)quinazolin-4-ylamino)benzoate (12f). The solution of compound **11a** (0.034 g, 0.1 mmol) and phenethyl isocyanate (0.074 g, 0.5 mmol) in dioxane (1 mL) was heated by microwave (power 150 W, temperature 120 °C) for 15 min. After filtration, compound **12f** (0.039 g, 80%) was obtained as yellow solid; mp 275–277 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.51 (s, 1H, NH), 9.97 (s, 1H, NH), 9.59 (d, J = 2.1 Hz, 1H, ArH), 9.44 (t, J = 6.0 Hz, 1H, NH) 8.46 (dd, J_1 = 9 Hz, J_2 = 2.1 Hz, 1H, ArH), 8.27 (d, J = 8.4 Hz, 2H, ArH), 7.96 (d, J = 8.7 Hz, 2H, ArH), 7.45 (d, J = 9.3 Hz, 1H, ArH), 7.23–7.34 (m, 5H, ArH), 3.84 (s, 3H, OCH_3), 3.49–3.55 (m, 2H, CH_2), 2.79–2.84 (t, J = 6.9 Hz, 2H, CH_2); HRMS (ESI): m/z, calcd for $C_{25}H_{23}N_6O_5$ [M+H$^+$]: 487.1724, found 487.1729.

5.1.18.2. 4-(6-Nitro-2-(3-phenethylureido)quinazolin-4-ylamino)benzoic acid (13f). The reaction mixture of compound **12f** (0.107 g, 0.22 mmol) and KOH (0.168 g, 2.99 mmol) in methanol (2 mL) and water (1.5 mL) was refluxed for 30 min, and then diluted with water (10 mL) and cooled with ice bath. The solution was adjusted to pH 2.5 with diluted HCl and stood for 1 h. The crude product was obtained after filtration and recrystalized with DMF/H_2O to give compound **13f** (0.088 g, 85%) as yellow solid; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 12.80 (br s, 1H, COOH), 10.50 (s, 1H, NH), 9.97 (s, 1H, NH), 9.44 (br s, 1H, CONH), 8.46 (d, J = 9.3 Hz, 1H, ArH), 8.22 (d, J = 9.0 Hz, 2H, ArH), 7.96 (d, J = 8.7 Hz, 2H, ArH), 7.46 (d, J = 9.0 Hz, 1H, ArH), 7.23–7.31 (m, 5H, ArH), 3.51–3.53 (m, 2H, CH_2), 2.84 (t, J = 7.2 Hz, 2H, CH_2); HRMS (ESI): m/z, calcd for $C_{24}H_{21}N_6O_5$ [M+H$^+$]: 473.1567, found 473.1565.

5.1.19. 4-(2-(3-Cyclohexylureido)-6-nitroquinazolin-4-ylamino) benzoic acid (13g)

5.1.19.1. Methyl 4-(2-(3-cyclohexylureido)-6-nitroquinazolin-4-ylamino)benzoate (12g). The solution of compound **11a** (0.034 g, 0.1 mmol) and cyclohexyl isocyanate (0.038 g, 0.3 mmol) in dioxane (1 mL) was heated by microwave (power 150 W, temperature 120 °C) for 105 min. After filtration, compound **12g** (0.035 g, 76%) was obtained as yellow solid; mp 272–275 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.55 (s, 1H, NH), 9.94 (s, 1H, NH), 9.63 (d, J = 2.4 Hz, 1H, ArH), 9.45 (d, J = 9.3 Hz,1H, NH), 8.50 (dd, J_1 = 9.3 Hz, J_2 = 2.4 Hz, 1H, ArH), 8.27 (d, J = 9.0 Hz, 2H, ArH), 7.99 (d, J = 8.7 Hz, 2H, ArH), 7.67 (d, J = 9.6 Hz, 1H, ArH), 3.85 (s, 3H, OCH_3), 3.65 (m, 1H, NCH), 1.82 (m, 2H, CH_2), 1.65 (m, 2H, CH_2), 1.22–1.57 (m, 6H, $(CH_2)_3$); HRMS (ESI): m/z, calcd for $C_{23}H_{25}N_6O_5$ [M+H$^+$]: 465.1881, found 465.1877.

5.1.19.2. 4-(2-(3-Cyclohexylureido)-6-nitroquinazolin-4-ylamino)benzoic acid (13g). The reaction mixture of compound **12g** (0.093 g, 0.2 mmol) and KOH (0.152 g, 2.72 mmol) in methanol (2 mL) and water (1.5 mL) was refluxed for 60 min, and then diluted with water (10 mL) and cooled with ice bath. The solution was adjusted to pH 2.5 with diluted HCl and extracted with EtOAc (50 mL × 3). The organic layer was concentrated to produce the crude product, which was recrystalized with DMF/H_2O to give compound **13g** (0.074 g, 82%) as yellow solid; mp >290 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.55 (s, 1H, NH), 9.93 (s, 1H, NH), 9.43 (s, 1H, ArH), 8.47 (d, J = 9.0 Hz, 1H, ArH), 8.20 (m, 2H, ArH), 7.94–7.98 (m, 3H, ArH), 7.67 (d, J = 9.0 Hz, 1H, ArH), 5.55 (m, 1H, CONH), 3.60 (m, 1H, NCH), 1.01–1.80 (m, 10H, CH_2); HRMS (ESI): m/z, calcd for $C_{22}H_{23}N_6O_5$ [M+H$^+$]: 451.1724, found 451.1711.

5.1.20. 3-(2-(3-(3,4-Dichlordrophenyl)ureido-6-nitroquinazolin-4-ylamino)propanoic acid (13h)

5.1.20.1. Methyl 3-(2-amino-6-nitroquinazolin-4ylamino)propanoate (11b). The reaction mixture of compound **4c** (0.093 g, 0.3 mmol) and the newly prepared saturated NH_3 ethanol solution (4 mL) was irradiated by microwave (power 50 W, temperature 90 °C) for 3 h. Compound **11b** (0.6 g, 69%) was obtained as yellow solid after filtration and washed with ethanol. Mp 265–266 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 9.05 (d, J = 2.1 Hz, 1H, ArNH), 8.54 (t, J = 4.8 Hz, 1H, NH), 8.19 (dd, J_1 = 9.3 Hz, J_2 = 2.4 Hz, 1H, ArH), 7.20 (d, J = 9.6 Hz, 1H, ArH), 6.86 (br s, 2H, NH_2), 3.68 (q, J = 6.6 Hz, 2H, CH_2), 3.61 (s, 3H, OCH_3), 2.74 (t, J = 7.2 Hz, 2H, CH_2).

5.1.20.2. Methyl 3-(2-(3-(3,4-dichlordrophenyl)ureido-6-nitroquinazolin-4-ylamino)propanate (12h). The solution of compound **11b** (0.035 g, 0.12 mmol) and 3,4-dichlorophenyl isocyanate (0.068 g, 0.36 mmol) in dioxane (1 mL) was heated by microwave (power 150 W, temperature 120 °C) for 5 min. After filtration, compound **12h** (0.056 g, 97%) was obtained as yellow solid; mp 259–261 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 12.29 (s, 1H, NH), 10.06 (s, 1H, NH), 9.22 (d, J = 2.4 Hz, 1H, ArH), 9.15 (t, 1H, NH), 8.39 (dd, J_1 = 9.3 Hz, J_2 = 2.1 Hz, 1H, ArH), 8.06 (d, J = 1.8 Hz, 1H, ArH), 7.82 (d, J = 9.0 Hz, 1H, ArH), 7.54 (s, 2H, ArH), 3.73 (q, J = 6.6 Hz, 2H, CH_2), 3.62 (s, 3H, OCH_3), 2.82 (t, J = 6.9 Hz, 2H, CH_2); HRMS (ESI): m/z, calcd for $C_{19}H_{17}Cl_2N_6O_5$ [M+H$^+$]: 479.0632, found 479.0634.

5.1.20.3. 3-(2-(3-(3,4-Dichlordrophenyl)ureido-6-nitroquinazolin-4-ylamino)propanoic acid (13h). The reaction mixture of compound **12h** (0.048 g, 0.1 mmol) and KOH (0.076 g, 1.36 mmol) in methanol (1 mL) and water (0.7 mL) was refluxed for 3.5 h, and then diluted with water (5 mL) and cooled with ice bath. The solution was adjusted to pH 2.0 with diluted HCl and stood for 1 h. The crude product was obtained after filtration and washed with water to give compound **13h** (0.043 g, 95%) as yellow solid; mp 240–242 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 12.22 (s, 1H, NH), 9.95 (s, 1H, NH), 9.28 (s, 1H, NH), 9.19 (d, J = 1.8 Hz, 1H, ArH), 8.30 (dd, J_1 = 9.0 Hz, J_2 = 1.8 Hz, 1H, , ArH), 8.04 (s, 1H, ArH), 7.68 (d, J = 9.3 Hz, 1H, ArH), 7.54 (s, 2H, ArH), 3.68 (m, 2H, CH_2), 2.65 (t,

J = 6.6 Hz, 2H, CH_2); HRMS (ESI): m/z, calcd for $C_{18}H_{15}Cl_2N_6O_5$ [M+H$^+$]: 465.0475, found 465.0478.

5.2. Biological evaluation

5.2.1. Protein expression and purification

The Pet28a-Pin1 plasmid was a gift from Professor Joseph P. Noel (The Salk Institute for Biological Studies, La Jolla, California). The N-terminally His_6-tagged Pin1 was expressed at 22 °C in *Escheirchia* coli strain BL21 following induction at an optical density of 0.6 (600 nm) with 0.5 mM IPTG for 20 h in terrific broth. Cells were resuspended in 25 mM Tris–Cl, 500 mM NaCl, 10 mM imidazole, 100 μg/mL cocktail, 0.5 mg/mL lysozyme. Following sonication at 4 °C, the soluble supernatant was loaded onto an Ni-NTA (Qiagen) column and washed with 10 bed volumes of washing buffer (50 mM imidazole, 500 mM NaCl, 20 mM Tris–Cl). His_6-Pin1 was eluted with 400 mM imidazole, 500 mM NaCl, 20 mM Tris–Cl and condensed by ultrafiltration (Millipore 5 kDa) with 20 mM Tris–Cl, 100 mM NaCl, 5 mM DTT.

5.2.2. Pin1 PPIase assay and IC_{50} measurements of Pin1 inhibitors

PPIase activities were measured at 10 °C JASCO V-650 spectrophotometer using protease-coupled assay according to Wang et al.[17,18] Suc-Ala-Glu-Pro-Phe-4-nitroanilide in 0.47 M LiCl/trifluoroethanol was used as the substrate. In brief, the assay buffer (840 μL of 35 mM HEPES at PH 7.8), Pin1 (10 μL of 850 μg/mL stock solution), and inhibitors (10 μL of varying concentrations in DMSO) were preequilibrated in the cuvette at 10 °C for 30 min. Then, 100 μL of ice-cooled chymotrypsin (60 mg/mL in 0.001 M HCl) was added and mixed immediately. Additional 40 μL of substrate was added to start the assay and the reaction was monitored by absorbance at 390 nM for 90 s. The data was analyzed by Graphpad Prism 5.01. DMSO was used as solvent for compounds **6a**–**6h** and **10a**–**10c**, and pyridine for compounds **13a**–**13h**.

5.3. Computational studies

All calculations and manipulations were performed using FlexX software package integrated in SYBYL 7.2[19], running on SGI Fuel workstation. The X-ray crystal structure of Pin1 complexed with a carboxylate inhibitor was retrieved from PDB (PDB code: 3JYJ).[13] In FlexX docking, the Receptor Description File (RDF) described the active site environment. It contains the information about the protein, its amino acids, the active site, non-amino acid residues, and specific torsion angles. The active site was defined as all residues within 6.5 Å radius of the cocrystallized reference molecule. The default Sybyl/FlexX parameters were used. The synthesized title compounds were built using the Sybyl Sketcher model and fully minimized with the Powell method (Tripos force field and Gasteiger–Huckel charges) to an energy gradient of 0.05 kcal/(mol Å). The 30 final docked conformations were ranked according to their binding free energy. The docking mode was chosen on the basis of binding affinity rank.

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