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Synthesis and Pin1 inhibitory activity of thiazole derivatives

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Abstract: Pin1 (Protein interacting with NIMA1) is a peptidyl prolyl *cis-trans* isomerase (PPIase) which specifically catalyze the conformational conversion of the amide bond of pSer/Thr-Pro motifs in its subtrate proteins and is a novel promising anticancer target. A series of new thiazole derivatives were designed and synthesized, and their inhibitory activites were measured agaist human Pin1 using a protease-coupled enzyme assay. Of all the tested compounds, a number of thiazole derivatives bearing an oxalic acid group at 4-position were found to be potent Pin1 inhibitors with IC_{50} values at low micromolar level. The detailed structure–activity relationships were analyzed and the binding features of compound **10b** (IC_{50} 5.38 μM) was predicted using CDOCKER program. The results of this research would provide informative guidance for further optimizing thiazole derivatives as potent Pin1 inhibitors.

Keywords: Thiazole derivatives; Pin1; Pin1 inhibitor; PPIase; Anti-cancer agents

1. Introduction

Pin1 (Protein interacting with NIMA1) is a member of the peptidyl prolyl *cis-trans* isomerase (PPIase) family which catalyze the isomerization of the amide bond prior to the proline in its target proteins[1-3]. Pin1 is structurally and fuctionally distinct from other PPIase members such as cyclophilins and FKBPs which were identified as the targets of immunosuppressive drugs cyclosporine A and FK506, respectively[4-11]. Pin1 has its own unique substrate specificity and it isomerizes specific phosphoSer/Thr-Pro motifs and induces conformational changes of its substrate proteins[1, 12-14]. Proline-directed phosphorylation on Ser or Thr is a common regulation mechanism in cells, thus Pin1 plays critical roles in many cellular process including cell cycle progression, cell signalling, cell proliferation and immune responses[15-18].

In comparision with pathological roles of Pin1 in other diseases, such as Alzheimer's disease and cardiovascular disease[19-21], its functions in tumorgenesis and tumor invasion have been investigated more extensively[22-25]. Pin1 is overexpressed in

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various types of cancer cells, including breast, prostate, colon, cervical, liver and esophagus cancers, and overexpression of Pin1 is associated with aggressive tumor progression and poor prognosis in cancer[2, 22-26]. Furthermore, it has been demonstrated that Pin1 as a molecular switch simultaneously impacts multiple oncogenic signaling pathways in tumor cells[3, 27]; therefore, inhibiting Pin1 is expected to be a more effective way for fighting against tumors, especially the aggressive and drug-resistant tumors[1, 28, 29].

Up to now, a number of structurally distinct small-molecule inhibitors of Pin1 have been reported[26, 30-39]. Among them, the most potent Pin1 inhibitor containing an aminophenylpropanol scaffold (**A, Fig. 1** K_i = 0.006 μM) was disclosed by Pfizer[33]. However, it lacked activity versus cells most likely because the phosphate group confers poor permeability[32-34]. In an effort to develop Pin1 inhibitors with improved physicochemical properties, several unnatural amino acid derivatives were found with enzymatic activity at submicromolar level, and their antiproliferative activity in cells at micromolar level (e.g. **B, Fig. 1** K_i = 0.138 μM)[14, 32]. Thus, it is challenging to discover druggable Pin1 inhibitors as anti-cancer agents. Until recently, it was reported that all-trans retinoic acid (ATRA), a therapeutic agent for acute promyelocytic leukemia, was a potent Pin1 inhibitor and its inhibitory effect on Pin1 was responsible for the anti-cancer activity[40]. These results gave more expectations on the development of Pin1 inhibitors for the treatment of cancers.

The crystal structures of some small molecule inhibitors complexed with Pin1 were reported[32-36]. The binding site of catalytic domain features three subpockets, including a prolyl pocket formed with His59, His157, Met130 and Phe13, a slightly shallow hydrophobic shelf lined with Ala118 and Leu22, and a unique phosphate binding pocket embracing Lys63, Arg68 and Arg69. In our previous work, we found that substituted pyrimidine derivatives possessed Pin1 inhibitory activity with IC_{50} values in the double digit micromolar level (e.g. **C, Fig. 1** IC_{50} = 26.7 μM)[39]. Based on the binding features of compound **C** and the known structure-activity relationships[32, 39], we envisioned that the pyrimidine scaffold could be replaced with a thiazole ring, and the thiazole scaffold may be preferable in terms of directing the pharmacophore group into the subpockets. Therefore, we designed a series of novel thiazole derivatives, in which an oxalic acid or acetic acid group was attached onto the 4-position, a small hydrophobic moiety was placed at the 2-position and a bulky aromatic substituent was incorporated into the 5-position. Herein, we present the synthesis and biochemical evaluation of a series of thiazol derivatives as Pin1 inhibitors.

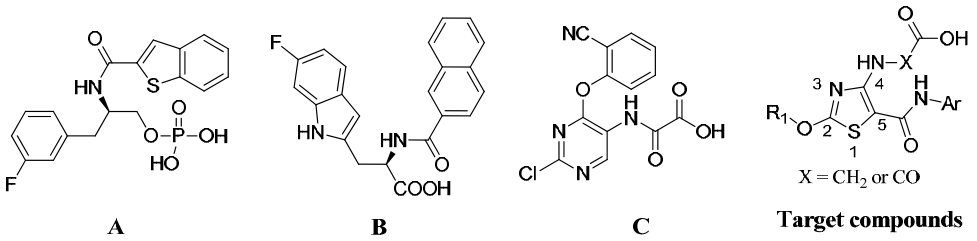
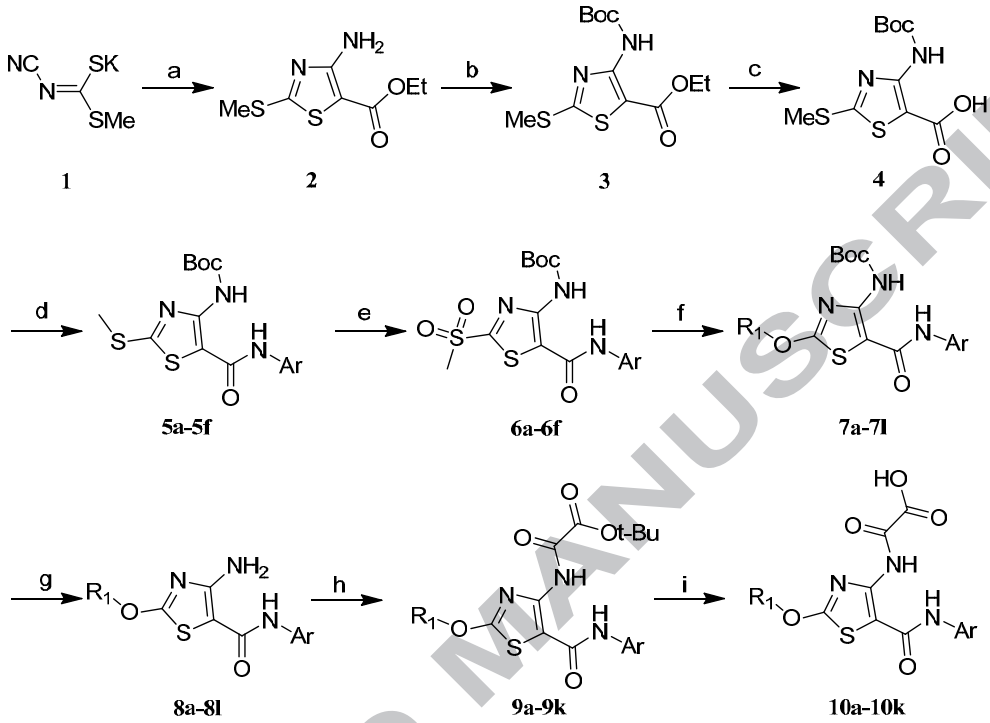


Figure 1 The chemical structures of some known Pin1 inhibitors and the general structure of

designed compounds

2. Chemistry



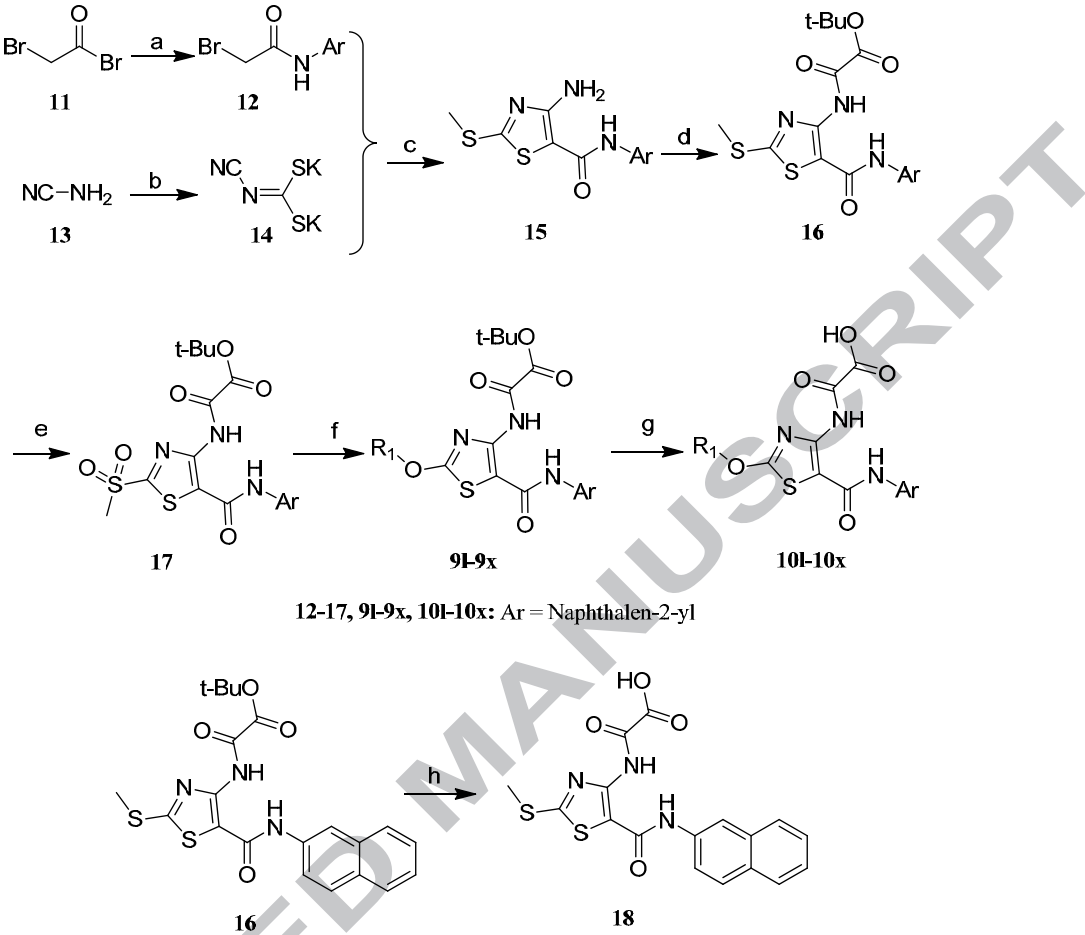
5a, 6a, 7a-7d, 8a-8d, 9a-9d, 10a-10d: Ar = Naphthalen-2-yl; **5b, 6b, 7e-10e, 7f-10f:** Ar = 4-PhOPh; **5c, 6c, 7g-10g, 7h-10h, 7l-8l:** Ar = 4-PhPh; **5d, 6d, 7i-10i:** Ar = 3,5-Dichlorobenzyl; **5e, 6e, 7j-10j:** Ar = 3,5-Bis(trifluoromethyl)benzyl; **5f, 6f, 7k-10k:** Ar = 2,6-Dichlorophenethyl.

Scheme 1: Reagents and conditions (a) ethyl bromoacetate, triethylamine, ethanol, 93%; (b) NaH, $(Boc)_2O$, THF, 90%; (c) NaOH, THF, H_2O 45°C, 90%; (d) EDC, HOAt, DMAP, DIEA, DCM, DMF, 50%-72%; (e) mCPBA, $CHCl_3$, reflux, 50%-78%; (f) R_1OH, NaH, DMF, 26%-86%; (g) TFA, DCM, 61%-94%; (h) tert-butyl 2-chloro-2-oxoacetate, toluene, 58%-93%; (i) TFA, DCM, 59%-92%.

The synthesis of target compounds (**10a–10x** and **19a–19e**) was depicted in Schemes 1–3, and their chemical structures were shown in Tables 1-2. Initially, we took the synthetic route as presented in scheme 1 to construct the target compounds.

A mixture of potassium methyl cyanimidodithiocarbonate **1,** ethyl bromoacetate and triethylamine in ethanol was heated to give rise to the key thiazole scaffold **2** in 93% yield. Upon subsequent Boc protection, hydrolysis of the ethyl ester, condensation with an array of primary amines, and oxidation of the methylthio group, compound **2** was converted into compounds **6a-6f** smoothly. In the presence of NaH and various substituted phenols in DMF, compounds **7a-7l** were prepared by the nucleophilic substitution reaction, which were further transformed into compounds **8a-8l** by removal of the Boc group with trifluoroacetic acid. The acylation of amines **8a-8k** with tert-butyl 2-chloro-2-oxoacetate in toluene afforded compounds **9a-9k**, which were subsequently transformed into target compounds **10a**-**10k** by treatment of

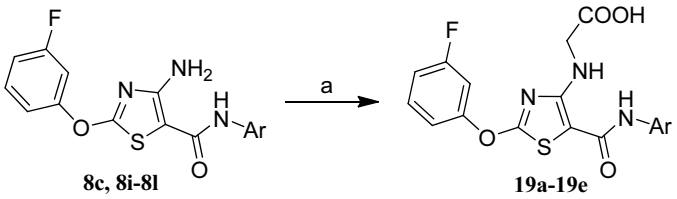
with TFA.



Scheme 2: Reagents and conditions (a) $ArNH_2$, Py, DCM, 41%; (b) 0°C, KOH, CS_2, ethanol, 72%; (c) i) 0-25°C, acetone, water, ii) ethanol, MeI, 73%; (d) tert-butyl 2-chloro-2-oxoacetate, tolueme, 92%; (e) m-CPBA, $CHCl_3$, 44%; (f) R_1OH, NaH, DMF, 41%-72%; (g) TFA, DCM, 69%-97%; (h) TFA, DCM, 69%.

During the course of synthesis using the first synthetic route, we found that methylsulfonyl compounds **6a-6f**, particularly **6a** had poor solubility that was troublesome for purification. Thus we tried an alternative synthetic route as shown in Scheme 2. In this approach, naphthylamine was first coupled with 2-bromoacetyl bromide to give rise to the intermediate **12** in 41% yield, and then 2-bromo-acetamide **12** was reacted with potassium cyanocarbonimidodithioate **14** [41] forming the key intermediate **15** in 72% yield. The acylation of amine **15** with tert-butyl 2-chloro-2-oxoacetate in toluene proceeded smoothly to afford compound **16**, which was subsequently transformed into target compounds **10l-10x** in three steps by oxidation, substitution and deprotection. In fact, compared with the first synthetic method, this approach was more concise and efficient.

In addition, with an aim to explore SAR on 2-position of thiazole ring, compound **18** was prepared by removing the t-butyl group from compound **16** using TFA.



8c, 19a: Ar = Naphthalen-2-yl
8i, 19b: Ar = 3,5-Dichlorobenzyl
8j, 19c: Ar = 3,5-Bis(trifluoromethyl)benzyl
8k, 19d: Ar = 2,6-Dichlorophenethyl
8l, 19e: Ar = 4-PhPh

Scheme 3: Reagents and conditions (a) glyoxylic acid, $NaBH_3CN$, HOAc, MeOH/THF, 38%-88%.

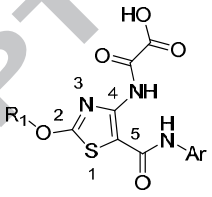
The acetic acid substituted target compounds **19a-19e** were synthesized starting from intermediates **8c** and **8i-8l** (Scheme 3). In the presence of sodium cyanoborohydride and acetic acid using MeOH and THF as solvents, compounds **8c** and **8i-8l** reacted with glyoxylic acid by reductive amination giving compounds **19a-19e** (38%-88% yield).

3. Biological results and discussion

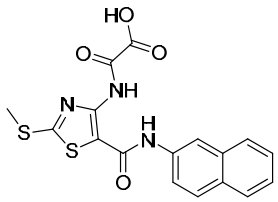
All target compounds were screened against Pin1 by a protease-coupled enzyme assay with Suc-Ala-Glu-Pro-Phe-pNA as the substrate[42, 43]. The reported Pin1 inhibitors compound **A** and compound **B** were used as reference molecules. The inhibitory activities were expressed as IC_{50} values and presented in Tables 1-2.

Table 1

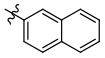
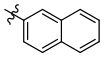
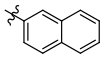
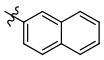
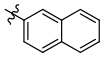
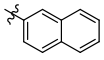
The chemical structures and inhibitory activities against hPin1 of compounds **10a**-**10x** and **18**

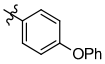
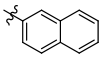
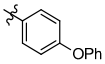
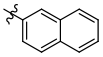
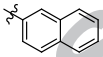
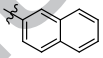
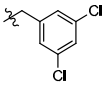
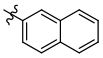
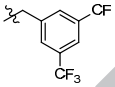
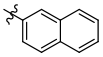
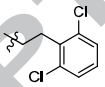
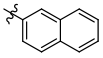
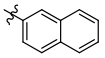
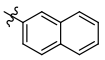


10



18

Cpd.	R_1	Ar	IC_{50}±SD(μM) [a]	Cpd.	R_1	Ar	IC_{50}±SD(μM) [a]
10a			4.57±1.14	**10n**			6.04±3.35
10b			5.38±0.35	**10o**			5.02±0.90
10c			5.50±4.23	**10p**			5.42±1.15

10d			4.68±0.41	**10q**			13.4±2.74
10e			9.44±2.96	**10r**			4.67±0.99
10f			5.18±2.32	**10s**			9.35±1.23
10g			3.15±0.65	**10t**			8.11±0.46
10h			2.93±0.39	**10u**			11.1±0.70
10i			25.8±27.2%[b]	**10v**			23.4±4.98
10j			51.1±6.43%[b]	**10w**			14.8±0.29
10k			53.3±3.56%[b]	**10x**			21.1±3.15
10l			48.9±2.26%[b]	**18**			-11.6±8.38%[b]
10m			3.22±1.20				

[a]. The measured IC_{50} for compound **A** was 0.05 μM, the reported K_i for compound **A** was 0.006 μM; the measured IC_{50} for compound **B** was 2.96 μM, the reported K_i for compound **B** was 0.138 μM; SD, standard deviation of two independent assays.

[b]. The percentage inhibition at the concentration of 100 μM of tested compounds.

As shown in Table 1, we initially investigated the SARs on the substituents at 2-position of thiazole ring by taking the naphthyl fragment as the bulky aromatic

group attached on the 5-position. When phenyl or mono-substituted phenyl group was introduced as R_1 group, the corresponding compounds (**10a**-**10d** and **10l-10t**) exhibited varied inhibitory activities against Pin1. Among them, most mono-substituted compounds showed similar potency with unsubstituted compound **10a**, they had IC_{50} values in the single digit micromolar level (IC_{50} 3.22-9.35 μM). It was noted that 2-chloro substituted compound **10q** had somewhat lower activity (IC_{50} 13.4 μM), and 2-methyl substituted compound **10l** displayed the weakest inhibition on Pin1 (48.9% @ 100 μM) in this series. Collectively, these results suggest that placement of a substituent on the 3-position of the benzene ring was allowed and incorporation of a fragment at the 2-position of the benzene ring is unfavorable for potency.

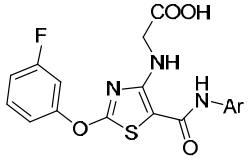
In addition, three 2, 5-disubstituted compounds (**10u**-**10w**) were also tested and their IC_{50}s varied from 11.1 μM to 23.4 μM. It further suggested that introducing a substituent on 2-position on the benzene ring would lead to reduction in potency in comparison with the corresponding mono-substituted compounds **10b** and **10c**.

Interestingly, when an isobutyl group substituted for the phenyl moiety as R_1 group, compound **10x** possessed inhibitory activity as well, although its potency (IC_{50} 21.1 μM) was lower than that of compound **10a**. This result gave an indication that alkyl moieties could be used as R_1 group to snugly fit into the prolyl pocket. This might offer a chance to further improve both potency and physicochemical properties. With an aim to expanding the SARs on 2-substitutents, we also tested the enzymatic activity of compound **18**. It had no inhibition on Pin1, presumably due to the methylthio group that cannot form a hydrogen bond with Pin1, compared with compound **10x**.

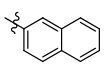
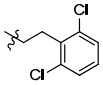
The preliminary SAR of 5-substituents on thiazole ring was also explored. The incorporation of bulky aromatic groups, such as 4-phenoxyphenyl (**10e** and **10f**) and 4-biphenyl moieties (**10g** and **10h**) through an amide linker resulted in comparable potency with naphthyl substituted compounds **10a** and **10b**. However, the replacement of naphthyl group of compound **10c** with 3, 5-dichlorobenzyl (**10i**), 3, 5-bis(trifluoromethyl)benzyl (**10j**) or 2, 6-dichlorophenethyl (**10k**) group led to reduction in inhibition markedly.

Table 2

The chemical structures and inhibitory activities against hPin1 of compounds **19a-19e**



19

Cpd.	Ar	IC_{50}±SD (μM) [a]	Cpd.	Ar	IC_{50}±SD (μM) [a]
19a		27.7±10.8%[b]	**19d**		5.41±7.64%[b]

19b	14.0	5.52	**19e**	9.40	2.43% [b]
19c	18.1	3.54			

[a]. The measured IC_{50} for compound **A** was 0.05 μM, the reported K_i for compound **A** was 0.006 μM; the measured IC_{50} for compound **B** was 2.96 μM, the reported K_i for compound **B** was 0.138 μM; SD, standard deviation of two independent assays.

[b]. The percentage inhibition at the concentration of 100 μM of tested compounds.

We substituted the acetic acid group for an oxalic acid moiety producing compounds **19a-19e** (Table 2). Among them, the potency of compounds **19a**, **19d** and **19e** were noticeably lost. Interestingly, compounds **19b** and **19c** had inhibitory activity and their IC_{50}s were 14.0 μM and 18.1 μM, respectively. From these data, it seemed that an oxalic acid group was more favorable than the acetic acid counterpart to interact with Pin1.

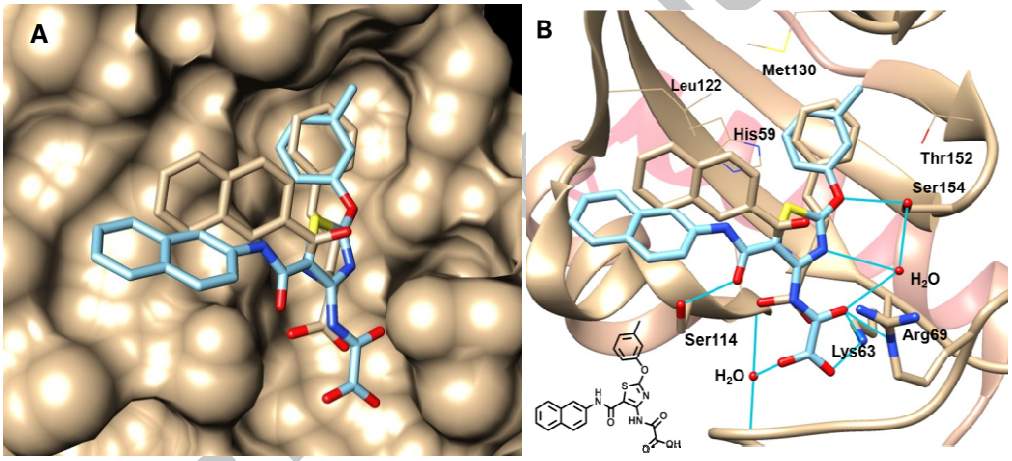


Figure 2. CDOCKER-modeled binding mode of compound **10b** (carbon atoms colored blue) in comparison with the co-crystal structure (3JYJ in PDB)[34] of a carboxylate Pin1 inhibitor (carbon atoms colored brown). (A) The binding pose of compound **10b** within the binding site of Pin1; (B) The interactions of compound **10b** with key amino acids within the binding stie. H-Bonding interactions were presented with blue lines. Molecular image was generated with UCSF Chimera[44].

With an aim to probe the binding features of the thiazole-based Pin1 inhibitors, molecular docking was performed using CDOCER protocol integrated in Accelrys Discovery Studio 2.5[45]. The coordinates of X-ray co-crystal structure of a carboxylate inhibitor (reference molecule) with Pin1 (PDB code: 3JYJ) reported by Pfizer in 2010[34] was employed for docking the thiazole derivatives. The binding modes of the oxalic acid substituted thiazoles were somewhat similar and exemplified by the representative compound **10b** as shown in Figure 2. Compound **10b** could nicely situate in the binding pocket and took a very similar binding pose with the reference molecule.

The carboxylate of oxalic acid interacted with the positive charged side chain of

Lys63 via the key charge-charge interaction. The 2-carbonyl oxygen of oxalic acid formed a hydrogen bond with Arg 69 and a hydrogen bond with Ser154 mediated by a water molecule. Therefore, the oxalic acid moiety played a critical role in the binding and could be a choice for replacement of the phosphorus acid group to improve the potency and physicochemical properties of Pin1 inhibitors. It was noticed that the oxygen atom as an ether linker could interact with Ser154 via a hydrogen bond as we intended, since the cocrystal structures of known Pin1 inhibitors have demonstrated that Ser154 had a key role in the binding[32, 34]. In fact, compound **18** bearing a methylthio group on the 2-poistion of the thiazole scaffold was inactive, a result consistent with the oxygen atom on the 2-position benefical for binding. In addition, the nitrogen atom on the thiazole ring also formed an H-bond with Ser154 via a water molecule. This may further contribute to the favorable binding affinity.

The hydrophobic 3-methylbenzene ring occupied the prolyl pocket consisting of Leu122, Phe134 and Met130 residues, the bulky naphthalene ring extended to the hydrophobic shelf including the side chains of Leu122 and Cys113. These hydrophobic interactions had positive contributions to the binding affinity as we and other groups have demonstrated[34, 39].

4. Conclusion

In summary, based on the pyrimidine Pin1 inhibitors we found previously, a series of novel thiazole derivatives containing an oxalic acid or an acetic acid group was designed and synthesized. The enzymatic assay was performed against Pin1 and some of compounds displayed potent inhibitory activity with IC_{50} values at the micromolar level. The preliminary SAR and the binding features of the thiazole derivatives were analyzed. These results suggest that an oxalic acid moiety could be useful in developing potent Pin1 inhibitors, and the thiazole ring could serve as an appropriate scaffold for discovering structurally novel and drug-like Pin1 inhibitors. These results will provide guidance for further modifications to achieve more potent thiazole derivatives as Pin1 inhibitors.

5. Experimental section

5.1. General

Melting points were measured on a Yanaco micro melting point apparatus and are uncorrected. ^{1}H NMR (300 MHz or 400 MHz) on a Varian Mercury 300 or 400 spectrometer was recorded in DMSO-d_6, acetone-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).

5.2. Synthesis of compounds 10a-10k

5.2.1

2-((5-(Naphthalen-2-ylcarbamoyl)-2-phenoxythiazol-4-yl)amino)-2-oxoacetic acid (10a)

To a stirred solution of **9a** (80 mg, 0.16 mmol) in DCM (15 mL) was added TFA (1.5 mL) dropwise, the reaction mixture was then allowed to stir at room temperature overnight. DCM and excess TFA were removed under reduced pressure, dichloromethane (2 mL) and petroleum ether (5 mL) were then added to the residue. After filtration and drying under high vacuum, compound **10a** was obtained as a yellow solid without further purification (50 mg, 74%); mp 155-157 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.94 (brs, 1H), 10.13 (s, 1H), 8.23 (s, 1H), 7.85 (t, J = 7.2 Hz, 3H), 7.67 (d, J = 8.8 Hz, 1H), 7.53-7.61 (m, 4H), 7.40-7.49 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.81, 161.18, 160.52, 154.22, 147.01, 135.71, 133.10, 130.71, 130.14, 128.12, 127.56, 127.42, 126.41, 125.05, 121.19, 120.92, 117.55; HRMS (ESI) Calcd. for $C_{22}H_{16}N_3O_5S$ $[M+H]^+$: 434.0805; Found: 434.0808.

5.2.2 2-((5-(Naphthalen-2-ylcarbamoyl)-2-(*m*-tolyloxy)thiazol-4-yl)amino)-2-oxoacetic acid (10b)

Following the preparation protocol of compound **10a**, starting from compound **9b** (90 mg, 0.18 mmol), the title compound **10b** was obtained as a yellow solid (70 mg, 88%); mp 123-125 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.96 (brs, 1H), 10.13 (s, 1H), 8.24 (s, 1H), 7.84-7.89 (m, 3H), 7.68 (dd, J_1 = 8.7 Hz, J_2 = 1.5 Hz,1H), 7.41-7.52 (m, 3H), 7.31-7.39 (m, 2H), 7.28 (d, J = 7.2 Hz, 1H), 2.39 (s, 3H); HRMS (ESI) Calcd. for $C_{23}H_{18}N_3O_5S$ $[M+H]^+$: 448.0962; Found: 448.0963.

5.2.3

2-((2-(3-Fluorophenoxy)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)amino)-2-oxo acetic acid (10c)

Following the preparation protocol of compound **10a**, starting from compound **9c** (70 mg, 0.14 mmol), the title compound **10c** was obtained as a yellow solid (54 mg, 87%); mp 98-100 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.92 (s, 1H), 10.18 (s, 1H), 8.24 (s, 1H), 7.84-7.89 (m, 3H), 7.68 (d, J = 9.9 Hz, 1H); 7.58-7.63 (m,

2H), 7.40-7.50 (m, 3H), 7.30 (t, J = 8.7 Hz, 1H); HRMS (ESI): *m/z*, calcd. for $C_{22}H_{15}N_3O_5FS$ $[M+H]^+$: 452.0711, Found: 452.0713.

5.2.4

2-((2-(3-Methoxyphenoxy)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)amino)-2-oxoacetic acid (10d)

Following the preparation protocol of compound **10a**, starting from compound **9d** (60 mg, 0.12 mmol), the title compound **10d** was obtained as a yellow solid (47 mg, 89%); mp 106-108 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.96 (brs, 1H), 10.13 (s, 1H), 8.24 (s, 1H), 7.83-7.88 (m, 3H), 7.68 (d, J = 7.2 Hz, 1H), 7.40-7.51 (m, 3H), 7.19 (s, 1H), 7.09 (d, J = 7.8 Hz, 1H), 7.01 (d, J = 6.6 Hz, 1H), 3.81 (s, 3H); HRMS (ESI) Calcd. for $C_{23}H_{18}N_3O_6S$ $[M+H]^+$: 464.0911; Found: 464.0900.

5.2.5 2-((2-Phenoxy-5-((4-phenoxyphenyl)carbamoyl)thiazol-4-yl)amino)-2-oxoacetic acid (10e)

Following the preparation protocol of compound **10a**, starting from compound **9e** (100 mg, 0.19 mmol), the title compound **10e** was obtained as a light yellow solid (67 mg, 75%); mp 123-125 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.95 (brs, 1H), 9.96 (s, 1H), 7.34-7.61 (m, 9H), 7.11 (t, J = 6.9 Hz, 1H), 6.97-6.99 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.71, 161.14, 160.23, 157.00, 154.20, 152.70, 146.90, 133.67, 130.70, 129.96, 127.54, 123.15, 122.92, 120.90, 119.00, 118.11; HRMS (ESI): *m/z*, calcd. for $C_{24}H_{18}N_3O_6S$ $[M+H]^+$: 476.0911, Found: 476.0904.

5.2.6

2-((5-((4-Phenoxyphenyl)carbamoyl)-2-(m-tolyloxy)thiazol-4-yl)amino)-2-oxoacetic acid (10f)

Following the preparation protocol of compound **10a**, starting from compound **9f** (45 mg, 0.08 mmol), the title compound **10f** was obtained as a yellow solid (37 mg, 92%); mp 124-127 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.94 (s, 1H), 9.94 (s, 1H), 7.60 (d, J = 8.7 Hz, 2H), 7.24-7.48 (m, 6H), 7.10 (t, J = 6.9 Hz, 1H), 6.98 (d, J = 8.7 Hz, 4H), 2.37 (s, 3H); HRMS (ESI): *m/z*, calcd. for $C_{25}H_{20}N_3O_6S$ $[M+H]^+$: 490.1067, Found: 490.1070.

5.2.7

2-((5-([1,1'-Biphenyl]-4-ylcarbamoyl)-2-phenoxythiazol-4-yl)amino)-2-oxoacetic acid (10g)

Following the preparation protocol of compound **10a**, starting from compound **9g** (70 mg, 0.14 mmol), the title compound **10g** was obtained as a yellow solid (50 mg, 81%); mp 139-141 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.96 (s, 1H), 10.02 (s, 1H), 7.52-7.78 (m, 10H), 7.42-7.45 (m, 3H), 7.32 (d, J = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.80, 160.35, 154.22, 147.08, 139.55, 137.58, 135.79, 130.72, 128.88, 127.56, 127.14, 126.75, 126.29, 121.33, 120.93; HRMS (ESI): m/z, calcd. for $C_{24}H_{18}N_3O_5S$ $[M+H]^+$: 460.0962, Found: 460.0961.

5.2.8 2-((5-([1,1'-Biphenyl]-4-ylcarbamoyl)-2-(*m*-tolyloxy)thiazol-4-yl)amino)-2--oxoacetic acid (10h)

Following the preparation protocol of compound **10a**, starting from compound **9h** (60 mg, 0.16 mmol), the title compound **10h** was obtained as a yellow solid (40 mg, 76%); mp 112-114 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.96 (brs, 1H), 10.01 (s, 1H), 7.63-7.72 (m, 6H), 7.41-7.49 (m, 3H), 7.22-7.35 (m, 4H), 2.38 (s, 3H); HRMS (ESI) Calcd. for $C_{25}H_{20}N_3O_5S$ $[M+H]^+$: 474.1118; Found: 474.1109.

5.2.9

2-((5-((3,5-Dichlorobenzyl)carbamoyl)-2-(3-fluorophenoxy)thiazol-4-yl)amino)-2-oxoacetic acid (10i)

Following the preparation protocol of compound **10a**, starting from compound **9i** (120 mg, 0.22 mmol), the title compound **10i** was obtained as a grey white solid (90 mg, 84%); mp 102-104 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.90 (brs, 1H), 8.84 (t, J = 5.6 Hz, 1H), 7.55-7.63 (m, 2H), 7.49 (s, 1H), 7.38 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H), 7.33 (d, J = 2.0 Hz, 1H), 7.28 (td, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), 4.39 (d, J = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 172.29, 162.38 (d, J = 259.1 Hz), 161.60, 161.23, 154.72 (d, J = 11.1 Hz), 146.05, 143.35 , 133.91, 131.86 (d, J = 9.2 Hz), 126.55 , 126.04 , 116.97 (d, J = 3.0 Hz), 114.21 (d, J = 20.7 Hz), 108.91 (d, J = 27.5 Hz), 41.64; HRMS (ESI) Calcd. for $C_{19}H_{13}N_3O_5Cl_2FS$ $[M+H]^+$: 483.9932; Found: 483.9923.

5.2.10

2-((5-((3,5-Bis(trifluoromethyl)benzyl)carbamoyl)-2-(3-fluorophenoxy)thiazol-4-yl)amino)-2-oxoacetic acid (10j)

Following the preparation protocol of compound **10a**, starting from compound **9j** (90 mg, 0.171 mmol), the title compound **10j** was obtained as a light yellow solid (50 mg, 59%); mp 101-103 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.85 (brs, 1H), 8.91 (t, J = 6.0 Hz, 1H), 8.00 (s, 3H), 7.56-7.63 (m, 2H), 7.39 (d, J = 8.0 Hz, 1H), 7.28 (t, J = 8.4 Hz, 1H), 4.57 (d, J = 5.6 Hz, 2H); HRMS (ESI) Calcd. for $C_{21}H_{13}N_3O_5Cl_2F_7S$ $[M+H]^+$: 552.0459; Found: 552.0450.

5.2.11 2-((5-((2,6-Dichlorophenethyl)carbamoyl)-2-(3-fluorophenoxy)thiazol-4-yl)-amino)-2-oxoacetic acid (10k)

Following the preparation protocol of compound **10a**, starting from compound **9k** (95 mg, 0.171mmol), the title compound **10k** was obtained as a pale yellow solid (50 mg, 59%); mp 104-106 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.94 (brs, 1H), 8.48 (brs, 1H), 7.54-7.64 (m, 2H), 7.45 (d, J = 8.0 Hz, 2H), 7.38 (d, J = 8.0 Hz, 1H), 7.28 (t, J = 8.0 Hz, 2H), 3.40-3.42 (m, 2H), 3.10 (t, J = 6.0 Hz, 2H); HRMS (ESI) Calcd. for $C_{20}H_{15}N_3O_5Cl_2FS$ $[M+H]^+$: 498.0088; Found: 498.0088.

5.3. Synthesis of compounds 10l-10x and 18

5.3.1

2-((5-(Naphthalen-2-ylcarbamoyl)-2-(*o*-tolyloxy)thiazol-4-yl)amino)-2-oxoacetic acid (10l)

Following the preparation protocol of compound **10a**, starting from compound **9l** (120 mg, 0.238 mmol), the title compound **10l** was obtained as a yellow solid (75 mg, 71.4%); mp 169-171 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.98 (brs, 1H), 10.11 (s, 1H), 8.24 (s, 1H), 7.84-7.88 (m, 3H), 7.67 (d, J = 8.8 Hz, 1H), 7.36-7.51 (m, 6H), 2.28 (s, 3H); HRMS (ESI) Calcd. for $C_{23}H_{18}N_3O_5S$ $[M+H]^+$: 448.0962; Found: 448.0948.

5.3.2

2-((5-(Naphthalen-2-ylcarbamoyl)-2-(*p*-tolyloxy)thiazol-4-yl)amino)-2-oxoacetic acid (10m)

Following the preparation protocol of compound **10a**, starting from compound **9m** (70 mg, 0.14 mmol), the title compound **10m** was obtained as a yellow solid (51 mg, 82%); mp 124-126 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.95 (brs, 1H), 10.09 (s, 1H), 8.23 (s, 1H), 7.83-7.87 (m, 3H), 7.65-7.68 (m, 1H), 7.36-7.50 (m, 6H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 174.40, 161.24, 160.59, 152.20, 147.34, 137.21, 135.76, 133.10, 131.02, 130.81, 130.12, 128.10, 127.41, 126.40, 125.02, 121.17, 120.77, 117.50, 20.44; HRMS (ESI) Calcd. for $C_{23}H_{18}N_3O_5S$ $[M+H]^+$: 448.0962; Found: 448.0952.

5.3.3

2-((2-(2-Methoxyphenoxy)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)amino)-2-oxoacetic acid (10n)

Following the preparation protocol of compound **10a**, starting from compound **9n** (70 mg, 0.14 mmol), the title compound **10n** was obtained as a light yellow solid (45 mg, 94%); mp 139-141 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.94 (brs, 1H), 10.09 (s, 1H), 8.24 (s, 1H), 7.83-7.87 (m, 3H), 7.68 (d, J = 8.4 Hz, 1H), 7.42-7.54 (m, 4H), 7.34 (d, J = 8.0 Hz, 1H), 7.12 (t, J = 7.2 Hz, 1H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 174.60, 161.23, 160.53, 150.79, 146.98, 142.48, 135.75, 133.10, 130.12, 129.07, 128.10, 127.41, 126.40, 125.02, 122.69, 121.41, 121.18, 117.48, 114.20, 109.29, 56.06; HRMS (ESI) Calcd. for $C_{23}H_{18}N_3O_6S$ $[M+H]^+$: 464.0911; Found: 464.0902.

5.3.4

2-((2-(2-Fluorophenoxy)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)amino)-2-oxoacetic acid (10o)

Following the preparation protocol of compound **10a**, starting from compound **9o** (80 mg, 0.16 mmol), the title compound **10o** was obtained as a yellow solid (65 mg, 92%); mp 124-126 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.85 (brs, 1H), 10.20 (s, 1H), 8.25 (s, 1H), 7.85-7.90 (m, 3H), 7.67-7.76 (m, 2H), 7.37-7.60 (m, 6H); HRMS (ESI) Calcd. for $C_{22}H_{15}N_3O_5FS$ $[M+H]^+$: 452.0711; Found: 452.0700.

5.3.5

2-((2-(3-Chlorophenoxy)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)amino)-2-oxo acetic acid (10p)

Following the preparation protocol of compound **10a**, starting from compound **9p** (80 mg, 0.15 mmol), the title compound **10p** was obtained as a yellow solid (69 mg, 97%); mp 177-180 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.94 (brs, 1H), 10.19 (s, 1H), 8.26 (s, 1H), 7.86-7.90 (m, 3H), 7.80 (s, 1H), 7.70 (d, J = 8.8 Hz, 1H), 7.62 (t, J = 8.0 Hz, 1H), 7.42-7.56 (m, 5H); HRMS (ESI) Calcd. for $C_{22}H_{15}N_3O_5ClS$ $[M+H]^+$: 468.0416; Found: 468.0407.

5.3.6

2-((2-(2-Chlorophenoxy)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)amino)-2-oxo acetic acid (10q)

Following the preparation protocol of compound **10a**, starting from compound **9q** (70 mg, 0.13 mmol), the title compound **10q** was obtained as a light yellow solid (58 mg, 94%); mp 132-134 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.89 (brs, 1H), 10.18 (s, 1H), 8.25 (s, 1H), 7.85-7.89 (m, 3H), 7.78 (dd, J_1 = 3.0 Hz, J_2 = 1.8 Hz, 1H), 7.75 (dd, J_1 = 3.3 Hz, J_2 = 1.8 Hz, 1H), 7.68 (dd, J_1 = 9.0 Hz, J_2 = 1.8 Hz, 1H), 7.58 (dt, J_1 = 7.8 Hz, J_2 = 1.8 Hz, 1H), 7.42-7.53 (m, 3H); HRMS (ESI) Calcd. for $C_{22}H_{15}N_3O_5ClS$ $[M+H]^+$: 468.0416; Found: 468.0407.

5.3.7

2-((2-(3-Cyanophenoxy)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)amino)-2-oxo acetic acid (10r)

Following the preparation protocol of compound **10a**, starting from compound **9r** (40 mg, 0.09 mmol), the title compound **10r** was obtained as a pale yellow solid (32 mg, 89%); mp 180-183 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.92 (brs, 1H), 10.23 (s, 1H), 8.26 (s, 1H), 8.21 (s, 1H), 7.86-7.95 (m, 5H), 7.78 (t, J = 8.1 Hz, 1H), 7.70 (d, J = 7.5 Hz, 1H), 7.37-7.53 (m, 3H); HRMS (ESI) Calcd. for $C_{23}H_{15}N_4O_5S$ $[M+H]^+$: 459.0758; Found: 459.0748.

5.3.8 2-((5-(Naphthalen-2-ylcarbamoyl)-2-(3-(trifluoromethyl) phenoxy)thiazol-4--yl)amino)-2-oxoacetic acid (10s)

Following the preparation protocol of compound **10a**, starting from compound **9s** (95 mg, 0.17 mmol), the title compound **10s** was obtained as a yellow solid (70 mg,

83%); mp 178-181 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.94 (brs, 1H), 10.20 (s, 1H), 8.25 (s, 1H), 8.06 (s, 1H), 7.81-7.90 (m, 7H), 7.70 (d, J = 8.8 Hz, 1H), 7.42-7.51 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 172.48, 161.15, 160.37, 154.12, 146.46, 135.67, 133.11, 131.88, 131.05 (d, J = 32.1 Hz), 130.19, 128.16, 127.44, 126.43, 125.27, 125.09, 123.93, (d, J = 2.9 Hz), 121.23, 118.17 (d, J = 4.4 Hz), 117.63; HRMS (ESI) Calcd. for $C_{23}H_{15}N_3O_5F_3S$ $[M+H]^+$: 502.0679; Found: 502.0669.

5.3.9 2-((5-(Naphthalen-2-ylcarbamoyl)-2-(2-(trifluoromethyl) phenoxy)thiazol-4--yl)amino)-2-oxoacetic acid (10t)

Following the preparation protocol of compound **10a**, starting from compound **9t** (130 mg, 0.23 mmol), the title compound **10t** was obtained as a light yellow solid (80 mg, 69%); mp 138-140 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.87 (brs, 1H), 10.22 (s, 1H), 8.26 (s, 1H), 7.85-7.98 (m, 6H), 7.67 (d, J = 8.1 Hz, 2H), 7.41-7.52 (m, 2H); HRMS (ESI) Calcd. for $C_{23}H_{15}F_3N_3O_5S$ $[M+H]^+$: 502.0679; Found: 502.0680.

5.3.10 2-((2-(2-Fluoro-5-methylphenoxy)-5-(naphthalen-2-ylcarbamoyl)thiazol--4-yl)amino)-2-oxoacetic acid (10u)

Following the preparation protocol of compound **10a**, starting from compound **9u** (100 mg, 0.19 mmol), the title compound **10u** was obtained as a pale yellow solid (72 mg, 81%); mp 124-126 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.88 (brs, 1H), 10.19 (s, 1H), 8.26 (s, 1H), 7.84-7.90 (m, 3H), 7.67 (d, J = 8.8 Hz, 1H), 7.54 (d, J = 7.2 Hz, 1H), 7.41-7.52 (m, 4H), 7.29 (brs, 1H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 172.77, 161.13, 160.21, 149.90, 146.16, 140.42 (d, J = 12.9 Hz), 135.78 (d, J = 3.4 Hz), 134.40 (d, J = 260.3 Hz), 130.16, 129.39 (d, J = 6.7 Hz), 128.15, 127.43, 126.43, 125.06, 123.67, 121.15, 117.50, 117.30 (d, J = 17.5 Hz), 20.15; HRMS (ESI) Calcd. for $C_{23}H_{17}N_3O_5FS$ $[M+H]^+$: 466.0868; Found: 466.0861.

5.3.11 2-((2-(5-Fluoro-2-methylphenoxy)-5-(naphthalen-2-ylcarbamoyl)thiazol--4-yl)amino)-2-oxoacetic acid (10v)

Following the preparation protocol of compound **10a**, starting from compound **9v** (80 mg, 0.15 mmol), the title compound **10v** was obtained as a yellow solid (65 mg, 92%); mp 124-126 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.94 (s, 1H), 10.15 (s, 1H), 8.25 (s, 1H), 7.84-7.90 (m, 3H), 7.68 (dd, J_1 = 8.7 Hz, J_2 = 1.8 Hz, 1H), 7.57 (dd, J_1 = 9.0 Hz, J_2 = 2.4 Hz, 1H), 7.41-7.52 (m, 3H), 7.25 (td, J_1 = 8.4 Hz, J_2 = 2.7 Hz, 1H), 2.24 (s, 3H); HRMS (ESI) Calcd. for $C_{23}H_{17}N_3O_5FS$ $[M+H]^+$: 466.0868; Found: 466.0860.

5.3.12 2-((2-(2,5-Difluorophenoxy)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl) amino)-2-oxoacetic acid (10w)

Following the preparation protocol of compound **10a**, starting from compound **9w** (60 mg, 0.11 mmol), the title compound **10w** was obtained as a yellow solid (50 mg, 93%); mp 118-120 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.83 (s, 1H), 10.25 (s, 1H), 8.26 (s, 1H), 7.84-7.91 (m, 4H), 7.59-7.72 (m, 3H), 7.37-7.53 (m, 3H); HRMS (ESI) Calcd. for $C_{22}H_{14}N_3O_5F_2S$ $[M+H]^+$: 470.0617; Found: 470.0609.

5.3.13 2-((2-Isobutoxy-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)amino)-2-oxoacetic acid (10x)

Following the preparation protocol of compound **10a**, starting from compound **9x** (140 mg, 0.30 mmol), the title compound **10x** was obtained as a light yellow solid (85 mg, 70%); mp 170-172 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.89 (brs, 1H), 10.14 (s, 1H), 8.26 (s, 1H), 7.87-7.91 (m, 3H), 7.72 (d, J = 8.4 Hz, 1H), 7.43-7.52 (m, 2H), 4.27 (d, J = 6.4 Hz, 2H), 2.16 (hept, J = 6.4 Hz, 1H), 0.99 (d, J = 6.8 Hz, 6H); HRMS (ESI) Calcd. for $C_{20}H_{20}N_3O_5S$ $[M+H]^+$: 414.1118; Found: 414.1107.

5.3.14. Synthesis of 2-((2-(Methylthio)-5-(naphthalen-2-ylcarbamoyl) thiazol-4-yl) amino)-2-oxoacetic acid (18)

Following the preparation protocol of compound **10a**, starting from compound **16a** (100 mg, 0.22 mmol), the title compound **18** was obtained as a yellow solid (60 mg, 69%); mp 146-148 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.78 (brs, 1H), 10.30 (s, 1H), 8.28 (s, 1H), 7.89 (t, J = 9.6 Hz, 3H), 7.73 (d, J = 8.8 Hz, 1H), 7.50 (t, J = 7.6 Hz, 1H), 7.45 (t, J = 7.6 Hz, 1H), 2.78 (s, 3H); HRMS (ESI) Calcd. for $C_{17}H_{14}N_3O_4S_2$ $[M+H]^+$: 388.0420; Found: 388.0407.

5.4. Synthesis of compounds 19a-19e

5.4.1. 2-((2-(3-Fluorophenoxy)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)amino) acetic acid (19a)

To a stirred solution of compound **8c** (80 mg, 0.211 mmol) in MeOH (5.0 mL) and THF (3.0 mL) was added glyoxylic acid (130 mg, 0.843 mmol) and acetic acid (30 mg, 0.43 mmol). The mixture was stirred at 45 °C for 1 h, then sodium

cyanoborohydride (78 mg, 1.27 mmol) was added, and the reaction mixture was continuously stirred at room temperature for 1 day. The solvent was evaporated under reduced pressure. The crude was purified by silica gel column chromatography (dichloromethane/isopropanol/HOAc 120:2:1) to afford the title compound **19a** (80 mg, 55.6%). Light yellow solid; mp 199-201 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.66 (s, 1H), 9.46 (s, 1H), 8.27 (s, 1H), 8.09 (t, J = 5.6 Hz, 1H), 7.81 (d, J = 8.8 Hz, 2H), 7.78 (d, J = 8.0 Hz, 1H), 7.68 (d, J = 8.8 Hz, 1H), 7.61 (dd, J_1 = 15.6 Hz, J_2 = 8.0 Hz, 1H), 7.54 (d, J = 9.6 Hz, 1H), 7.45 (t, J = 7.2 Hz, 1H), 7.38 (t, J = 7.2 Hz, 2H), 7.30 (t, J = 8.4 Hz, 1H), 4.11 (d, J = 6.0 Hz, 2H); HRMS (ESI) Calcd. for $C_{22}H_{17}N_3O_4FS$ $[M+H]^+$: 438.0918; Found: 438.0902.

5.4.2.

2-((5-((3,5-Dichlorobenzyl)carbamoyl)-2-(3-fluorophenoxy)thiazol-4-yl)amino)acetic acid (19b)

Following the preparation protocol of compound **19a**, starting from compound **8i** (150 mg, 0.364 mmol), the title compound **19b** was obtained as a white solid (65 mg, 38%); mp 150-152 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.60 (brs, 1H), 8.09 (t, J = 6.0 Hz, 1H), 7.79 (t, J = 6.0 Hz, 1H), 7.57 (dd, J_1 = 15.6 Hz, J_2 = 8.0 Hz, 1H), 7.46-7.50 (m, 2H), 7.24-7.343 (m, 4H), 4.29 (d, J = 5.6 Hz, 2H), 4.01 (d, J = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 172.58, 171.85, 163.48, 162.39 (d, J = 244.9 Hz), 158.38, 154.66 (d, J = 11.3 Hz), 144.53, 133.79, 131.73 (d, J = 9.6 Hz), 126.26, 126.01, 117.10 (d, J = 3.4 Hz), 114.14 (d, J = 20.9 Hz), 109.00 (d, J = 24.7 Hz), 44.75, 41.38; HR-MS (ESI): m/z, calcd. for $C_{19}H_{15}O_4N_3Cl_2FS$ $[M+H]^+$ 470.0139, Found: 470.0133.

5.4.3.

2-((5-((3,5-Bis(trifluoromethyl)benzyl)carbamoyl)-2-(3-fluorophenoxy)thiazol-4-yl)amino)acetic acid (19c)

Following the preparation protocol of compound **19a**, starting from compound **8j** (170 mg, 0.36 mmol), the title compound **19c** was obtained as a white solid (90 mg, 48%); mp 115-117 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.55 (brs, 1H), 8.17 (t, J = 5.6 Hz, 1H), 7.93-7.99 (m, 3H), 7.77 (t, J = 5.6 Hz, 1H), 7.58 (dd, J_1 = 15.2 Hz, J_2 = 8.0 Hz, 1H), 7.48 (d, J = 9.6 Hz, 1H), 7.31-7.36 (m, 1H), 7.26 (t, J = 8.4 Hz, 1H), 4.47 (d, J = 5.6 Hz, 2H), 4.01 (d, J = 5.6 Hz, 2H); HR-MS (ESI): m/z, calcd. for $C_{21}H_{15}O_4N_3F_7S$ $[M+H]^+$ 538.0666, Found: 538.0657.

5.4.4. 2-((5-((2,6-Dichlorophenethyl)carbamoyl)-2-(3-fluorophenoxy) thiazol-4-yl) amino)acetic acid (19d)

Following the preparation protocol of compound **19a**, starting from compound **8k** (200 mg, 0.47 mmol), the title compound **19d** was obtained as a yellow solid (120 mg, 53%); mp 167-169 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ (ppm): 10.98 (brs, 1H), 7.91 (brs, 1H), 7.54 (dd, J_1 = 14.4 Hz, J_2 = 7.6 Hz, 1H), 7.38-7.40 (m, 2H), 7.27-7.30 (m, 3H), 7.16-7.18 (m, 1H), 6.91 (brs, 1H), 4.19 (d, J = 5.2 Hz, 2H), 3.54 (q, J = 6.4 Hz, 2H), 3.19-3.23 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 172.18, 171.95, 163.38, 162.38 (d, J = 245.6 Hz), 157.92, 154.70 (d, J = 11.1 Hz), 134.86, 134.78 , 131.68 (d, J = 9.5 Hz), 128.95, 128.41, 117.04 (d, J = 2.1 Hz), 114.02 (d, J = 21.2 Hz), 108.94 (d, J = 25 Hz), 44.70, 36.94, 31.45; HR-MS (ESI): m/z, calcd. for $C_{20}H_{17}O_4N_3Cl_2FS$ $[M+H]^+$ 484.0295, Found: 484.0300.

5.4.5. 2-((5-([1,1'-Biphenyl]-4-ylcarbamoyl)-2-(3-fluorophenoxy)thiazol-4-yl)amino)acetic acid (19e)

Following the preparation protocol of compound **19a**, starting from compound **8l** (80 mg, 0.20 mmol), the title compound **19e** was obtained as a light yellow solid (80 mg, 88%); mp 215-217 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.62 (brs, 1H), 9.34 (s, 1H), 8.05 (t, J = 5.6 Hz, 1H), 7.20 (d, J = 8.4 Hz, 2H), 7.58-7.65 (m, 5H), 7.53 (d, J = 9.6 Hz, 1H), 7.43 (t, J = 7.2 Hz, 2H), 7.37 (d, J = 8.0 Hz, 1H), 7.32 (t, J = 7.2 Hz, 1H), 7.29 (d, J = 8.4 Hz, 1H), 4.10 (d, J = 6.0 Hz, 2H); HR-MS (ESI): m/z, calcd. for $C_{24}H_{19}O_4N_3FS$ $[M+H]^+$ 464.1075, Found: 464.1066.

Note: Except for target compounds, the synthesis and characterization of all other compounds mentioned in the Schemes 1-3 were described in the supporting information.

5.5. Biological evaluation

5.5.1. 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 g/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.

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

PPIase activities were measured at 6 °C JASCO V-650 spectrophotometer using protease-coupled assay according to Wang et al[42,43]. Suc-Ala-Glu-Pro-Phe-4-nitroanilide in 0.47 M LiCl/trifluoroethanol was used as the substrate. In brief, the assay buffer (855 μL of 35 mM HEPES at pH 7.8), Pin1 (0.5 μL of 850 μg/mL stock solution), and inhibitors (5 μL of varying concentrations in DMSO) were pre-equilibrated in the 1.0 mL quartz cuvette in spectrophotometer for 10 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 (2.5 mM in 0.47 M LiCl/trifluoroethanol) was added to the cuvette and the reaction was monitored by absorbance at 390 nm for 90 s. For each compound, three concentrations (100 μM, 10 μM, 1μM) were chosen, and the assay was performed in duplicate. The data was analyzed by Graphpad Prism 5.01. The inhibition at each concentration was calculated according the following equation: Inhibition ratio (%) = $[1-(k_X-k_1)/(k_D-k_1)]x100$, where k_X represents the reaction rate in the presence of tested compound; k_D is the reaction rate of DMSO control without the tested compound; k_1 means the reaction rate of blank control without Pin1 protein, it represents the reaction rate of thermal isomerization without any catalysis. The IC_{50} was measured according to the inhibition ratio at each concentration of tested compound.

5.6. Computational studies

All molecular computation studies were performed using CDOCER protocol integrated in Accelrys Discovery Studio Client 2.5[45] (Accelrys Software Inc., San Diego, CA). The co-crystal structure of Pin1 complexed with a non-phosphate small molecule inhibitor (PDB ID: 3JYJ) was chosen for molecular modeling. Using Clean Protein tool of DS, the water molecules in protein were removed and the protein was added hydrogen and corrected the incomplete residues, then the protein was refined with CHARMm force field. The active site was defined as all residues within 10 Å radius of the co-crystallized reference molecule. Compound **10Ab** was minimized using Prepare Ligands tool of DS and refined with CHARMm force field. Then it was docked into the prepared Pin1 protein with CDOCKER using the default parameters except that the Random Conformation was defined as 20. The 20 final docked conformations were ranked according to their binding free energy. The docking mode was chosen on the basis of binding rationality.

6. Acknowledgement

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Graphical Abstract:

Synthesis and Pin1 inhibitory activity of thiazole derivatives

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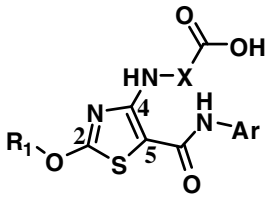

R_1 = small hydrophobic groups
Ar = bulky aromatic substituents
X = CH_2 or CO
IC_{50} = 3.22 -23.4 uM