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Synthesis and biological evaluation of novel human Pin1 inhibitors with benzophenone skeleton

Chang Liu [a,b,†], Jing Jin [a,†], Liang Chen [a,c], Jie Zhou [a], Xiaoguang Chen [a], Decai Fu [b], Hongrui Song [c], Bailing Xu [a,*]

[a] Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China
[b] Hebei University of Science and Technology, Shijiazhuang 050018, China
[c] School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, China

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ABSTRACT

A series of novel benzophenone derivatives were prepared and their inhibitory activities were evaluated on hPin1. Of all the synthesized compounds, the most active compound displayed inhibitory activities with an IC_{50} value of 5.99 μmol/L. Preliminary structure–activity relationships were analyzed in details and the binding mode of the titled compounds was predicted using FlexX algorithm. The results of this research will shed light on further design and optimization of novel small molecule Pin1 inhibitors.

1. Introduction

Peptidyl-prolyl cis/trans isomerase (PPIase) consists of three major families, namely, cyclophilins, FK-506 binding proteins (FKBPs) and parvulins. Pin1 (Protein interaction with NIMA1) belongs to the parvulin family and has distinctive substrate specificity. Pin1 specifically catalyzes the conformational switch between *cis*- and *trans*- amide bond in pSer-Pro/pThr-Pro motif, which occurred exclusively in many key protein kinases involved in cell cycle.[1–3] Pin1 is frequently up-regulated in various tumors and its over-expression level is also associated with tumor grades and clinical outcomes in prostate cancer.[4,5] Depletion of Pin1 by over-expression of Pin1antisense RNA induces mitotic arrest and apoptosis in human tumor cells.[1,6] Therefore, it was expected that Pin1 may hold a promise as a novel anticancer target and inhibition of Pin1 with small molecules may become a new anticancer treatment strategy.

So far, a number of studies on small molecule inhibitors of Pin1 have been reported.[7–16] Juglone (**A**, Fig. 1), identified by screening a collection of pure secondary metabolites, irreversibly inhibited the enzymatic activity of several parvulins including human Pin1.[10] Mimicking the 'twisted-amide' transition state of the substrate bound with Pin1, aryl indanyl ketones (B, Fig. 1) has been developed as reversible inhibitors with inhibition constants in submicromolar range.[11] Structure-based strategy was successfully employed to design the small molecule inhibitors of Pin1 by Vernalis and Pfizer.[12–15] The most potent inhibitor (**C**, Fig. 1) with the aminophenylpropanol scaffold was found with nanomolar inhibition activity. However, it is not active in cancer cells due to its poor permeability.[12] In the present, it is a utmost need for the development of small molecules as Pin1 inhibitors to validate Pin1 as an anticancer target.

In our early efforts to search for novel Pin1 inhibitors, quinazoline-based Pin1 inhibitors (**D**, Fig. 1) were discovered by screening our in-house library.[16] Another chemical entity (compound **7a**) containing benzophenone motif in the library was also identified as Pin1 inhibitor with an IC_{50} value of 10.11 μM. In order to investigate the structure–activity relationships (SAR), a series of benzophenone derivatives were synthesized by diversifying the substituents at 4-position of A ring and 3- or 4-position of B ring. The active site of Pin1 in its crystal structure featured two hydrophobic domains and one basic cluster formed by the side chains of Lys63, Arg68 and Arg69 residues.[12–15,17] The hydrophobic prolyl pocket is formed by His59, His157, Met130 and Phe134 residues and the slightly shallow hydrophobic shelf embraces His59, Ala118 and Leu122 residues. We envisioned that the oxalic acid group is anchored to the subpocket constructed by basic amino acid residues, the hydrophobic aromatic fragments on A ring may

* Corresponding author. Tel./fax: +86 10 63166764.
E-mail address: xubl@imm.ac.cn (B. Xu).
† The first two authors contributed equally.

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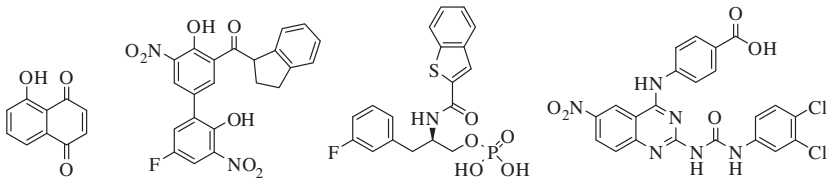


Figure 1. Several known Pin1 inhibitor structures.

occupy the two hydrophobic domains. Herein, the chemical synthesis of these new benzophenone derivatives is described in details. The inhibitory activities on Pin1 of these new chemical entities are presented along with their SAR analysis as follows.

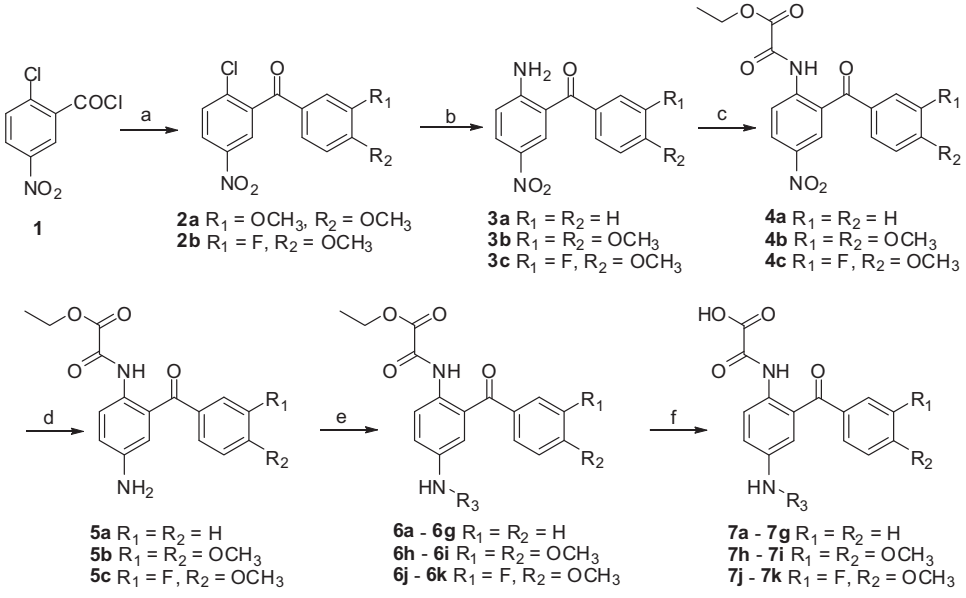
2. Chemistry

The synthesis of benzophenone derivatives (**7a–7k**) was depicted in Scheme 1. The benzophenone scaffolds (**2a** and **2b**) were constructed through Friedel–Crafts reaction between 2-chloro-5-nitrobenzoyl chloride and substituted benzene. Amination of intermediates **2a** and **2b** with aqueous ammonia under microwave irradiation furnished compounds **3b** and **3c** in 54% and 47% yields, respectively. The acylation of amines **3a**–**3c** with ethyl oxalyl monochloride in toluene underwent smoothly to provide the corresponding products in 64–83% yields. Compounds **4a**–**4c** were reduced under catalytic hydrogenation in trifluoroethanol to the corresponding amines **5a**–**5c** in 76–91% yields. It was noted that the reduction of compound **4b** with $SnCl_2$, Fe/CH_3COOH, or catalytic hydrogenation in methanol gave rise to compound **5b** in relatively low yield (30–50%). Nevertheless, compound **5b** could be prepared in excellent yield (91%) when the catalytic hydrogenation was performed in trifluoroethaonl. It has been demonstrated that the trifluoroethanol or hexafluoroisopropanol was a better solvent for the catalytic hydrogenation reduction of nitro compounds.[16] The conversion of amines **5a–5c** to amides **6a**–**6k** with various carboxylic chloride in the presence of hunig's base proceeded smoothly in moderate to good yields. Upon treatment with basic reaction conditions, compounds **6a–6k** were hydrolyzed into the corresponding oxoacetic acid derivatives **7a–7k** in 39–91% yields.

3. Biological results and discussion

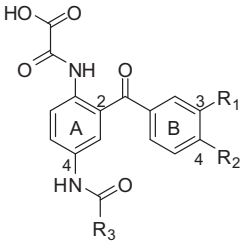
The inhibitory activities on Pin 1 of all target compounds (**7a–7k**) were evaluated by a protease-coupled enzyme assay with Suc-Ala-Glu-Pro-Phe-pNA as the substrate.[18,19] The corresponding results are expressed as IC_{50} values and presented in Table 1.

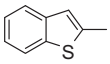
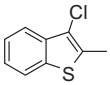
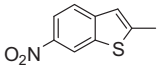
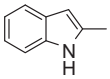
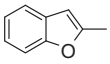
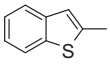
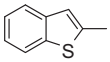
As shown in Table 1, the SAR was investigated initially on the substituents at 4-position of A ring. The target compounds with aromatic hydrophobic fragments (compound **7a**–**7f**) exhibited varied inhibitory activities on Pin1 with an IC_{50} value ranging from 5.99 μM to 18.30 μM. Among which, 7-nitro benzothiophene (compound **7d**) and indole (compound **7e**) motifs are the most favorable variations and lead to inhibitory activities with an IC_{50} value of 5.99 μM and 6.31 μM, respectively. Substitutions at 4-position with benzothiophene-2-carboxamido (compound **7a**), benzothiophene-3-carboxamido (compound **7b**) and 3-chlorobenzothiophene-2-carboxamido (compound **7c**) functional groups produced comparable inhibition effects on Pin1 at the 10 μM level. In comparison with indole-2-carboxamido substitution (compound **7e**), the inhibitory potency of compound **7f** with benzofuran-2-carboxamido fragment decreased to about 1/3. When the 4-aromatic group was replaced by the acetyl substituent (compound **7g**), the inhibition effect was completely lost. It was indicated that introduction of bulky aromatic group to the 4-position of A ring is beneficial to the binding affinity.



Scheme 1. Reagents and conditions: (a) $AlCl_3$, 1,2-dimethoxybenzene or *o*-fluoroanisole; (b) NH_3/H_2O, microwave; (c) ethyl oxalyl monochloride, toluene, reflux; (d) H_2/10% Pd-C, trifluoroehtanol, rt; (e) RCOCl, DIEA, toluene, reflux; (f) NaOH, THF/H_2O, rt.

Table 1
The chemical structures and inhibitory activities on hPin1 of compounds **7a**–**7k**.



Compound	R_1	R_2	R_3	IC_{50}[a] (μM)
7a	H	H		10.11
7b	H	H		11.49
7c	H	H		8.93
7d	H	H		5.99
7e	H	H		6.31
7f	H	H		18.30
7g	H	H	CH_3CO–	>100
7h	OCH_3	OCH_3		54.33
7i	OCH_3	OCH_3		53.55
7j	F	OCH_3		10.36
7k	F	OCH_3		14.52

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

The preliminary SAR of 3- and 4-substituents on B ring was also explored. When 3,4-dimethoxyl benzene was used as B ring instead of benzene, the inhibitory potency dramatically decreased to 1/5 (compounds **7h** and **7i** vs **7a** and **7b**). While 3-fluoro-4-methoxylbenzene served as B ring, compounds **7j** and **7k** displayed somewhat similar inhibition effect to that of compounds **7a** and **7b**. It was rationalized that the bulky methoxyl group on 3-position may result in unfavorable contact with Pin1. In comparison with 3-position of B ring, the variation on 4-position was allowed to a greater extent.

In order to get some insights for further structure-based modification of benzophenones Pin1 inhibitor, the binding mode was investigated using FlexX algorithm implemented in SYBYL 7.2.[21] 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 the synthesized benzophenones are somewhat similar and exemplified by the representative compound **7e** as shown in Figure 2. The carboxylate of 2-oxoacetic acid interacted with the positive charged amino side chain of Arg69 and Lys63 via the key charge-charge interaction. In addition, the hydrogen bond was formed between 2-carbonyl oxygen of oxalic acid and hydroxyl group of Ser114. We rationalized that 2-oxoacetic acid may serve as an alternative for the phosphorus acid group generally used in Pin1 inhibitor. The benzene ring A of benzophenone situated in a small hydrophobic area consisting of His59 and Cys113 residues. The benzene ring B did not occupy the prolyl pocket in which the benzene ring of reference molecule located. The carbonyl oxygen between A and B ring formed a hydrogen bond with the hydroxyl group of Ser154. It was suggested that installment of a hydrogen bond acceptor on the appropriate position of A ring may have a positive contribution to the binding affinity. The bulky aromatic fragment on A ring extended to the hydrophobic shelf including the side chains of Leu122 and Met130. The orientation of this aromatic hydrophobic group was somewhat different from the naphthalene ring of the reference molecule. Nonetheless, both of them could bring important hydrophobic interaction with Pin1 due to a large shallow hydrophobic region existed.

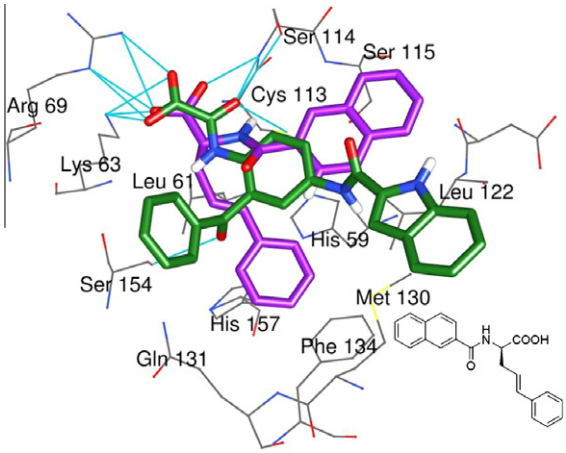


Figure 2. FlexX-modeled binding mode of compound **7e** (carbon atoms colored green) in comparison with the crystal structure (3JYJ in PDB)[13] of a carboxylate Pin1 inhibitor (carbon atoms colored violet). H-Bonding interactions are presented with blue line. The chemical structure of reference molecule is presented. Molecular image was generated with UCSF Chimera.[21]

4. Conclusion

In summary, a series of novel benzophenone-based chemical entities was synthesized as potent Pin1 inhibitors with IC_{50} values 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. Experimental

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 sil-

ica gel G plates at 254 nm under a UV lamp. Column chromatography separations were performed with silica gel (200–300 mesh).

5.1.2. 2-(4-(Benzo[*b*]thiophene-2-carboxamido)-2-benzoylphenylamino)-2-oxoacetic acid (7a)

5.1.2.1. Ethyl 2-(2-benzoyl-4-nitrophenylamino)-2-oxoacetate (4a). To a stirred solution of (2-amino-5-nitrophenyl)(phenyl)methanone (5.0 g, 0.02 mol) dissolved in toluene (70 mL) at 110 °C was added chlorooxalic acid ethyl ester (2.82 g, 0.02 mol). The reaction mixture was stirred for 3 h and then diluted with EtOAc (200 mL) and saturated $NaHCO_3$ aqueous solution (200 mL). The organic layer was washed with water (100 mL × 2) and brine (100 mL × 2), and dried over anhydrous Na_2SO_4. The crude product was obtained after concentration and recrystalized with EtOAc to afford compound **4a** as white solid (4.53 g, 64%); mp 157–159 °C; ^{1}H NMR (acetone-d_6) δ (ppm): 12.13 (brs, 1H, NH), 8.86 (d, J = 9.3 Hz, 1H, ArH), 8.56 (dd, J_1 = 9.0 Hz, J_2 = 2.7 Hz, 1H, ArH), 8.49 (d, J = 2.7 Hz, 1H, ArH), 7.85–7.88 (m, 2H, ArH), 7.75 (t, J = 7.2 Hz, 1H, ArH), 7.62 (t, J = 7.5 Hz, 1H, ArH), 4.39 (q, J = 6.9 Hz, 2H, CH_2), 1.38 (t, J = 6.9 Hz, 3H, CH_3); HRMS (ESI): m/z, Calcd for $C_{17}H_{15}N_2O_6$ [M+H$^+$]: 343.0925, Found 343.0919.

5.1.2.2. Ethyl 2-(4-amino-2-benzoylphenylamino)-2-oxoacetate (5a). The mixture of compound **4a** (2.0 g, 5.84 mmol) and 10% Pd-C (0.4 g) in trifluroethanol (50 mL) was hydrogenated at room temperature and 1 atm for 3 h. Pd-C was filtered off and the filtrate was concentrated to give the crude product, which was recrystalized with EtOAc to afford compound **5a** as yellow solid (1.49 g, 81.6%); mp 129–131 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.96 (s, 1H, NH), 7.62–7.69 (m, 4H, ArH), 7.51 (t, J = 7.5 Hz, 2H, ArH), 6.79 (dd, J_1 = 8.7 Hz, J_2 = 2.4 Hz, 1H, ArH), 6.67(d, J = 2.4 Hz, 1H, ArH), 5.38 (s, 2H, NH_2), 4.20 (q, J = 7.2 Hz, 2H, CH_2), 1.24 (t, J = 7.2 Hz, 3H, CH_3); HRMS (ESI): m/z, Calcd for $C_{17}H_{17}N_2O_4$ [M+H$^+$]: 313.1183, Found 313.1197.

5.1.2.3. Ethyl 2-(4-(Benzo[*b*]thiophene-2-carboxamido)-2-benzoylphenylamino)-2-oxoacetate (6a). To a stirred solution of compound **5a** (0.20 g, 0.64 mmol) dissolved in toluene (4 mL) at 110 °C was added benzo[*b*]thiophene-2-carboxachloride (0.19 g, 0.96 mmol) and DIEA (0.12 g, 0.96 mmol). The reaction mixture was heated for 2 h and then cooled to room temperature. The reaction mixture was diluted with EtOAc (80 mL) and saturated $NaHCO_3$ aq (80 mL). The organic layer was separated and washed with water (80 mL × 1) and brine (80 mL × 1), and then dried over anhydrous Na_2SO_4. The crude product was obtained after concentration and recrystalized with EtOAc to afford compound **6a** as yellow solid (0.21 g, 69%); mp 228–230 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.39 (s, 1H, NH), 10.72 (s, 1H, NH), 8.34 (s, 1H, ArH), 7.99–8.14 (m, 5H, ArH), 7.66 (d, J = 7.2 Hz, 2H, ArH), 7.70 (t, J = 7.5 Hz, 1H, ArH), 7.56 (t, J = 7.5 Hz, 2H, ArH), 7.46–7.50 (m, 2H, ArH), 4.26 (q, J = 7.2 Hz, 2H, CH_2), 1.28 (t, J = 7.2 Hz, 3H, CH_3); HRMS (ESI): m/z, Calcd for $C_{26}H_{21}N_2O_5S$ [M+H$^+$]: 473.1166, Found 473.1165.

5.1.2.4. 2-(4-(Benzo[*b*]thiophene-2-carboxamido)-2-benzoylphenylamino)-2-oxoacetic acid (7a). The reaction mixture of compound **6a** (0.10 g, 0.32 mmol) and NaOH (0.064 g, 1.6 mmol) in water (2.0 mL), THF (2.0 mL) and acetone (4.0 mL) was stirred at room temperature for 1 h. The solvent was removed by evaporation and the residue was dissolved in water (10.0 mL), which was cooled in ice-water bath and adjusted to pH = 2.0 with diluted HCl solution. The resulting mixture was stirred for 0.5 h and the precipitate was filtered to give the title compound **7a** as yellow solid (0.075 g, 53%); mp 223–225 °C; ^{1}H NMR (acetone-d_6) δ (ppm): 11.40 (s, 1H, NH), 10.72 (s, 1H, NH), 8.34 (s, 1H, ArH), 8.13 (m, 2H, ArH), 7.99–8.07 (m, 3H, ArH), 7.78 (d, J = 7.2 Hz, 2H, ArH), 7.71 (t, J = 7.2 Hz, 1H, ArH), 7.58 (t, J = 7.5 Hz, 2H, ArH), 7.44–7.52 (m, 2H, ArH); HRMS (ESI): m/z, Calcd for $C_{24}H_{17}N_2O_5S$ [M + H$^+$]: 445.0853, Found 445.0863.

5.1.3. 2-(4-(benzo[*b*]thiophene-3-carboxamido)-2-benzoylphenylamino)-2-oxoacetic acid (7b)

5.1.3.1. Ethyl 2-(4-(benzo[*b*]thiophene-3-carboxamido)-2-benzoylphenylamino)-2-oxoacetate (6b). Following the preparation protocol of Section 5.1.2.3, the reaction mixture of compound **5a** (0.20 g, 0.64 mmol), benzo[b]thiophene-3-carboxachloride (0.19 g, 0.96 mmol) and DIEA (0.12 g, 0.96 mmol) in toluene (4 mL) was heated at 110 °C for 1.0 h to give compound **6b** as yellow solid (0.22 g, 73%); mp 204–206 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.36 (s, 1H, NH), 10.55 (s, 1H, NH), 8.56 (s, 1H, ArH), 8.37–8.40 (m, 1H, ArH), 7.98–8.15 (m, 4H, ArH), 7.76 (d, J = 7.2 Hz, 1H, ArH), 7.85 (d, J = 6.9 Hz, 1H, ArH), 7.69 (t, 1H, J = 7.5 Hz, ArH), 7.56 (t, J = 7.2 Hz, 2H, ArH), 7.43–7.49 (m, 2H, ArH), 4.26 (q, J = 7.5 Hz, 2H, CH_2), 1.28 (t, J = 7.5 Hz, 3H, CH_3); HRMS (ESI): m/z, Calcd for $C_{26}H_{21}N_2O_5S$ [M + H$^+$]: 473.1166, Found 473.1174.

5.1.3.2. 2-(4-(Benzo[*b*]thiophene-3-carboxamido)-2-benzoylphenylamino)-2-oxoacetic acid (7b). Following the preparation protocol of Section 5.1.2.4, the reaction mixture of compound **6b** (0.10 g, 0.21 mmol) and NaOH (0.042 g, 1.05 mmol) in water (2.0 mL) and THF (2.0 mL) was stirred at room temperature for 50 min to give compound **7b** as yellow solid (0.081 g, 86%); mp 206–208 °C; ^{1}H NMR (acetone-d_6) δ (ppm): 11.80 (s, 1H, NH), 9.86 (s, 1H, NH), 8.60 (d, J = 8.7 Hz, 1H, ArH), 8.51–8.54 (m, 1H, ArH), 8.43 (s, 1H, ArH), 8.21 (d, J = 8.7 Hz, 2H, ArH), 7.98–8.01 (m, 1H, ArH), 7.84 (d, J = 7.2 Hz, 2H, ArH), 7.63–7.72 (m, 1H, ArH), 7.56 (t, J = 7.5 Hz, 2H, ArH), 7.42–7.49 (m, 2H, ArH); HRMS (ESI): m/z, Calcd for $C_{24}H_{17}N_2O_5S$ [M + H$^+$]: 445.0853, Found 445.0861.

5.1.4. 2-(2-Benzoyl-4-(3-chlorobenzo[b]thiophene-2-carboxamido)phenylamino)-2-oxoacetic acid (7c)

5.1.4.1. Ethyl 2-(2-benzoyl-4-(3-chlorobenzo[*b*]thiophene-2-carboxamido)phenylamino)-2-oxoacetate (6c). Following the preparation protocol of Section 5.1.2.3, the reaction mixture of compound **5a** (0.10 g, 0.32 mmol), benzo[*b*]thiophene-3-chloro-2-carboxachloride (0.11 g, 0.48 mmol) and DIEA (0.06 g, 0.48 mmol) in toluene (4 mL) was heated at 110 °C for 0.5 h to give compound **6c** as yellow solid (0.18 g, 74%); mp 238–240 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.39 (s, 1H, NH), 10.78 (s, 1H, NH), 8.14–8.17 (m, 1H, ArH), 8.00–8.08 (m, 2H, ArH), 7.92–7.97 (m, 2H, ArH), 7.54–7.77 (m, 5H, ArH), 4.26 (q, J = 7.2 Hz, 2H, CH_2), 1.28 (t, J = 7.2 Hz, 3H, CH_3); HRMS (ESI): m/z, Calcd for $C_{26}H_{20}ClN_2O_5S$ [M+H$^+$]: 507.0776, Found 507.0781.

5.1.4.2. 2-(2-Benzoyl-4-(3-chlorobenzo[b]thiophene-2-carboxamido)phenylamino)-2-oxoacetic acid (7c). Following the preparation protocol of Section 5.1.2.4, the reaction mixture of compound **6c** (0.05 g, 0.10 mmol) and NaOH (0.02 g, 0.49 mmol) in water (2.0 mL), THF (2 mL) and acetone (4.0 mL) was stirred at room temperature for 30 min to give compound **7c** as yellow solid (0.038 g, 81%); mp 235–237 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.41 (s, 1H, NH), 10.77 (s, 1H, NH), 8.09–8.17 (m, 2H, ArH), 7.92–8.03 (m, 3H, ArH), 7.76 (d, J = 6.9 Hz, 2H, ArH), 7.54–7.71 (m, 5H, ArH); HRMS (ESI): m/z, Calcd for $C_{24}H_{16}ClN_2O_5S$ [M+H$^+$]: 479.0463, Found 479.0470.

5.1.5. 2-(2-Benzoyl-4-(6-nitrobenzo[*b*]thiophene-2-carboxamido)phenylamino)-2-oxoacetic acid (7d)

5.1.5.1. Ethyl 2-(2-benzoyl-4-(6-nitrobenzo[b]thiophene-2-carboxamido)phenylamino)-2-oxoacetate (6d). Following the preparation protocol of Section 5.1.2.3, the reaction mixture of

compound **5a** (0.20 g, 0.64 mmol), benzo[*b*]thiophene-6-nitro-2-carboxachloride (0.19 g, 0.76 mmol) and DIEA (0.10 g, 0.76 mmol) in toluene (4 mL) was heated at 110 °C for 0.5 h to give compound **6d** as yellow solid (0.29 g, 90%); mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.39 (s, 1H, NH), 11.18 (s, 1H, NH), 8.92 (d, J = 2.1 Hz, 1H, ArH), 8.61(s, 1H, ArH), 8.35 (d, 1H, J = 8.7 Hz, ArH), 8.27 (dd, J_1 = 8.7 Hz, J_2 = 2.4 Hz, 1H, ArH), 8.15 (d, J = 8.7 Hz, 1H, ArH), 8.06 (d, 1H, J = 8.7 Hz, ArH), 8.03 (d, J = 2.4 Hz, 2H, ArH), 7.77 (d, J = 7.5 Hz, 2H, ArH), 7.69 (t, J = 7.5 Hz, 1H, ArH), 7.58 (t, J = 7.5 Hz, 2H, ArH), 4.27 (q, J = 7.2 Hz, 2H, CH_2), 1.28 (t, J = 7.2 Hz, 3H, CH_3); HRMS (ESI): *m/z*, Calcd for $C_{26}H_{20}N_3O_7S$ [M+H$^+$]: 518.1016, Found 518.1048.

5.1.5.2. 2-(2-Benzoyl-4-(6-nitrobenzo[b]thiophene-2-carboxamido)phenylamino)-2-oxoacetic acid (7d). Following the preparation protocol of Section 5.1.2.4, the reaction mixture of compound **6d** (0.15 g, 0.29 mmol) and NaOH (0.058 g, 1.45 mmol) in water (2.0 mL), THF (2 mL) and acetone (2.0 mL) was stirred at room temperature for 60 min to give compound **7d** as yellow solid (0.12 g, 84%); mp 200–201 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.39 (s, 1H, NH), 10.92 (s, 1H, NH), 8.93 (d, J = 1.8 Hz, 1H, ArH), 8.49 (s, 1H, ArH), 8.34 (d, J = 9.0 Hz, 1H, ArH), 8.26 (dd, J_1 = 9.3 Hz, J = 1.8 Hz, 1H, ArH), 8.12 (m, 2H, ArH), 7.98 (s, 1H, ArH), 7.77 (d, J = 7.2 Hz, 2H, ArH), 7.69 (d, J = 7.2 Hz, 1H, ArH), 7.57 (t, J = 7.2 Hz, 2H, ArH); HRMS (ESI): *m/z*, Calcd for $C_{24}H_{16}N_3O_7S$ [M+H$^+$]: 490.0703, Found 490.0707.

5.1.6. 2-(2-Benzoyl-4-(1*H*-indole-2-carboxamido)phenylamino) -2-oxoacetic acid (7e)

5.1.6.1. Ethyl 2-(2-benzoyl-4-(1*H*-indole-2-carboxamido)phenylamino)-2-oxoacetate (6e). The mixture of 1*H*-indole-2-carboxalic acid (0.1 g, 0.62 mmol), $SOCl_2$ (0.22 g, 1.86 mmol) and one drop DMF in CH_2Cl_2 (2 mL) was refluxed for 2 h. The crude product of 1*H*-indole-2-carboxachloride was obtained as yellow solid after evaporation and directly used in the next step without further purification.

Following the preparation protocol of Section 5.1.2.3, the reaction mixture of compound **5a** (0.13 g, 0.41 mmol), 1*H*-indole-2-carboxachloride and DIEA (0.08 g, 0.62 mmol) in toluene (3 mL) was heated at 110 °C for 2.0 h to give compound **6e** as yellow solid (0.135 g, 66%); mp 118–120 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.76 (s, 1H, NH), 11.41 (s, 1H, NH), 10.44 (s, 1H, NH), 8.09–8.15 (m, 3H, ArH), 7.78 (d, J = 7.2 Hz, 2H, ArH), 7.68 (t, J = 7.5 Hz, 2H, ArH), 7.58 (t, J = 7.8 Hz, 2H, ArH), 7.40–7.46 (m, 2H, ArH), 7.22 (t, J = 6.9 Hz, 1H, ArH), 7.06 (t, J = 7.5 Hz, 1H, ArH), 4.27 (q, J = 6.9 Hz, 2H, CH_2), 1.28 (t, J = 7.2 Hz, 3H, CH_3); HRMS (ESI): *m/z*, Calcd for $C_{26}H_{22}N_3O_5$ [M+H$^+$]: 456.1554, Found 456.1564.

5.1.6.2. 2-(2-Benzoyl-4-(1*H*-indole-2-carboxamido)phenylamino)-2-oxoacetic acid (7e). Following the preparation protocol of Section 5.1.2.4, the reaction mixture of compound **6e** (0.05 g, 0.11 mmol) and NaOH (0.022 g, 0.55 mmol) in water (2.0 mL), THF (1 mL) and acetone (2.0 mL) was stirred at room temperature for 60 min to give compound **7e** as brown solid (0.043 g, 91%); mp 197–199 °C; ^{1}H NMR (acedone-d_6) δ (ppm): 11.77 (s, 1H, NH), 11.43 (s, 1H, NH), 10.45(s, 1H, NH), 8.10–8.14 (m, 3H, ArH), 7.79 (d, J = 6.9 Hz, 2H, ArH), 7.70 (t, J = 8.1 Hz, 2H, ArH), 7.58 (t, J = 8.7 Hz, 2H, ArH), 7.43 (m, 2H, ArH), 7.22 (t, J = 7.2 Hz, 1H, ArH), 7.06 (t, J = 7.2 Hz, 1H, ArH); HRMS (ESI): *m/z*, Calcd for $C_{24}H_{18}N_3O_5$ [M+H$^+$]: 428.1241, Found 428.1252.

5.1.7. 2-(4-(Benzofuran-2-carboxamido)-2-benzoylphenylamino)-2-oxoacetic acid (7f)

5.1.7.1. Ethyl 2-(4-(benzofuran-2-carboxamido)-2-benzoylphenylamino)-2-oxoacetate (6f). The mixture of benzofuran-2-carboxalic acid (0.20 g, 1.23 mmol), $SOCl_2$ (0.44 g, 3.70 mmol) and one drop DMF in CH_2Cl_2 (5 mL) was refluxed for 5 h. The crude product of benzofuran-2-carboxachloride was obtained after evaporation and directly used in the next step without further purification.

Following the preparation protocol of Section 5.1.2.3, the reaction mixture of compound **5a** (0.26 g, 0.82 mmol), benzofuran-2-carboxachloride and DIEA (0.16 g, 1.23 mmol) in toluene (7 mL) was heated at 110 °C for 1 h to give compound **6f** as yellow solid (0.34 g, 90%); mp 231–232 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.40 (s, 1H, NH), 10.82 (s, 1H, NH), 8.17 (dd, J_1 = 9.0 Hz, J_2 = 2.4 Hz,1H, ArH), 8.02–8.09 (m, 2H, ArH), 7.76–7.83 (m, 4H, ArH), 7.69–7.71 (m, 2H, ArH), 7.49–7.60 (m, 3H, ArH), 7.37 (d, J = 7.2 Hz, 1H, ArH), 4.26 (q, J = 6.9 Hz, 2H, CH_2), 1.28 (t, J = 7.2 Hz, 3H, CH_3); HRMS (ESI): *m/z*, Calcd for $C_{26}H_{21}N_2O_6$ [M+H$^+$]: 457.1394, Found 457.1405.

5.1.7.2. 2-(2-Benzoyl-4-(1*H*-indole-2-carboxamido)phenylamino)-2-oxoacetic acid (7f). Following the preparation protocol of Section 5.1.2.4, the reaction mixture of compound **6f** (0.15 g, 0.33 mmol) and NaOH (0.066 g, 1.64 mmol) in water (5.0 mL) and THF (5.0 mL) was stirred at room temperature for 30 min to give compound **7f** as yellow solid (0.095 g, 67%); mp 210–212 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.41 (s, 1H, NH), 10.78 (s, 1H, NH), 8.08–8.13 (m, 3H, ArH), 7.69–7.83 (m, 6H, ArH), 7.58 (t, J = 7.5 Hz, 2H, ArH), 7.51 (d, J = 7.2 Hz, 1H, ArH), 7.37 (d, J = 7.8 Hz, 1H, ArH); HRMS (ESI): *m/z*, Calcd for $C_{24}H_{17}N_2O_6$ [M+H$^+$]: 429.1081, Found 429.1092.

5.1.8. 2-(4-Acetamido-2-benzoylphenylamino)-2-oxoacetic acid (7g)

5.1.8.1. Ethyl 2-(4-acetamido-2-benzoylphenylamino)-2-oxoacetate (6g). The reaction mixture of compound **5a** (0.10 g, 0.32 mmol) and acetic anhydride (0.065 g, 0.64 mmol) in acetic acid (5 mL) was stirred at 110 °C for 1 h. Then the reaction mixture was poured into ice water, after filtration and recrystalization, the title compound **6g** was obtained as yellow solid (0.083 g, 73%); mp 181–182 °C; ^{1}HNMR (DMSO-d_6) δ (ppm): 11.29 (s,1H, NH), 10.14 (s,1H, NH), 7.93 (d, J = 8.7 Hz, 1H, ArH), 7.84 (d, J = 8.7 Hz, 1H, ArH), 7.66 (m, 4H, ArH), 7.53 (m, 2H, ArH), 4.24 (q, J = 6.9 Hz, 2H, CH_2), 2.00 (s,3H, CH_3), 1.25 (t, J = 7.2 Hz, 3H, CH_3); HRMS (ESI): *m/z*, Calcd for $C_{19}H_{19}N_2O_5$ [M+H$^+$]: 355.1288, found 355.1282.

5.1.8.2. 2-(4-Acetamido-2-benzoylphenylamino)-2-oxoacetic acid (7g). Following the preparation protocol of Section 5.1.2.4, the reaction mixture of compound **6g** (0.05 g, 0.14 mmol) and NaOH (0.028 g, 0.70 mmol) in water (1.5 mL) and THF (3.0 mL) was stirred at room temperature for 90 min to give compound **7g** as yellow solid (0.039 g, 85%); mp 178–179 °C; ^{1}HNMR (DMSO-d_6) δ (ppm): 11.32 (s,1H, NH), 10.14 (s,1H, NH), 8.01 (d, J = 8.7 Hz, 1H, ArH), 7.84 (d, J = 8.7 Hz, 1H, ArH), 7.78 (s, 1H, ArH), 7.64–7.72 (m, 3H, ArH), 7.51–7.56 (m, 2H, ArH), 2.00 (s,3H, CH_3); HRMS (ESI): *m/z*, Calcd for $C_{17}H_{15}N_2O_5$ [M+H$^+$] 327.0975, Found 327.0992.

5.1.9. 2-(4-(Benzo[*b*]thiophene-2-carboxamido)-2-(3,4-dimethoxybenzoyl)phenylamino)-2-oxoacetic acid (7h)

5.1.9.1. (2-Chloro-5-nitrophenyl)(3,4-dimethoxyphenyl)methanone (2a). The mixture of 2-chloro-5-nitrobenzoyl chloride (3.0 g, 13.6 mmol), 1,2-dimethoxybenzene (10 mL, 78 mmol) and $AlCl_3$ (2.72 g, 20.5 mmol) was stirred for a while, which was separated into three reaction tubes and heated by microwave (power 50 W, temperature 50 °C) for 30 min. The reaction mixture was poured into 10% HCl aqueous solution cooled in ice bath, which was extracted with EtOAc (100 mL × 3). The combined organic layer was washed with brine (50 mL × 1) and dried over anhydrous Na_2SO_4. The crude product was provided after evaporation and

recrystalized with EtOAc to give compound **2a** as white solid (0.71 g, 16%); mp 135–137 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 8.29 (dd, J_1 = 8.4 Hz, J_2 = 2.7 Hz, 1H, ArH), 8.24 (d, J = 2.4 Hz, 1H, ArH), 7.67 (d, J = 9.0 Hz, 1H, ArH), 7.59 (d, J = 2.1 Hz, 1H, ArH), 7.15 (dd, J_1 = 8.1 Hz, J_2 = 1.5 Hz, 1H, ArH), 6.86 (d, J = 8.4 Hz, 1H, ArH), 3.97 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3); HRMS (ESI): *m/z*, Calcd for $C_{15}H_{13}ClNO_5$ [M+H$^+$]: 322.0477, Found 322.0460.

5.1.9.2 (2-Amino-5-nitrophenyl)(3,4-dimethoxyphenyl)methanone (**3b**).

The reaction mixture of compound **2a** (0.50 × 2 g, 1.55 mmol) and aqueous ammonia (2 × 2 mL) was loaded into two reaction tubes and irradiated by microwave (power 100 W, temperature 150 °C, pressure 180 psi) for 3 h respectively. The reaction mixture was diluted with water (20 mL) and adjusted to pH = 2.0 with 10% HCl aqueous solution. After stirring for 30 min, the precipitate was filtered and recrystalized with EtOAc to afford compound **3b** as yellow solid (0.51 g, 54%); mp 224–226 °C; ^{1}H NMR (acetone-d_6) δ (ppm): 8.44 (d, J = 2.4 Hz, 1H, ArH), 8.13 (dd, J_1 = 9.3 Hz, J_2 = 2.7 Hz, 1H, ArH), 7.61 (brs, 2H, NH), 7.30–7.36 (m, 2H, ArH), 7.10 (d, J = 7.5 Hz, 1H, ArH), 7.02 (d, J = 9.3 Hz, 1H, ArH), 3.93 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3); HRMS (ESI): *m/z*, Calcd for $C_{15}H_{15}N_2O_5$ [M+H$^+$]: 303.0975, Found 303.0945.

5.1.9.3. Ethyl 2-(2-(3,4-dimethoxybenzoyl)-4-nitrophenylamino)-2-oxoacetate (4b). Following the preparation protocol of Section 5.1.2.1, the reaction mixture of compound **3a** (0.50 g, 1.66 mmol), chlorooxalic acid ethyl ester (0.23 g, 1.66 mol) in toluene (10 mL) was heated at 110 °C for 3 h to give compound **4b** as yellow solid (0.51 g, 76%); mp 134–136 °C; ^{1}H NMR (acetone-d_6) δ (ppm): 11.87 (brs, 1H, NH), 8.82 (d, J = 9.3 Hz, 1H, ArH), 8.52–8.56 (m, 2H, ArH), 7.50 (d, J = 1.5 Hz, 1H, ArH), 7.44 (dd, J_1 = 8.7 Hz, J_2 = 2.1 Hz, 1H, ArH), 7.12 (d, J = 8.1 Hz, 1H, ArH), 4.38 (q, J = 6.9 Hz, 2H, CH_2), 3.95 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 1.37 (t, J = 7.2 Hz, 3H, CH_3); HRMS (ESI): *m/z*, Calcd for $C_{19}H_{19}N_2O_8$ [M + H$^+$]: 403.1136, Found 403.1139.

5.1.9.4. Ethyl 2-(4-amino-2-(3,4-dimethoxybenzoyl)phenylamino)-2-oxoacetate (5b). Following the preparation protocol of Section 5.1.2.2, the reaction mixture of compound **4b** (0.05 g, 0.12 mmol) and 10% Pd-C (0.01 g) in trifluoroethanol (5.0 mL) was hydrogenated at room temperature and 1 atm for 10 h to provide compound **5b** as yellow solid (0.042 g, 91%); mp 187–189 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.65 (d, J = 8.7 Hz, 1H, ArH), 7.27–7.30 (m, 1H, ArH), 7.07 (d, J = 8.1 Hz, 1H, ArH), 6.77 (dd, J_1 = 8.7 Hz, J_2 = 2.7 Hz, 1H, ArH), 6.72 (d, J = 2.4 Hz, 1H, ArH), 4.21 (q, J = 7.2 Hz, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 1.24 (t, J = 6.9 Hz, 3H, CH_3); HRMS (ESI): *m/z*, Calcd for $C_{19}H_{21}N_2O_6$ [M+H$^+$]: 373.1394, Found 373.1355.

5.1.9.5. Ethyl 2-(4-(benzo[b]thiophene-2-carboxamido)-2-(3,4-dimethoxybenzoyl)phenyl-amino)-2-oxoacetate (6h). Following the preparation protocol of Section 5.1.2.3, the reaction mixture of compound **5b** (0.05 g, 0.13 mmol), benzo[*b*]thiophene-2-carboxachloride (0.032 g, 0.16 mmol) and DIEA (0.021 g, 0.16 mmol) in toluene (3 mL) was heated at 110 °C for 2 h to give compound **6h** as yellow solid (0.03 g, 42%); mp 262–263 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.23 (s, 1H, NH), 10.72 (s, 1H, NH), 8.34 (s, 1H, ArH), 7.98–8.05 (m, 5H, ArH), 7.43–7.49 (m, 2H, ArH), 7.37 (d, J = 7.8 Hz, 2H, ArH), 7.12 (d, J = 8.4 Hz, 1H, ArH), 4.25 (q, J = 7.2 Hz, 2H, CH_2), 3.86 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 1.27 (t, J = 7.2 Hz, 3H, CH_3); HRMS (ESI): *m/z*, Calcd for $C_{28}H_{25}N_2O_7S$ [M+H$^+$]: 533.1377, Found 533.1388.

5.1.9.6. 2-(4-(Benzo[*b*]thiophene-2-carboxamido)-2-(3,4-dimethoxybenzoyl)phenylamino)-2-oxoacetic acid (7h). Following the preparation protocol of Section 5.1.2.4, the reaction mixture of compound **6h** (0.10 g, 0.19 mmol) and NaOH (0.025 g, 0.58 mmol) in water (5.0 mL) and acetone (5.0 mL) was stirred at room temperature for 2 h to give compound **7h** as yellow solid (0.038 g, 39%); mp 225–227 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.26 (s, 1H, NH), 10.74 (brs, 1H, NH), 8.36 (s, 1H, ArH), 7.99–8.18 (m, 5H, ArH), 7.45–7.49 (m, 2H, ArH), 7.37–7.41 (m, 2H, ArH), 7.13 (d, J = 8.4 Hz, 1H, ArH), 3.88 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3); HRMS (ESI): *m/z*, Calcd for $C_{26}H_{21}N_2O_7S$ [M+H$^+$]: 505.1064, Found 505.1066.

5.1.10. 2-(4-(Benzo[*b*]thiophene-3-carboxamido)-2-(3,4-dimethoxybenzoyl)phenylamino)-2-oxoacetic acid (7i)

5.1.10.1. Ethyl 2-(4-(benzo[*b*]thiophene-3-carboxamido)-2-(3,4-dimethoxybenzoyl)phenyl-amino)-2-oxoacetate (6i). Following the preparation protocol of Section 5.1.2.3, the reaction mixture of compound **5b** (0.05 g, 0.13 mmol), benzo[*b*]thiophene-3-carboxachloride (0.032 g, 0.16 mmol) and DIEA (0.021 g, 0.16 mmol) in toluene (3 mL) was heated at 110 °C for 2 h to give compound **6i** as yellow solid (0.022 g, 31%); mp 237–239 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.22 (s, 1H, NH), 10.54 (s, 1H, NH), 8.56 (s, 1H, ArH), 8.38 (dd, J_1 = 8.4 Hz, J_2 = 2.1 Hz, 1H, ArH), 8.07 (m, 4H, ArH), 7.44–7.46 (m, 2H, ArH), 7.37 (d, J = 7.2 Hz, 2H, ArH), 7.11 (d, J = 8.4 Hz, 1H, ArH), 4.26 (q, J = 6.9 Hz, 2H, CH_2), 3.86 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 1.27 (t, J = 6.9 Hz, 3H, CH_3); HRMS (ESI): *m/z*, Calcd for $C_{28}H_{25}N_2O_7S$ [M+H$^+$]: 533.1377, Found 533.1380.

5.1.10.2. 2-(4-(Benzo[*b*]thiophene-3-carboxamido)-2-(3,4-dimethoxybenzoyl)phenylamino)-2-oxoacetic acid (7i). Following the preparation protocol of Section 5.1.2.4, the reaction mixture of compound **6i** (0.08 g, 0.16 mmol) and NaOH (0.031 g, 0.78 mmol) in water (3.0 mL) and THF (5.0 mL) was stirred at room temperature for 2 h to give compound **7i** as yellow solid (0.062 g, 78%); mp 232–234 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.54 (brs, 1H, NH), 8.57 (s, 1H, ArH), 8.37 (m, 1H, ArH), 8.07–8.17 (m, 4H, ArH), 7.37–7.46 (m, 4H, ArH), 7.12 (d, J = 8.7 Hz, 1H, ArH), 3.86 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3); HRMS (ESI): *m/z*, Calcd for $C_{26}H_{21}N_2O_7S$ [M+H$^+$]: 505.1064, Found 505.1066.

5.1.11. 2-(4-(Benzo[*b*]thiophene-2-carboxamido)-2-(3-fluoro-4-methoxybenzoyl)phenyl-amino)-2-oxoacetic acid (7j)

5.1.11.1. (2-Chloro-5-nitrophenyl) (3-fluoro-4-methoxyphenyl)methanone (2b). The mixture of 2-chloro-5-nitrobenzoyl chloride (4.0 g, 18.18 mmol), 2-fluoroanisole (5.0 mL) and $AlCl_3$ (3.63 g, 27.27 mmol) was stirred at room temperature for 24 h. The reaction mixture was poured into 10% HCl aqueous solution cooled in ice bath, which was extracted with EtOAc (100 mL × 3). The combined organic layer was washed with brine (100 mL × 1) and dried over anhydrous Na_2SO_4. The crude product was provided after evaporation and recrystalized with EtOAc to give compound **2b** as white solid (5.40 g, 96%); mp 113–115 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 8.41 (dd, J_1 = 8.7 Hz, J_2 = 2.4 Hz, 1H, ArH), 8.35 (d, J = 2.4 Hz, 1H, ArH), 7.88 (d, J = 8.7 Hz, 1H, ArH), 7.60–7.67 (m, 2H, ArH), 7.28 (t, J = 8.4 Hz, 1H, ArH), 4.01 (s, 3H, OCH_3); HRMS (ESI): *m/z*, Calcd for $C_{14}H_{10}ClFNO_4$ [M+H$^+$]: 310.0277, Found 310.0297.

5.1.11.2. (2-Amino-5-nitrophenyl) (3-fluoro-4-methoxyphenyl) methanone (3c). The reaction mixture of compound **2b** (2.9 g, 9.38 mmol) and aqueous ammonia (12 mL) was separated into six tubes and irradiated by microwave (power 100 W, temperature 150 °C, pressure 180 psi) for 3 h respectively. The reaction mixture was diluted with water (20 mL) and adjusted to pH = 7.0 with 10% HCl aqueous solution. After stirring for 30 min, the precipitate was filtered and recrystalized with EtOAc to afford com-

pound **3c** as yellow solid (1.28 g, 47%); mp 182–184 °C; ^{1}H NMR (acetone-d_6) δ (ppm): 8.39 (d, J = 2.4 Hz, 1H, ArH), 8.14 (dd, J_1 = 9.3 Hz, J_2 = 2.7 Hz, 1H, ArH), 7.54–7.58 (m, 2H, ArH), 7.31 (t, J = 8.1 Hz, 1H, ArH), 7.03 (d, J = 9.0 Hz, 1H, ArH), 4.02 (s, 3H, OCH_3); HRMS (ESI): m/z, Calcd for $C_{14}H_{12}FN_2O_4$ [M+H$^+$]: 291.0776, Found 291.0762.

5.1.11.3. Ethyl 2-(2-(3-fluoro-4-methoxybenzoyl)-4-nitrophenylamino)-2-oxoacetate (4c). Following the preparation protocol of Section 5.1.2.1, the reaction mixture of compound **3b** (0.85 g, 2.93 mmol), chlorooxalic acid ethyl ester (0.40 g, 2.93 mol) in toluene (25 mL) was heated at 110 °C for 2 h to give compound **4c** as white solid (0.95 g, 83%); mp 152–154 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm): 11.83 (brs, 1H, NH), 8.82 (d, J = 9.2 Hz, 1H, ArH), 8.56 (d, J = 9.2 Hz, 1H, ArH), 8.52 (d, J = 2.4 Hz, 1H, ArH), 7.71 (m, 2H, ArH), 7.35 (t, J = 8.4 Hz, 1H, ArH), 4.39 (q, J = 7.2 Hz, 2H, CH_2), 4.05 (s, 3H, OCH_3), 1.38 (t, J = 7.2 Hz, 3H, CH_3); HRMS (ESI): m/z, Calcd for $C_{18}H_{16}FN_2O_7$ [M+H$^+$]: 391.0936, Found 391.0948.

5.1.11.4. Ethyl 2-(4-amino-2-(3-fluoro-4-methoxybenzoyl)phenylamino)-2-oxoacetate (5c). Following the preparation protocol of Section 5.1.2.2, the reaction mixture of compound **4c** (0.05 g, 0.12 mmol) and 10% Pd-C (0.01 g) in trifluoroethanol (5.0 mL) was hydrogenated at room temperature and 1 atm for 10 h to provide compound **5c** as yellow solid (0.035 g, 76%); mp 194–195 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.77 (s, 1H, NH), 7.48–7.57 (m, 3H, ArH), 7.29 (t, J = 8.4 Hz, 1H, ArH), 6.78 (dd, J_1 = 8.4 Hz, J_2 = 2.1 Hz, 1H, ArH), 6.67(d, J = 2.4 Hz, 1H, ArH), 5.37 (brs, 1H, NH), 4.20 (q, J = 7.2 Hz, 2H, CH_2), 3.92 (s, 3H, OCH_3), 1.23 (t, J = 7.2 Hz, 3H, CH_3); HRMS (ESI): m/z, Calcd for $C_{18}H_{18}FN_2O_5$ [M+H$^+$]: 361.1194, Found 361.1197.

5.1.11.5. Ethyl 2-(4-(benzo[b]thiophene-2-carboxamido)-2-(3-fluoro-4-methoxybenzoyl)-phenylamino)-2-oxoacetate (6j). Following the preparation protocol of Section 5.1.2.3, the reaction mixture of compound **5c** (0.20 g, 0.56 mmol) and benzo[*b*]thiophene-2-carboxachloride (0.11 g, 0.56 mmol) in toluene (10 mL) was heated at 110 °C for 2 h to give compound **6j** as yellow solid (0.23 g, 79%); mp 242–244 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.21 (brs, 1H, NH), 10.71 (brs, 1H, NH), 8.34 (s, 1H, ArH), 7.96–8.09 (m, 5H, ArH), 7.59 (d, J = 9.6 Hz, 2H, ArH), 7.43–7.51 (m, 2H, ArH), 7.34 (t, J = 8.4 Hz, 1H, ArH), 4.25 (q, J = 6.9 Hz, 2H, CH_2), 3.95 (s, 3H, OCH_3), 1.26 (t, J = 6.9 Hz, 3H, CH_3); HRMS (ESI): m/z, Calcd for $C_{27}H_{22}FN_2O_6S$ [M+H$^+$]: 521.1177, Found 521.1166.

5.1.11.6. 2-(4-(Benzo[*b*]thiophene-2-carboxamido)-2-(3-fluoro-4-methoxybenzoyl)phenyl-amino)-2-oxoacetic acid (7j). Following the preparation protocol of Section 5.1.2.4, the reaction mixture of compound **6j** (0.10 g, 0.20 mmol) and NaOH (0.038 g, 0.96 mmol) in water (5.0 mL) and THF (10.0 mL) was stirred at room temperature for 2 h to give compound **7j** as yellow solid (0.065 g, 68%); mp > 300 °C; ^{1}H NMR(DMSO-d_6) δ (ppm): 11.24 (brs, 1H, NH), 10.73 (brs, 1H, NH), 8.36 (s, 1H, ArH), 8.15 (m, 1H, ArH), 7.97–8.07 (m, 4H, ArH), 7.60–7.64 (m, 2H, ArH), 7.43–7.49 (m, 2H, ArH), 7.34 (t, J = 8.7 Hz, 1H, ArH), 3.95 (s, 3H, OCH_3); HRMS (ESI): m/z, Calcd for $C_{25}H_{18}FN_2O_6S$ [M+H$^+$]: 493.0864, Found 493.0883.

5.1.12. 2-(4-(Benzo[*b*]thiophene-3-carboxamido)-2-(3-fluoro-4-methoxybenzoyl)phenyl-amino)-2-oxoacetic acid (7k)

5.1.12.1. Ethyl 2-(4-(benzo[*b*]thiophene-3-carboxamido)-2-(3-fluoro-4-methoxybenzoyl)-phenylamino)-2-oxoacetate (6k). Following the preparation protocol of Section 5.1.2.3, the reaction mixture of compound **5c** (0.05 g, 0.14 mmol) and benzo[*b*]thiophene-3-carboxachloride (0.027 g, 0.14 mmol) in toluene (3 mL) was heated at 110 °C for 1 h to give compound **6l** as yellow solid (0.058 g, 80%); mp 210–211 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.19 (brs, 1H, NH), 10.56 (brs, 1H, NH), 8.58 (s, 1H, ArH), 8.38 (m, 1H, ArH), 8.06–8.10 (m, 2H, ArH), 7.98 (d, 1H, J = 1.8 Hz, ArH), 7.93 (d, 1H, J = 8.4 Hz, ArH), 7.58 (d, J = 10.2 Hz, 2H, ArH), 7.44 (m, 2H, ArH), 7.33 (t, J = 7.2 Hz, 1H, ArH), 4.24 (q, J = 6.9 Hz, 2H, OCH_2), 3.94 (s, 3H, OCH_3), 1.26 (t, J = 6.9 Hz, 3H, CH_3); HRMS (ESI): m/z, Calcd for $C_{27}H_{22}FN_2O_6S$ [M+H$^+$]: 521.1177, Found 521.1196.

5.1.12.2. 2-(4-(Benzo[*b*]thiophene-3-carboxamido)-2-(3-fluoro-4-methoxybenzoyl)phenyl-amino)-2-oxoacetic acid (7k). Following the preparation protocol of Section 5.1.2.4, the reaction mixture of compound **6k** (0.04 g, 0.08 mmol) and NaOH (0.015 g, 0.38 mmol) in water (4.0 mL) and THF (3.0 mL) was stirred at room temperature for 2 h to give compound **7k** as yellow solid (0.027 g, 71%); mp 262–264 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.20 (brs, 1H, NH), 10.55 (brs, 1H, NH), 8.58 (s, 1H, ArH), 8.38 (m, 1H, ArH), 8.00–8.11 (m, 4H, ArH), 7.61 (d, J = 9.9 Hz, 2H, ArH), 7.44–7.49 (m, 2H, ArH), 7.33 (t, J = 8.4 Hz, 1H, ArH), 3.94 (s, 3H, OCH_3); HRMS (ESI): m/z, Calcd for $C_{25}H_{18}FN_2O_6S$ [M+H$^+$]: 493.0864, Found 493.0878.

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 *E. 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.[18,19] 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.

5.3. Computational studies

All calculations and manipulations were performed using FlexX software package integrated in Sybyl 7.2[20], running on SGI Fuel workstation. The X-ray crystal structure of Pin1 complexed with a carboxylate inhibtor 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 was 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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