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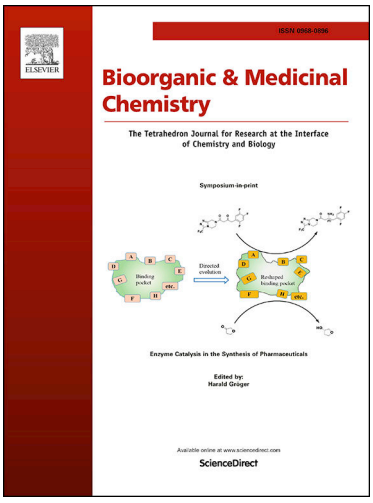

Design, synthesis and biological evaluation of novel thiazole-based derivatives as human Pin1 inhibitors

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Design, synthesis and biological evaluation of novel thiazole-based derivatives as human Pin1 inhibitors

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Abstract

Pin1 is a peptidyl prolyl *cis-trans* isomerase (PPIase) and inhibiting Pin1 is a potential way for discovering anti-tumor agents. With an aim to find potent Pin1 inhibitors with a novel scaffold, a series of thiazole derivatives with an alicyclic heterocycles on the 2-position were designed, synthesized and tested against human Pin1. Compound **9p** bearing a 2-oxa-6-azaspiro [3,3] heptane moiety on the thiazole scaffold was identified as the most potent Pin1 inhibitor of this series with an IC_{50} value of 0.95 μM. The structure-activity relationship (SAR) and molecular modeling study indicated that introducing an alicyclic ring with an H-bond acceptor would be a viable way to improve the binding affinity.

Keywords: PPIase, Pin1, thiazole derivatives, Pin1 inhibitor

1. Introduction

Proline-directed phosphorylation on Ser or Thr is a common regulation mechanism in diverse physiological processes. Pin1(protein interacting with NIMA1) is the only enzyme, functioning as a conformational switch, specifically isomerizing phosphorylated Ser/Thr-Pro peptide bonds in a variety of substrate proteins,[1-5] and its dysregulation is implicated in a number of diseases, including cancer and Alzheimer disease.[6, 7] It was observed that Pin1 is overexpressed in various human cancer cells including breast, prostate, colon, cervical cancer cells.[8, 9] It was reported that Pin1

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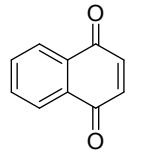
activates numerous oncogenic proteins or growth-promoting proteins and also inactivates multiple tumor suppressors or growth-inhibitory proteins.[10] Thus, inhibiting Pin1 is expected to be a potential strategy for the treatment of cancer.

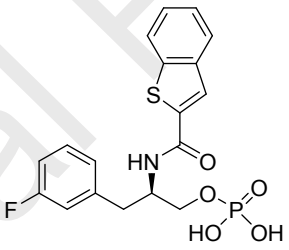
During the past more than two decades, many efforts have been made on the discovery of Pin1 inhibitors and a number of structurally distinct small molecules have been reported, and these inhibitors can be divided into non-covalent inhibitors and covalent inhibitors.[11-47] The research on non-covalent inhibitors was represented by Pfizer, which developed several structurally novel Pin1 inhibitors using structure guided design strategies.[34-36] In 2009, Pfizer disclosed a phosphate-containing derivative, which was the most potent Pin1 inhibitor to date (Fig. 1 compound **A**, K_i = 0.006 μM). However, its membrane permeability was supposed to be poor and there was no activity at the cellular level due to the presence of a phosphate group. Therefore, Pfizer designed novel derivatives containing carboxylic acid moiety instead of the phosphate group in the subsequent structural optimization.[34] In 2010, Compound **B** was reported to be active against Pin1 (Fig. 1 K_i = 0.89 μM), however, it was still not active at the cellular level.[35] In 2014, Pfizer took advantage of scaffold hopping approach and designed compound **C**, which was active at both enzymatic and cellular levels (Fig. 1, K_i = 0.58 μM， HT29 cells IC_{50} = 1.9 μM).[36] Obviously, it is challenging to develop drug-like Pin1 inhibitors with high potency. Interestingly, all-trans retinoic acid (Fig. 1, **ATRA**) is a drug for the treatment of acute promyelocytic leukemia (APL). In 2015, Kunping Lu's team utilized a mechanism-based high-throughput screening method and found that ATRA can directly bind to Pin1, inhibiting Pin1 with a K_i value of 0.82 μM.[37] This result further indicated that Pin1 was involved in cancer development and was a potential anticancer target.

Recently, the research on Pin1 covalent inhibitors was gradually increasing. Juglone (Fig. 1) was the first covalent binding inhibitor of Pin1, and it irreversibly inhibited Pin1.[38] The SH of Cys113 in the catalytic domain can undergo a Michael addition with Juglone, which led to the partial opening of the binding cavity and inhibited Pin1, but

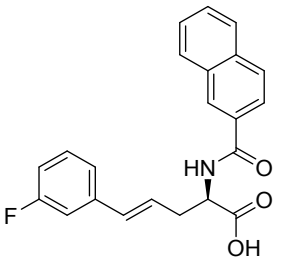
Juglone is a non-specific inhibitor.[39] Different from Juglone, KPT-6566, reported in 2016, is a specific covalent inhibitor of Pin1 (Fig. 1, IC_{50} = 0.64 μM).[40] The results of molecular docking showed that the electrophilic thioglycolic acid fragment was close to Cys113, forming a disulfide bond with Cys113, and releasing the quinone structure which induced apoptosis of tumor cells by alkylation of DNA. These two mechanisms could synergistically kill the tumor cells. In 2019, Ieda et. al successfully engineered the covalent binding inhibitor **E** of Pin1 by introducing a 2-naphthalene acryl group to the lead compound **D** and this inhibitor could covalently bind to Cys113.[41] Based on a highly potent peptide inhibitor, D-peptide (Ac-Phe-D-phosThr-Pip-Nal-Gln-NH2; K_i = 20 nM), a selective and potent covalent inhibitor BJP-06-005-3 without a phosphate group was achieved by using α-chloroacetamide fragment as an electrophile to interact with Cys113. However, it had poor mouse liver microsome stability. [42] Although various chemical entities were disclosed as Pin1 inhibitors, discovering of drug-like new Pin1 inhibitors with distinct scaffolds is still in quest for the development of potential anti-cancer drugs.



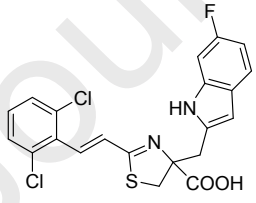
Juglone IC_{50} = 5 μM



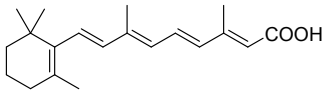
A. K_i = 0.006 μM



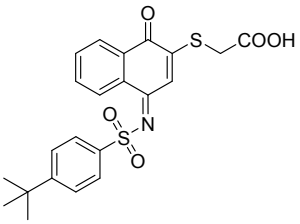
B. K_i = 0.89 μM



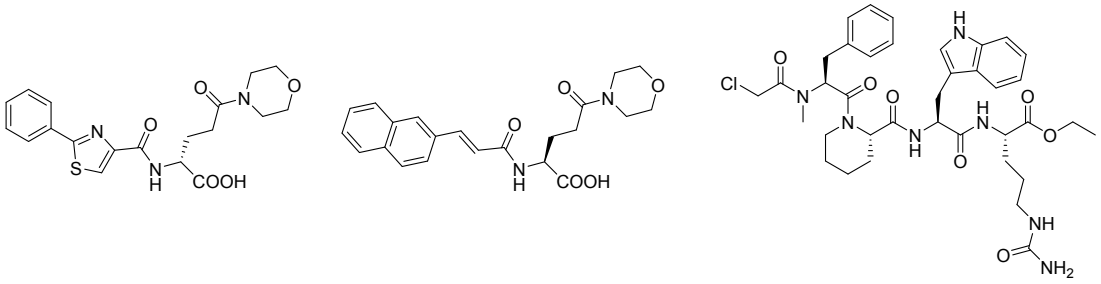
C. K_i = 0.58 μM
IC_{50} = 1.9 μM (HT29 cells)



ATRA K_i= 0.82 μM



KPT6566 IC_{50} = 0.64 μM



D. IC_{50} = 5.4 μM **E.** IC_{50} = 3.2 μM **BJP-06-005-3** K_i = 48 nM

Figure 1. Chemical structures of several reported Pin1 inhibitors

The crystal structures of some small molecule inhibitors complexed with Pin1 revealed that the binding site of catalytic domain consisted of three sub-pockets, namely, a prolyl pocket, a slightly shallow hydrophobic shelf and a unique phosphate binding pocket.[43-46] The prolyl site is a characteristic region of Pin1 and was exploited by numerous inhibitors. In our previous work, a series of thiazole-based inhibitors (Figure 2. structure **I**) were constructed and showed micromolar inhibitory activity.[47] In order to improve the potency and drug-like properties of the thiazole derivatives, we tentatively used the alicyclic moieties to replace the aromatic ring on the 2-position to fully exploit the prolyl pocket, while employing the acetic acid substituted for oxalic acid for creating hydrogen binding within phosphate binding pocket. We conjectured that the designed structure **II** (Figure 2) was somewhat less rigid than structure **I** and this would be beneficial to the physicochemical properties. In addition, an appropriate substitution on the alicyclic ring might bring about hydrogen bindings to enhance the inhibition. Herein, the synthesis and enzymatic activity of the newly designed thiazole derivatives were described, and the structure-activity relationship analysis and the molecular docking were performed for guiding further structural optimizations.

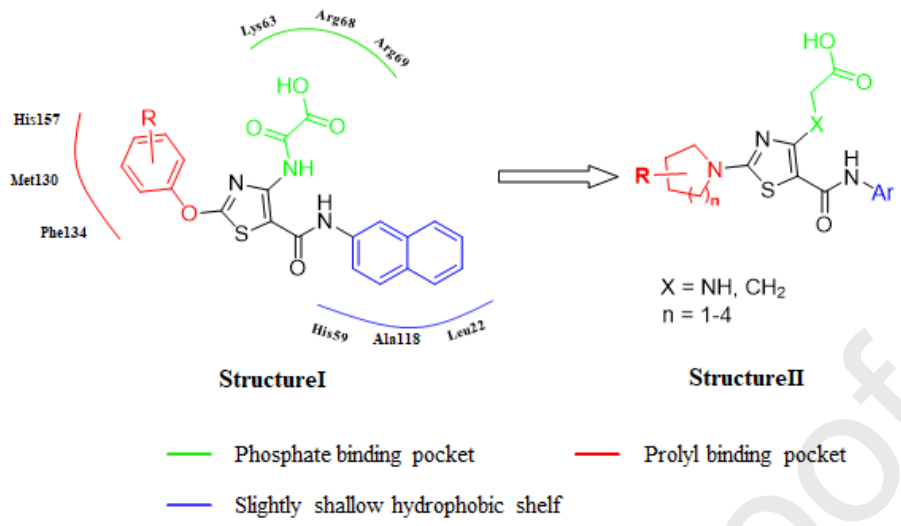
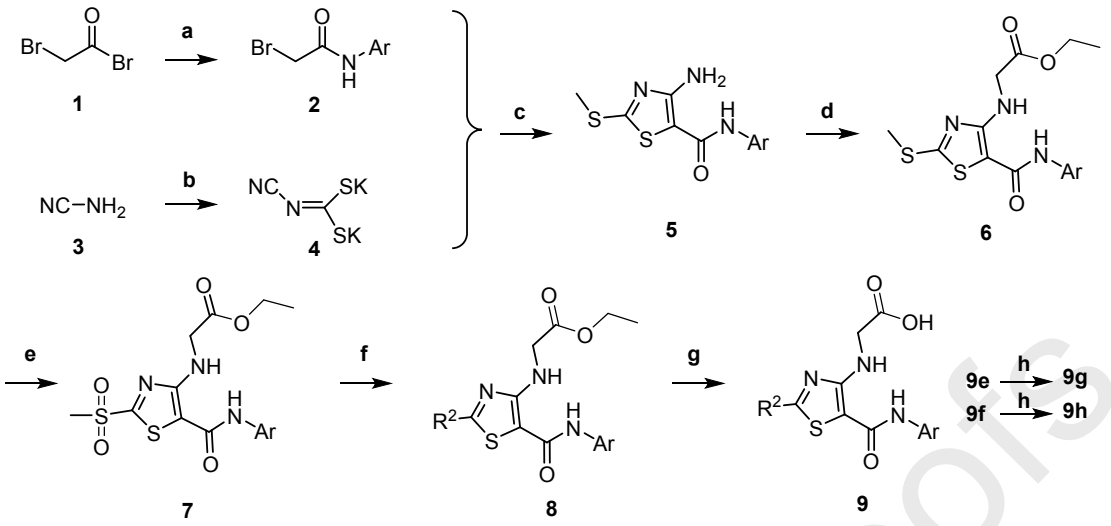


Figure 2. The design of new thiazole-based Pin1 inhibitors

2. Chemistry

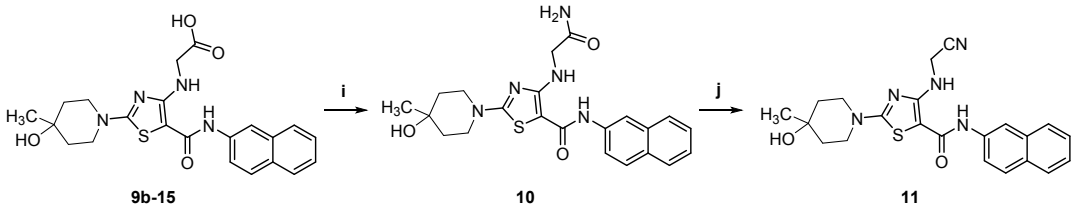
The designed compounds were prepared according to **Schemes 1-3** and their structures were listed in **Tables 1-5**. As shown in **Scheme 1**, 2-naphthylamine was first acylated with bromoacetyl bromide to form compound **2,** which was reacted with potassium cyanocarbonimidodithiate **4** obtained from cyanamide to give rise to the key thiazole intermediate **5** in 76% yield. The reductive amination reaction between 4-amino subsituted thiazole **5** and ethyl glyoxylate provided compound **6,** which was oxidized in the presence of $Na_2WO_4.2H_2O$ and H_2O_2 to give the 2-methylsulfonyl thiazole derivatives **7**. Treatment of compound **7** with a variety of alicyclic amines resulted in the compounds **8**, which were hydrolyzed to afford the corresponding target compounds **9**. In addition, removal the Boc group of compounds **9e** and **9f** using TFA gave compounds **9g** and **9h**.



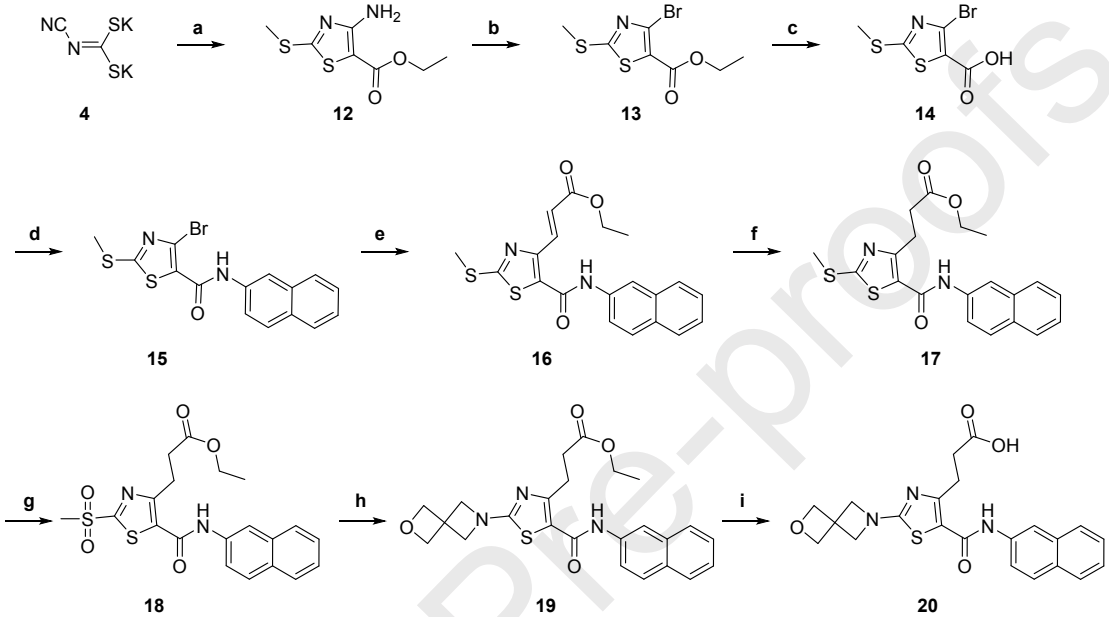
Scheme 1. Reagents and conditions (a) $ArNH_2$, pyridine, DCM, 53-99%; (b) KOH, CS_2, ethanol, 85%; (c) i: H_2O, acetone; ii: NaH, ethanol; iii: MeI, ethanol, 46-81%; (d) Ethyl glyoxalate, $NaBH_4$, toluene, 40-55%; (e) $Na_2WO_4.2H_2O$, H_2O_2, ethanol, 74-88%; (f) Alicyclic amine, TEA, dioxane, 20-90%; (g) NaOH, H_2O, ethanol, 90%; (h) TFA, DCM, 94%.

In order to explore the SAR on the 4-position of thiazole scaffold, a number of derivatives were prepared as outlined in Schemes 2 and 3. The carboxyl amide derivative **10** was readily obtained through a condensation reaction of compound **9b-15** with $NH_3.H_2O$. The elimination reaction of compound **10** was conducted in the presence of ethyl dichlorophosphate and DBU to generate the cyano substituded compound **11**.

Intermediate **12** was obtained by ring closure of compound **4** and ethyl bromoacetate in a 3-step one-pot reaction. Using Sandmeyer reaction, compound **12** was converted into 4-bromo substituted thiazole derivative **13,** which was subjected to hydrolysis and condensation reaction to give compound **15**. Then the key intermediate **16** was prepared by Suzuki-Miyaura reaction, which was further transformed into compound **17** by the reduction reaction in the presence of $NaBH_4$ and $NiCl_2$. Upon subsequent oxidation of the methylthio group and the nucleophilic substitution with 2-oxa-6-azaspiro[3.3]heptane, compound **17** was smoothly converted into intermediate **19,** which was hydrolyzed to afford the target compound **20**.



Scheme 2. Reagents and conditions (i) $NH_3.H_2O$, EDCI, HOBt, THF, 58%; (j) Ethyl dichlorophosphate, DBU, DCM, 85%.



Scheme 3. Reagents and conditions (a) i: Ethyl bromoacetate, H_2O, acetone; ii: NaH, ethanol; iii: MeI, ethanol, 78%; (b) $CuBr_2$, *t*-BuONO, acetonitrile, 73%; (c) NaOH, H_2O, ethanol, 90%; (d) 2-Naphthylamine, oxalyl chloride, Py, DCM, 50%; (e) 2-Ethoxylcarbonylvinyl boronic ester, $Pd(OAc)_2$, K_3PO_4, 1,1'-bis(di-tert-butylphosphino)ferrocene, toluene, 82%; (f) $NaBH_4$, $NiCl_2$, MeOH, 34%; (g) $Na_2WO_4.2H_2O$, H_2O_2, ethanol, 64%; (h) 2-Oxa-6-azaspiro[3.3]heptane hemioxalate, TEA, dioxane, 38%; (i) NaOH, H_2O, ethanol, THF, 67%.

3. Biological results and discussion

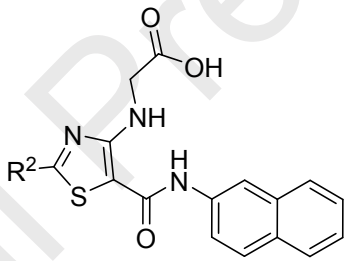
All target compounds were tested for their inhibitory potency against hPin1 and the corresponding results were expressed as IC_{50} values and presented in **Tables 1-5**.

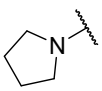
In this work, we mainly focused on the exploration of the SAR on the 2-position of the thiazole ring. Initially, the impact of alicyclic ring size on the inhibition was evaluated as shown in **Table 1**. It was observed that the inhibitory activity gradually increased from the five-membered ring (compound **9a**, IC_{50} = 16.61 μM) to the seven-membered ring (compound **9c**, IC_{50} = 5.30 μM). In contrast, compound **9d** (IC_{50} = 13.70 μM) with an eight-membered ring gave reduced inhibition, suggesting that the binding

site had a limited space. With an aim to guide the further variation of alicyclic ring, molecular docking was performed with compounds **9a-9d**. These compounds showed similar binding poses, which were illustrated by compound **9b** (**Figure 3**). The acetic acid extended into the phosphate binding pocket and formed H-bonds and charge-charge interactions with lys63, Arg69 and Ser114. As expected, the piperidine ring occupied the proline pocket composed of Leu122, Gln131, Phe134 and Met130. The bulky naphthalene ring was bound to a hydrophobic region consisting of Leu122 and Cys113. It was reported that these hydrophobic interactions were beneficial for the binding affinity.[35, 48] As the proline pocket is one of the key binding regions within Pin1, and the results in **Table 1** demonstrated that it could be exploited by the alicyclic ring with an appropriate size. Considering the diversity and availability of the ring derivatives, we further explored the SAR of pyrrolidine and piperidine derivatives.

Table 1

The chemical structures and inhibitory activities against hPin1 of compounds **9a - d**



Compd.	R^2	$IC_{50} \pm SD$ (μM)[a]
9a		16.61 ± 0.99
9b		10.79 ± 2.33
9c		5.30 ± 0.92
9d		13.70 ± 3.09

[a] The measured IC_{50} for compound **B** was 0.09 μM, the reported K_i for compound **B** was 0.006 μM; SD, standard deviation of two independent assays.

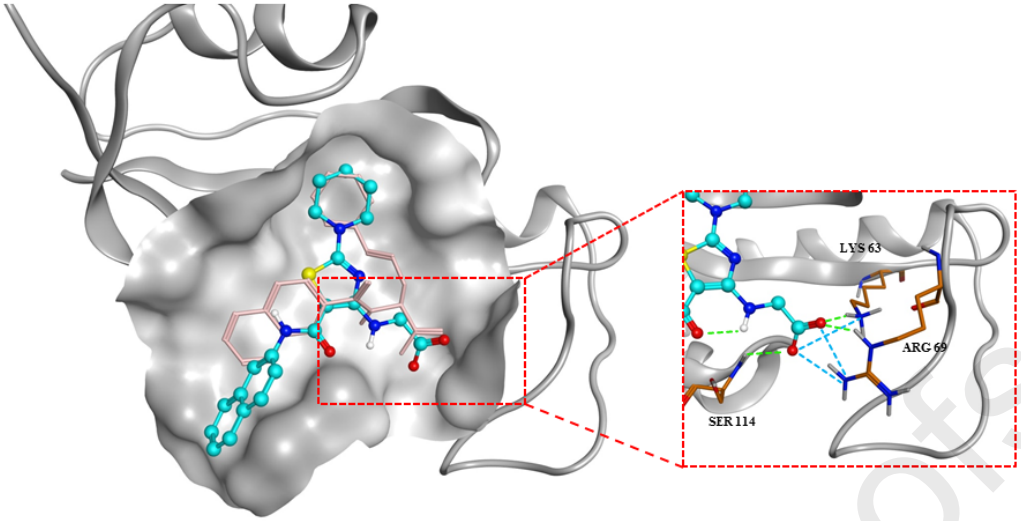
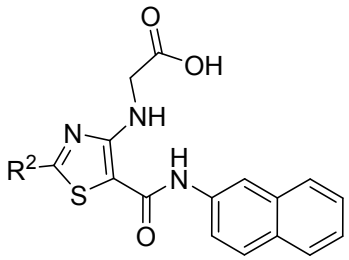


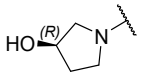
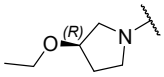
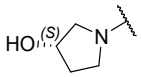
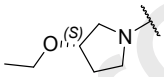
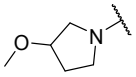
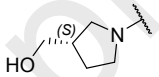
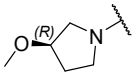
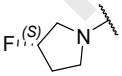
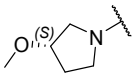
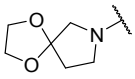
Figure 3. Predicted binding mode of compound **9b** (carbon atoms colored blue) was shown in comparison with a carboxylate Pin1 inhibitor (carbon atoms colored pink) within the co-crystal structure (3JYJ in PDB) using CDOCKER protocol integrated in Accelrys Discovery Studio.[49] The binding pocket of Pin1 was shown in grey. The residues interacting with compound **9b** through hydrogen bonds (green lines) and charge-charge interactions (blue lines) were shown as sticks with yellow carbon atoms. The image was created by MOE 2019.

The enzymatic activity of the 3-substitued pyrrolidine derivatives were presented in **Table 2**. Most of tested compounds exhibited inhibition with IC_{50} values of 3.75 μM - 28.52 μM. It was observed that the configuration of the substituted pyrrolidine ring had a pronounced effect on the inhibition. When a 3-hydroxyl or 3-methoxyl substituted pyrrolidine was incorporated, the R-enatiomer was more active than the S-enatiomer, and compound **(R)-9a-2** showed more than 5-fold increase than compound **(S)-9a-2**. However, when a 3-ethoxyl pyrrolidine was placed on the thiazole ring, the S-enatiomer was more active than the R-enatiomer. When a F atom or a ketal moiety was used as a 3-substituent on the pyrrolidine ring, the resultant compounds **9a-5** and **9a-6** exhibited inhibition as well. In contrast to other compounds in **Table 2**, compound **9a-4** with a hydroxylmethyl group had no inhibition at all.Collectively, an H-bond acceptor linked to pyrrolidine ring was beneficial to the binding and a key feature of this series of inhibitors.

Table 2

The chemical structures and inhibitory activities against hPin1 of compounds **9a-1** - **9a-6**



Compd.	R^2	IC_{50} ± SD (μM)[a]	Compd.	R^2	IC_{50} ± SD (μM)[a]
(R)-9a-1		3.75 ± 0.64	(R)-9a-3		23.18 ± 2.62
(S)-9a-1		8.46 ± 1.36	(S)-9a-3		5.26 ± 0.18
9a-2		7.15 ± 0.06	9a-4		>100
(R)-9a-2		5.04 ± 0.08	9a-5		10.11 ± 0.38
(S)9a-2		28.52 ± 1.94	9a-6		12.78 ± 1.65

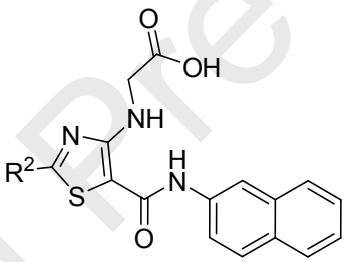
[a] The measured IC_{50} for compound **B** was 0.09 μM, the reported K_i for compound **B** was 0.006 μM; SD, standard deviation of two independent assays.

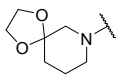
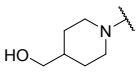
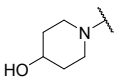
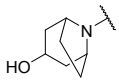
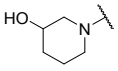
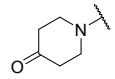
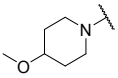
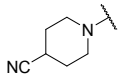
A variety of piperidine derivatives were evaluated as well and the results were listed in **Table 3**. Except for compounds **9b-11**, all other compounds displayed inhibitory activity with IC_{50} values ranging from 3.04 μM to 19.73 μM. It was noted that compounds **9b-3** and **9b-4** bearing a hydroxyl group on the 3- or 4-position of piperidine ring showed stronger inhibition than the corresponding compounds with a methyl or methoxyl group (compounds **9b-1**, **9b-2**, **9b-5**, and **9b-6**), these two molecules had IC_{50} values at a low micromolar level, suggesting that the OH group might create an H-bond to benefit the binding. Interestingly, compound **9b-14** (IC_{50} = 3.63 μM) with a gem-dimethyl group on the piperidine, compound **9b-15** (IC_{50} = 3.78 μM) with a 4-hydroxyl-4-methyl on the piperidine ring, and compound **9b-16** (IC_{50} = 4.71 μM) with a tetrahydropiperidine on the 2-position of thiazole scaffold displayed

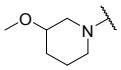
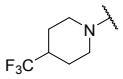
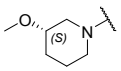
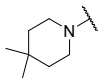
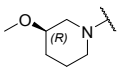
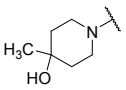
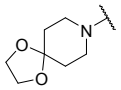
potent inhibition as well. Presumably, these compounds might possess an appropriate shape matching with the proline pocket and thus the hydrophobic interactions bolstered the binding. It was also observed that a somewhat bulky group on the 4-position of piperidine ring was not preferred, as compounds **9b-7**, **9b-12** and **9b-13** showed lower potency with IC_{50} values of > 10 μM. In addition, different from 3-methoxyl substituted pyrrolidine derivatives, the R-enatiomer of 3-methoxyl substituted piperidine derivative (**(R)-9b-6**) showed similar potency to the S-enatiomer (**(S)-9b-6**). Taken together, the results demonstrated that a 4-hydroxyl, 4-hydroxyl-4-methyl, or a gem-dimethyl group substituted on the 4-position of piperidine ring would be beneficial to the binding.

Table 3

The chemical structures and inhibitory activities against hPin1 of compounds **9b-1** - **9b-16**



Compd.	R^2	IC_{50} ± SD (μM)[a]	Compd.	R^2	IC_{50} ± SD (μM)[a]
9b-1		7.64 ± 1.59	9b-8		6.78 ± 0.05
9b-2		16.42 ± 0.42	9b-9		8.30 ± 0.59
9b-3		3.04 ± 0.00	9b-10		7.43 ± 0.93
9b-4		5.07 ± 0.14	9b-11		>100
9b-5		16.12 ± 1.16	9b-12		13.98 ± 0.38

9b-6		8.74 ± 0.65	9b-13		19.73 ± 2.19
(S)-9b-6		8.50 ± 0.42	9b-14		3.63 ± 1.37
(R)-9b-6		9.09 ± 1.85	9b-15		3.78 ± 0.54
9b-7		12.91 ± 3.79	9b-16		4.71 ± 0.67

[a] The measured IC_{50} for compound **B** was 0.09 μM, the reported K_i for compound **B** was 0.006 μM; SD, standard deviation of two independent assays.

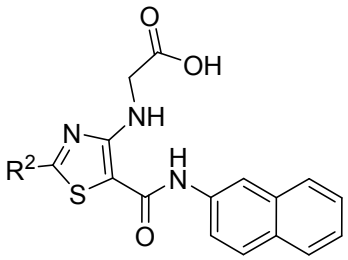
The piperazine derivatives and its close isosteres were also synthesized and tested (**Table 4**). Compound **9g** bearing a piperazine moiety had a weak inhibition (IC_{50} = 46.89 μM). Introducing a 3-methyl piperazine ring on the thiazole ring produced a 7-fold enhancement in potency (compound **9h**, IC_{50} = 6.55 μM), whereas incorporating a 3,3-dimethyl piperazine, 3,5-dimethyl piperazine or *N*-methyl piperazine fragment resulted in a loss of activity (compounds **9i**, **9j**, **9k**), and compound **9l** bearing a 3-methyl-*N*-methyl piperazine showed similar potency to compound **9g**. These results suggested that very limited modifications could be allowed on the piperazine ring and the proline pocket had a restricted space. Compounds **9n** with a morpholine ring and **9o** with a thiomorpholine ring, the close analogs of compound **9g**, displayed a 2.6-fold and 5-fold increase in potency, respectively, suggesting that an H-bond acceptor (O or S) benefited to the binding. Remarkably, it was found that compound **9p** with a 2-oxa-6-azaspiro[3,3]heptane fragment presented a strong inhibition with an IC_{50} value of 0.95 μM, which is the most potent inhibitor of this series. In contrast, compound **9q** bearing a 2-azaspiro[3,3]heptane fragment produced a 5.6-fold reduction in potency, suggesting that the oxygen atom in compound **9p** played a key role in the binding, and it was supposed to be a critical H-bond acceptor.

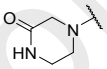
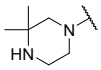
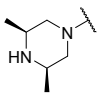
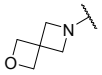
With an aim to probe the binding features of compound **9p**, molecular docking was conducted and the binding mode was illustrated in **Figure 4**. As expected, the binding pose of compound **9p** was the same as that of compound **9b**, however, the oxygen atom

on the ring formed a hydrogen bond with the backbone NH of Gln131, and this hydrogen binding greatly contributed to the binding affinity.

Table 4

The chemical structures and inhibitory activities against hPin1of compounds **9g-9q**



Compd.	R^2	IC_{50} ± SD (μM)[a]	Compd.	R^2	IC_{50} ± SD (μM)[a]
9g		46.89 ± 14.17	9m		11.38 ± 0.47
9h		6.55 ± 0.91	9n		17.71 ± 0.92
9i		>100	9o		9.32 ± 0.82
9j		>100	9p		0.95 ± 0.11
9k		>100	9q		5.34 ± 0.35
9l		37.07 ± 9.96			

[a] The measured IC_{50} for compound **B** was 0.09 μM, the reported K_i for compound **B** was 0.006 μM; SD, standard deviation of two independent assays.

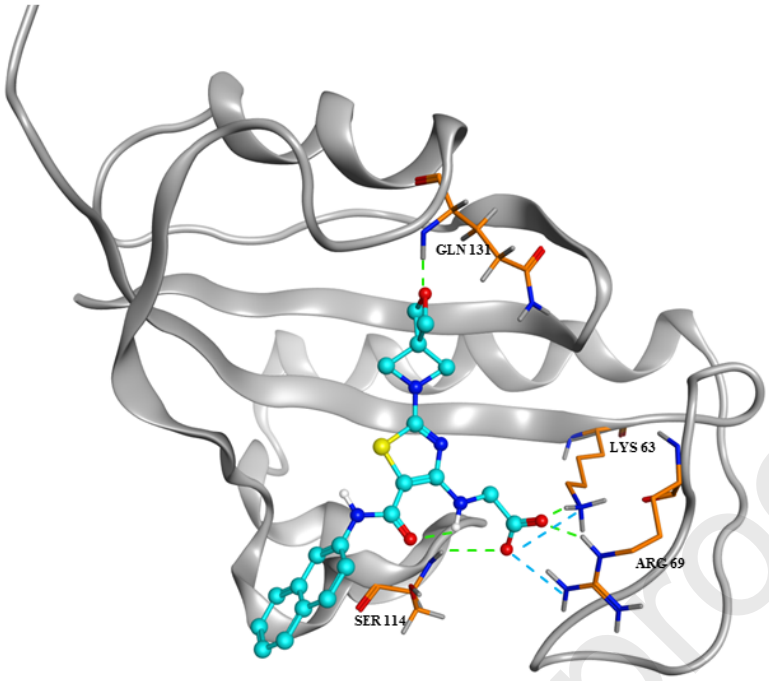
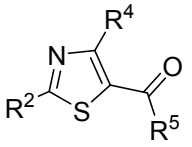


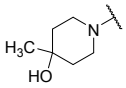
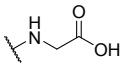
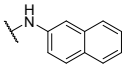
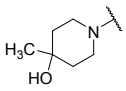
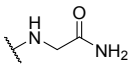
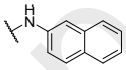
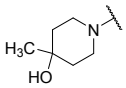
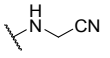
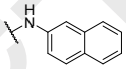
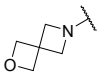
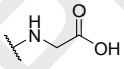
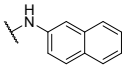
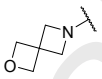
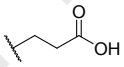
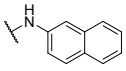
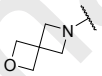
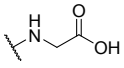
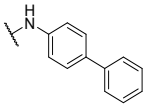
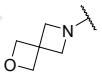
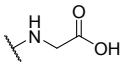
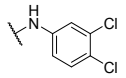
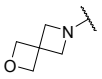
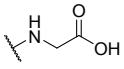
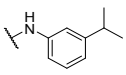
Figure 4. Predicted binding mode of compound **9p** using CDOCKER protocol integrated in Accelrys Discovery Studio. [49] The enzyme was shown in grey, compound **9p** was shown as sticks with blue carbon atoms. The residues that interacted with compound **9p** were shown as sticks with yellow carbon atoms, and hydrogen bonds were indicated with green lines and charge-charge interactions (blue lines) were shown as sticks with yellow carbon atoms. The image was created by MOE 2019.

We also preliminarily investigated the SAR of 4- and 5-substitution of the thiazole ring. Changing the carboxylic acid group of compound **9b-15** to the corresponding carboxylic amide (compound **10**) or acetonitrile analog (compound **11**) led to loss of potency, demonstrating that the carboxylic acid group is a characteristic structural moiety as well for this series of inhibitors, and this result is consistent with the predicted binding mode (**Figure 4)**. The replacement of the NH group on the side chain of compound **9p** with a CH_2 moiety afforded compound **20**, which displayed a 12-fold reduction in potency, demonstrating that this NH group was critical for the binding. As shown in **Figure 4**, presumably, partly because the intramolecular hydrogen bond between the NH and carbonyl group on the ortho position was favorable for the binding through a less entropy cost. When the naphthyl fragment in compound **9p** was substituted by other aromatic segments including the biphenyl, 3,4-dichlorophenyl, or 3-isopropylphenyl group, the inhibition of the resulted compounds (**9r**, **9s** and **9t**)

declined drastically, indicating that the fused aromatic ring was preferred, and this hydrophobic interaction made a pronounced contribution to the inhibition.

Table 5
The chemical structures and inhibitory activities against hPin1 of compounds **9b-15, 10, 11, 9p, 20, 9r-9t**



Compd	R^2	R^4	R^5	$IC_{50} \pm SD$ (μM)[a]
9b-15				3.77±0.54
10				>100
11				>100
9p				0.95±0.11
20				11.69±3.16
9r				13.35±1.29
9s				28.00±4.40
9t				16.83±1.83

[a] The measured IC_{50} for compound **B** was 0.09 μM, the reported K_i for compound **B** was 0.006 μM; SD, standard deviation of two independent assays.

4. Conclusion

In summary, a series of novel thiazole derivatives bearing alicyclic heterocycles on

the 2-position were prepared to explore the proline binding pocket of Pin1. As a result, compound **9p** was disclosed as the most potent inhibitor with an IC_{50} value of 0.95 μM, and it possessed drug-like properties with ClogP value of 3.20 and tPSA of 103.26. It provides a new template for further structural modifications. The SAR and molecular docking results indicated that introducing an H-bond acceptor to form an H-bond with Gln131 was accessible and greatly benefited to the binding affinity. The proline pocket could accommodate various hydrophobic alicyclic heterocycles, however, the space was somewhat restricted. In addition, it has been demonstrated that the carboxylic acid and the bulky aromatic moiety were also the key pharmacophoric groups, which were consistent with the known SAR. The above results would facilitate to discover new Pin1 inhibitors for probing the potential of Pin1 as an anticancer target.

5.Experimental

5.1. General

^{1}H NMR spectra on a Varian Mercury 400 or 500 spectrometer were recorded using tetramethylsilane (TMS) as the internal standard in Acetone-d_6, DMSO-d_6, MeOD or $CDCl_3$. High resolution mass spectra (HRMS) were obtained on an Agilent Technologies LC/MSD TOF spectrometer. Melting points were measured on a Yanaco micro melting point apparatus and were uncorrected. All chemicals and solvents used were of reagent grade without further purification or dried before used. All the reactions were monitored by thin-layer chromatography (TLC) under a UV lamp at 254 nm. Column chromatography separations were performed with silica gel (200-300 mesh).

5.2. Synthesis of compounds

*5.2.1 (5-(Naphthalen-2-ylcarbamoyl)-2-(pyrrolidin-1-yl)thiazol-4-yl)glycine (**9a**)*

To a stirred solution of **8a** (79 mg, 0.19mmol) in ethanol (2 mL) was added NaOH (22 mg, 0.56 mmol) dissolved in H_2O (1 mL) dropwise, the reaction mixture was then stirred at room temperature for 3 h. Ethanol was removed under reduced pressure, then adjusting pH of the residue to 2-3 with dilute hydrochloric acid. After filtration and drying under high vacuum, compound **9a** was obtained as a white solid (70 mg, 93%); mp 162-164 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.97 (s, 1H), 8.30 (d, J = 1.9 Hz, 1H), 8.09 (t, J = 5.7 Hz, 1H), 7.83 - 7.71 (m, 4H), 7.46 - 7.31 (m, 2H), 4.09 (d, J = 5.6 Hz, 2H), 3.42 (brs, 4H), 2.03 - 1.95 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.54, 166.07, 163.74, 163.29, 138.47, 134.04, 129.66, 128.14, 127.81, 127.55, 126.56, 124.40, 121.57, 115.73, 81.96, 49.47, 46.06, 25.59. HRMS (ESI): m/z, Calcd. for $C_{20}H_{21}O_3N_4S$ $[M+H]^+$: 397.1329, Found 397.1345.

*5.2.2 (5-(Naphthalen-2-ylcarbamoyl)-2-(piperidin-1-yl)thiazol-4-yl)glycine (**9b**)*

Following the preparation protocol of compound **9a**, starting from compound **8b** (50 mg, 0.11 mmol), LiOH instead of NaOH was used, the title compound **9b** was obtained as a white solid (10 mg, 25%); mp 132-134 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.00 (s, 1H), 8.28 (d, J = 2.0 Hz, 1H), 8.04 (t, J = 5.9 Hz, 1H), 7.84 - 7.69 (m, 4H), 7.47 - 7.31 (m, 2H), 4.12 (d, J = 5.8 Hz, 2H), 3.50 (brs, 4H), 1.62 (brs, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.59, 169.28, 163.49, 163.27, 138.33, 134.01, 129.73, 128.18, 127.83, 127.56, 126.60, 124.48, 121.60, 115.87, 82.08, 48.90, 45.61, 25.21, 23.90; HRMS (ESI): m/z, Calcd. for $C_{21}H_{23}O_3N_4S$ $[M+H]^+$: 411.1485, Found 411.1469.

*5.2.3 (2-(Azepan-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9c**)*

Following the preparation protocol of compound **9a**, starting from compound **8c** (130 mg, 0.33 mmol), the title compound **9c** was obtained as a grayish white solid (93 mg, 76%); mp 131-133 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ (ppm) 8.36 (s, 1H), 8.18 (t, J = 5.2 Hz, 1H), 8.11 (s, 1H), 7.82 - 7.72 (m, 4H), 7.45 - 7.30 (m, 2H), 4.30 (d, J = 4.7 Hz, 2H), 3.76 - 3.48(m, 4H), 1.89 - 1.78 (m, 4H), 1.65 - 1.55 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.54, 168.63, 163.54, 163.21, 138.32, 133.96, 129.65, 128.11, 127.77, 127.50, 126.53, 124.40, 121.52, 115.76, 81.90, 45.31, 27.32, 27.26, 15.61; HRMS (ESI): m/z, Calcd. for $C_{22}H_{25}O_3N_4S$ $[M+H]^+$: 425.1642, Found 425.1670.

*5.2.4 (2-(Azepan-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9d**)*

Following the preparation protocol of compound **9a**, starting from compound **8d** (50 mg, 0.11 mmol), the title compound **9d** was obtained as a white solid (24 mg, 76%); mp 133-135 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.97 (s, 1H), 8.29 (d, J = 2.0 Hz, 1H), 8.04 (t, J = 5.8 Hz, 1H), 7.83 - 7.69 (m, 4H), 7.46 - 7.30 (m, 2H), 4.11 (d, J = 5.7 Hz, 2H), 3.55 (brs, 4H), 1.79 (brs, 4H), 1.60 - 1.40 (m, 6H); HRMS (ESI): m/z, Calcd. for $C_{23}H_{27}O_3N_4S$ $[M+H]^+$: 439.1798, Found 439.1801.

*5.2.5 (R)-(2-(3-Hydroxypyrrolidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**(R)-9a-1**)*

Following the preparation protocol of compound **9a**, starting from compound **8a-1R** (100 mg, 0.22 mmol), LiOH was used, the title compound **(R)-9a-1** was obtained as a white solid (50 mg, 53%); mp 158-160 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.00 (s, 1H), 8.29 (d, J = 2.0 Hz, 1H), 8.09 (t, J = 5.9 Hz, 1H), 7.84 - 7.69 (m, 4H), 7.47 - 7.31 (m, 2H), 4.43 (brs, 1H), 4.15 (d, J = 5.0 Hz, 2H), 3.59 - 3.45 (m, 4H), 2.17 - 1.88 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.56, 166.35, 163.72, 163.30, 138.33, 133.99, 129.70, 128.16, 127.82, 127.55, 126.58, 124.46, 121.57, 115.85, 82.24, 69.73, 57.85, 47.69, 45.27, 34.08; HRMS (ESI): m/z, Calcd. for $C_{20}H_{21}O_4N_4S$ $[M+H]^+$: 413.1278, Found 413.1314.

*5.2.6 (S)-(2-(3-Hydroxypyrrolidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**(S)-9a-1**)*

Following the preparation protocol of compound **9a**, starting from compound **8a-1S** (100 mg, 0.22 mmol), LiOH was used, the title compound **(S)-9a-1** was obtained as a

white solid (66 mg, 70%); mp 149-151 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.00 (s, 1H), 8.29 (s, 1H), 8.20 - 7.98 (m, 1H), 7.94 - 7.69 (m, 4H), 7.53 - 7.30 (m, 2H), 4.43 (s, 1H), 4.15 (s, 2H), 3.53 (s, 4H), 2.19 - 1.85 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.57, 166.37, 163.74, 163.32, 138.34, 134.01, 129.72, 128.17, 127.83, 127.56, 126.59, 124.48, 121.58, 115.87, 82.25, 69.74, 57.87, 47.71, 45.29, 34.09; HRMS (ESI): m/z, Calcd. for $C_{20}H_{21}O_4N_4S$ $[M+H]^+$: 413.1278, Found 413.1255.

*5.2.7 ((2-(3-Methoxypyrrolidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9a-2**)*

Following the preparation protocol of compound **9a**, starting from compound **8a-2** (50 mg, 0.11 mmol), the title compound **9a-2** was obtained as a yellow solid (40 mg, 94%); mp 136-137 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.576 (s, 1H), 9.02 (s, 1H),8.29 (d, J = 2.0 Hz, 1H), 8.09 (t, J = 6.0 Hz, 1H), 7.84 - 7.69 (m, 4H), 7.48 - 7.29 (m, 2H), 4.15 (d, J = 5.9 Hz, 2H), 4.11 (m, 1H), 3.61 - 3.84 (m, 4H),3.28 (s, 3H), 2.18-2.05 (m, 2H); HRMS (ESI): m/z, Calcd. for $C_{21}H_{23}O_4N_4S$ $[M+H]^+$: 427.1435, Found 427.1409.

*5.2.8 (R)-(2-(3-Methoxypyrrolidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**(R)-9a-2**)*

Following the preparation protocol of compound **9a**, starting from compound **8a-2R** (80 mg, 0.18 mmol), the title compound **(R)-9a-2** was obtained as a white solid (52 mg, 69%); mp 126-128 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.02 (s, 1H), 8.30 (d, J = 2.4 Hz, 1H), 8.10 (brs, 1H), 7.87 - 7.69 (m, 4H), 7.47 - 7.32 (m, 2H), 4.23 - 4.07 (m, 3H), 3.60 - 3.36 (m, 4H), 3.28 (s, 3H), 2.19 - 2.07 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.55, 166.30, 163.65, 163.29, 138.31, 133.99, 129.72, 128.16, 127.82, 127.56, 126.58, 124.48, 121.57, 115.88, 82.38, 79.51, 56.38, 54.52, 47.65, 45.29, 30.65; HRMS (ESI): m/z, Calcd. for $C_{21}H_{23}O_4N_4S$ $[M+H]^+$: 427.1435, Found 427.1405.

*5.2.9 (S)-(2-(3-Methoxypyrrolidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**(S)-9a-2**)*

Following the preparation protocol of compound **9a**, starting from compound **8a-2S** (100 mg, 0.22 mmol), the title compound **(S)-9a-2** was obtained as a light pink solid (88 mg, 95%); mp 126-128 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.03 (s, 1H), 8.29 (s, 1H), 7.96 - 7.68 (m, 4H), 7.54 - 7.29 (m, 2H), 4.15 (s, 2H), 4.12 (s, 1H), 3.63 - 3.38 (m, 4H), 3.28 (s, 3H), 2.14 (s, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.54, 166.30, 163.64, 163.29, 138.30, 133.98, 129.71, 128.16, 127.82, 127.56, 126.58, 124.48, 121.57, 115.88, 82.38, 79.51, 56.38, 54.51, 47.65, 45.29, 30.65; HRMS (ESI): m/z, Calcd. for $C_{21}H_{23}O_4N_4S$ $[M+H]^+$: 427.1435, Found 427.1407.

*5.2.10 (R)-(2-(3-Ehoxypyrrolidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**(R)-9a-3**)*

Following the preparation protocol of compound **9a**, starting from compound **8a-3R** (70 mg, 0.18 mmol), the title compound **(R)-9a-3** was obtained as a light pink solid (31

mg, 48%); mp 130-132 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 8.92 (s, 1H), 8.33 (s, 1H), 8.11 (brs, 1H), 7.82 - 7.69 (m, 3H), 7.46 - 7.29 (m, 2H), 4.21 (s, 1H), 3.71 (m, 2H), 3.64 - 3.33 (m, 6H), 2.12 (s, 2H), 1.12 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.23, 166.22, 163.55, 163.07, 138.80, 134.11, 129.50, 128.04, 127.77, 127.51, 126.48, 124.20, 121.50, 115.30, 81.07, 77.73, 64.03, 54.84, 48.98, 47.59, 31.13, 15.79. HRMS (ESI): m/z, Calcd. for $C_{22}H_{25}O_4N_4S$ $[M+H]^+$: 441.1591, Found 441.1561.

*5.2.11 (S)-(2-(3-Ethoxypyrrolidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**(S)-9a-3**)*

Following the preparation protocol of compound **9a**, starting from compound **(S)-8a-3** (100 mg, 0.21 mmol), the title compound **(S)-9a-3** was obtained as a white solid (79 mg, 84%); mp 131-133 °C; ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) δ 8.04 (d, J = 2.1 Hz, 1H), 7.77 (t, J = 7.6 Hz, 3H), 7.65 - 7.57 (m, 1H), 7.53 - 7.32 (m, 2H), 4.26 (s, 3H), 3.70 - 3.39 (m, 6H), 2.25 - 2.09 (m, 2H), 1.21 (t, J = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.57, 166.28, 163.67, 163.31, 138.32, 134.00, 129.73, 128.17, 127.83, 127.56, 126.59, 124.49, 121.58, 115.89, 82.37, 77.72, 64.04, 54.95, 47.69, 45.29, 31.09, 15.78; HRMS (ESI): m/z, Calcd. for $C_{22}H_{25}O_4N_4S$ $[M+H]^+$: 441.1591, Found 441.1633.

*5.2.12 (S)-(2-(3-(Hydroxymethyl)pyrrolidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9a-4**)*

Following the preparation protocol of compound **9a**, starting from compound **8a-4** (60 mg, 0.13 mmol), the title compound **9a-4** was obtained as a white solid (34 mg, 74%); mp 183-185 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm) 8.87 (s, 1H), 8.32 (d, J = 1.8 Hz, 1H), 8.06 (t, J = 4.0 Hz, 1H), 7.82 - 7.69 (m, 4H), 7.46 - 7.27 (m, 2H), 4.81 (brs, 1H), 3.66 (d, J = 4.3 Hz, 2H), 3.55 - 3.39 (m, 5H), 3.27 - 3.19 (m, 1H), 2.48 - 2.42 (m, 1H), 2.13 - 1.99 (m, 1H), 1.86 - 1.74 (m, 1H); HRMS (ESI): m/z, Calcd. for $C_{21}H_{23}O_4N_4S$ $[M+H]^+$: 427.1435, Found 424.1478.

*5.2.13 (S)-(2-(3-Fluoropyrrolidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9a-5**)*

Following the preparation protocol of compound **9a**, starting from compound **8a-5** (50 mg, 0.11 mmol), the title compound **9a-5** was obtained as a white solid (39 mg, 83%); mp 176-178 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.59 (s, 1H), 9.07 (s, 1H), 8.30 (d, J = 2.0 Hz, 1H), 8.10 (t, J = 5.9 Hz, 1H), 7.85 - 7.70 (m, 4H), 7.48 - 7.31 (m, 2H), 5.47 (d, J = 52.8 Hz, 1H), 4.16 (d, J = 5.8 Hz, 2H), 3.83 - 3.47 (m, 4H), 2.44 - 2.17 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.54, 166.17, 163.54, 163.28, 138.25, 133.98, 129.74, 128.18, 127.82, 127.56, 126.59, 124.51, 121.57, 115.95, 94.10, 92.95, 82.64, 56.04, 55.89, 47.38, 45.30, 32.26, 32.12; HRMS (ESI): m/z, Calcd. for $C_{20}H_{20}O_3N_4SF$ $[M+H]^+$: 415.1235, Found 415.1242.

5.2.14 (5-(Naphthalen-2-ylcarbamoyl)-2-(1,4-dioxa-7-azaspiro[4.4]nonan-7-

*yl)thiazol-4-yl)glycine (**9a-6**)*

Following the preparation protocol of compound **9a**, starting from compound **8a-6** (76 mg, 0.16 mmol), the title compound **9a-6** was obtained as a yellow solid (59 mg, 82%); mp 135-137 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.07 (s, 1H), 8.29 (s, 1H), 8.08 (brs, 1H), 7.84 - 7.67 (m, 4H), 7.39 (m, 2H), 4.14 (s, 2H), 3.98 - 3.95 (m, 4H), 3.53 (t, *J* = 6.4 Hz, 2H), 3.49 (s, 2H), 2.21 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.54, 166.49, 163.50, 163.29, 138.24, 133.98, 129.75, 128.18, 127.82, 127.57, 126.59, 124.52, 121.58, 115.96, 113.35, 82.62, 64.95, 56.22, 47.75, 45.29, 34.36; HRMS (ESI): m/z, Calcd. for $C_{22}H_{23}O_5N_4S$ $[M+H]^+$: 455.1384, Found 455.1382.

*5.2.15 (2-(4-Methylpiperidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9b-1**)*

Following the preparation protocol of compound **9a**, starting from compound **8b-1** (80 mg, 0.18 mmol), the title compound **9b-1** was obtained as a white solid (33 mg, 44%); mp 148-150 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.99 (s, 1H), 8.28 (d, *J* = 2.1 Hz, 1H), 8.04 (brs, 1H), 7.82 - 7.70 (m, 4H), 7.48 - 7.30 (m, 2H), 4.12 (s, 2H), 3.95 - 3.84 (m, 2H), 3.19 - 3.05 (m, 2H), 1.79 - 1.58 (m, 3H), 1.29 - 1.08 (m, 2H), 0.94 (d, *J* = 6.4 Hz, 4H); HRMS (ESI): m/z, Calcd. for $C_{22}H_{25}O_3N_4S$ $[M+H]^+$: 425.1642, Found 425.1634.

*5.2.16 (2-(3-Methylpiperidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9b-2**)*

Following the preparation protocol of compound **9a**, starting from compound **8b-2** (80 mg, 0.18 mmol), the title compound **9b-2** was obtained as a white solid (33 mg, 44%); mp 134-135 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ (ppm) 8.35 (s, 1H), 8.16 (t, *J* = 6.0 Hz, 1H), 8.11 (brs, 1H), 7.82 - 7.74 (m, 4H), 7.45 - 7.31 (m, 2H), 4.28 (d, *J* = 4.4 Hz, 2H), 4.00 - 3.84 (m, 2H), 3.13 - 3.02 (m, 1H), 2.84- 2.72 (m, 1H), 1.92 - 1.82 (m, 1H), 1.82 - 1.67 (m, 2H), 1.66 - 1.50 (m, 1H), 1.31 - 1.16 (m, 1H), 0.96 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.57, 169.17, 163.47, 163.25, 138.28, 133.98, 129.72, 128.18, 127.82, 127.55, 126.59, 124.49, 121.54, 115.86, 82.12, 45.27, 32.31, 30.67, 24.54, 19.17; HRMS (ESI): m/z, Calcd. for $C_{22}H_{25}O_3N_4S$ $[M+H]^+$: 425.1642, Found 425.1637.

*5.2.17 (2-(4-Hydroxypiperidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9b-3**)*

Following the preparation protocol of compound **9a**, starting from compound **8b-3** (100 mg, 0.22 mmol), the title compound **9b-3** was obtained as a white solid (84 mg, 89%); mp 158-160 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.03 (s, 1H), 8.29 (d, *J* = 2.1 Hz, 1H), 8.05 (t, *J* = 4.8 Hz, 1H), 7.82 - 7.69 (m, 4H), 7.46 - 7.32 (m, 2H), 4.12 (d, *J* = 4.6 Hz, 2H), 3.81 - 3.68 (m, 3H), 3.32 - 3.25 (m, 2H), 1.92 - 1.77 (m, 2H), 1.53 - 1.39 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.52, 169.09, 163.42, 163.21, 138.20, 133.94, 129.70, 128.14, 127.78, 127.51, 126.55, 124.47, 121.55, 115.89, 82.35, 65.29, 45.23, 33.57; HRMS (ESI): m/z, Calcd. for $C_{21}H_{23}O_4N_4S$ $[M+H]^+$:

427.1435, Found 427.1433.

*5.2.18 (2-(3-Hydroxypiperidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9b-4**)*

Following the preparation protocol of compound **9a**, starting from compound **8b-4** (100 mg, 0.22 mmol), the title compound **9b-4** was obtained as a white solid (87 mg, 92%); mp 159-161 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.55 (s, 1H), 8.99 (s, 1H), 8.28 (s, 1H), 8.05 (t, J = 6.0 Hz, 1H), 7.85 - 7.68 (m, 4H), 7.48 - 7.29 (m, 2H), 5.06 (d, J = 4.2 Hz, 1H), 4.13 (d, J = 5.8 Hz, 2H), 3.87 - 3.71 (m, 1H), 3.62 (brs, 2H), 3.28 - 3.19 (m, 1H), 3.10 - 2.97 (m, 1H), 1.95 - 1.71 (m, 2H), 1.47 (m, 2H); HRMS (ESI): m/z, Calcd. for $C_{21}H_{23}O_4N_4S$ $[M+H]^+$: 427.1435, Found 427.1454.

*5.2.19 (2-(4-Methoxypiperidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9b-5**)*

Following the preparation protocol of compound **9a**, starting from compound **8b-5** (140 mg, 0.30 mmol), the title compound **9b-5** was obtained as a white solid (47 mg, 36%); mp 163-165 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.06 (s, 1H), 8.29 (d, J = 2.0 Hz, 1H), 8.04 (t, J = 5.9 Hz, 1H), 7.83 - 7.71 (m, 4H), 7.47 - 7.31 (m, 2H), 4.12 (d, J = 5.8 Hz, 2H), 3.76 - 3.63 (m, 2H), 3.53 - 3.44 (m, 1H), 3.42 - 3.37 (m, 2H), 3.29 (s, 3H), 2.00 - 1.85 (m, 2H), 1.62 - 1.48 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.51, 169.16, 163.39, 163.26, 138.24, 133.97, 129.74, 128.16, 127.82, 127.55, 126.58, 124.50, 121.61, 115.95, 82.52, 74.72, 55.61, 45.33, 29.95; HRMS (ESI): m/z, Calcd. for $C_{22}H_{25}O_4N_4S$ $[M+H]^+$: 441.1591, Found 441.1592.

*5.2.20 (2-(3-Methoxypiperidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9b-6**)*

Following the preparation protocol of compound **9a**, starting from compound **8b-6** (100 mg, 0.22 mmol), LiOH instead of NaOH was used, the title compound **9b-6** was obtained as a white solid (24 mg, 50%); mp 107-109 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.54 (s, 1H), 8.99 (s, 1H), 8.28 (d, J = 2.0 Hz, 1H), 8.04 (t, J = 5.9 Hz, 1H), 7.83 - 7.68 (m, 4H), 7.47 - 7.31 (m, 2H), 4.12 (d, J = 5.9 Hz, 2H), 3.70 - 3.53 (m, 2H), 3.51 - 3.44 (m, 2H), 3.42 - 3.37 (m, 1H), 3.29 (s, 3H), 1.92 - 1.45 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 172.57, 169.38, 163.40, 163.24, 138.26, 133.98, 129.73, 128.17, 127.82, 127.54, 126.59, 124.49, 121.57, 115.91, 82.08, 73.96, 56.12, 45.26, 28.97, 21.34; HRMS (ESI): m/z, Calcd. for $C_{22}H_{25}O_4N_4S$ $[M+H]^+$: 441.1591, Found 441.1590.

*5.2.21 (S)-(2-(3-Methoxypiperidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**(S)-9b-6**)*

Following the preparation protocol of compound **9a**, starting from compound **(S)-8b-6** (50 mg, 0.11 mmol), the title compound **(S)-9b-6** was obtained as a white solid (40 mg, 91%); mp 115-117 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.99 (s, 1H), 8.28 (d, J = 2.0 Hz, 1H), 8.04 (brs, 1H), 7.85 - 7.66 (m, 4H), 7.46 - 7.32 (m, 2H), 4.12 (s, 2H), 3.66 - 3.60 (m, 2H), 3.49 - 3.36 (m, 3H), 3.29 (s, 3H), 1.91 - 1.73 (m, 2H), 1.73

- 1.59 (m, 1H), 1.58 - 1.47 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.56, 169.38, 163.40, 163.24, 138.26, 133.98, 129.73, 128.17, 127.82, 127.54, 126.58, 124.49, 121.57, 115.91, 82.08, 73.96, 56.12, 50.65, 48.45, 45.25, 28.97, 21.34; HRMS (ESI): m/z, Calcd. for $C_{22}H_{25}O_4N_4S$ $[M+H]^+$: 441.1591, Found 441.1585.

*5.2.22 (R)-(2-(3-Methoxypiperidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**(R)-9b-6**)*

Following the preparation protocol of compound **9a**, starting from compound **(R)-8b-6** (45 mg, 0.10 mmol), the title compound **(R)-9b-6** was obtained as a white solid (40 mg, 95%); mp 123-125 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.99 (s, 1H), 8.32 - 8.24 (m, 1H), 8.05 (brs, 1H), 7.83 - 7.69 (m, 3H), 7.48 - 7.32 (m, 2H), 4.12 (s, 2H), 3.67 - 3.59 (m, 2H), 3.51 - 3.36 (m, 3H), 3.29 (s, 3H), 1.93 - 1.72 (m, 2H), 1.69 - 1.60 (m, 1H), 1.57 - 1.45 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.56, 169.39, 163.40, 163.24, 138.26, 133.98, 129.73, 128.17, 127.82, 127.54, 126.59, 124.49, 121.58, 115.91, 82.08, 73.96, 56.12, 50.66, 48.42, 45.26, 28.97, 21.34; HRMS (ESI): m/z, Calcd. for $C_{22}H_{25}O_4N_4S$ $[M+H]^+$: 441.1591, Found 441.1584.

*5.2.23 (5-(Naphthalen-2-ylcarbamoyl)-2-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)thiazol-4-yl)glycine (**9b-7**)*

Following the preparation protocol of compound **9a**, starting from compound **8b-7** (100 mg, 0.20 mmol), the title compound **9b-7** was obtained as a white solid (85 mg, 90%); mp 131-133 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.56 (s, 1H), 9.05 (s, 1H), 8.28 (d, J = 2.1 Hz, 1H), 8.09 - 8.01 (m, 1H), 7.85 - 7.67 (m, 4H), 7.48 - 7.31 (m, 2H), 4.13 (d, J = 5.8 Hz, 2H), 3.94 (s, 4H), 3.59 (t, J = 5.6 Hz, 4H), 1.75 (t, J = 5.9 Hz, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.56, 169.34, 163.31, 163.22, 138.25, 133.98, 129.74, 128.17, 127.82, 127.55, 126.59, 124.50, 121.57, 115.91, 105.51, 82.12, 64.72, 49.38, 47.73, 45.29, 33.68, 22.68; HRMS (ESI): m/z, Calcd. for $C_{23}H_{25}O_4N_5S$ $[M+H]^+$: 469.1540, Found 469.1529.

*5.2.24 (5-(Naphthalen-2-ylcarbamoyl)-2-(1,4-dioxa-7-azaspiro[4.5]decan-7-yl)thiazol-4-yl)glycine (**9b-8**)*

Following the preparation protocol of compound **9a**, starting from compound **8b-8** (100 mg, 0.20 mmol), the title compound **9b-8** was obtained as a white solid (88 mg, 93%); mp 143-145 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.99 (s, 1H), 8.28 (d, J = 2.1 Hz, 1H), 8.06 (s, 1H), 7.83 - 7.67 (m, 4H), 7.47 - 7.29 (m, 2H), 4.12 (s, 2H), 3.98 - 3.92 (m, 4H), 3.51 (s, 4H), 1.84 - 1.71 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.53, 169.01, 163.33, 163.23, 138.19, 133.96, 129.76, 128.19, 127.82, 127.56, 126.60, 124.53, 121.58, 115.97, 106.44, 82.73, 64.42, 46.36, 45.28, 34.11; HRMS (ESI): m/z, Calcd. for $C_{23}H_{25}O_4N_5S$ $[M+H]^+$: 469.1540, Found 469.1538.

*5.2.25 (2-(4-(Hydroxymethyl)piperidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9b-9**)*

Following the preparation protocol of compound **9a**, starting from compound **8b-9** (100 mg, 0.21 mmol), the title compound **9b-9** was obtained as a yellow solid (85 mg,

90%); mp 155-157 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.59 (s, 1H), 8.99 (s, 1H), 8.28 (d, J = 2.0 Hz, 1H), 8.04 (t, J = 5.9 Hz, 1H), 7.86 - 7.66 (m, 4H), 7.49 - 7.30 (m, 2H), 4.55 (brs, 1H), 4.12 (d, J = 5.9 Hz, 2H), 4.02 - 3.85 (m, 2H), 3.31 - 3.26 (m, 2H), 3.19 - 3.02 (m, 2H), 1.85 - 1.73 (m, 2H), 1.73 - 1.59 (m, 1H), 1.29 - 1.13 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.56, 169.21, 163.47, 163.27, 138.26, 133.98, 129.74, 128.17, 127.82, 127.55, 126.59, 124.50, 121.58, 115.91, 82.23, 65.80, 48.12, 45.29, 38.32, 28.26; HRMS (ESI): m/z, Calcd. for $C_{22}H_{25}O_4N_4S$ $[M+H]^+$: 441.1591, Found 441.1600.

*5.2.26 (2-(3-Hydroxy-8-azabicyclo[3.2.1]octan-8-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9b-10**)*

Following the preparation protocol of compound **9a**, starting from compound **8b-10** (80 mg, 0.17 mmol), the title compound **9b-10** was obtained as a yellow solid (54 mg, 74%); mp 159-161 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.53 (s, 1H), 8.99 (s, 1H), 8.28 (d, J = 2.0 Hz, 1H), 8.06 (t, J = 6.0 Hz, 1H), 7.82 - 7.68 (m, 4H), 7.49 - 7.28 (m, 2H), 4.76 (brs, 1H), 4.23 - 4.08 (m, 4H), 3.92 (brs, 1H), 2.36 - 2.24 (m, 2H), 2.16 - 2.04 (m, 2H), 2.03 - 1.91 (m, 2H), 1.79 - 1.67 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.55, 165.20, 163.61, 163.28, 138.29, 133.98, 129.72, 128.16, 127.81, 127.54, 126.58, 124.48, 121.57, 115.87, 82.25, 63.21, 57.12, 45.32, 36.95, 28.28; HRMS (ESI): m/z, Calcd. for $C_{23}H_{25}O_4N_4S$ $[M+H]^+$: 453.1591, Found 453.1571.

*5.2.27 (5-(Naphthalen-2-ylcarbamoyl)-2-(4-oxopiperidin-1-yl)thiazol-4-yl)glycine (**9b-11**)*

To a stirred solution of **8b-11** (193 mg, 0.39 mmol) in actone (2 mL) was added 1M HCl aqueous solution (2 mL), the reaction mixture was then refluxed for 2 h. Then the mixture was adjusted pH to 7 with saturated Na_2CO_3 solution. The aqueous layer was washed with **EA** (5 mL × 3), crystallized and filtered to obtain **9b-11** as a light pink solid (81 mg, 49%); mp 175-177 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.02 (s, 1H), 8.32 (d, J = 2.0 Hz, 1H), 8.04 (t, J = 4.4, 1H), 7.81 - 7.69 (m, 4H), 7.46 - 7.29 (m, 2H), 3.86 (t, J = 6.3 Hz, 4H), 3.68 (d, J = 4.3 Hz, 2H), 2.57 (t, J = 6.2 Hz, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 207.12, 171.92, 168.70, 163.16, 163.05, 138.67, 134.09, 129.58, 128.08, 127.79, 127.53, 126.51, 124.28, 121.52, 115.44, 81.87, 48.94, 45.97, 40.54; HRMS (ESI): m/z, Calcd. for $C_{21}H_{21}O_4N_4S$ $[M+H]^+$: 425.1278, Found 425.1259.

*5.2.28 (2-(4-Cyanopiperidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9b-12**)*

Following the preparation protocol of compound **9a**, starting from compound **8b-12** (50 mg, 0.11 mmol), the title compound **9b-12** was obtained as a white solid (28 mg, 60%); mp 146-148 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.07 (s, 1H), 8.28 (d, J = 2.0 Hz, 1H), 7.83 - 7.67 (m, 3H), 7.47 - 7.31 (m, 2H), 4.13 (s, 2H), 3.77 - 3.62 (m, 2H), 3.50 - 3.37 (m, 2H), 3.23 - 3.12 (m, 1H), 2.08 - 1.95 (m, 2H), 1.90 - 1.76 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.52, 169.22, 163.23, 163.20, 138.15, 133.95, 129.78, 128.19, 127.83, 127.56, 126.61, 124.55, 122.12, 121.59, 116.03, 82.76,

46.25, 45.28, 27.63, 25.43; HRMS (ESI): m/z, Calcd. for $C_{22}H_{22}O_3N_5S$ $[M+H]^+$: 436.1438, Found 436.1441.

*5.2.29 (5-(Naphthalen-2-ylcarbamoyl)-2-(4-(trifluoromethyl)piperidin-1-yl)thiazol-4-yl)glycine (**9b-13**)*

Following the preparation protocol of compound **9a**, starting from compound **8b-13** (100 mg, 0.20 mmol), the title compound **9b-13** was obtained as a yellow solid (71 mg, 75%); mp 160-162 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.02 (s, 1H), 8.29 (d, J = 2.0 Hz, 1H), 8.03 (t, J = 5.2 Hz, 1H), 7.84 - 7.68 (m, 4H), 7.47 - 7.30 (m, 2H), 4.09 - 3.97 (m, 2H), 3.94 (d, J = 5.1 Hz, 2H), 3.24 - 3.11 (m, 2H), 2.67 (s, 1H), 2.01 - 1.88 (m, 2H), 1.60 - 1.43 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.48, 169.21, 163.21, 163.18, 138.35, 134.01, 127.93 (q), 129.70, 128.15, 127.80, 127.55, 126.56, 124.44, 121.57, 115.80, 82.25, 46.67, 46.60, 38.85 (q), 23.87; HRMS (ESI): m/z, Calcd. for $C_{22}H_{22}O_3N_4SF_3$ $[M+H]^+$: 479.1359, Found 479.1353.

*5.2.30 (2-(4,4-Dimethylpiperidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9b-14**)*

Following the preparation protocol of compound **9a**, starting from compound **8b-14** (100 mg, 0.21 mmol), the title compound **9b-14** was obtained as a white solid (78 mg, 83%); mp 172-174 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.58 (s, 1H), 9.01 (s, 1H), 8.29 (d, J = 2.0 Hz, 1H), 8.05 (t, J = 5.9 Hz, 1H), 7.85 - 7.68 (m, 4H), 7.48 - 7.31 (m, 2H), 4.12 (d, J = 5.9 Hz, 2H), 3.51 (t, J = 5.9 Hz, 4H), 1.43 (t, J = 5.9 Hz, 4H), 0.99 (s, 7H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.55, 169.21, 163.47, 163.26, 138.26, 133.98, 129.73, 128.17, 127.81, 127.54, 126.58, 124.49, 121.57, 115.89, 82.22, 45.26, 37.56, 29.25, 27.77; HRMS (ESI): m/z, Calcd. for $C_{23}H_{27}O_3N_4S$ $[M+H]^+$: 439.1798, Found 439.1791.

*5.2.31 (2-(4-Hydroxy-4-methylpiperidin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9b-15**)*

Following the preparation protocol of compound **9a**, starting from compound **8b-15** (100 mg, 0.21 mmol), the title compound **9b-15** was obtained as a white solid (85 mg, 90%); mp 180-182 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.99 (s, 1H), 8.28 (d, J = 2.0 Hz, 1H), 8.04 (s, 1H), 7.83 - 7.69 (m, 4H), 7.48 - 7.31 (m, 2H), 4.12 (d, J = 3.6 Hz, 2H), 3.75 - 3.59 (m, 2H), 3.47 - 3.41 (m, 2H), 1.64 - 1.50 (m, 4H), 1.18 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.37, 169.06, 163.46, 163.17, 138.49, 134.04, 129.63, 128.11, 127.79, 127.53, 126.53, 124.35, 121.54, 115.62, 81.68, 66.37, 46.93, 44.85, 37.72, 30.06; HRMS (ESI): m/z, Calcd. for $C_{22}H_{25}O_4N_4S$ $[M+H]^+$: 441.1591, Found 425.1604.

*5.2.32 (2-(3,6-Dihydropyridin-1(2H)-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9b-16**)*

Following the preparation protocol of compound **9a**, starting from compound **8b-16** (50 mg, 0.11 mmol), the title compound **9b-16** was obtained as a white solid (34 mg, 74%); mp 131-133 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.53 (s, 1H), 9.06 (s,

1H), 8.29 (d, *J* = 2.0 Hz, 1H), 8.06 (t, *J* = 6.0 Hz, 1H), 7.84 - 7.69 (m, 4H), 7.50 - 7.31 (m, 2H), 5.93 (d, *J* = 10.4 Hz, 1H), 5.82 (d, *J* = 10.4 Hz, 1H), 4.14 (d, *J* = 5.5 Hz, 2H), 4.00 - 3.92 (m, 2H), 3.64 (t, *J* = 5.8 Hz, 2H), 2.26 (brs, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.56, 169.22, 163.28, 163.26, 138.22, 133.97, 129.75, 128.18, 127.82, 127.55, 126.59, 125.72, 124.52, 123.93, 121.60, 115.97, 82.23, 47.02, 45.27, 44.53, 24.40; HRMS (ESI): m/z, Calcd. for $C_{21}H_{21}O_3N_4S$ $[M+H]^+$: 409.1329, Found 409.1311.

*5.2.33 (5-(Naphthalen-2-ylcarbamoyl)-2-(piperazin-1-yl)thiazol-4-yl)glycine (**9g**)*

To a stirred solution of **9e** (46 mg, 0.08 mmol) in DCM (2 mL) was added TFA (0.1 mL) dropwise, the reaction mixture was then allowed to stir at room temperature for 5 h. DCM and excess TFA were removed under reduced pressure, diethyl ether (2 mL) were then added to the residue. After filtration and drying under high vacuum, compound **9g** was obtained as a yellow solid (35 mg, 94%); mp 131-133 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.23 (s, 1H), 8.29 (d, *J* = 2.0 Hz, 1H), 8.06 (t, *J* = 5.9 Hz, 1H), 7.84 - 7.70 (m, 4H), 7.47 - 7.33 (m, 2H), 4.14 (d, *J* = 6.0 Hz, 2H), 3.85 - 3.65 (m, 4H), 3.29 - 3.18 (m, 4H).

*5.2.34 (2-(3-Methylpiperazin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9h**)*

Following the preparation protocol of compound **9g**, starting from compound **9f** (50 mg, 0.11 mmol), the title compound **9h** was obtained as a yellow solid (30 mg, 64%); mp 139-140 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.18 (s, 1H), 8.28 (d, *J* = 2.0 Hz, 1H), 8.06 (t, *J* = 5.1 Hz, 1H), 7.84 - 7.79 (m, 2H), 7.77 (d, *J* = 8.2 Hz, 1H), 7.72 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.48 - 7.32 (m, 2H), 4.16 (d, *J* = 4.9 Hz, 2H), 3.97 (t, *J* = 12.7 Hz, 2H), 3.51 - 3.43 (m, 2H), 3.41 - 3.35 (m, 1H), 3.27 - 3.14 (m, 2H), 1.28 (d, *J* = 6.5 Hz, 3H); HRMS (ESI): m/z, Calcd. for $C_{21}H_{24}O_3N_5S$ $[M+H]^+$: 426.1594, Found 426.1607.

*5.2.35 (2-(3,3-Dimethylpiperazin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9i**)*

Following the preparation protocol of compound **9a**, starting from compound **8i** (79 mg, 0.19 mmol), the title compound **9i** was obtained as a white solid (70 mg, 94%); mp 179-181 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.98 (s, 1H), 8.28 (d, *J* = 2.0 Hz, 1H), 8.04 (t, *J* = 5.8 Hz, 1H), 7.79 (dd, *J* = 8.5, 3.1 Hz, 2H), 7.75 (d, *J* = 8.2 Hz, 1H), 7.71 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.48 - 7.29 (m, 2H), 4.07 (d, *J* = 5.7 Hz, 2H), 3.50 - 3.42 (m, 2H), 3.26 (s, 2H), 2.89 (t, *J* = 5.1 Hz, 2H), 1.10 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.54, 169.60, 163.36, 163.23, 138.27, 133.98, 129.72, 128.17, 127.81, 127.54, 126.59, 124.48, 121.54, 115.84, 81.97, 58.27, 50.46, 48.02, 45.50, 40.53, 25.23 ; HRMS (ESI): m/z, Calcd. for $C_{22}H_{26}O_3N_5S$ $[M+H]^+$: 440.1751, Found 440.1764.

*5.2.36 (2-((3S,5R)-3,5-Dimethylpiperazin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9j**)*

Following the preparation protocol of compound **9a**, starting from compound **8j** (140 mg, 0.30 mmol), the title compound **9j** was obtained as a white solid (47 mg, 36%); mp 180-182 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.98 (s, 1H), 8.29 (d, J = 2.0 Hz, 1H), 8.03 (t, J = 5.5 Hz, 1H), 7.82 - 7.69 (m, 4H), 7.46 - 7.31 (m, 2H), 4.04 (d, J = 5.4 Hz, 2H), 3.73 (brs, 2H), 2.86 - 2.76 (m, 2H), 2.69 - 2.59 (m, 2H), 1.04 (d, J = 6.2 Hz, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.45, 169.18, 163.30, 163.19, 138.36, 134.00, 129.68, 128.16, 127.80, 127.55, 126.56, 124.42, 121.51, 115.73, 81.94, 54.13, 50.26, 46.28, 19.19; HRMS (ESI): m/z, Calcd. for $C_{22}H_{26}O_3N_5S$ $[M+H]^+$: 440.1751, Found 440.1752.

*5.2.37 (2-(4-Methylpiperazin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9k**)*

Following the preparation protocol of compound **9a**, starting from compound **8k** (130 mg, 0.30 mmol), the title compound **9k** was obtained as a white solid (91 mg, 77%); mp 215-217 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.08 (s, 1H), 8.29 (d, J = 2.0 Hz, 1H), 8.05 (t, J = 5.9 Hz, 1H), 7.85 - 7.78 (m, 2H), 7.76 (d, J = 8.1 Hz, 1H), 7.72 (dd, J = 8.9, 2.1 Hz, 1H), 7.47 - 7.32 (m, 2H), 4.12 (d, J = 5.8 Hz, 2H), 3.49 (t, J = 4.8, 4H), 2.44 (t, J = 5.1 Hz, 4H), 2.24 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.52, 169.45, 163.27, 163.24, 138.20, 133.97, 129.76, 128.18, 127.82, 127.56, 126.59, 124.52, 121.60, 115.98, 82.56, 53.99, 47.65, 46.09, 45.44; HRMS (ESI): m/z, Calcd. for $C_{21}H_{24}O_3N_5S$ $[M+H]^+$: 426.1594, Found 426.1594.

*5.2.38 (2-(3,4-Dimethylpiperazin-1-yl)-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9l**)*

Following the preparation protocol of compound **9a**, starting from compound **8l** (130 mg, 0.28 mmol), the title compound **9l** was obtained as a white solid (112 mg, 97%); mp 187-189 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 (s, 1H), 8.28 (d, J = 2.0 Hz, 1H), 8.04 (t, J = 5.9 Hz, 1H), 7.84 - 7.77 (m, 2H), 7.76 (d, J = 8.1 Hz, 1H), 7.72 (dd, J = 8.8, 2.1 Hz, 1H), 7.48 - 7.31 (m, 2H), 4.13 (d, J = 5.8 Hz, 2H), 3.83 - 3.61 (m, 2H), 3.27 - 3.21 (m, 1H), 2.96 - 2.78 (m, 2H), 2.29 - 2.11 (m, 5H), 1.05 (d, J = 6.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.55, 169.20, 163.29, 163.24, 138.19, 133.97, 129.76, 128.20, 127.82, 127.56, 126.60, 124.53, 121.56, 115.96, 82.58, 56.85, 53.91, 53.83, 47.67, 45.32, 42.35, 16.47 ; HRMS (ESI): m/z, Calcd. for $C_{22}H_{26}O_3N_5S$ $[M+H]^+$: 440.1751, Found 440.1751.

*5.2.39 (5-(Naphthalen-2-ylcarbamoyl)-2-(3-oxopiperazin-1-yl)thiazol-4-yl)glycine (**9m**)*

Following the preparation protocol of compound **9a**, starting from compound **8m** (30 mg, 0.06 mmol), the title compound **9m** was obtained as a yellow solid (22 mg, 78%); mp 108-110 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm)δ 9.13 (s, 1H), 8.65 (brs, 1H), 8.29 (t, J = 2.6 Hz, 2H), 8.06 (q, J = 5.6 Hz, 1H), 7.84 - 7.75 (m, 3H), 7.73 (dd, J = 8.9, 2.0 Hz, 1H), 7.47 - 7.31 (m, 2H), 4.16 (m, 2H), 4.01 (s, 1H), 3.82 (s, 2H), 3.67 (m, 1H), 3.40 - 3.35 (m, 1H), 3.16 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 168.15, 168.10, 165.90, 163.22, 163.05, 138.17, 133.96, 129.77, 128.19, 127.82, 127.56,

126.60, 124.55, 121.60, 116.03, 82.70, 63.27, 50.26, 45.82, 44.62; HRMS (ESI): m/z, Calcd. for $C_{20}H_{20}O_4N_5S$ $[M+H]^+$: 426.1231, Found 426.1232.

*5.2.40 (2-Morpholino-5-(naphthalen-2-ylcarbamoyl)thiazol-4-yl)glycine (**9n**)*

Following the preparation protocol of compound **9a**, starting from compound **8n** (130 mg, 0.29 mmol), the title compound **9n** was obtained as a white solid (55 mg, 45%); mp 174-176 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.03 (s, 1H), 8.31 (d, J = 2.0 Hz, 1H), 8.01 (t, J = 4.4, 1H), 7.82 - 7.73 (m, 3H), 7.72 (dd, J = 8.9, 2.0 Hz, 1H), 7.47 - 7.28 (m, 2H), 3.72 (t, J = 4.9 Hz, 4H), 3.67 (d, J = 4.2 Hz, 2H), 3.48 (t, J = 4.9 Hz, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.53, 169.82, 163.27, 163.14, 138.13, 133.95, 129.79, 128.19, 127.83, 127.57, 126.61, 124.57, 121.62, 116.08, 82.72, 65.76, 47.78, 45.27; HRMS (ESI): m/z, Calcd. for $C_{20}H_{21}O_4N_4S$ $[M+H]^+$: 413.1278, Found 413.1276.

*5.2.41 (5-(Naphthalen-2-ylcarbamoyl)-2-thiomorpholinothiazol-4-yl)glycine (**9o**)*

Following the preparation protocol of compound **9a**, starting from compound **8o** (70 mg, 0.15 mmol), the title compound **9o** was obtained as a white solid (63 mg,96%); mp 131-133 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.60 (s, 1H), 9.03 (s, 1H), 8.31 (d, J = 2.0 Hz, 1H), 8.05 (t, J = 6.0 Hz, 1H), 7.81 - 7.70 (m, 4H), 7.46 - 7.29 (m, 2H), 4.12 (d, J = 5.6 Hz, 2H), 3.89 - 3.72 (m, 4H), 2.80 - 2.66 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.51, 169.04, 163.24, 163.19, 138.17, 133.96, 129.78, 128.20, 127.83, 127.56, 126.61, 124.55, 121.57, 115.99, 82.65, 50.61, 45.30, 26.21; HRMS (ESI): m/z, Calcd. for $C_{20}H_{21}O_3N_4S_2$ $[M+H]^+$: 429.1050, Found 429.1044.

*5.2.42 (5-(Naphthalen-2-ylcarbamoyl)-2-(2-oxa-6-azaspiro[3.3]heptan-6-yl)thiazol-4-yl)glycine (**9p**)*

Following the preparation protocol of compound **9a**, starting from compound **8p** (51 mg, 0.11 mmol), the title compound **9p** was obtained as a yellow solid (36 mg, 75%); mp 181-183 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.05 (s, 1H), 8.28 (d, J = 2.0 Hz, 1H), 8.08 (s, 1H), 7.84 - 7.78 (m, 2H), 7.76 (d, J = 8.2 Hz, 1H), 7.72 (dd, J = 8.9, 2.1 Hz, 1H), 7.47 - 7.31 (m, 2H), 4.73 (s, 4H), 4.27 (s, 4H), 4.12 (s, 2H); HRMS (ESI): m/z, Calcd. for $C_{21}H_{21}O_4N_4S$ $[M+H]^+$: 425.1278, Found 425.1276.

*5.2.43 (5-(Naphthalen-2-ylcarbamoyl)-2-(2-azaspiro[3.3]heptan-2-yl)thiazol-4-yl)glycine (**9q**)*

Following the preparation protocol of compound **9a**, starting from compound **8q** (50 mg, 0.11 mmol), the title compound **9q** was obtained as a yellow solid (46 mg, 95%); mp 144-146 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.03 (s, 1H), 8.28 (d, J = 2.1 Hz, 1H), 8.08 (t, J = 5.5 Hz, 1H), 7.84 - 7.77 (m, 2H), 7.76 (d, J = 8.1 Hz, 1H), 7.72 (dd, J = 8.9, 2.1 Hz, 1H), 7.47 - 7.31 (m, 2H), 4.11 (d, J = 4.5 Hz, 2H), 4.06 (s, 4H), 2.22 (t, J = 7.6 Hz, 4H), 1.80 (p, J = 7.7 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.46, 169.35, 163.57, 163.25, 138.19, 133.96, 129.76, 128.18, 127.82, 127.56, 126.60, 124.53, 121.59, 115.99, 83.17, 64.94, 45.30, 32.73, 32.11, 16.10; HRMS (ESI): m/z, Calcd. for $C_{22}H_{23}O_3N_4S$ $[M+H]^+$: 423.1485, Found 423.1488.

*5.2.44 (5-([1,1'-Biphenyl]-4-ylcarbamoyl)-2-(2-oxa-6-azaspiro[3.3]heptan-6-yl)thiazol-4-yl)glycine (**9r**)*

Following the preparation protocol of compound **9a**, starting from compound **8r** (75 mg, 0.16 mmol), the title compound **9r** was obtained as a white solid (64 mg, 90%); mp 144-146 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.94 (s, 1H), 8.03 (s, 1H), 7.74 (d, *J*= 8.4 Hz, 2H), 7.64 (d, *J* = 7.7 Hz, 2H), 7.57 (d, *J* = 8.4 Hz, 2H), 7.43 (t, *J* = 7.6 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 1H), 4.73 (s, 4H), 4.26 (s, 4H), 4.11 (s, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.44, 169.19, 163.41, 163.06, 140.37, 140.03, 134.29, 129.34, 127.32, 127.28, 126.97, 126.79, 126.59, 120.72, 83.30, 79.89, 62.02, 45.30, 30.51; HRMS (ESI): m/z, Calcd. for $C_{23}H_{23}O_4N_4S$ $[M+H]^+$: 451.1435, Found 451.1408.

*5.2.45 (5-((3,4-Dichlorophenyl)carbamoyl)-2-(2-oxa-6-azaspiro[3.3]heptan-6-yl)thiazol-4-yl)glycine (**9s**)*

Following the preparation protocol of compound **9a**, starting from compound **8s** (75 mg, 0.16mmol), the title compound **9s** was obtained as a white solid (69 mg, 97%); mp 147-149 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.08 (s, 1H), 8.06 (d, *J* = 2.4 Hz, 1H), 8.03 (t, *J* = 5.8 Hz, 1H), 7.60 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.48 (d, *J* = 8.8 Hz, 1H), 4.72 (s, 4H), 4.26 (s, 4H), 4.07 (d, *J* = 5.8 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.26, 169.32, 163.96, 162.91, 140.83, 130.99, 130.64, 123.72, 121.15, 120.03, 82.75, 79.85, 61.97, 45.52, 30.18; HRMS (ESI): m/z, Calcd. for $C_{17}H_{17}O_4N_4SCl_2$ $[M+H]^+$: 443.0342, Found 443.0341.

*5.2.46 (5-((3-Isopropylphenyl)carbamoyl)-2-(2-oxa-6-azaspiro[3.3]heptan-6-yl)thiazol-4-yl)glycine (**9t**)*

Following the preparation protocol of compound **9a**, starting from compound **8t** (100 mg, 0.23 mmol), the title compound **9t** was obtained as a white solid (76 mg, 81%); mp 136-138 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.52 (s, 1H), 8.75 (s, 1H), 7.98 (t, *J* = 6.0 Hz, 1H), 7.52 (t, *J* = 1.9 Hz, 1H), 7.45 (dd, *J* = 7.6, 2.1 Hz, 1H), 7.14 (t, *J* = 7.8 Hz, 1H), 6.85 (d, *J* = 7.6 Hz, 1H), 4.72 (s, 4H), 4.25 (s, 4H), 4.09 (d, *J* = 5.9 Hz, 2H), 2.82 (hept, *J* = 6.8 Hz, 1H), 1.19 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.48, 169.11, 163.21, 163.06, 148.93, 140.41, 128.61, 120.84, 118.43, 118.04, 83.21, 79.89, 62.01, 45.47, 34.01, 28.90, 24.39; HRMS (ESI): m/z, Calcd. for $C_{20}H_{25}O_4N_4S$ $[M+H]^+$: 417.1591, Found 417.1597.

*5.2.47 4-((2-Amino-2-oxoethyl)amino)-2-(4-hydroxy-4-methylpiperidin-1-yl)-N-(naphthalen-2-yl)thiazole-5-carboxamide (**10**)*

To a stirred solution of **9b-15** (367 mg, 0.80 mmol) in THF (20 mL) was added EDCI (335 mg, 1.75 mmol) and HOBt (237 mg, 1.75 mmol), the reaction mixture was then allowed to stir at room temperature for 1 h. Ammonia (145 mg,2.4 mmol) was added and stirred at room temperature for 2 h. The reaction solution was diluted with EA (5 mL) and washed with saturated NaCl solution (10 mL × 2) and water (10 mL × 2). The organic layer was concentrated and the crude product was purified by column chromatography (D/M = 50:1-20:1) to give the title compound **10** (218 mg, 58%) as a

white solid; mp 121-123 °C; ^{1}H NMR (400 MHz, Methanol-d_4) δ (ppm) 8.04 (s, 1H), 7.81 - 7.72 (m, 3H), 7.64 - 7.58 (m, 1H), 7.46 - 7.32 (m, 2H), 4.13 - 4.09 (m, 2H), 3.79 (d, J = 13.1 Hz, 2H), 3.53 - 3.45 (m, 3H), 1.73 - 1.60 (m, 4H), 1.27 (s, 3H); HRMS (ESI): m/z, Calcd. for $C_{22}H_{26}O_3N_5S$ $[M+H]^+$: 440.1751, Found 440.1728.

*5.2.48 2-(4-Amino-4-hydroxypiperidin-1-yl)-4-((cyanomethyl)amino)-N-(naphthalen-2-yl)thiazole-5-carboxamide (**11**)*

To a stirred solution of **10** (112 mg, 0.25 mmol) in DCM (5 mL) was added DBU (197 mg, 1.27 mmol) and stirred at room temperature for 10 min. Ethyl dichlorophosphate (210 mg, 1.27 mmol) was added. The reaction mixture was then allowed to stir at room temperature for 3 h. The reaction solution was diluted with DCM (5 mL) and washed with saturated NaCl solution (10 mL × 2) and water (10 mL × 2). The organic layer was concentrated and the crude product was purified by column chromatography (P/E= 1:1) to give the title compound **11** (25 mg, 23%) as a white solid; mp 149-151 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 8.08 (s, 1H), 7.92 - 7.67 (m, 4H), 7.52 - 7.34 (m, 3H), 6.66 (s, 1H), 4.37 (d, J = 6.2 Hz, 2H), 3.80 (s, 2H), 3.61 - 3.46 (m, 2H), 1.71 (s, 4H), 1.33 (s, 3H); ^{13}C NMR (150MHz, DMSO-d_6) δ (ppm) 169.13, 162.95, 161.91, 137.94, 133.87, 129.82, 128.20, 127.81, 127.53, 126.62, 124.63, 121.55, 119.15, 116.20, 84.04, 66.30, 44.97, 37.63, 32.11, 30.02; HRMS (ESI): m/z, Calcd. for $C_{22}H_{24}O_2N_5S$ $[M+H]^+$: 422.1645, Found 422.1652.

*5.2.49 3-(5-(Naphthalen-2-ylcarbamoyl)-2-(2-oxa-6-azaspiro[3.3]heptan-6-yl)thiazol-4-yl)propanoic acid (**20**)*

Following the preparation protocol of compound **9a**, starting from compound **19** (30 mg, 0.07 mmol), the title compound **20** was obtained as a yellow solid (19 mg, 67%); mp 143-145 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 10.17 (s, 1H), 8.28 (d, J = 2.1 Hz, 1H), 7.90 - 7.77 (m, 3H), 7.69 (dd, J = 8.9, 2.1 Hz, 1H), 7.52 - 7.36 (m, 2H), 4.73 (s, 4H), 4.26 (s, 4H), 3.07 (t, J = 7.2 Hz, 2H), 2.68 (t, J = 7.1 Hz, 2H); HRMS (ESI): m/z, Calcd. for $C_{22}H_{22}O_4N_3S$ $[M+H]^+$: 424.1326, Found 424.1340.

5.3. Biological evaluation

5.3.1. Protein expression and purification

The pET-28a-Pin1 plasmid was a gift from Professor Joseph P. Noel (The Salk Institute for Biological Studies, La Jolla, California). The Pin1 gene fragment was amplified with PCR and inserted into pET-28a plasmid after a His-tag and a Prescission protease cleavage site (His-tag-Leu-Phe-Gln-Gly-Pro), enabling to remove the N-terminal His-tag by Prescission protease. The Pin1 was expressed in *E.coli* BL21(DE3) induced by IPTG. Cells were harvested via centrifugation and resuspended in 25 mM Tris-HCl, 200 mM NaCl, pH 7.8. Following sonication at 4 °C, the crude protein solution was sequencially purified with HisTrap™ HP, Hitrap™ Q HP and Superdex G75 (GE health care). After first purified with HisTrap™ HP, the collected Pin1 was incubated with Precission at 4 °C for 16 h.

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

PPIase activities were measured at 6 ˚C Thermo Scientific NanoDrop One^C Microvolume UV-Vis Spectrophotometer using protease-coupled assay according to Wang et al.[50, 51] Initially, 5 μL DMSO (Control) or test compounds desolved in DMSO with varying concerntrations (Sample) was incubated with 850 μL assay buffer (HEPES-Na 35 mM, pH 7.8) and 5 μL Pin1 (12 μM) on ice for 10 min. Before mixing with 100 μL chymotrypsin (60 mg/mL in 0.001 M HCl) and 40 μL substrate (2.5 mM Suc-Ala-Glu-Pro-Phe-4-nitroanilide in 0.47 M LiCl/trifluoroethanol), the pre-mixture was transferred to a quartz cuvette and set blanking. After a fast and fine mixing, the assay was measured with kinetic method by monitoring the absorbance at 390 nm once per 2 seconds during 90 s. Three concentrations (100 μM, 10 μM, 1 μM) were chosen for all compounds, except for **9q** (100 μM, 10 μM, 1 μM, 0.1 μM), and the assay was performed in duplicate. An independent assay without Pin1, tested compound and DMSO must be run to determine the kinetics of spontaneous isomerization of substrate.

The data was analyzed with One Phase Decay model in Graphpad Prism 5.01 to obtain reaction rate. The inhibition at each concentration was calculated according the following equation: Inhibition ratio(%)=[1-(k_X-k_1)/(k_D-k_1)]×100, 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 without Pin1, tested compound and DMSO. The IC_{50} was calculated by modelling the relationship between inhibition ratio and the corresponded concentration of tested compounds.

5.4 Computational studies

All molecular computation studies were performed using CDOCKER protocol integrated in Accelrys Discovery Studio Client 2018 (Accelrys Software Inc., San Diego, CA). The 3D structure of Compound **9b** and **9p** was generated using Prepare Ligand tools of DS and refined with CHARMm forcefield. The co-crystal structure of Pin1 with a small molecular inhibitor (PDB ID: 3JYJ, B chain) was chosen for molecular docking. Using Prepare Protein tool of DS, the water molecules in protein were removed and the protein were added hydrogen, corrected the incomplete residues and refined with CHARMm force field. The co-crystallized reference molecule was chosen as the center to construct the binding site within 9.34 Å. To dock those compounds into the binding site, CDOCKER was performed using the default parameters. The 20 final docked conformations were ranked according to their binding free energy. The docking mode was chosen on the basis of binding rationality.

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Graphical Abstract:

Design, synthesis and biological evaluation of novel thiazole-based derivatives as human Pin1 inhibitors

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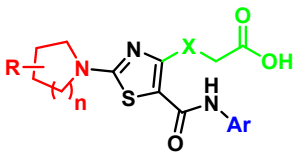

X = NH, CH_2

Ar = aromatic rings

Various alicyclic rings on the 2-position of thiazole scaffold

IC_{50} = 0.95 - 46.89 μM

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: