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Article

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A Potent, Selective, and Orally Bioavailable HCV NS5A Inhibitor for Treatment of Hepatitis C Virus: (*S*)-1-((*R*)-2-(Cyclopropanecarboxamido)-2-phenylacetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide

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ABSTRACT

Starting from the initial lead 4-phenylthiazole **18**, a modest HCV inhibitor (EC_{50} = 9440 nM), a series of structurally related thiazole derivatives has been identified as a novel chemical class of potent and selective HCV NS5A inhibitors. The introduction of a carboxamide group between the thiazole and pyrrolidine ring (**42**) of compound **18** resulted in a dramatic increase in activity (EC_{50} = 0.92 nM). However, **42** showed only moderate pharmacokinetic properties and limited oral bioavalability of 18.7% in rats. Further optimization of the substituents at the 4-position of the thiazole ring and pyrrolidine nitrogen of the lead compound **42** led to the identification of compound **57**, a highly potent and selective NS5A inhibitor of HCV (EC_{50} = 4.6 nM), with greater therapeutic index (CC_{50}/EC_{50} > 10,000). Pharmacokinetic studies revealed that compound **57** had a superior oral exposure and desired bioavailability of 45% after oral administration in rats.

INTRODUCTION

Hepatitis C is a liver disease caused by the hepatitis C virus (HCV) that was first identified in 1989.[1] HCV has at least six major genotypes, each containing multiple subtypes, with genotype 1 being the most common worldwide.[2] An estimated 170 million people globally have chronic hepatitis C (CHC) that eventually develops into cirrhosis, hepatocellular carcinoma or liver failure.[3] Genotype 1 has been historically the most difficult to treat due to the fact that traditional dual therapy (pegylated interferon plus ribavirin) produces relatively low sustained virological response (SVR) rates (42% - 46%).[4]

In May 2011, the combination use of the first generation protease inhibitors telaprevir and boceprevir (Figure 1) with pegylated interferon and ribavirin (triple therapy) was approved by the Food and Drug Administration (FDA) for the treatment of HCV genotype 1.[5] Telaprevir- or boceprevir-based triple therapy is more effective than traditional dual therapy and has an increased SVR rate, higher than 70%, in genotype-1-infected patients.[5c-e, 6] However, these triple therapies are limited by treatment-related adverse effects and poor tolerability, especially in difficult-to-treat patients, such as those with cirrhosis or advanced liver fibrosis.[5c-e, 6a-e, 7

Pharmaceutical developer Vertex Pharmaceuticals Inc. and Merck & Co. Inc. decide to stop selling telaprevir and boceprevir on October 2014 and December 2015, respectively, due to the alternative direct-acting antivirals (DAAs) with better efficacy and tolerability, approved by FDA since 2013.[8]

Insert Figure 1

Recently, newly approved DAAs (Figure 2), such as the NS3/4A protease inhibitors simeprevir[9] and paritaprevir,[10] the NS5A inhibitors ledipasvir,[11] ombitasvir[12] and daclatasvir,[13] the NS5B polymerase inhibitors sofosbuvir[14] and dasabuvir[15] have led to dramatic progress in treating CHC. These emerging DAAs are being investigated in interferon-free regimens consisting of one to three DAAs with or without ribavirin.[16] However, the efficacy of monotherapy with single DAAs is always insufficient and increases the risk of emerging resistant strains.[17] Combination therapy with DAAs exhibiting different mechanisms is often necessary to reduce drug resistance and dose-related toxicity as well as to enhance the effectiveness of the antivirals. Currently, the NS5A inhibitor ledipasvir is most commonly used in combination with the NS5B polymerase inhibitor sofosbuvir for treatment in chronic hepatitis C genotype 1 patients. This drug has been tested and shown efficacy in

treatment-naive and treatment experienced patients. However, the cost of the fixed-dose combination (ledipasvir 90 mg/sofosbuvir 400 mg) has been a controversial topic. It costs $1,125 per pill in the US, translating to $94,500 for a 12-week treatment course. Therefore, it was aimed to discover and develop a novel, potent, and orally bioavailable HCV NS5A inhibitor with lower molecular weight and cost.

Insert Figure 2

As shown in Figure 2, ledipasvir, ombitasvir, and daclatasvir are potent inhibitors of HCV NS5A, which contain hetero-dimeric and homo-dimeric structures with large molecular weights. In fact, the extremely high cost of this class of HCV NS5A inhibitors may be due to their complicated structures and difficult synthesis. To simplify the chemical structure and reduce the molecular weight of the HCV NS5A inhibitor, daclatasvir, several heterocyclic compounds **6**, **12**, **18**, and **22** (Figure 3) have been designed, synthesized, and evaluated for their inhibitory activity against 1b replicon assay in our anti-HCV screening program. Interestingly, the imidazole **6**, and thiazoles **18** and **22** were identified as the initial leads of HCV inhibitors.

Insert Figure 3

In addition to daclatasvir, ombitasvir is also a potent HCV NS5A inhibitor with a specific carboxamide group between the phenyl and pyrrolidine ring (Figure 2). This interesting structure prompts us to further investigate a series of new amide analogs based on the scaffold of the thiazole compound **18**. The introduction of a carboxamide group between the thiazole and pyrrolidine ring (**42**) resulted in a dramatic increase in activity (EC_{50} = 0.92 nM). Further optimization of the substituents at the 4-position of the thiazole ring and pyrrolidine nitrogen of the lead compound **42** led to the identification of compound **57** (Figure 4), a highly potent and selective NS5A inhibitor of HCV (EC_{50} = 4.6 nM), with greater therapeutic index (CC_{50}/EC_{50} > 10,000).

Insert Figure 4

RESULTS AND DISCUSSION

Chemistry

The synthesis of the imidazole derivative **6** was carried out as shown in Scheme 1.

Coupling reaction of 2-aminoacetophenone hydrochloride **1** with N-Boc-*L*-proline in the presence of EDC/HOBt·H_2O at room temperature gave the corresponding amide **2** in 40% yield. Reaction of **2** with ammonium acetate in the presence of acetic acid at 160 ^oC led to the formation of the imidazole derivative **3** in 74% yield. Deprotection of **3** with trifluoroacetic acid gave the pyrrolidine **4** in 82% yield, which was then coupled with N-Boc-*D*-phenylglycine to give the protected pyrrolidine **5** in 67% yield. Deprotection of **5** with trifluoroacetic acid gave the corresponding amine, which was reacted with 4-morpholinecarbonyl chloride in the presence of triethylamine to give the desired imidazole **6** in 31% overall yield.

Insert Scheme 1

The oxadiazole derivative **12** was prepared as outlined in Scheme 2. Reaction of the benzonitrile **7** (Scheme 2) with hydroxylamine hydrochloride in the presence of DIPEA gave the amidoxime **8** in 85% yield, which was then coupled with N-Boc-*L*-proline in the presence of TBTU/HOBt·H_2O followed by thermal cyclization of the O-acylated intermediate to give the oxadiazole intermediate **9** in 53% yield. Deprotection of **9** with trifluoroacetic acid gave the corresponding pyrrolidine **10** in 90% yield, which was then coupled with N-Boc-*D*-phenylglycine in

the presence of EDC/HOBt·H_2O to give the protected pyrrolidine **11** in 89% yield. Deprotection of **11** with trifluoroacetic acid gave the corresponding amine, which was reacted with 4-morpholinecarbonyl chloride in the presence of triethylamine to give the desired oxadiazole **12** in 77% overall yield.

Insert Scheme 2

The 4-phenylthiazole derivative **18** was prepared as outlined in Scheme 3. Reaction of N-Boc-*L*-proline with $(Boc)_2O$ and $(NH_4)_2CO_3$ in the presence of pyridine gave the corresponding amide **13** in 95% yield, which was reacted with Lawesson's reagent in THF at 70 ℃ to give the thioamide **14** in 89% yield. Condensation of **14** with phenacyl bromide at refluxing ethanol provided the thiazole intermediate **15** in 74% yield. Similarly deprotection of **15** with trifluoroacetic acid gave the pyrrolidine **16** in 82% yield, which was then coupled with N-Boc-*D*-phenylglycine in the presence of EDC/HOBt·H_2O to give the protected pyrrolidine **17** in 71% yield. Deprotection of **17** with trifluoroacetic acid gave the corresponding amine, which was reacted with 4-morpholinecarbonyl chloride in the presence of triethylamine to give the desired thiazole **18** in 58% overall yield.

Insert Scheme 3

The 5-phenylthiazole derivative **22** was prepared as outlined in Scheme 4. Reaction of the amide **2** with Lawesson's reagent at refluxing THF gave the thiazole intermediate **19** in 81% yield. Similarly deprotection of **19** with trifluoroacetic acid gave the pyrrolidine **20** in 88% yield, which was then coupled with N-Boc-*D*-phenylglycine in the presence of EDC/HOBt·H_2O to give the protected pyrrolidine **21** in 72% yield. Deprotection of **21** with trifluoroacetic acid gave the corresponding amine, which was reacted with 4-morpholinecarbonyl chloride in the presence of triethylamine to give the desired thiazole **22** in 56% overall yield.

Insert Scheme 4

To search for more potent HCV inhibitors, exploration of the substituents at the 4-position of the thiazole ring and the pyrrolidine nitrogen of the amide analog of the thiazole **18** was carried out. A series of new amide derivatives **42-51** were designed and synthesized according to the procedures as shown in Scheme 5 beginning from the commercially available 4-substituted-2-aminothiazole **23a-e**. The coupling reaction of **23a-e** with N-Boc-*L*-proline in the presence of HATU/DIPEA in DMF at

50 °C gave the corresponding amides **24a-e**. Deprotection of **24a-e** with trifluoroacetic acid at room temperature gave the pyrrolidine derivatives **25a-e**, which was then coupled with N-Boc protected glycine derivatives **26-31** in the presence of EDC/HOBt·H_2O to give the corresponding pyrrolidines **32-41** in good yields (76-93%). Deprotection of **32-41**with trifluoroacetic acid gave the corresponding amines, which was reacted with 4-morpholinecarbonyl chloride in the presence of triethylamine to give the target compounds **42-51** in moderate to good yields (41-89%).

Insert Scheme 5

The synthesis of amides **52-68**, ureas **69-76**, and thioureas **77-81** were performed according to the procedures as shown in Scheme 6. Deprotection of the Boc protected thiazole **32** with trifluoroacetic acid at room temperature gave the corresponding amine, which was reacted with a variety of commercially available acyl chlorides in the presence of triethylamine at room temperature to give the corresponding amides **52-68** in moderate to good yields (21-90%). In addition, the ureas **69-76** were prepared by reacting the amine intermediate with the corresponding isocyanates, while the thioureas **77-81** were prepared by reacting the amine

intermediate with the corresponding isothiocyanates in the presence of triethylamine. Alternatively, the urea derivatives can also be successfully synthesized by the treatment of the amine intermediate with CDI and primary amine or triphosgene in the presence of triethylamine.

Insert Scheme 6

Biological Evaluation

In order to simplify the chemical structure of the HCV NS5A inhibitor, daclatasvir, and find better therapeutic agents, several heterocyclic compounds **6**, **12**, **18**, and **22** (Figure 3) have been designed, synthesized, and evaluated for their inhibitory activity against 1b replicon assay in our anti-HCV screening program. As shown in Table 1, the imidazole **6**, and thiazoles **18** and **22** showed potent inhibitory activity against HCV genotype 1b with an EC_{50} value of 98, 9440 and 2970 nM, respectively. All of the above compounds (**6**, **18** and **22**) exhibit low cytotoxicity (CC_{50} >50 μM), indicating a good therapeutic window. Surprisingly, a complete loss in activity was observed when A ring was replaced with a 1,2,4-oxadiazole ring (**12**). This result suggests that the type of ring A is important for the activity of this class of inhibitors. Compound **18** was selected for further optimization due to its convenient synthesis

with better yield and excellent thermal and chemical stability though compound **6** and **22** also show inhibitory activity.

Insert Table 1

As shown in Table 2, it is very interesting to note that the introduction of a carboxamide group between the thiazole and pyrrolidine ring resulted in a dramatic increase in inhibitory activity, compound **42** was found to be 10000-fold more potent than **18** (**18** vs **42**, EC_{50} = 9440 vs 0.92 nM). The reason for this is probably that the amide group of **42** can increase the strength of intermolecular interactions via H bonding (since the secondary amide group has an H atom as H-bond donor and a carbonyl group as the H-bond acceptor). With this result in hand, we turned our investigation to the phenyl ring of **42** in order to assess the effect of relatively flexible alkyl groups (Table 2). As shown in compound **43**, removal of phenyl group at position 4 of the thiazole ring of **42** resulted in drastic loss in activity. (EC_{50} from 0.92 nM of **42** $\rightarrow$ 9650 nM of **43**). Furthermore, replacement of the phenyl group at position 4 of the thiazole ring with three other types of alkyl groups, that is, methyl (**44**), *tert*-butyl (**45**), and cyclohexyl (**46**) resulted in significant loss of activity. To gain a deeper insight in the analysis of the activity-flexibility/rigidity relationship for

substituents at the 4-position of the thiazole ring (**42**-**46**), an interesting trend was observed. It was found that analogues with more rigid substituents such as phenyl (**42**) or cyclohexyl (**46**) at the 4-position of the thiazole ring displayed higher potency. When the R_2 group of **42** was changed from phenyl (**42**) to methyl (**47**), a more than 1400 times decrease in activity was observed (**42** vs **47**, EC_{50} = 0.92 vs 1310 nM). Further replacement of the methyl group (**47**) by the ethyl (**48**), *n*-propyl (**49**), *i*-propyl (**50**) and *tert*-butyl (**51**) had no noticeable effect on activity. These above results imply that a more rigid phenyl ring in both R_1 and R_2 positions (**42**) should be necessary for improving the inhibitory activity against HCV (**42** vs **43-51)**.

Insert Table 2

The next step in our design was to investigate if conversion of the morpholine moiety in **42** to different alkyl, alicyclic, aromatic or heterocyclic groups could provide additional anti-HCV potency (Table 3). Replacement of the morpholino urea with a variety of terminal amides (**52**-**56** for methyl, ethyl, *n*-propyl, *i*-propyl and *tert*-butyl, respectively), a double-digit decrease in activity was observed compared with **42** (**42** vs **52-56**, EC_{50} = 0.92 vs 15-86 nM). This decreased activity was also seen in cyclopentyl (**58**) and cyclohexyl (**59**) analogues. (**58**, EC_{50} = 13 nM; **59**, EC_{50} = 14

nM). Interestingly, cyclopropyl derivative **57** showed only a single-digit drop in activity compared with **42** (**42** vs **57**, EC_{50} = 0.92 vs 4.6 nM). Replacing the alicyclic cyclohexyl ring of **59** with an aromatic phenyl ring (**60**) showed no significant change in activity (**59** vs **60**, EC_{50} = 14 vs 15 nM). Introduction of a nitrogen atom into the phenyl ring of **60** yielded three positional isomers, 2'-pyridyl (**61**), 3'-pyridyl (**62**) and 4'-pyridyl (**63**) derivatives. Interestingly, the 4′-pyridyl derivative **63** was more active compared with the corresponding phenyl ring derivative **60** (**63** vs **60**, EC_{50} = 3.8 vs 15 nM), although the other two isomers **61** and **62** showed no increase in activity (**61**, EC_{50} = 22 nM; **62**, EC_{50} = 13 nM). A comparison of activity data from pyridine substituted amides (**61-63**) suggested that the nitrogen atom at the 4′-position of the terminal pyridine ring is optimal.

It would be of interest to study the effect of different nitrogen-containing alkyl substituents (**64-67**) at the R_3 position. Replacement of the isopropyl group (**55**) with a dimethylamino group (**64**) gave a 3-fold enhancement in activity (**55** vs **64**, EC_{50} = 36 vs 11 nM). Increasing the alkyl chain length of the dimethylamine from methyl (**64**) to ethyl (**65**) resulted in equipotent anti-HCV activity (**65**, EC_{50} = 18 nM). Interestingly, the replacement of the *N*,*N*-diethylamino group (**65**) by a bioisostere such as pyrrolidine (**66**) or piperidine (**67**) ring enhanced the activity by 5.8-fold (**65**

vs **66**-**67**, EC_{50} = 18 vs 3.1 nM). Further modification to this moiety by placing a *N*-methyl group at the 4-position of the piperidine ring of **67** generated compound **68** that was as active as **42**. (**68**, EC_{50} = 0.85 nM). These findings suggest that 6-membered heterocycles with two heteroatoms such as morpholine (**42**) and piperazine (**68**) at the R3 position are preferred.

Insert Table 3

Structure activity relationship (SAR) studies upon changing the amide moiety of **57**-**63** to a urea group led to synthesis the urea derivatives **69**-**71** (Table 4). Cyclopropyl (**69**) and cyclopentyl (**70**) urea compounds did not significantly change the potency comparing to their amide counterpart **57** and **58** (**69** vs **57** and **70** vs **58**, EC_{50} = 3.5 vs 4.6 and 13 vs 13 nM), while cyclohexyl urea **71** led to 4-fold increased potency as compared to its corresponding amide **59** (**71** vs **59**, EC_{50} = 3.3 vs 14 nM). Modification of the cyclohexyl ring (**71**) by increasing the ring size to a 7-membered cycloheptyl ring (**72**) or replacement with a phenyl ring (**73**) was leading to slightly increased activity compared with **71** (**72**, EC_{50} = 3.8 nM; **73**, EC_{50} = 2.3 nM). The 3-pyridine substituted urea **75** was found to be 11- to 15-fold more active than its corresponding 2-pyridyl (**74**) and 4-pyridyl (**76**) derivatives (**75** vs **74** and **76**, EC_{50} =

1.9 vs 30 and 22 nM). It was interesting to observe that the phenyl (**73**) and 3-pyridyl (**75**) substituted ureas exhibited a better inhibitory activity compared to their corresponding amide analogues (**60** and **62**). Further bioisosteric replacement of the urea oxygen with sulfur gave the corresponding thioureas **77**-**81** (Table 4). Cyclopentyl substituted thiourea **78** was found to be more active than the oxygen counterpart **70** (**78** vs **70**, EC_{50} = 5.2 vs 13 nM). The remaining cyclopropyl (**77**), cyclohexyl (**79**), phenyl (**80**) and 3-pyridyl (**81**) substituted thioureas possessed similar or reduced activity when compared to their corresponding urea analogues (**69**, **71**, **73** and **75**).

Insert Table 4

In vivo studies

Following *in vitro* studies, five compounds (**42**, **57**, **60**, **63** and **66**) were selected for further studies *in vivo* based on the *in vitro* results and availability. The *in vivo* rat PK data for the compounds selected has been summarized in Table 5 and 6.

As can be seen from Table 5, compound **57** showed a favorable PK profile after a single iv administration of 1 mg/kg. Low clearance (Cl = 27.1 ± 4.6 mL/min/kg)

associated with a relatively low volume of distribution at steady state (V_{ss} = 1.32 ± 0.04 L/kg), moderate half-life ($t_{1/2}$ = 1.3 ± 0.3 h), and high AUC (635 ± 116 ng/mL×h) was seen with **57** iv administration. Compound **63** showed highest clearance (Cl = 62.2 ± 8.9 mL/min/kg) and lowest AUC (295.6 ± 33 ng/mL×h). Compound **60** displayed highest V_{ss} (4.2 ± 0.9 L/kg), longest $t_{1/2}$ (2.3 ± 0.3 h), moderate CL (44.8 ± 3.2 mL/min/kg) and moderate AUC (367 ± 27 ng/mL×h).

Insert Table 5

As shown in Table 6, Compound **57** exhibited rapid absorption (T_{max} = 0.8 ± 0.4 h), high *C*max (247.3 ± 93.6 ng/mL) and AUC (1436 ± 293 ng/mL×h) as well as relatively high bioavailability (F = 45%) in rats after oral administration of 5 mg/kg. Compound **42** has similar *C*max (247.3 ± 93.6 ng/mL) and T_{max} (0.8 ± 0.3 h) but lower AUC (404 ± 76 ng/mL×h) and bioavailability (F = 18.7%). Lower bioavailability was also reported for other inhibitors, including **60** (F = 31%), **63** (F = 10.1%), and **66** (20.4%). These results indicate that compound **57** had a superior PK profile compared to the other compounds evaluated, characterized by significantly higher oral bioavailability and AUC.

Insert Table 6

Furthermore, drug resistance studies have been carried out to elucidate how compound **57** works in the HCV RNA replication. The resistance profile showed that the N terminus of NS5A is the region responsible for the **57**-mediated inhibition of HCV1b replicon activity. It may be possible that **57** exert inhibitory activity by directly binding to NS5A due to it produce very similar resistance data to daclatasvir, a NS5A inhibitor, in the drug resistance mutations within the N terminus of NS5A.[18] Of course, there is still a need to clarify the binding site and molecular mechanism of action of this class of inhibitors. Nevertheless, the findings from this study provide very useful information to develop novel anti-HCV agents.

CONCLUSION

In this paper, we described the discovery of (*S*)-1-((*R*)-2-(cyclopropanecarboxamido)-2-phenylacetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (**57)** as a potent, selective, and orally bioavailable HCV NS5A inhibitor for the treatment of HCV infection. A medicinal chemistry program based on the scaffold of 4-phenylthiazole **18** has led to the identification of **57**, a

potent HCV NS5A inhibitor with EC_{50} value of 4.6 nM, which showed high therapeutic index (CC_{50}/EC_{50} > 10,000). In addition, compound **57** displayed promising pharmacokinetic properties in rats following oral administration. Previously drug resistance data suggested that **57** is likely to inhibit HCV replication by directly binding to HCV NS5A, and it is currently under preclinical development for the treatment of HCV infection. Further SAR and mechanistic studies are still in progress and will be reported elsewhere.

EXPERIMENTAL SECTION

General Methods

All commercial chemicals and solvents are reagent grade and were used without further treatment unless otherwise noted. ^{1}H NMR spectra were obtained with a Varian Mercury-300 or a Varian Mercury-400 spectrometer. Chemical shifts were recorded in parts per million (ppm, δ) and were reported relative to the solvent peak or TMS. Coupling constants (*J*) are reported in hertz (Hz). Splitting patterns are described by using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; br, broad; m, multiplet. LC/MS data were measured on an Agilent MSD-1100 ESI-MS/MS System. All tested compounds were detected at UV 254nm unless otherwise stated. Column chromatography was performed with silica gel (Merck Kieselgel 60, 230-400 mesh). Reactions were monitored by TLC using Merck 60 F_{254} silica gel glass backed plates and visualized under ultraviolet irradiation (254 nm and 360 nm) or by spraying with phosphomolybdic acid reagent (Aldrich) followed by heating at 80 ^{o}C. Melting points were determined on an Electrothermal IA9000 Series Digital Melting Point Apparatus. Purity of the final compounds was determined on a Hitachi 2000 series HPLC system with a reverse phase C_{18} column (Agilent ZORBAX Eclipse XDB-C18 5 μm, 4.6 mm x 150 mm),

operating at 25 ºC. Mobile phase A was acetonitrile. Mobile phase B was 10 mM NH_4OAc aqueous solution containing 0.1% formic acid. The gradient system started from A/B (10%/90%) at 0 min to A/B (90%/10%) at 45 min. The flow rate of the mobile phase was 0.5 mL/min, and the injection volume of the sample was 5 μL. Peaks were detected at 254 nm. The purity of all tested compounds is >95% purity.

A.1. Preparation of Compound 6, 12, 18 and 22

(*S*)-tert-butyl 2-(2-oxo-2-phenylethylcarbamoyl)pyrrolidine-1-carboxylate (2)

To a solution of Boc-L-proline (5.64 g, 2.62 mmol) in CH_2Cl_2 (30 mL) at room temperature, 1-hydroxybenzotriazole monohydrate ($HOBt·H_2O$, 4 g, 2.62 mmol) and ethyl-(N',N'-dimethylamino)propylcarbodiimide hydrochloride (EDC, 5 g, 2.62 mmol) was added and then stirred for 30 min. To the above reaction mixture, 2-aminoacetophenone hydrochloride **1** (3 g, 1.74 mmol) and N, N-diisopropylethylamine (DIPEA, 3.4 mL, 2.62 mmol) was added in one portion respectively, and then stirred at room temperature for 18 hours. The solution was washed by 10% citric $acid_{(aq.)}$ and $NaHCO_{3(aq.)}$. The result mixture was then extracted with CH_2Cl_2, dried over $MgSO_4$, filtered and concentrated to give viscous yellow liquid. The liquid was purified with column chromatography (Hexane:EtOAc = 2:1) to yield white solid (2.19 g, 40%): mp 96-97 ℃; ^1H NMR

(300MHz, $CDCl_3$) δ 1.48 (br s, 9H), 1.85-1.99 (m, 2H), 2.02-2.18 (m, 2H), 3.42 (br s, 0.4H), 3.53 (br s, 1.6H), 4.31 (br s, 0.5H), 4.40 (br s, 0.5H), 4.08-4.85 (m, 2H), 7.05 (br s, 1H), 7.47-7.65 (m, 3H), 7.97 (d, *J* = 3.7 Hz, 2H); LC/MS(ESI) *m/z*: 233.2 $[M\text{-}Boc + H]^+$.

(*S*)-tert-butyl 2-(5-phenyl-1H-imidazol-2-yl)pyrrolidine-1-carboxylate (3)

To a solution of compound **2** (2.19 g, 6.6 mmol) and ammonium acetate (12.7 g) in xylenes (80 mL), acetic acid was added and then stirred at refluxed temperature (~160 ℃) for 3 hours. After cooling to room temperature, the reaction mixture was extracted with EtOAc/H_2O. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified with column chromatography (Hexane:EtOAc = 2:1) to provide compound **3** (1.52 g, 73.5%): mp 63-65 ℃; ^1H NMR (300MHz, $CDCl_3$) δ 1.47 (br s, 9H), 1.93-1.99 (m, 2H), 2.07-2.15 (m, 2H), 2.97 (br s, 1H), 3.36-3.40 (m, 1H), 4.95-4.97 (m, 1H), 7.19-7.23 (m, 2H), 7.34 (t, *J* = 7.5 Hz, 3H), 7.63 (br s, 2H); LC/MS(ESI) *m/z*: 314.1 $[M + H]^+$, 336.1 $[M + Na]^+$.

(*S*)-5-phenyl-2-(pyrrolidin-2-yl)-1H-imidazole (4)

To a solution of compound **3** (1.52 g, 4.85 mmol) in CH_2Cl_2 (15 mL) at 0 ℃, trifluoroacetic acid (7.5 mL) was added. Then, the reaction was stirred at room

temperature for 1 hour. Basification of the solution by $NaHCO_{3(sat.)}$ was accomplished until pH value was about 8. The solution was extracted with CH_2Cl_2. The combined organic layers were dried by $MgSO_4$ and concentrated in vacuo. The crude product was used as starting material for next step without further purification. Compound **4** (0.845 g, 82%): mp 196-198 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.84-1.98 (m, 2H), 2.21-2.28 (m, 2H), 3.02-3.14 (m, 2H), 4.48 (t, *J* = 7.9 Hz, 1H), 7.21 (d, *J* = 4.2 Hz, 2H), 7.33 (t, *J* = 7.6 Hz, 2H), 7.61 (d, *J* = 3.6 Hz, 2H); LC/MS(ESI) *m/z*: 214.1 $[M + H]^+$, 236.1 $[M + Na]^+$.

tert-butyl

(*R*)-2-oxo-1-phenyl-2-((*S*)-2-(5-phenyl-1H-imidazol-2-yl)pyrrolidin-1-yl)ethylcarbamate (5)

To a solution of N-Boc-D-phenylglycine (1.09 g, 4.36 mmol) in CH_2Cl_2 (25 mL) at room temperature, $HOBt·H_2O$ (0.73 g, 4.75 mmol) was added in one portion and then the mixture was stirred for 10 min. To the above reaction mixture were added EDC (0.91 g, 4.75 mmol) and compound **4** (0.84 g, 3.96 mmol) respectively, and then stirred for 18 hours at room temperature. The solution was washed by 10% citric $acid_{(aq.)}$ and $NaHCO_{3(aq.)}$. The result mixture was then extracted with CH_2Cl_2, dried over $MgSO_4$, filtered and concentrated to give viscous yellow liquid. The liquid was

purified with column chromatography (Hexane:EtOAc = 2:1) to yield brown gel (1.18 g, 67%): mp 99-100 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.21 (s, 9H), 1.87-2.19 (m, 4H), 2.89 (br s, 1H), 3.19-3.25 (m, 1H), 3.60-3.79 (m, 1H), 5.31 (d, *J* = 4.0 Hz, 1H), 5.34 (d, *J* = 3.4 Hz, 1H), 5.66 (br s, 1H), 7.20-7.25 (m, 3H), 7.27-7.45 (m, 6H), 7.68 (br s, 2H); LC/MS(APCI) *m/z*: 447.3 $[M + H]^+$, 347.3 $[M\text{-}Boc + H]^+$; HPLC t_R = 43.75 min, 96.8%.

N-((*R*)-2-oxo-1-phenyl-2-((*S*)-2-(5-phenyl-1H-imidazol-2-yl)pyrrolidin-1-yl)ethyl) morpholine-4-carboxamide (6)

To a solution of compound **5** (0.36 g, 0.81 mmol) in CH_2Cl_2 (10 mL) at 0℃, trifluoroacetic acid (5 mL) was added. Then, the reaction was stirred at room temperature for 1 hour. Basification of the solution by $NaHCO_{3(sat.)}$ was accomplished until pH value was about 8. The solution was extracted with CH_2Cl_2. The combined organic layers were dried by $MgSO_4$ and concentrated in vacuo to afford product as starting material for next step without further purification. To the above crude product in CH_2Cl_2 (10 mL) at ice bath, morpholine-4-carbonyl chloride (0.18 mL, 1.62 mmol) and Et_3N (0.23 mL, 1.62 mmol) were added respectively and then stirred for 10 min. The result mixture was concentrated under reduced pressure and then extracted with EtOAc. The organic layer was washed with brine, dried

over $MgSO_4$, filtered and concentrated to yield crude product. The residue was purified with column chromatography (Acetone:Hexane = 1:5) to afford the final viscous solid **6** (0.113 g, 31%): ^{1}H NMR (300MHz, $CDCl_3$) δ 1.83-2.15 (m, 4H), 2.61-2.72 (m, 1H), 3.21-3.38 (m, 5H), 3.41-3.69 (m, 4H), 3.83-3.89 (m, 1H), 5.35 (d, *J* = 3.0 Hz, 1H), 5.46 (s, 1H), 7.16-7.23 (m, 2H), 7.24-7.49 (m, 8H), 7.67 (d, *J* = 3.7 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.35, 30.01, 43.93, 46.70, 55.72, 56.76, 66.27, 124.85, 126.59, 127.72, 128.27, 128.51, 128.92, 129.27, 135.21, 148.25, 157.79, 170.68; LC/MS(APCI) *m/z*: 460.2 $[M + H]^+$; HRMS (*m/z*): calcd for $C_{26}H_{30}N_5O_3$ $[M + H]^+$ 460.2349, found 460.2346; HPLC t_R = 18.60 min, 96.8%.

N'-hydroxybenzimidamide (8)

To a solution of benzonitrile **7** (3 g, 29 mmol) in ethanol (50 mL) at room temperature, hydroxylamine hydrochloride (2.02 g, 29 mmol) and DIPEA (5.1 mL, 29 mmol) were added respectively. Then the reaction mixture was stirred at 90 ℃ for 5 hours. After cooling to room temperature and removing the solvent, the result colorless viscous liquid was extracted with EtOAc/H_2O. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The crude compound was washed with n-Hexane and filtered to give white solid **8** (3.36 g, 85%) for next step without further purification: mp 182-185 ℃; ^{1}H NMR (400MHz, DMSO-d_6) δ 7.56-7.59 (m, 2H),

7.67-7.76 (m, 3H), 9.09 (br s, 2H), 11.36 (br s, 1H); LC/MS(ESI) *m/z*: 137.1 [M + H]$^+$.

(*S*)-tert-butyl 2-(3-phenyl-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (9)

To a solution of Boc-L-proline (2.15g, 10 mmol) in DMF (18 mL), O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU, 3.21 g, 10 mmol), HOBt·H_2O (0.31g, 2 mmol) and DIPEA (8.8 mL, 50 mmol) was added respectively. The reaction mixture was stirred at room temperature for 5 min. To the above reaction mixture, compound **8** (1.36 g, 10 mmol) was added, and then stirred at room temperature for 1 hour, and at 110 ℃ for 2.5 hours. After cooling to room temperature, the mixture was extracted with EtOAc/H_2O, dried over $MgSO_4$, then filtered and concentrated to give crude yellow liquid. The crude compound was purified with column chromatography (EtOAc:n-Hexane = 1:10) to provide compound **9** (1.67 g, 53%): mp 109-110 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.30 (s, 7H), 1.47 (s, 2H), 1.99-2.20 (m, 3H), 2.36-2.44 (m, 1H), 3.50-3.60 (m, 1H), 3.68-3.74 (m, 1H), 5.06-5.09 (m, 0.7H), 5.20 (br s, 0.3H), 7.49 (d, *J* = 3.6 Hz, 3H), 8.07 (d, *J* = 3.8 Hz, 2H); LC/MS(ESI) *m/z*: 338.1 [M + Na]$^+$.

(*S*)-3-phenyl-5-(pyrrolidin-2-yl)-1,2,4-oxadiazole (10)

To a solution of compound **9** (1 g, 3.2 mmol) in CH_2Cl_2 (10 mL) at 0 ℃, trifluoroacetic acid (5 mL) was added. Then, the reaction was stirred at room temperature for 1 hour. Basification of the solution by $NaHCO_{3(sat.)}$ was accomplished until pH value was about 8. The solution was extracted with CH_2Cl_2. The combined organic layers were dried by Na_2SO_4 and concentrated in vacuo. The crude product was used as starting material for next step without further purification. Viscous solid compound **10** (0.61 g, 90%): ^{1}H NMR (400MHz, $CDCl_3$) δ 1.87-2.02 (m, 2H), 2.10-2.20 (m, 2H), 2.28-2.37 (m, 1H), 3.06-3.12 (m, 1H), 3.19-3.25 (m, 1H), 4.55 (dd, *J* = 5.6, 8.4 Hz, 1H), 7.44-7.52 (m, 3H), 8.07-8.10 (m, 2H); LC/MS(ESI) *m/z*: 216.1 $[M+H]^+$, 238.1 $[M + Na]^+$.

tert-butyl

(*R*)-2-oxo-1-phenyl-2-((*S*)-2-(3-phenyl-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)ethylc

arbamate (11)

To a solution of N-Boc-D-phenylglycine (0.58 g, 2.3 mmol) in CH_2Cl_2 (10 mL) at room temperature, $HOBt{\cdot}H_2O$ (0.43g, 2.8 mmol) was added in one portion; the mixture was then stirred for 10 min. To the above reaction mixture were added EDC (0.53 g, 2.8 mmol) and compound **10** (0.5g, 2.3 mmol) respectively, and then stirred for 18 hours at room temperature. The solution was washed by 10% citric $acid_{(aq.)}$

and $NaHCO_{3(aq.)}$. The result mixture was then extracted with CH_2Cl_2, dried over Na_2SO_4, filtered and concentrated to give viscous yellow liquid. The liquid was purified with column chromatography (Hexane:EtOAc = 2:1) to yield white solid (0.93 g, 89%): mp 75-77 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.37 (s, 4.5H), 1.41 (s, 4.5H), 1.94-2.40 (m, 4H), 3.18-3.39 (m, 1H), 3.83-3.95 (m, 1H), 5.34 (d, *J* = 2.2 Hz, 1H), 5.46-5.58 (m, 1H), 6.01 (d, *J* = 3.6 Hz, 1H), 7.30-7.52 (m, 8H), 7.78-8.10 (m, 2H); LC/MS(ESI) *m/z*: 349.1 $[M\text{-Boc} + H]^+$, 471.2 $[M + Na]^+$.

N-((*R*)-2-oxo-1-phenyl-2-((*S*)-2-(3-phenyl-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)ethyl)morpholine-4-carboxamide (12)

To a solution of compound **11** (0.5 g, 1.1 mmol) in CH_2Cl_2 (10 mL) at 0℃, trifluoroacetic acid (5 mL) was added. Then, the reaction was stirred at room temperature for 1 hour. Basification of the solution by $NaHCO_{3(sat.)}$ was accomplished until pH value was about 8. The solution was extracted with CH_2Cl_2. The combined organic layers were dried by Na_2SO_4 and concentrated in vacuo to afford crude product as starting material for next step without further purification. To the above crude product in CH_2Cl_2 (10 mL) at ice bath, morpholine-4-carbonyl chloride (0.16 mL, 1.3 mmol) and Et_3N (0.18 mL, 1.3 mmol) were added respectively and then stirred for 10 min. The result mixture was concentrated under reduced

pressure and then extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated to yield crude product. The residue was purified with column chromatography (Hexane:EtOAc = 4:1, and then Hexane:EtOAc = 2:1) to afford the final product **12** (0.39 g, 76.6%): mp 89-92 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.93-2.45 (m, 4H), 3.14-3.27 (m, 1H), 3.31-3.48 (m, 4H), 3.59-3.67 (m, 4H), 3.80-3.94 (m, 1H), 5.34 (dd, *J* = 3.0, 7.8 Hz, 1H), 5.62-5.72 (m, 1H), 6.12 (d, *J* = 3.2 Hz, 1H), 7.30-7.53 (m, 8H), 8.05-8.11 (m, 2H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.41, 30.51, 43.72, 46.70, 53.99, 56.74, 66.33, 126.59, 127.52, 128.11, 128.30, 128.75, 129.01, 131.20, 137.65, 156.28, 168.39, 169.61, 179.06; LC/MS(ESI) *m/z*: 462.2 $[M + H]^+$, 484.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{25}H_{28}N_5O_4$ $[M + H]^+$ 462.2141, found 462.2136; HPLC t_R = 29.47 min, 98.4%.

(*S*)-tert-butyl 2-carbamoylpyrrolidine-1-carboxylate (13)

To a solution of compound Boc-L-proline (5 g, 23 mmol) in 1,4-dioxane (90 mL) at room temperature, pyridine (1.16 mL, 13.9 mmol), ammonium carbonate (2.9 g, 30.2 mmol) and $(Boc)_2O$ (6.59 g, 30.2 mmol) was added and then stirred for 18 hours. After removed the solvent, the mixture was extracted with 20% citric acid/brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude compound was washed with n-Hexane and filtered to give white solid **13** (4.7 g, 95%)

for next step without further purification: mp 102-104 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.47 (s, 9H), 1.86-2.35 (m, 4H), 3.35 (br s, 0.67H), 3.45 (br s, 1.33H), 4.20 (br s, 0.36H), 4.31 (br s, 0.64H), 5.52 (br s, 0.5H), 5.64 (br s, 0.5H), 6.07 (br s, 0.5H), 6.85 (br s, 0.5H); LC/MS(ESI) *m/z*: 237.1 $[M + Na]^+$.

(*S*)-tert-butyl 2-carbamothioylpyrrolidine-1-carboxylate (14)

A flask of compound **13** (2.5 g, 12 mmol) and Lawesson's reagent (5.66 g, 14 mmol) was flushed with nitrogen, and then added dry-THF (40 mL) as solvent. The reaction mixture was stirred at 70 ℃ under nitrogen for 8 hours. After removed the solvent in vacuo, the residue was purified with column chromatography (Hexane:EtOAc = 2:1) to yield white solid **14** (2.4 g, 89%): mp 196-198 ℃; ^{1}H NMR (300MHz, DMSO-d_6) δ 1.32 (s, 6H), 1.38 (s, 3H), 1.71-1.88 (m, 3H), 2.17-2.22 (m, 1H), 3.30-3.46 (m, 2H), 4.39 (dd, *J* = 3.6, 8.7 Hz, 1H), 9.05 (br s, 0.4H), 9.09 (br s, 0.6H), 9.50 (br s, 1H); LC/MS(ESI) *m/z*: 253.1 $[M + Na]^+$.

(*S*)-tert-butyl 2-(4-phenylthiazol-2-yl)pyrrolidine-1-carboxylate (15)

To a solution of compound **14** (2.2 g, 9.5 mmol) and phenacyl bromide (1.9 g, 9.5 mmol) in EtOH (50 mL) was stirred at room temperature for 1 hour. The mixture was extracted with EtOAc/H_2O, dried over $MgSO_4$, then filtered and concentrated to

give crude yellow liquid. The residue was purified with column chromatography (EtOAc:Hexane = 1:3) to provide compound **15** (2.33 g, 74%): mp 101-103 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.34 (s, 6.5H), 1.49 (s, 2.5H), 1.90-2.02 (m, 2H), 2.29-2.36 (m, 2H), 3.45-3.65 (m, 2H), 5.18-5.28 (m, 1H), 7.31-7.40 (m, 4H), 7.86-7.88 (m, 2H); LC/MS(ESI) *m/z*: 331.1 $[M + H]^+$, 353.1 $[M + Na]^+$.

(*S*)-4-phenyl-2-(pyrrolidin-2-yl)thiazole (16)

To a solution of compound **15** (2 g, 6 mmol) in CH_2Cl_2 (5 mL) at 0 ℃, trifluoroacetic acid (2.5 mL) was added. Then, the reaction was stirred at room temperature for 1 hour. Basification of the solution by $NaHCO_{3(sat.)}$ was accomplished until pH value was about 8. The solution was extracted with CH_2Cl_2. The combined organic layers were dried by Na_2SO_4 and concentrated in vacuo. The crude product was used as starting material for next step without further purification. Compound **16** (1.14 g, 82%): mp 43-46 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.82-1.94 (m, 2H), 2.01-2.09 (m, 2H), 2.16 (br s, 1H), 2.29-2.36 (m, 1H), 3.06-3.20 (m, 2H), 4.63 (dd, *J* = 5.6, 8.0 Hz, 1H), 7.28-7.43 (m, 4H), 7.86-7.89 (m, 2H); LC/MS(ESI) *m/z*: 231.0 $[M + H]^+$.

tert-butyl

(*R*)-2-oxo-1-phenyl-2-((*S*)-2-(4-phenylthiazol-2-yl)pyrrolidin-1-yl)ethylcarbamate (17)

To a solution of N-Boc-D-phenylglycine (0.65 g, 2.6 mmol) in CH_2Cl_2 (10 mL) at room temperature, $HOBt \cdot H_2O$ (0.40 g, 2.6 mmol) was added in one portion; the mixture was then stirred for 10 min. To the above reaction mixture were added EDC (0.5 g, 2.6 mmol) and compound **16** (0.5 g, 2.2 mmol) respectively, and then stirred for 18 hours at room temperature. The solution was washed by 10% citric $acid_{(aq.)}$ and $NaHCO_{3(aq.)}$. The result mixture was then extracted with CH_2Cl_2, dried over Na_2SO_4, filtered and concentrated to give viscous yellow liquid. The liquid was purified with column chromatography to yield white solid (0.72 g, 71%): mp 65-68 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.39 (s, 9H), 1.86-2.44 (m, 4H), 3.15-3.24 (m, 1H), 3.71-3.88 (m, 1H), 5.45-5.51 (m, 2H), 6.00 (d, *J* = 3.9Hz, 1H), 7.29-7.40 (m, 9H), 7.78-7.89 (m, 2H); LC/MS(ESI) *m/z*: 464.2 $[M + H]^+$, 486.2 $[M + Na]^+$.

N-((*R*)-2-oxo-1-phenyl-2-((*S*)-2-(4-phenylthiazol-2-yl)pyrrolidin-1-yl)ethyl)morpholine-4-carboxamide (18)

To a solution of compound **17** (0.6 g, 1.3 mmol) in CH_2Cl_2 (10 mL) at 0℃, trifluoroacetic acid (5 mL) was added. Then, the reaction was stirred at room temperature for 1 hour. Basification of the solution by $NaHCO_{3(sat.)}$ was

accomplished until pH value was about 8. The solution was extracted with CH_2Cl_2. The combined organic layers were dried by Na_2SO_4 and concentrated in vacuo to afford crude product as starting material for next step without further purification. To the above crude product in CH_2Cl_2 (10 mL) at ice bath, morpholine-4-carbonyl chloride (0.18 mL, 1.6 mmol) and Et_3N (0.22 mL, 1.6 mmol) were added respectively and then stirred for 10 min. The result mixture was concentrated under reduced pressure and then extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated to yield crude product. The residue was purified with column chromatography to afford the final product **18** (0.36 g, 58%): mp 90-93 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.84-2.37 (m, 4H), 3.12-3.19 (m, 1H), 3.28-3.38 (m, 4H), 3.59-3.66 (m, 4H), 3.76-3.87 (m, 1H), 5.01 (d, *J* = 3.2Hz, 1H), 5.67 (d, *J* = 3.6Hz, 1H), 5.70 (d, *J* = 2.0Hz, 1H), 6.12 (d, *J* = 3.4Hz, 1H), 7.30-7.48 (m, 9H), 7.86-7.88 (m, 2H); ^{13}C NMR ($CDCl_3$, 100MHz) δ 23.94, 31.94, 43.83, 46.81, 56.90, 59.38, 66.39, 112.62, 126.38, 128.06, 128.19, 128.32, 128.69, 129.06, 134.49, 137.60, 155.32, 156.50, 169.63, 171.55; LC/MS(ESI) *m/z*: 477.2 $[M + H]^+$, 499.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{26}H_{29}N_4O_3S$ $[M + H]^+$ 477.1960, found 477.1958; HPLC t_R = 31.29 min, 98.6%.

(*S*)-tert-butyl 2-(5-phenylthiazol-2-yl)pyrrolidine-1-carboxylate (19)

A flask of compound **2** (1 g, 3 mmol) and Lawesson's reagent (1.46 g, 3.6 mmol) was flushed with nitrogen, and then added dry-THF (20 mL) as solvent. The reaction mixture was stirred at refluxed temperature (~80 ℃) under nitrogen for 6 hours. After removed the solvent in vacuo, the residue was purified with column chromatography (Hexane:EtOAc = 4:1) to yield yellow viscous solid **19** (0.8 g, 80.6%): ^{1}H NMR (400MHz, $CDCl_3$) δ 1.36 (s, 6.3H), 1.49 (s, 2.7H), 1.92-2.04 (m, 2H), 2.25-2.33 (m, 2H), 3.46-3.63 (m, 2H), 5.11-5.23 (m, 1H), 7.32-7.39 (m, 3H), 7.53 (d, *J* = 3.8Hz, 2H), 7.84 (s, 1H); LC/MS(ESI) *m/z*: 331.1 $[M + H]^+$, 353.1 $[M + Na]^+$.

(*S*)-5-phenyl-2-(pyrrolidin-2-yl)thiazole (20)

To a solution of compound **19** (0.6 g, 1.8 mmol) in CH_2Cl_2 (10 mL) at 0 ℃, trifluoroacetic acid (5 mL) was added. Then, the reaction was stirred at room temperature for 1 hour. Basification of the solution by $NaHCO_{3(sat.)}$ was accomplished until pH value was about 8. The solution was extracted with CH_2Cl_2. The combined organic layers were dried by Na_2SO_4 and concentrated in vacuo. The crude product was used as starting material for next step without further purification. Viscous solid compound **20** (0.37 g, 88%): ^{1}H NMR (300MHz, $CDCl_3$) δ 1.82-2.09 (m, 4H), 2.26-2.37 (m, 1H), 3.05-3.21 (m, 2H), 4.55-4.59 (m, 1H), 7.27-7.41 (m, 3H),

7.52-7.56 (m, 2H), 7.85 (s, 1H); LC/MS(ESI) *m/z*: 231.1 $[M + H]^+$, 253.0 $[M + Na]^+$.

tert-butyl

(*R*)-2-oxo-1-phenyl-2-((*S*)-2-(5-phenylthiazol-2-yl)pyrrolidin-1-yl)ethylcarbamate

(21)

To a solution of N-Boc-D-phenylglycine (0.48 g, 1.9 mmol) in CH_2Cl_2 (10 mL) at room temperature, HOBt·H_2O (0.29 g, 1.9 mmol) was added in one portion; the mixture was then stirred for 10 min. To the above reaction mixture were added EDC (0.4 g, 1.9 mmol) and compound **20** (0.37 g, 1.6 mmol) respectively, and then stirred for 18 hours at room temperature. The solution was washed by 10% citric $acid_{(aq.)}$ and $NaHCO_{3(aq.)}$. The result mixture was then extracted with CH_2Cl_2, dried over Na_2SO_4, filtered and concentrated to give viscous yellow liquid. The liquid was purified with column chromatography to yield white solid (0.54 g, 72.3%): mp 67-69 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.39 (s, 9H), 1.87-1.91 (m, 1H), 1.99-2.22 (m, 2H), 2.33-2.38 (m, 1H), 3.17-3.23 (m, 1H), 3.73-3.87 (m, 1H), 5.43-5.48 (m, 2H), 6.01 (d, *J* = 3.8Hz, 1H), 7.29-7.45 (m, 8H), 7.53 (d, *J* = 3.6Hz, 2H), 7.84 (s, 1H); LC/MS(ESI) *m/z*: 464.2 $[M + H]^+$, 486.2 $[M + Na]^+$.

N-((*R*)-2-oxo-1-phenyl-2-((*S*)-2-(5-phenylthiazol-2-yl)pyrrolidin-1-yl)ethyl)morph

oline-4-carboxamide (22)

To a solution of compound **21** (0.5 g, 1.1 mmol) in CH_2Cl_2 (10 mL) at 0 ℃, trifluoroacetic acid (5 mL) was added. Then, the reaction was stirred at room temperature for 1 hour. Basification of the solution by $NaHCO_{3(sat.)}$ was accomplished until pH value was about 8. The solution was extracted with CH_2Cl_2. The combined organic layers were dried by Na_2SO_4 and concentrated in vacuo to afford product. The crude product was used as starting material for next step without further purification. To the above crude product in CH_2Cl_2 (10 mL) at ice bath, morpholine-4-carbonyl chloride (0.15 mL, 1.3 mmol) and Et_3N (0.18 mL, 1.3 mmol) were added respectively and then stirred for 18 hours. The result mixture was concentrated under reduced pressure and then extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated to yield crude product. The residue was purified with column chromatography to afford the final product **22** (0.29 g, 56%): mp 191-193 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.83-1.99 (m, 1H), 2.00-2.17 (m, 2H), 2.20-2.25 (m, 1H), 3.08-3.17 (m, 1H), 3.24-3.37 (m, 4H), 3.58-3.64 (m, 4H), 3.71-3.83 (m, 1H), 5.43 (d, *J* = 3.9 Hz, 1H), 5.67 (d, *J* = 3.3 Hz, 1H), 6.13 (d, *J* = 3.3 Hz, 1H), 7.25-7.57 (m, 10H), 7.82 (s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 23.93, 31.70, 43.77, 46.74, 56.82, 59.25, 66.34, 126.60, 126.66, 128.13, 128.25, 128.33, 128.98, 131.29, 137.62, 137.76, 156.37, 169.50,

170.68; LC/MS(ESI) *m/z*: 477.2 $[M + H]^+$, 499.1 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{26}H_{29}N_4O_3S$ $[M + H]^+$ 477.1960, found 477.1959; HPLC t_R = 29.22 min, 100%.

A.2. General Procedure for the synthesis of compounds 42-51

A.2.1. General Procedure of the Synthesis of Compound 32-36 from 23a-e

To a solution of corresponding 4-substituted thiozole amine (**23a-e**, 2 mmol) and Boc-L-proline (2 mmol) in DMF (10 mL) were added DIPEA (3.4 mmol) and O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU, 3 mmol) sequentially under N_2. Then, the solution was heated at 50 ℃ for 6 hours. 50 mL H_2O was added to the reaction and then the mixture was extracted with EtOAc. The combined organic layers were washed with H_2O (3x) and brine. After the solvent was dried over anhydrous Na_2SO_4 and evaporated in vacuo, the product **24a-e** was prepared for next step without purification.

To a solution of compound **24a-e** in CH_2Cl_2 (5 mL) at 0℃, trifluoroacetic acid (2.5 mL) was added. Then, the reaction was stirred at room temperature for 1 hour. Basification of the solution by $NaHCO_{3(sat.)}$ was accomplished until pH value was about 8. The solution was extracted with CH_2Cl_2. The combined organic layers were dried by Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column to provide compound **25a-e**.

To a solution of N-Boc-D-phenylglycine (**26**, 1.6 mmol) in CH_2Cl_2 (10 mL) at room temperature, HOBt·H_2O (1.44 mmol) was added in one portion; the mixture was then stirred for 10 min. To the above reaction mixture were added EDC (1.44 mmol) and compound **25a-e** (1.3 mmol) respectively, and then stirred for 18 hours at room temperature. The solution was washed by 10% citric $acid_{(aq.)}$ and $NaHCO_{3(aq.)}$. The result mixture was then extracted with CH_2Cl_2, dried over Na_2SO_4, filtered and concentrated to give viscous yellow liquid. The liquid was purified with column chromatography to yield solid **32-36**.

tert-butyl

(*R*)-2-oxo-1-phenyl-2-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)ethyl carbamate (32)

The title compound was obtained from commercially available 4-phenyl-thiazol-2-ylamine (**23a**) according to the general procedure to afford the title compound in 89% yield: mp 129-131 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.4 (s, 9H), 2.05-2.10 (m, 1H), 2.44-2.48 (m, 1H), 3.18-3.24 (m, 1H), 3.81-3.86 (m, 1H), 4.82-4.84 (m, 1H), 5.43-5.45 (m, 1H), 5.80 (d, *J* = 3.6 Hz, 1H), 7.16 (s, 1H), 7.27-7.44 (m, 9H), 7.84-7.90 (m, 2H), 10.70 (brs, 1H); LC/MS(ESI) *m/z*: 507 $[M + H]^+$, 529 $[M + Na]^+$; HPLC t_R = 40.97 min, 93.8%.

tert-butyl (*R*)-2-oxo-1-phenyl-2-((*S*)-2-(thiazol-2-ylcarbamoyl)pyrrolidin-1-yl) ethylcarbamate (33)

The title compound was obtained from commercially available thiazol-2-ylamine (**23b**) according to the general procedure to afford the title compound in 91% yield: mp 104-106 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.83-2.11 (m, 3H), 2.33-2.40 (m, 1H), 3.13-3.21 (m, 1H), 3.74-3.80 (m, 1H), 4.76 (d, *J* = 3.0 Hz, 1H), 5.46 (d, *J* = 3.8 Hz, 1H), 6.00 (d, *J* = 3.6 Hz, 1H), 6.96 (d, *J* = 1.8 Hz, 1H), 7.30-7.50 (m, 6H); LC/MS(ESI) *m/z*: 431.1 $[M + H]^+$, 453.1 $[M + Na]^+$; HPLC t_R = 30.45 min, 98.6%.

tert-butyl (*R*)-2-((*S*)-2-(4-methylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)-2-oxo-1-phenylethyl carbamate (34)

The title compound was obtained from commercially available 4-methyl-thiazol-2-ylamine (**23c**) according to the general procedure to afford the title compound in 91.2% yield: mp 105-108 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.82-2.17 (m, 3H), 2.32 (s, 3H), 2.33-2.47 (m, 1H), 3.15-3.24 (m, 1H), 3.77-3.83 (m, 1H), 4.79 (d, *J* = 4.0 Hz, 1H), 5.42 (d, *J* = 4.6 Hz, 1H), 5.87 (d, *J* = 4.6 Hz, 1H), 6.51

(s, 1H), 7.30-7.42 (m, 5H), 10.67 (br s, 1H); LC/MS(ESI) *m/z*: 445.1 $[M + H]^+$, 467.1 $[M + Na]^+$; HPLC t_R = 32.90 min, 99.6%.

tert-butyl

(*R*)-2-((*S*)-2-(4-tert-butylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)-2-oxo-1-phenylethylcarbamate (35)

The title compound was obtained from commercially available 4-tert-butyl-thiazol-2-ylamine (**23d**) according to the general procedure to afford the title compound in 92.9% yield: mp 111-114 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.28 (s, 9H), 1.42 (s, 9H), 1.83-1.99 (m, 1H), 2.01-2.08 (m, 1H), 2.36-2.46 (m, 2H), 3.14-3.20 (m, 1H), 3.79-3.84 (m, 1H), 4.76-4.78 (m, 1H), 5.45 (d, *J* = 3.8 Hz, 1H), 5.89 (d, *J* = 3.8 Hz, 1H), 6.51 (s, 1H), 7.28-7.41 (m, 5H), 10.47 (brs, 1H); LC/MS(ESI) *m/z*: 487.3 $[M + H]^+$, 509.3 $[M + Na]^+$; HPLC t_R = 42.72 min, 98.0%.

tert-butyl

(*R*)-2-((*S*)-2-(4-cyclohexylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)-2-oxo-1-phenylethylcarbamate (36)

The title compound was obtained from commercially available 4-cyclohexyl-thiazol-2-ylamine (**23e**) according to the general procedure to afford the

title compound in 93.1% yield: mp 107-110 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.21-1.56 (m, 13H), 1.70-2.25 (m, 9H), 2.40-2.43 (m, 1H), 2.58-2.61 (m, 1H), 3.14-3.19 (m, 1H), 3.77-3.82 (m, 1H), 4.77 (d, *J* = 3.0 Hz, 1H), 5.43 (d, *J* = 3.6 Hz, 1H), 5.95 (d, *J* = 3.6 Hz, 1H), 6.46 (s, 1H), 7.32-7.42 (m, 5H), 10.55 (br s, 1H); LC/MS(ESI) *m/z*: 513.3 $[M + H]^+$, 535.3 $[M + Na]^+$; HPLC t_R = 45.35 min, 92.4%.

A.2.2 General Procedure of the Synthesis of Compound 37-41 from Substituted Glycine 27-31

To a solution of **27-31** (1.6mmol) in CH_2Cl_2 (10 mL) at room temperature, HOBt·H_2O (1.44mmol) was added in one portion; the mixture was then stirred for 10 min. To the above reaction mixture were added EDC (1.44mmol) and compound **25a** (1.3mmol) respectively, and then stirred for 18 hours at room temperature. The solution was washed by 10% citric $acid_{(aq.)}$ and $NaHCO_{3(aq.)}$. The result mixture was then extracted with CH_2Cl_2, dried over Na_2SO_4, filtered and concentrated to give viscous yellow liquid. The liquid was purified with column chromatography to yield solid **37-41**.

tert-butyl

(*R*)-1-oxo-1-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)propan-2-ylca

rbamate (37)

Intermediate **25a** was reacted with commercially available (*R*)-2-(tert-butoxycarbonylamino)propanoic acid (**27**) according to the general procedure to afford the title compound in 83% yield: mp 226-228 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.33 (d, *J* = 3.4 Hz, 3H), 1.37 (s, 9H), 1.97-2.14 (m, 3H), 2.49-2.52 (m, 1H), 3.46-3.54 (m, 1H), 3.87-3.92 (m, 1H), 4.42-4.46 (m, 1H), 4.82-4.85 (m, 1H), 5.31 (d, *J* = 3.4 Hz, 1H), 7.11 (s, 1H), 7.25-7.39 (m, 3H), 7.82-7.85 (m, 2H), 10.61 (brs, 1H); LC/MS(ESI) *m/z*: 445.1 $[M + H]^+$, 467.1 $[M + Na]^+$.

tert-butyl

(*R*)-1-oxo-1-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)butan-2-ylcarbamate (38)

Intermediate **25a** was reacted with commercially available (*R*)-2-(tert-butoxycarbonylamino)butanoic acid (**28**) according to the general procedure to afford the title compound in 85% yield; mp 218-219 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.01 (t, *J* = 7.4 Hz, 3H), 1.39 (s, 9H), 1.62-1.84 (m, 2H), 1.98-2.16 (m, 3H), 2.52-2.57 (m, 1H), 3.56 (dd, *J* = 9.6, 16.8 Hz, 1H), 3.93-3.96 (m, 1H), 4.36 (dd, *J* = 7.6, 14 Hz, 1H), 4.84-4.86 (m, 1H), 5.21 (d, *J* = 3.8 Hz, 1H), 7.13

(s, 1H), 7.25-7.40 (m, 3H), 7.85-7.87 (m, 2H), 10.65 (br s, 1H); LC/MS(ESI) *m/z*: 459.2 $[M + H]^+$, 481.2 $[M + Na]^+$.

tert-butyl

(*R*)-1-oxo-1-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)pentan-2-ylcarbamate (39)

Intermediate **25a** was reacted with commercially available (*R*)-2-(tert-butoxycarbonylamino)pentanoic acid (**29**) according to the general procedure to afford the title compound in 91% yield: mp 100-102 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 0.94 (t, *J* = 7.3 Hz, 3H), 1.23-1.68 (m, 13H), 1.96-2.15 (m, 3H), 2.49-2.52 (m, 1H), 3.52 (dd, *J* = 9.0, 16.5 Hz, 1H), 3.93 (t, *J* = 7.3 Hz, 3H), 4.38 (dd, *J* = 7.8, 13.8 Hz, 1H), 4.81-4.84 (m, 1H), 5.20 (d, *J* = 3.9 Hz, 1H), 7.10 (s, 1H), 7.26-7.38 (m, 3H), 7.82-7.85 (m, 2H), 10.65 (br s, 1H); LC/MS(APCI) *m/z*: 473.2 $[M + H]^+$.

tert-butyl

(*R*)-3-methyl-1-oxo-1-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)butan-2-ylcarbamate (40)

Intermediate **25a** was reacted with commercially available

(*R*)-2-(tert-butoxycarbonylamino)-3-methylbutanoic acid (**30**) according to the general procedure to afford the title compound in 88% yield: mp 103-106 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.00 (d, *J* = 3.3 Hz, 6H), 1.36 (s, 9H), 1.93-2.17 (m, 4H), 2.51-2.57 (m, 1H), 3.59 (dd, *J* = 9.0, 16.5 Hz, 1H), 3.96 (t, *J* = 7.9 Hz, 1H), 4.21 (t, *J* = 7.9 Hz, 1H), 4.83-4.86 (m, 1H), 5.20 (d, *J* = 4.2 Hz, 1H), 7.12 (s, 1H), 7.26-7.40 (m, 3H), 7.84-7.87 (m, 2H), 10.72 (br s, 1H); LC/MS(APCI) *m/z*: 473.2 $[M + H]^+$.

tert-butyl

(*R*)-3,3-dimethyl-1-oxo-1-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)butan-2-ylcarbamate (41)

Intermediate **25a** was reacted with commercially available (*R*)-2-(tert-butoxycarbonylamino)-3,3-dimethylbutanoic acid (**31**) according to the general procedure to afford the title compound in 76% yield: mp 81-83 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.06 (s, 9H), 1.35 (s, 9H), 1.94-2.18 (m, 3H), 2.52-2.56 (m, 1H), 3.63-3.72 (m, 1H), 4.01 (t, *J* = 8.4 Hz, 1H), 4.26 (d, *J* = 4.4 Hz, 1H), 4.83 (d, *J* = 3.6 Hz, 1H), 5.22 (d, *J* = 4.2 Hz, 1H), 7.12 (s, 1H), 7.27-7.40 (m, 3H), 7.85-7.88 (m, 2H), 10.71 (br s, 1H); LC/MS(ESI) *m/z*: 487.2 $[M + H]^+$, 509.2 $[M + Na]^+$.

A.2.3 General Procedure of the Synthesis of Compound 42-51 from 32-41

To a solution of compound **32-41** (2.9 mmol) in CH_2Cl_2 (20 mL) at 0 ℃, trifluoroacetic acid (10 mL) was added. Then, the reaction was stirred at room temperature for 1 hour. Basification of the solution by $NaHCO_{3(sat.)}$ was accomplished until pH value was about 8. The solution was extracted with CH_2Cl_2. The combined organic layers were dried by Na_2SO_4 and concentrated in vacuo to afford crude product as starting material for next step without further purification. To the above crude product in CH_2Cl_2 (10 mL) at ice bath, morpholine-4-carbonyl chloride (3.5 mmol) and Et_3N (3.5 mmol) were added respectively and then stirred for 10 min. The result mixture was concentrated under reduced pressure and then extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated to yield crude product. The residue was purified with column chromatography to afford the final product.

N-((*R*)-2-oxo-1-phenyl-2-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)ethyl)morpholine-4-carboxamide (42)

The title compound was obtained from compound **32** according to the general procedure to afford the title compound in 41.2% yield: mp 140-141 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.92-2.11 (m, 3H), 2.39-2.42 (m, 1H), 3.26-3.56 (m, 9H), 4.06-4.15 (m, 1H), 4.83 (d, *J* = 4.0 Hz, 1H), 5.46 (d, *J* = 3.4 Hz, 1H), 5.54 (d, *J* = 3.4

Hz, 1H), 7.12 (s, 1H), 7.27-7.52 (m, 8H), 7.80 (d, *J* = 3.6 Hz, 2H), 10.82 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.38, 29.14, 43.98, 47.02, 57.49, 60.79, 66.21, 107.75, 126.02, 127.81, 128.28, 128.48, 128.83, 129.17, 134.46, 135.23, 149.85, 157.68, 157.87, 169.87, 171.46; LC/MS(ESI) *m/z*: 520.3 $[M + H]^+$, 542.3 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{27}H_{30}N_5O_4S$ $[M + H]^+$ 520.2019, found 520.2014; HPLC t_R = 31.63 min, 95.6%.

N-((*R*)-2-oxo-1-phenyl-2-((*S*)-2-(thiazol-2-ylcarbamoyl)pyrrolidin-1-yl)ethyl)morpholine-4-carboxamide (43)

The title compound was obtained from compound **33** according to the general procedure to afford the title compound in 51.3% yield: mp 88-91 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.82-2.13 (m, 3H), 2.30-2.40 (m, 1H), 3.26-3.38 (m, 1H), 3.40-3.49 (m, 4H), 3.55-3.77 (m, 4H), 3.96-4.05 (m, 1H), 4.77 (d, *J* = 2.6 Hz, 1H), 5.60 (d, *J* = 3.4 Hz, 1H), 5.81 (d, *J* = 3.6 Hz, 1H), 6.94 (d, *J* = 1.8 Hz, 1H), 7.27-7.46 (m, 6H), 11.03 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.38, 28.99, 43.93, 47.03, 57.23, 60.73, 66.31, 113.52, 125.41, 128.13, 128.59, 129.04, 135.88, 137.18, 157.30, 169.56, 171.30; LC/MS(ESI) *m/z*: 444.1 $[M + H]^+$, 466.1 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{21}H_{26}N_5O_4S$ $[M + H]^+$ 444.1706, found 444.1702; HPLC t_R = 20.25 min, 95.6%.

N-((*R*)-2-((*S*)-2-(4-methylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)-2-oxo-1-phenylethyl)morpholine-4-carboxamide (44)

The title compound was obtained from compound **34** according to the general procedure to afford the title compound in 80.34% yield: mp 109-111 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.91-2.19 (m, 2H), 2.23 (s, 3H), 2.28-2.38 (m, 1H), 3.26-3.32 (m, 1H), 3.42-3.59 (m, 4H), 3.61-3.69 (m, 4H), 3.99-4.13 (m, 1H), 4.74 (d, *J* = 2.8 Hz, 1H), 5.59 (d, *J* = 3.4 Hz, 1H), 5.91 (d, *J* = 3.4 Hz, 1H), 6.47 (s, 1H), 7.29-7.45 (m, 5H), 10.97 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 16.87, 24.32, 29.05, 43.96, 47.02, 57.28, 60.76, 66.33, 107.98, 125.41, 128.14, 128.59, 129.03, 135.76, 146.83, 157.39, 169.55, 171.32; LC/MS(ESI) *m/z*: 458.2 $[M + H]^+$, 480.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{22}H_{28}N_5O_4S$ $[M + H]^+$ 458.1862, found 458.1856; HPLC t_R = 22.08 min, 98.4%.

N-((*R*)-2-((*S*)-2-(4-tert-butylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)-2-oxo-1-phenylethyl)morpholine-4-carboxamide (45)

The title compound was obtained from compound **35** according to the general procedure to afford the title compound in 85.3% yield: mp 238-239 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.28 (s, 9H), 1.84-1.89 (m, 1H), 1.97-2.07 (m, 2H), 2.24-2.28 (m,

1H), 3.21-3.27 (m, 1H), 3.31-3.46 (m, 4H), 3.59-3.66 (m, 4H), 3.90-3.95 (m, 1H), 4.75-4.77 (m, 1H), 5.59 (d, *J* = 3.4 Hz, 1H), 5.92 (d, *J* = 3.4 Hz, 1H), 6.51 (s, 1H), 7.31-7.46 (m, 5H), 10.94 (brs, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.38, 28.65, 29.83, 34.36, 43.99, 46.99, 57.37, 60.70, 66.33, 104.97, 128.19, 128.80, 129.23, 135.65, 156.92, 157.36, 160.95, 169.15, 171.35; LC/MS(ESI) *m/z*: 500.3 $[M + H]^+$, 522.3 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{25}H_{34}N_5O_4S$ $[M + H]^+$ 500.2332, found 500.2328; HPLC t_R = 32.29 min, 99.4%.

N-((*R*)-2-((*S*)-2-(4-cyclohexylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)-2-oxo-1-phenylethyl)morpholine-4-carboxamide (46)

The title compound was obtained from compound **36** according to the general procedure to afford the title compound in 78.6% yield: mp 118-121 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.20-1.39 (m, 4H), 1.70-2.18 (m, 9H), 2.34-2.37 (m, 1H), 2.40-2.59 (m, 1H), 3.26-3.33 (m, 1H), 3.38-3.50 (m, 4H), 3.60-3.70 (m, 4H), 3.95-3.99 (m, 1H), 4.77-4.79 (m, 1H), 5.54-5.58 (m, 1H), 5.64 (d, *J* = 3.4 Hz, 1H), 6.48 (s, 1H), 7.29-7.47 (m, 5H), 10.64 (brs, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.35, 26.12, 26.32, 28.85, 32.61, 32.74, 40.35, 44.02, 46.99, 57.41, 60.73, 66.34, 105.70, 128.22, 128.78, 129.18, 135.52, 157.09, 157.18, 157.49, 169.29, 171.39; LC/MS(ESI) *m/z*: 526.3 $[M + H]^+$, 548.3 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{27}H_{36}N_5O_4S$ [M +

H]$^+$ 526.2488, found 526.2482; HPLC t_R = 35.18 min, 98.6%.

N-((*R*)-1-oxo-1-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)propan-2-yl)morpholine-4-carboxamide (47)

The title compound was obtained from compound **37** according to the general procedure to afford the title compound in 89% yield: mp 138-140 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.33 (d, *J* = 3.6 Hz, 3H), 2.02-2.20 (m, 3H), 2.23-2.39 (m, 1H), 3.36-3.43 (m, 8H), 3.54 (dd, *J* = 7.8, 17.4 Hz, 1H), 4.11-4.18 (m, 1H), 4.43-4.48 (m, 1H), 4.76-4.80 (m, 1H), 5.34 (d, *J* = 3.6 Hz, 1H), 7.07 (s, 1H), 7.25-7.35 (m, 3H), 7.72-7.76 (m, 2H), 10.82 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 16.22, 24.36, 29.58, 44.07, 47.11, 48.85, 60.62, 66.22, 107.75, 126.11, 127.81, 128.42, 134.59, 149.96, 157.81, 158.30, 170.34, 174.39; LC/MS(APCI) *m/z*: 458.2 [M + H]$^+$; HRMS (*m/z*): calcd for $C_{22}H_{28}N_5O_4S$ [M + H]$^+$ 458.1862, found 458.1858; HPLC t_R = 25.59 min, 99.7%.

N-((*R*)-1-oxo-1-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)butan-2-yl)morpholine-4-carboxamide (48)

The title compound was obtained from compound **38** according to the general procedure to afford the title compound in 84% yield: mp 132-136 ℃; ^{1}H NMR

(300MHz, $CDCl_3$) δ 1.03 (t, *J* = 7.5 Hz, 3H), 1.69-1.82 (m, 2H), 2.03-2.41 (m, 4H), 3.37-3.44 (m, 8H), 3.61 (q, *J* = 8.7 Hz, 1H), 4.19-4.26 (m, 1H), 4.32 (q, *J* = 7.5 Hz, 1H), 4.83 (dd, *J* = 2.1, 8.7 Hz, 1H), 5.31 (d, *J* = 3.6 Hz, 1H), 7.09 (s, 1H), 7.25-7.37 (m, 3H), 7.75-7.78 (m, 2H), 10.82 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 10.41, 24.28, 24.32, 29.69, 44.12, 47.22, 54.67, 60.59, 66.21, 107.75, 126.11, 127.78, 128.40, 134.62, 149.98, 157.75, 158.57, 170.40, 174.02; LC/MS(ESI) *m/z*: 472.2 $[M + H]^+$, 494.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{23}H_{30}N_5O_4S$ $[M + H]^+$ 472.2019, found 472.2014; HPLC t_R = 27.89 min, 100%.

N-((*R*)-1-oxo-1-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)pentan-2-yl)morpholine-4-carboxamide (49)

The title compound was obtained from compound **39** according to the general procedure to afford the title compound in 78% yield: mp 131-134 ℃; ^1H NMR (400MHz, $CDCl_3$) δ 0.94 (t, *J* = 7.4 Hz, 3H), 1.32-1.50 (m, 2H), 1.63-1.69 (m, 2H), 2.04-2.37 (m, 4H), 3.23-3.38 (m, 8H), 3.42-3.60 (m, 1H), 4.18-4.23 (m, 1H), 4.36 (q, *J* = 7.2 Hz, 1H), 4.79 (d, *J* = 4.4 Hz, 1H), 5.21 (d, *J* = 3.6 Hz, 1H), 7.06 (s, 1H), 7.22-7.33 (m, 3H), 7.74 (d, *J* = 4.2 Hz, 2H), 10.77 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 13.92, 19.12, 24.30, 29.66, 33.14, 44.13, 47.15, 53.11, 60.60, 66.21, 107.75, 126.11, 127.76, 128.40, 134.63, 149.98, 157.73, 158.63, 170.37, 174.19;

LC/MS(APCI) *m/z*: 486.2 $[M + H]^+$; HRMS (*m/z*): calcd for $C_{24}H_{32}N_5O_4S$ $[M + H]^+$ 486.2175, found 486.2173; HPLC t_R = 30.71 min, 97.8%.

N-((*R*)-3-methyl-1-oxo-1-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)butan-2-yl)morpholine-4-carboxamide (50)

The title compound was obtained from compound **40** according to the general procedure to afford the title compound in 87% yield: mp 117-120 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.01 (dd, *J* = 6.6, 24.9 Hz, 6H), 1.94-2.37 (m, 5H), 3.23-3.42 (m, 8H), 3.56-3.67 (m, 1H), 4.01-4.06 (m, 1H), 4.20-4.27 (m, 1H), 4.78-4.82 (m, 1H), 5.33 (d, *J* = 3.7 Hz, 1H), 7.06 (s, 1H), 7.21-7.34 (m, 3H), 7.71-7.75 (m, 2H), 10.75 (brs, 1H); LC/MS(APCI) *m/z*: 486.2 $[M + H]^+$; HRMS (*m/z*): calcd for $C_{24}H_{32}N_5O_4S$ $[M + H]^+$ 486.2175, found 486.2169; HPLC t_R = 30.93 min, 97.2%.

N-((*R*)-3,3-dimethyl-1-oxo-1-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)butan-2-yl)morpholine-4-carboxamide (51)

The title compound was obtained from compound **41** according to the general procedure to afford the title compound in 75% yield: mp 122-124 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.09 (s, 9H), 2.03-2.38 (m, 4H), 3.37-3.49 (m, 8H), 3.63-3.71 (m, 1H), 4.18 (d, *J* = 4.2 Hz, 1H), 4.25-4.32 (m, 1H), 4.79-4.87 (m, 2H), 7.08 (s, 1H),

7.25-7.38 (m, 3H), 7.75-7.79 (m, 2H), 10.61 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100MHz) δ 24.48, 26.68, 29.61, 33.64, 44.14, 47.89, 60.00, 60.73, 66.11, 107.80, 126.14, 127.76, 128.44, 134.71, 149.87, 157.74, 159.01, 170.39, 172.91; LC/MS(ESI) *m/z*: 500.2 [M + H]$^+$, 522.2 [M + Na]$^+$; HRMS (*m/z*): calcd for $C_{25}H_{34}N_5O_4S$ [M + H]$^+$ 500.2332, found 500.2330; HPLC t_R = 33.78 min, 99.8%.

A.3. Preparation of Compounds 52-81

To a solution of compound **32** (2.9 mmol) in CH_2Cl_2 (20 mL) at 0 ℃, trifluoroacetic acid (10 mL) was added. Then, the reaction was stirred at room temperature for 1 hour. Basification of the solution by $NaHCO_{3(sat.)}$ was accomplished until pH value was about 8. The solution was extracted with CH_2Cl_2. The combined organic layers were dried by Na_2SO_4 and concentrated in vacuo to afford intermediate. The crude intermediate was used as starting material for next step without further purification.

(1) General Procedure A：

To the above crude amine intermediate (0.29 mmol) in CH_2Cl_2 (5 mL) at ice bath, corresponding acid chloride (0.32 mmol) and Et_3N (0.05 mL, 0.35 mmol) were added respectively and then stirred for 18 hours. The result mixture was concentrated

under reduced pressure and then extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated to yield crude product. The residue was purified with column chromatography to afford the final product.

(2) General Procedure B：

To the crude amine intermediate (0.29 mmol) in CH_2Cl_2 (5 mL) at ice bath, corresponding isocyanate or isothiocyanate (0.32 mmol) was added respectively and then stirred for 18 hours. The result mixture was concentrated under reduced pressure and then extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated to yield crude product. The residue was purified with column chromatography to afford the final product.

(3) General Procedure C：

A solution of corresponding amine (0.32 mmol) in 2.5 mL CH_2Cl_2 was added dropwise into a solution of N,N′-carbonyldiimidazole (CDI, 0.44 mmol) in CH_2Cl_2 (5 mL) at ice bath via addition funnel. After 10 minutes, the solvent was removed and the reaction mixture was extracted with EtOAc/H_2O. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated to get crude product. The

crude product in CH_2Cl_2 (2.5 mL) was added slowly into a solution of intermediate (0.29 mmol) and Et_3N (0.35 mmol) in CH_2Cl_2 (5 mL) at ice bath. The reaction was stirred at room temperature for 18 hours. The result mixture was concentrated under reduced pressure and then extracted with EtOAc. The residue was purified with column chromatography to afford the final product.

(4) General Procedure D：

To a solution of corresponding amine (0.49 mmol) in THF (5 mL), triphosgene (0.25 mmol) was added at 0 ~ -10℃. After Et_3N (0.98 mmol) was added via addition funnel in 5 minutes at 0 ~ -10℃, the intermediate (0.25 mmol) in THF (5 mL) was reacted with the above solution at room temperature for 18 hours. The result mixture was concentrated under reduced pressure and then extracted with EtOAc. The residue was purified with column chromatography to afford the final product.

(*S*)-1-((*R*)-2-acetamido-2-phenylacetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (52)

The title compound was obtained from commercially available acetyl chloride according to the general procedure **A** to afford the title compound in 90.3% yield: mp 150-153 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.81-1.94 (m, 2H), 1.96 (s, 3H), 1.98-2.17

(m, 1H), 2.32-2.38 (m, 1H), 3.14-3.22 (m, 1H), 3.78-3.85 (m, 1H), 4.75-4.79 (m, 1H), 5.80 (d, *J* = 3.6 Hz, 1H), 7.13 (s, 1H), 7.16 (d, *J* = 3.7 Hz, 1H), 7.28-7.60 (m, 8H), 7.70-7.78 (m, 2H), 10.91 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 22.96, 24.51, 28.47, 47.17, 55.73, 60.85, 107.76, 126.04, 127.91, 128.07, 128.62, 128.69, 129.20, 134.25, 135.99, 149.93, 157.79, 169.24, 169.88, 170.39; LC/MS(ESI) *m/z*: 449.1 $[M + H]^+$, 471.1 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{24}H_{25}N_4O_3S$ $[M + H]^+$ 449.1647, found 449.1642; HPLC t_R = 29.08 min, 99.4%.

(*S*)-1-((*R*)-2-phenyl-2-propionamidoacetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (53)

The title compound was obtained from commercially available propionyl chloride according to the general procedure **A** to afford the title compound in 89.7% yield: mp 134-136 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.11 (t, *J* = 7.5 Hz, 3H), 1.81-2.41 (m, 6H), 3.16-3.25 (m, 1H), 3.73-3.87 (m, 1H), 4.78 (d, *J* = 2.8 Hz, 1H), 5.77 (d, *J* = 3.4 Hz, 1H), 6.98 (d, *J* = 3.4 Hz, 1H), 7.12 (s, 1H), 7.27-7.46 (m, 8H), 7.71-7.82 (m, 2H), 10.82 (brs, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 9.35, 24.53, 28.22, 29.20, 47.15, 55.70, 60.77, 107.76, 126.07, 127.90, 128.08, 128.60, 128.71, 129.20, 134.31, 135.90, 149.96, 157.64, 169.09, 170.52, 173.55; LC/MS(ESI) *m/z*: 463.2 $[M + H]^+$, 485.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{25}H_{27}N_4O_3S$ $[M + H]^+$ 463.1804, found 463.1801;

HPLC t_R = 31.90 min, 99.5%.

(*S*)-1-((*R*)-2-butyramido-2-phenylacetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (54)

The title compound was obtained from commercially available butyryl chloride according to the general procedure **A** to afford the title compound in 90% yield: mp 125-127 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 0.85 (t, *J* = 7.5 Hz, 3H), 1.57-1.69 (m, 2H), 1.84-2.26 (m, 5H), 2.34-2.43 (m, 1H), 3.18-3.27 (m, 1H), 3.73-3.90 (m, 1H), 4.79 (d, *J* = 3.2 Hz, 1H), 5.78 (d, *J* = 3.6 Hz, 1H), 6.96 (d, *J* = 3.4 Hz, 1H), 7.13 (s, 1H), 7.27-7.46 (m, 8H), 7.71-7.83 (m, 2H), 10.83 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 13.55, 18.82, 24.53, 28.25, 38.07, 47.15, 55.69, 60.79, 107.72, 126.07, 127.88, 128.07, 128.59, 128.71, 129.20, 134.31, 135.84, 149.94, 157.67, 169.13, 170.51, 172.91; LC/MS(ESI) *m/z*: 477.2 $[M + H]^+$, 499.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{26}H_{29}N_4O_3S$ $[M + H]^+$ 477.1960, found 477.1953; HPLC t_R = 34.52 min, 100%.

(*S*)-1-((*R*)-2-isobutyramido-2-phenylacetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (55)

The title compound was obtained from commercially available isobutyryl chloride according to the general procedure **A** to afford the title compound in 81.75% yield:

mp 120-123 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.12 (dd, *J* = 6.0, 11.2 Hz, 6H), 1.85-2.10 (m, 3H), 2.38-2.45 (m, 2H), 3.21-3.28 (m, 1H), 3.85-3.91 (m, 1H), 4.80 (d, *J* = 4.2 Hz, 1H), 5.73 (d, *J* = 3.4 Hz, 1H), 6.87 (d, *J* = 3.4 Hz, 1H), 7.14 (s, 1H), 7.27-7.46 (m, 8H), 7.83 (d, *J* = 3.6 Hz, 2H), 10.75 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 19.19, 19.35, 24.51, 28.15, 35.14, 47.14, 55.70, 60.74, 107.70, 126.05, 127.85, 128.05, 128.57, 128.72, 129.21, 134.33, 135.70, 149.94, 157.58, 169.09, 170.59, 176.95; LC/MS(ESI) *m/z*: 477.2 $[M + H]^+$, 499.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{26}H_{29}N_4O_3S$ $[M + H]^+$ 477.1960, found 477.1956; HPLC t_R = 34.59 min, 100%.

(*S*)-1-((*R*)-2-phenyl-2-pivalamidoacetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (56)

The title compound was obtained from commercially available 2,2-dimethyl-propionyl chloride according to the general procedure **A** to afford the title compound in 83% yield: mp 123-126 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.22 (s, 9H), 1.85-2.11 (m, 3H), 2.43-2.49 (m, 1H), 3.24-3.33 (m, 1H), 3.89-3.96 (m, 1H), 4.82-4.84 (m, 1H), 5.65 (d, *J* = 3.3 Hz, 1H), 6.84 (d, *J* = 3.2 Hz, 1H), 7.14 (s, 1H), 7.27-7.48 (m, 8H), 7.71-7.88 (m, 2H), 10.67 (brs, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.48, 27.26, 28.01, 38.62, 47.06, 56.07, 60.68, 107.64, 126.07, 127.79, 128.08,

128.53, 128.83, 129.24, 134.40, 135.41, 149.96, 157.43, 169.04, 170.65, 178.74; LC/MS(ESI) *m/z*: 491.2 $[M + H]^+$, 513.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{27}H_{31}N_4O_3S$ $[M + H]^+$ 491.2117, found 491.2115; HPLC t_R = 37.79 min, 99.8%.

(*S*)-1-((*R*)-2-(cyclopropanecarboxamido)-2-phenylacetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (57)

The title compound was obtained from commercially available cyclopropanecarbonyl chloride according to the general procedure **A** to afford the title compound in 76.6% yield: mp 146-149 ℃; ^{1}H NMR ($CDCl_3$, 300MHz) δ 0.67-0.74 (m, 2H), 0.95-1.09 (m, 3H), 1.37-1.45 (m, 1H), 1.84-2.40 (m, 6H), 3.20-3.29 (m, 1H), 3.81-3.88 (m, 1H), 4.75-4.82 (m, 1H), 5.75 (d, *J* = 3.5 Hz, 1H), 7.13 (s, 1H), 7.25-7.49 (m, 9H), 7.74-7.85 (m, 2H), 10.79 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100MHz) δ 7.64, 7.89, 14.49, 24.50, 28.29, 47.11, 56.14, 60.72, 107.74, 126.10, 127.86, 128.13, 128.56, 128.74, 129.20, 134.32, 135.61, 149.94, 157.61, 169.18, 170.64, 173.79; LC/MS(ESI): 475 $[M + H]^+$, 497 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{26}H_{27}N_4O_3S$ $[M + H]^+$ 475.1804, found 475.1799; HPLC t_R = 34.12 min, 99.3%.

(*S*)-1-((*R*)-2-(cyclopentanecarboxamido)-2-phenylacetyl)-N-(4-phenylthiazol-2-yl) pyrrolidine-2-carboxamide (58)

The title compound was obtained from commercially available cyclopentanecarbonyl chloride according to the general procedure **A** to afford the title compound in 21.3% yield: mp 114-116 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.43-2.11 (m, 11H), 2.43-2.46 (m, 1H), 2.48-2.61 (m, 1H), 3.26 (dd, *J* = 9.6, 16.4 Hz, 1H), 3.88-3.92 (m, 1H), 4.82 (d, *J* = 3.0 Hz, 1H), 5.71 (d, *J* = 3.4 Hz, 1H), 6.74 (d, *J* = 3.4 Hz, 1H), 7.14 (s, 1H), 7.29-7.52 (m, 8H), 7.85 (d, *J* = 4.0 Hz, 2H), 10.74 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100MHz) δ 24.56, 25.91, 27.97, 30.23, 30.34, 45.30, 47.15, 55.95, 60.73, 107.71, 126.10, 127.85, 128.09, 128.56, 128.79, 129.25, 134.41, 135.62, 150.02, 157.48, 168.99, 170.73, 176.35; LC/MS(ESI) *m/z*: 503.3 $[M + H]^+$, 525.3 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{28}H_{31}N_4O_3S$ $[M + H]^+$ 503.2117, found 503.2113; HPLC t_R = 38.07 min, 100%.

(*S*)-1-((*R*)-2-(cyclohexanecarboxamido)-2-phenylacetyl)-N-(4-phenylthiazol-2-yl) pyrrolidine-2-carboxamide (59)

The title compound was obtained from commercially available cyclohexanecarbonyl chloride according to the general procedure **A** to afford the title compound in 45.4% yield: mp 107-108 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.23-1.51 (m, 7H), 1.83-2.19 (m, 7H), 2.44-2.49 (m, 1H), 3.22-3.31 (m, 1H), 3.91-3.95 (m, 1H), 4.81 (d, *J* = 2.8 Hz, 1H), 5.71 (d, *J* = 3.3 Hz, 1H), 6.72 (d, *J* = 3.4 Hz, 1H), 7.14 (s, 1H), 7.28-7.47 (m,

8H), 7.86 (d, *J* = 3.7 Hz, 2H), 10.74 (brs, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.51, 25.49, 25.57, 28.12, 29.22, 29.38, 44.88, 47.11, 55.67, 60.71, 107.69, 126.08, 127.84, 128.05, 128.54, 128.71, 129.18, 134.37, 135.70, 149.96, 157.61, 169.12, 170.65, 176.10; LC/MS(ESI) *m/z*: 517.3 $[M + H]^+$, 539.3 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{29}H_{33}N_4O_3S$ $[M + H]^+$ 517.2273, found 517.2272; HPLC t_R = 40.05 min, 99.7%.

(*S*)-1-((*R*)-2-benzamido-2-phenylacetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (60)

The title compound was obtained from commercially available benzoyl chloride according to the general procedure **A** to afford the title compound in 53.3% yield: mp 196-200 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.88-2.25 (m, 3H), 2.38-2.64 (m, 1H), 3.29-3.37 (m, 1H), 3.95-4.01 (m, 1H), 4.84 (d, *J* = 3.2 Hz, 1H), 5.89 (d, *J* = 3.3 Hz, 1H), 7.13 (s, 1H), 7.29-7.60 (m, 12H), 7.86 (d, *J* = 4.0 Hz, 4H), 10.78 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100MHz) δ 24.50, 28.48, 47.16, 56.55, 60.82, 107.80, 126.14, 127.39, 127.85, 128.33, 128.37, 128.58, 128.85, 129.22, 131.75, 133.31, 134.37, 135.42, 149.95, 157.76, 167.24, 169.28, 170.31; LC/MS(ESI) *m/z*: 511.2 $[M + H]^+$, 533.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{29}H_{27}N_4O_3S$ $[M + H]^+$ 511.1804, found 511.1796; HPLC t_R = 37.87 min, 99.5%.

N-((*R*)-2-oxo-1-phenyl-2-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)ethyl)picolinamide (61)

The title compound was obtained from commercially available picolinoyl chloride hydrochloride according to the general procedure **A** to afford the title compound in 77% yield: mp 136-139 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.89-2.15 (m, 3H), 2.37-2.45 (m, 1H), 3.33-3.39 (m, 1H), 4.00-4.07 (m, 1H), 4.83-4.86 (m, 1H), 5.84 (d, *J* = 3.2 Hz, 1H), 7.12 (s, 1H), 7.25-7.45 (m, 7H), 7.54-7.74 (m, 3H), 7.82-7.85 (m, 1H), 8.26 (d, *J* = 3.9 Hz, 1H), 8.50 (d, *J* = 1.9 Hz, 1H), 8.92 (d, *J* = 3.2 Hz, 1H), 10.76 (brs, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.59, 28.41, 47.20, 56.39, 60.83, 107.76, 122.74, 126.14, 126.42, 127.79, 128.33, 128.54, 128.97, 129.30, 134.54, 135.14, 137.15, 148.25, 149.09, 149.93, 157.61, 164.45, 169.35, 170.02; LC/MS(ESI) *m/z*: 512.2 $[M + H]^+$, 534.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{28}H_{26}N_5O_3S$ $[M + H]^+$ 512.1756, found 512.1748; HPLC t_R = 36.89 min, 98.7%.

N-((*R*)-2-oxo-1-phenyl-2-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)ethyl)nicotinamide (62)

The title compound was obtained from commercially available nicotinoyl chloride hydrochloride according to the general procedure **A** to afford the title compound in 79.5% yield: mp 143-145 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.91-2.16 (m, 3H),

2.38-2.43 (m, 1H), 3.23-3.31 (m, 1H), 3.89-3.96 (m, 1H), 4.82-4.85 (m, 1H), 5.91 (d, *J* = 3.3 Hz, 1H), 7.13 (s, 1H), 7.27-7.38 (m, 5H), 7.40-7.54 (m, 2H), 7.72-7.81 (m, 4H), 8.20-8.24 (m, 1H), 8.66 (br s, 1H), 9.02 (brs, 1H), 10.79 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.54, 28.50, 47.18, 56.54, 60.82, 107.85, 123.35, 126.07, 127.93, 128.31, 128.63, 128.72, 128.95, 129.29, 134.30, 135.18, 135.67, 148.27, 149.96, 152.23, 157.72, 165.32, 169.29, 169.99; LC/MS(ESI) *m/z*: 512.2 $[M + H]^+$, 534.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{28}H_{26}N_5O_3S$ $[M + H]^+$ 512.1756, found 512.1757; HPLC t_R = 30.80 min, 99.7%.

N-((*R*)-2-oxo-1-phenyl-2-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)ethyl)isonicotinamide (63)

The title compound was obtained from commercially available isonicotinoyl chloride hydrochloride according to the general procedure **A** to afford the title compound in 39.8% yield: mp 105-108 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.83-2.18 (m, 3H), 2.38-2.48 (m, 1H), 3.23-3.31 (m, 1H), 4.03-4.09 (m, 1H), 5.05 (d, *J* = 2.1 Hz, 1H), 5.93 (d, *J* = 3.3 Hz, 1H), 7.10 (s, 1H), 7.25-7.34 (m, 3H), 7.39-7.45 (m, 4H), 7.52-7.55 (m, 1H), 7.62-7.72 (m, 4H), 7.83 (d, *J* = 3.2 Hz, 1H), 8.65 (d, *J* = 2.1 Hz, 1H), 11.05 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.57, 28.74, 47.25, 56.47, 60.94, 108.04, 121.17, 125.33, 128.07, 128.30, 128.68, 128.97, 129.30, 134.33, 135.69,

140.63, 149.98, 150.40, 158.19, 164.89, 169.41, 170.92; LC/MS(ESI) *m/z*: 512.2 [M + H]$^+$, 534.2 [M + Na]$^+$; HRMS (*m/z*): calcd for $C_{28}H_{26}N_5O_3S$ [M + H]$^+$ 512.1756, found 512.1753; HPLC t_R = 30.96 min, 98.8%.

(*S*)-1-((*R*)-2-(3,3-dimethylureido)-2-phenylacetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (64)

The title compound was obtained from commercially available dimethylcarbamoyl chloride according to the general procedure **A** to afford the title compound in 75.5% yield: mp 131-133 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.91-2.11 (m, 3H), 2.38-2.41 (m, 1H), 2.95 (s, 6H), 3.35-3.40 (m, 1H), 4.04-4.10 (m, 1H), 4.83 (dd, *J* = 2.4, 7.2 Hz, 1H), 5.34 (d, *J* = 3.4 Hz, 1H), 5.57 (d, *J* = 3.4 Hz, 1H), 7.14 (s, 1H), 7.28-7.51 (m, 8H), 7.85 (d, *J* = 3.6 Hz, 2H), 10.89 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.41, 29.00, 36.27, 47.02, 57.46, 60.77, 107.67, 126.05, 127.69, 128.19, 128.46, 128.75, 129.20, 134.54, 135.67, 149.85, 157.82, 158.30, 169.87, 171.67; LC/MS(ESI) *m/z*: 478.2 [M + H]$^+$, 500.2 [M + Na]$^+$; HRMS (*m/z*): calcd for $C_{25}H_{28}N_5O_3S$ [M + H]$^+$ 478.1913, found 478.1918; HPLC t_R = 32.11 min, 98.8%.

(*S*)-1-((*R*)-2-(3,3-diethylureido)-2-phenylacetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (65)

The title compound was obtained from commercially available diethylcarbamoyl chloride according to the general procedure **A** to afford the title compound in 68.2% yield: mp 122-125 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.11 (t, *J* = 7.2 Hz, 6H), 2.02-2.08 (m, 3H), 2.40-2.42 (m, 1H), 3.12-3.24 (m, 2H), 3.31-3.49 (m, 3H), 4.07 (t, *J* = 7.2 Hz, 1H), 4.85 (d, *J* = 2.8 Hz, 1H), 5.29 (d, *J* = 3.6 Hz, 1H), 5.59 (d, *J* = 3.6 Hz, 1H), 7.14 (s, 1H), 7.27-7.51 (m, 8H), 7.86 (d, *J* = 3.9 Hz, 2H), 10.93 (brs, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 13.63, 24.38, 28.86, 41.22, 46.94, 57.41, 60.67, 107.58, 126.07, 127.67, 128.16, 128.42, 128.74, 129.23, 134.57, 135.65, 149.82, 157.41, 157.73, 169.82, 171.85; LC/MS(APCI) *m/z*: 506.3 $[M + H]^+$; HRMS (*m/z*): calcd for $C_{27}H_{32}N_5O_3S$ $[M + H]^+$ 506.2226, found 506.2228; HPLC t_R = 37.03 min, 99.2%.

N-((*R*)-2-oxo-1-phenyl-2-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)ethyl)pyrrolidine-1-carboxamide (66)

The title compound was obtained from commercially available pyrrolidine-1-carbonyl chloride according to the general procedure **A** to afford the title compound in 72.6% yield: mp 130-133 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.80-2.09 (m, 7H), 2.36-2.39 (m, 1H), 3.30-3.38 (m, 5H), 4.05-4.12 (m, 1H), 4.82 (dd, *J* = 2.4, 7.5 Hz, 1H), 5.21 (d, *J* = 3.6 Hz, 1H), 5.64 (d, *J* = 3.6 Hz, 1H), 7.13 (s, 1H), 7.27-7.39 (m, 6H), 7.40-7.56 (m, 2H), 7.84 (d, *J* = 3.6 Hz, 2H), 11.01 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100MHz) δ 24.40,

25.33, 28.99, 45.71, 47.06, 57.02, 60.86, 107.64, 126.08, 127.70, 128.14, 128.45, 128.70, 129.19, 134.52, 135.90, 149.82, 156.42, 157.89, 169.87, 171.61; LC/MS(ESI) *m/z*: 504.3 $[M + H]^+$, 526.3 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{27}H_{30}N_5O_3S$ $[M + H]^+$ 504.2069, found 504.2066; HPLC t_R = 33.81 min, 99.7%.

N-((*R*)-2-oxo-1-phenyl-2-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidin-1-yl)ethyl)piperidine-1-carboxamide (67)

The title compound was obtained from commercially available piperidine-1-carbonyl chloride according to the general procedure **A** to afford the title compound in 85.3% yield: mp 135-138 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.30-1.94 (m, 6H), 2.03-2.13 (m, 3H), 2.37-2.40 (m, 1H), 3.29-3.53 (m, 5H), 4.09-4.15 (m, 1H), 4.85 (dd, *J* = 2.4, 7.5 Hz, 1H), 5.33 (d, *J* = 3.6 Hz, 1H), 5.53 (d, *J* = 3.6 Hz, 1H), 7.13 (s, 1H), 7.27-7.43 (m, 8H), 7.85 (d, *J* = 3.4 Hz, 2H), 10.94 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.19, 24.35, 25.40, 29.20, 44.97, 46.94, 57.57, 60.71, 107.59, 126.07, 127.64, 128.25, 128.42, 128.77, 129.18, 134.59, 135.35, 149.85, 157.70, 157.81, 170.08, 171.87; LC/MS(ESI) *m/z*: 518.3 $[M + H]^+$, 540.3 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{28}H_{32}N_5O_3S$ $[M + H]^+$ 518.2226, found 518.2229; HPLC t_R = 38.11 min, 99.5%.

4-methyl-N-((*R*)-2-oxo-1-phenyl-2-((*S*)-2-(4-phenylthiazol-2-ylcarbamoyl)pyrroli

din-1-yl)ethyl)piperazine-1-carboxamide (68)

The title compound was obtained from commercially available 4-methyl-piperazine-1-carbonyl chloride according to the general procedure **A** to afford the title compound in 71.2% yield: mp 130-132 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.92-2.11 (m, 3H), 2.18 (s, 3H), 2.19-2.27 (m, 4H), 2.38-2.41 (m, 1H), 3.35-3.48 (m, 5H), 4.08 (t, *J* = 6.9 Hz, 1H), 4.84 (d, *J* = 2.7 Hz, 1H), 5.42 (d, *J* = 3.4 Hz, 1H), 5.52 (d, *J* = 3.4 Hz, 1H), 7.13 (s, 1H), 7.28-7.39 (m, 6H), 7.41-7.50 (m, 2H), 7.82 (d, *J* = 3.6 Hz, 2H), 10.86 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.35, 29.15, 43.67, 45.87, 46.97, 54.28, 57.46, 60.76, 107.67, 126.02, 127.73, 128.24, 128.45, 128.74, 129.14, 134.48, 135.35, 149.82, 157.47, 157.87, 169.94, 171.52; LC/MS(ESI) *m/z*: 533.2 $[M + H]^+$, 555.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{28}H_{33}N_6O_3S$ $[M + H]^+$ 533.2335, found 533.2341; HPLC t_R = 21.73 min, 99.6%.

(*S*)-1-((*R*)-2-(3-cyclopropylureido)-2-phenylacetyl)-N-(4-phenylthiazol-2-yl)pyrro lidine-2-carboxamide (69)

The title compound was obtained from commercially available cyclopropylamine according to the general procedure **C** to afford the title compound in 45% yield: mp 144-146 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 0.86-0.90 (m, 1H), 1.25-1.26 (m, 1H), 1.88-2.48 (m, 8H), 3.38-3.41 (m, 1H), 4.27-4.28 (m, 1H), 4.71-4.75 (m, 1H), 5.84 (d,

J = 3.4 Hz, 1H), 6.63 (d, *J* = 3.7 Hz, 1H), 7.07 (s, 1H), 7.27-7.40 (m, 6H), 7.43-7.48 (m, 2H), 7.74 (d, *J* = 3.6 Hz, 2H), 11.66 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 7.14, 7.29, 22.33, 24.45, 29.29, 47.34, 56.31, 61.20, 107.98, 126.34, 127.79, 127.98, 128.46, 128.51, 129.06, 134.51, 137.21, 149.85, 158.71, 159.14, 169.67, 170.16; LC/MS(ESI) *m/z*: 490.2 $[M + H]^+$, 512.1 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{26}H_{28}N_5O_3S$ $[M + H]^+$ 490.1913, found 490.1917; HPLC t_R = 31.64 min, 95.7%.

(*S*)-1-((*R*)-2-(3-cyclopentylureido)-2-phenylacetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (70)

The title compound was obtained from commercially available cyclopentyl isocyanate according to the general procedure **B** to afford the title compound in 78% yield: mp 149-152 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 0.98-2.03 (m, 9H), 2.25-2.28 (m, 1H), 2.68 (brs, 2H), 3.18-3.26 (m, 1H), 3.90-4.06 (m, 2H), 4.83 (d, *J* = 2.7 Hz, 1H), 5.17-5.41 (m, 1H), 5.70 (d, *J* = 3.6 Hz, 1H), 6.22-6.37 (m, 1H), 7.10 (s, 1H), 7.25-7.40 (m, 8H), 7.79 (d, *J* = 3.9 Hz, 2H), 11.15 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 23.29, 23.37, 24.32, 29.23, 32.90, 33.44, 47.06, 51.80, 56.76, 60.92, 107.59, 126.02, 127.85, 128.04, 128.48, 128.56, 129.21, 134.31, 135.79, 149.88, 157.90, 157.96, 169.87, 172.66; LC/MS(ESI) *m/z*: 518.2 $[M + H]^+$, 540.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{28}H_{32}N_5O_3S$ $[M + H]^+$ 518.2226, found 518.2232; HPLC t_R = 36.59 min, 98.1%.

(*S*)-1-((*R*)-2-(3-cyclohexylureido)-2-phenylacetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (71)

The title compound was obtained from commercially available cyclohexylamine according to the general procedure **C** to afford the title compound in 26.4% yield: mp 151-154 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 0.66-2.03 (m, 11H), 2.22-2.27 (m, 1H), 3.01 (brs, 2H), 3.11-3.20 (m, 1H), 3.39-3.49 (m, 1H), 3.88-3.93 (m, 1H), 4.79 (d, *J* = 2.8 Hz, 1H), 5.25 (dd, *J* = 7.5, 27 Hz, 1H), 5.72 (d, *J* = 3.9 Hz, 1H), 6.48 (d, *J* = 3.9 Hz, 1H), 7.10 (s, 1H), 7.27-7.41 (m, 6H), 7.79 (d, *J* = 3.6 Hz, 2H), 11.29 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.36, 24.70, 24.76, 25.28, 29.05, 33.31, 33.73, 47.05, 48.93, 56.70, 60.91, 107.64, 126.10, 127.88, 128.05, 128.45, 128.57, 129.20, 134.34, 136.08, 149.99, 157.41, 157.88, 169.75, 172.66; LC/MS(ESI) *m/z*: 532.2 $[M + H]^+$, 554.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{29}H_{34}N_5O_3S$ $[M + H]^+$ 532.2382, found 532.2385; HPLC t_R = 38.61 min, 97.2%.

(*S*)-1-((*R*)-2-(3-cycloheptylureido)-2-phenylacetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (72)

The title compound was obtained from commercially available cycloheptylamine according to the general procedure **C** to afford the title compound in 67.7% yield: mp

155-158 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.01-1.39 (m, 8H), 1.61-2.03 (m, 6H), 2.24-2.29 (m, 1H), 2.78 (brs, 2H), 3.14-3.23 (m, 1H), 3.65-3.71 (m, 1H), 3.84-3.90 (m, 1H), 4.81-4.83 (m, 1H), 5.26 (d, *J* = 3.9 Hz, 1H), 5.71 (d, *J* = 3.9 Hz, 1H), 6.43 (d, *J* = 3.9 Hz, 1H), 7.11 (s, 1H), 7.25-7.41 (m, 8H), 7.79 (d, *J* = 3.4 Hz, 2H), 11.19 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 23.84, 24.35, 27.75, 27.78, 29.09, 35.20, 35.54, 47.05, 51.14, 56.70, 60.88, 107.63, 126.08, 127.85, 128.02, 128.39, 128.56, 129.17, 134.33, 136.13, 149.96, 157.33, 157.91, 169.81, 172.66; LC/MS(ESI) *m/z*: 546.2 $[M + H]^+$, 568.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{30}H_{36}N_5O_3S$ $[M + H]^+$ 546.2539, found 546.2534; HPLC t_R = 40.77 min, 96.4%.

(*S*)-1-((*R*)-2-phenyl-2-(3-phenylureido)acetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (73)

The title compound was obtained from commercially available phenyl isocyanate according to the general procedure **B** to afford the title compound in 79.4% yield: mp 152-155 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.67-2.10 (m, 3H), 2.28 (brs, 3H), 3.17-3.25 (m, 1H), 3.85-3.91 (m, 1H), 4.87 (d, *J* = 2.5 Hz, 1H), 5.79 (d, *J* = 3.6 Hz, 1H), 6.71-6.94 (m, 4H), 7.08-7.15 (m, 3H), 7.24-7.35 (m, 6H), 7.68 (brs, 1H), 7.75 (d, *J* = 3.3 Hz, 2H), 11.15 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100MHz) δ 24.43, 29.06, 47.22, 56.77, 61.04, 107.88, 119.20, 122.42, 126.14, 127.91, 128.21, 128.58, 128.63, 128.76,

129.29, 134.19, 135.54, 138.58, 149.94, 155.51, 157.84, 169.62, 172.40; LC/MS(ESI) *m/z*: 526.2 $[M + H]^+$, 548.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{29}H_{28}N_5O_3S$ $[M + H]^+$ 526.1913, found 526.1916; HPLC t_R = 37.59 min, 99.6%.

(*S*)-1-((*R*)-2-phenyl-2-(3-pyridin-2-ylureido)acetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (74)

The title compound was obtained from commercially available 2-aminopyridine according to the general procedure **D** to afford the title compound in 47.6% yield: mp 155-158 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.86-2.24 (m, 6H), 3.26-3.30 (m, 1H), 3.84-4.02 (m, 1H), 4.87-4.89 (m, 1H), 5.87 (d, *J* = 3.4 Hz, 1H), 6.93-7.06 (m, 3H), 7.22-7.30 (m, 5H), 7.43-7.53 (m, 3H), 7.66-7.84 (m, 2H), 8.30 (brs, 1H), 8.79 (brs, 1H), 10.67 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.61, 29.15, 47.51, 56.57, 60.47, 107.64, 112.51, 117.37, 126.08, 127.66, 128.11, 128.39, 128.45, 129.12, 134.54, 136.82, 138.53, 146.60, 149.87, 152.69, 154.63, 157.78, 170.10, 170.40; LC/MS(ESI) *m/z*: 527.1 $[M + H]^+$, 549.1 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{28}H_{27}N_6O_3S$ $[M + H]^+$ 527.1865, found 527.1858; HPLC t_R = 34.07 min, 99.9%.

(*S*)-1-((*R*)-2-phenyl-2-(3-pyridin-3-ylureido)acetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (75)

The title compound was obtained from commercially available 3-aminopyridine according to the general procedure **D** to afford the title compound in 50.4% yield: mp 192-194 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.68-2.07 (m, 3H), 2.57 (brs, 3H), 3.16-3.21 (m, 1H), 3.86-3.89 (m, 1H), 4.73-4.75 (m, 1H), 5.72 (d, *J* = 3.6 Hz, 1H), 6.70-6.98 (m, 2H), 7.04-7.52 (m, 7H), 7.69-7.78 (m, 3H), 7.93 (br s, 1H), 8.02 (br s, 1H), 8.31 (br s, 1H), 11.82 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.64, 29.11, 47.54, 56.19, 60.77, 107.92, 123.32, 126.01, 126.20, 127.56, 127.99, 128.40, 128.66, 128.98, 134.19, 135.75, 136.24, 139.90, 142.48, 149.78, 154.72, 157.75, 170.34, 171.65; LC/MS(ESI) *m/z*: 527.1 $[M + H]^+$, 549.1 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{28}H_{27}N_6O_3S$ $[M + H]^+$ 527.1865, found 527.1860; HPLC t_R = 28.37 min, 99.3%.

(*S*)-1-((*R*)-2-phenyl-2-(3-pyridin-4-ylureido)acetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (76)

The title compound was obtained from commercially available 4-aminopyridine according to the general procedure **D** to afford the title compound in 29.6% yield: mp 164-166 ℃; ^{1}H NMR (400MHz, $CDCl_3$) δ 1.19-1.34 (m, 2H), 1.59 (brs, 2H), 1.94 (brs, 2H), 2.39-2.46 (m, 2H), 2.89 (br s, 1H), 4.63 (br s, 1H), 5.57 (brs, 1H), 6.90-7.11 (m, 3H), 7.24-7.40 (m, 9H), 7.42-7.61 (m, 2H), 7.69-8.05 (m, 2H); ^{13}C NMR ($CDCl_3$, 100MHz) δ 24.30, 29.39, 47.19, 56.89, 61.30, 108.24, 112.41, 126.23, 127.90, 128.10,

128.67, 128.74, 129.35, 134.29, 135.26, 146.62, 149.20, 149.97, 154.00, 157.78, 169.73, 172.29; LC/MS(ESI) *m/z*: 527.1 $[M + H]^+$, 549.1 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{28}H_{27}N_6O_3S$ $[M + H]^+$ 527.1865, found 527.1861; HPLC t_R = 23.07 min, 98.7%.

(*S*)-1-((*R*)-2-(3-cyclopropylthioureido)-2-phenylacetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (77)

The title compound was obtained from commercially available cyclopropyl isothiocyanate according to the general procedure **B** to afford the title compound in 89% yield: mp 134-137 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 0.48-0.85 (m, 4H), 1.97-2.16 (m, 4H), 2.38-2.42 (m, 2H), 4.01 (t, *J* = 7.5 Hz, 1H), 4.79-4.82 (m, 1H), 6.34 (d, *J* = 3.6 Hz, 1H), 6.89 (br s, 1H), 7.12 (s, 1H), 7.27-7.39 (m, 6H), 7.41-7.62 (m, 2H), 7.73-7.84 (m, 2H), 10.92 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 7.09, 7.28, 23.55, 24.47, 28.27, 47.14, 60.67, 60.92, 107.75, 126.17, 127.85, 128.45, 128.59, 128.92, 129.18, 134.46, 135.38, 150.02, 157.47, 168.98, 170.63, 181.74; LC/MS(ESI) *m/z*: 506.2 $[M + H]^+$, 528.1 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{26}H_{28}N_5O_2S_2$ $[M + H]^+$ 506.1684, found 506.1680; HPLC t_R = 36.25 min, 95.1%.

(*S*)-1-((*R*)-2-(3-cyclopentylthioureido)-2-phenylacetyl)-N-(4-phenylthiazol-2-yl)py

rrolidine-2-carboxamide (78)

The title compound was obtained from commercially available cyclopentyl isothiocyanate according to the general procedure **B** to afford the title compound in 75.3% yield: mp 178-180 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.22-1.54 (m, 6H), 1.80-2.04 (m, 7H), 2.35-2.36 (m, 1H), 3.15-3.20 (m, 1H), 3.95-4.01 (m, 1H), 4.81 (d, *J* = 3.2 Hz, 1H), 6.33 (d, *J* = 3.4 Hz, 1H), 6.65 (brs, 1H), 7.13 (s, 1H), 7.24-7.73 (m, 8H), 7.86 (d, *J* = 3.4 Hz, 2H), 10.78 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 23.48, 24.21, 29.02, 32.47, 32.82, 46.99, 55.75, 60.59, 60.83, 107.55, 126.10, 127.87, 128.14, 128.57, 128.68, 129.18, 134.30, 134.94, 149.96, 157.15, 169.23, 171.88, 180.80; LC/MS(ESI) *m/z*: 534.2 $[M + H]^+$, 556.2 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{28}H_{32}N_5O_2S_2$ $[M + H]^+$ 534.1997, found 534.1991; HPLC t_R = 42.05 min, 98.1%.

(*S*)-1-((*R*)-2-(3-cyclohexylthioureido)-2-phenylacetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (79)

The title compound was obtained from commercially available cyclohexyl isothiocyanate according to the general procedure **B** to afford the title compound in 25.6% yield: mp 154-158 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 0.83-1.60 (m, 6H), 1.84-2.05 (m, 9H), 2.36-2.37 (m, 1H), 3.14-3.19 (m, 1H), 3.99-4.05 (m, 1H), 4.83 (d, *J* = 3.3 Hz, 1H), 6.39 (d, *J* = 3.7 Hz, 1H), 6.54 (brs, 1H), 7.13 (s, 1H), 7.28-7.43 (m,

8H), 7.87 (d, *J* = 3.6 Hz, 2H), 10.80 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 24.09, 24.79, 24.90, 25.11, 29.34, 32.24, 32.64, 46.91, 53.63, 60.41, 60.77, 107.49, 126.08, 127.84, 127.98, 128.56, 128.60, 129.23, 134.28, 134.75, 150.07, 157.14, 169.38, 172.59, 181.33; LC/MS(ESI) *m/z*: 548.1 $[M + H]^+$, 570.1 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{29}H_{34}N_5O_2S_2$ $[M + H]^+$ 548.2154, found 548.2149; HPLC t_R = 43.87 min, 98.0%.

(*S*)-1-((*R*)-2-phenyl-2-(3-phenylthioureido)acetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (80)

The title compound was obtained from commercially available Phenyl isothiocyanate according to the general procedure **B** to afford the title compound in 90.8% yield: mp 150-153 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.84-2.07 (m, 5H), 2.34-2.37 (m, 1H), 3.16-3.24 (m, 1H), 3.97 (t, *J* = 7.5 Hz, 1H), 4.79 (d, *J* = 3.3 Hz, 1H), 6.36 (d, *J* = 3.3 Hz, 1H), 7.01-7.35 (m, 10H), 7.45-7.48 (m, 2H), 7.57-7.66 (m, 1H), 7.75 (d, *J* = 3.3 Hz, 2H), 8.30 (brs, 1H), 10.89 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100MHz) δ 24.32, 28.71, 47.07, 60.83, 60.93, 107.71, 124.30, 126.14, 126.31, 127.76, 128.45, 128.49, 128.86, 129.13, 129.40, 134.29, 134.80, 136.51, 149.98, 157.43, 169.13, 170.76, 179.60; LC/MS(ESI) *m/z*: 542.1 $[M + H]^+$, 564.1 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{29}H_{28}N_5O_2S_2$ $[M + H]^+$ 542.1684, found 542.1680; HPLC t_R = 40.35 min, 96.3%.

(*S*)-1-((*R*)-2-phenyl-2-(3-pyridin-3-ylthioureido)acetyl)-N-(4-phenylthiazol-2-yl)pyrrolidine-2-carboxamide (81)

The title compound was obtained from commercially available 3-pyridyl isothiocyanate according to the general procedure **B** to afford the title compound in 66.1% yield: mp 146-149 ℃; ^{1}H NMR (300MHz, $CDCl_3$) δ 1.84-2.09 (m, 5H), 2.29-2.63 (m, 1H), 3.18-3.24 (m, 1H), 4.04 (t, *J* = 7.2 Hz, 1H), 4.77 (d, *J* = 2.7 Hz, 1H), 6.35 (d, *J* = 3.3 Hz, 1H), 6.59-6.64 (m, 1H), 7.07 (s, 1H), 7.15-7.42 (m, 6H), 7.51 (t, *J* = 9.0 Hz, 1H), 7.70 (d, *J* = 3.9 Hz, 1H), 8.12 (d, *J* = 2.4 Hz, 1H), 8.23 (d, *J* = 3.2 Hz, 1H), 8.60 (s, 1H), 8.67 (s, 1H), 10.67 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100MHz) δ 24.27, 29.16, 47.10, 60.84, 60.98, 107.81, 122.97, 126.04, 127.93, 128.19, 128.68, 128.97, 129.35, 131.91, 133.95, 134.10, 134.99, 145.66, 146.01, 149.80, 157.11, 169.05, 171.60, 181.50; LC/MS(ESI) *m/z*: 543.1 $[M + H]^+$, 565.1 $[M + Na]^+$; HRMS (*m/z*): calcd for $C_{28}H_{27}N_6O_2S_2$ $[M + H]^+$ 543.1637, found 543.1635; HPLC t_R = 33.57 min, 98.0%.

Biology

Huh-7 cells containing HCV subgenomic replicons (Ava5) were provided by Apath, LLC (St. Louis, MO). The reporter-based HCV subgenomic replicon,

Ava5-EG(D4AB)SEAP, has previously been described.[11] Cell culture reagents were obtained from Life Technologies (Gaithersburg, MD). Cell viability was determined by the MTS assay that was essentially as described.

Subgenomic HCV inhibitory assay

In 96-well plates, Ava5-EG(D4AB)SEAP cells were seeded at a density of 7×10^3 cells per well. After incubation at 37 ^{o}C for 1 day, cells were treated with various drugs at final 10 μM. Two days later, culture medium was replaced with fresh phenol red-free DMEM/10% FBS containing the same concentration of drugs and cells were incubated for one more day. Culture supernatants were collected from each well and SEAP activities were measured using Phospha-Light assay kit (Tropix, Foster City, CA), according to the manufacturer's instruction.

Pharmacokinetic study

Pharmacokinetic analysis in Sprague-Dawley rats

The SD rats for the pharmacokinetic study were obtained from BioLASCO Taiwan Co., Ltd. (Ilan, Taiwan, ROC), and housed in the animal facility at the National Health Research Institutes, Taiwan, ROC. The animal studies were performed according to committee approved procedures. Male rats, each weighing 330–380 g

(9–10 weeks old), were quarantined for 1 week before use. The animals were surgically implanted with a jugular-vein cannula 1 day before treatment, and were fasted before treatment. The compound was given to the rats (n = 3) as an intravenous (1 mg/kg) or oral (5 mg/kg) dose prepared in a mixture of dosing vehicles. The volume of the dosing solution given was adjusted according to the body weight recorded before the drug was administered. At 0 (immediately before dosing), 2, 5 (intravenous only), 15 and 30 min and 1, 2, 4, 6, 8 and 24 h after dosing, a blood sample (~150 mL) was taken from each animal via the jugular-vein cannula and stored in ice (0–4 ^{o}C). The processing of the plasma and analysis by high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) was carried out as described.[13] The plasma concentration data were analyzed with a standard non-compartmental method.

ASSOCIATED CONTENT

Supporting Information

^{1}H NMR and ESMS spectra for all target compounds, HPLC purity, and elemental analysis data for tested compounds (PDF)

Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

APCI, atmospheric pressure chemical ionization; CDI, N,N′-carbonyldiimidazole; CHC, chronic hepatitis C; CL, clearance; Cmax, maximum concentration; DAAs, direct-acting antivirals; DIPEA, N, N-diisopropylethylamine; DMEM, Dulbecco's

modified Eagle's medium; EDC, ethyl-(N',N'-dimethylamino)propylcarbodiimide hydrochloride; FBS, Fetal Bovine Serum; HATU, O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate; HOBt.H2O, 1-hydroxybenzotriazole monohydrate; Huh-7, human hepatoma cell line; NS5A, non-structural protein 5A; NS3/4A, non-structural protein 3/4A; NS5B, non-structural protein 5B; SD rat, Sprague Dawley® rat; SEAP, Secretory Alkaline Phosphatase; SVR, sustained virological response; TBTU, O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate; Tmax, time to reach Cmax; Vss, volume of distribution at steady state.

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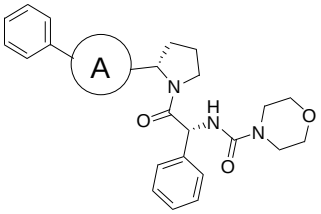
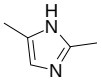
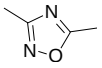
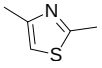
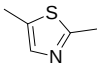
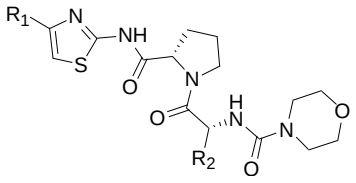
Table 1. Cell-based HCV inhibitory activity and cytotoxicity for morpholino urea derivatives **6, 12, 18** and **22**



Compound	A ring	EC_{50} (nM)[a]	CC_{50} (μM)[a]
6		98	>50
12		>50000	>50
18		9440	>50
22		2970	>50

[a] Mean of triplicate well values. All experiments were performed at least twice. EC_{50} stands for 50% effective concentration; CC_{50} stands for 50% cytotoxic concentration. The genotype 1b subgenomic replicon cells were applied to evaluate the inhibitory activity of the compounds.

Table 2. Cell-based HCV inhibitory activity and cytotoxicity for a series of morpholino urea derivatives **42-51**

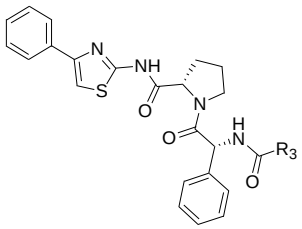


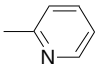
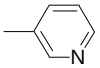
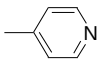
Compound	R_1	R_2	EC_{50} (nM)[a]	CC_{50} (μM)[a]
18	-	-	9440	>50
42	Ph	Ph	0.92	>50
43	H	Ph	9650	>50
44	CH_3	Ph	400	>50
45	*t*-Bu	Ph	21	>50
46	Cyclohexyl	Ph	6	>50
47	Ph	CH_3	1310	>50
48	Ph	Et	1630	>50
49	Ph	*n*-Pr	270	>50
50	Ph	*i*-Pr	990	>50
51	Ph	*t*-Bu	7060	>50

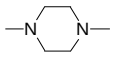
[a] Mean of triplicate well values. All experiments were performed at least twice. EC_{50} stands for 50% effective concentration; CC_{50} stands for 50% cytotoxic concentration.

The genotype 1b subgenomic replicon cells were applied to evaluate the inhibitory activity of the compounds.

Table 3. Cell-based HCV inhibitory activity and cytotoxicity for a series of amide derivatives **52-68**

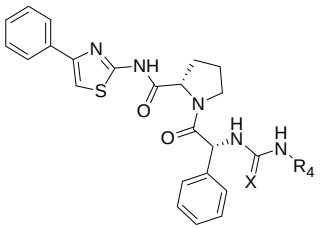


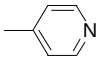
Compound	R_3	EC_{50} (nM)[a]	CC_{50} (μM)[a]
52	CH_3	16	>50
53	Et	23	>50
54	*n*-Pr	15	>50
55	*i*-Pr	36	>50
56	*t*-Bu	86	>50
57		4.6	>50
58		13	>50
59		14	>50
60		15	>50
61		22	>50
62		13	>50
63		3.8	>50
64		11	>50

65		18	>50
66		3.1	>50
67		3.1	>50
68		0.85	>50
42		0.92	>50

[a] Mean of triplicate well values. All experiments were performed at least twice. EC_{50} stands for 50% effective concentration; CC_{50} stands for 50% cytotoxic concentration. The genotype 1b subgenomic replicon cells were applied to evaluate the inhibitory activity of the compounds.

Table 4. Cell-based HCV inhibitory activity and cytotoxicity for a series of urea and thiourea derivatives **69-81**



Compound	X	R_4	EC_{50} (nM)[a]	CC_{50} (μM)[a]
57	-	-	4.6	>50
69	O		3.5	>50
70	O		13	>50
71	O		3.3	>50
72	O		3.8	>50
73	O		2.3	>50
74	O		30	>50
75	O		1.9	>50
76	O		22	>50
77	S		34	>50
78	S		5.2	>50
79	S		3.2	>50

80	S		46	>50
81	S		23	>50

[a] Mean of triplicate well values. All experiments were performed at least twice. EC_{50} stands for 50% effective concentration; CC_{50} stands for 50% cytotoxic concentration. The genotype 1b subgenomic replicon cells were applied to evaluate the inhibitory activity of the compounds.

Table 5. Pharmacokinetic parameters of **42, 57, 60, 63,** and **66** following intravenous administration[a] to rats[b]

Parameter	Compound				
	42	**57**	**60**	**63**	**66**
CL (mL/min/kg)	38.4 ± 1.5	27.1 ± 4.6	44.8 ± 3.2	62.2 ± 8.9	55.1 ± 4.2
V_{ss} (L/kg)	1.04 ± 0.02	1.32 ± 0.04	4.2 ± 0.9	1.8 ± 0.1	1.1 ± 0.1
$t_{1/2}$ (h)	0.46 ± 0.03	1.3 ± 0.3	2.3 ± 0.3	0.5 ± 0.1	0.5 ± 0.1
AUC (ng/mL×h)	436 ± 17	635 ± 116	367 ± 27	295.6 ± 33	311.4 ± 23

[a] Compound was formulated as a solution in DMA/propylene glycol (20/80, v/v) and administered at 1 mg/kg. [b] n = 3

Table 6. Pharmacokinetic parameters of **42, 57, 60, 63,** and **66** following oral administration[a] to rats[b]

Parameter	Compound				
	42	**57**	**60**	**63**	**66**
C_{max} (ng/mL)	247.3 ± 93.6	260.3 ± 75.3	109.3 ± 56.0	58.3 ± 30.7	63.9 ± 35.7
T_{max} (h)	0.8 ± 0.3	0.8 ± 0.4	2.3 ± 3.3	0.7 ± 0.3	2.8 ± 2.9
$t_{1/2}$ (h)	1.6 ± 0.3	2.8 ± 0.2	2.7 ± 0.1	4.5 ± 2.8	2.4 ± 1.3
AUC (ng/mL×h)	404 ± 76	1436 ± 293	588 ± 142	125 ± 62	288 ± 122
bioavailability (%)	18.7	45	31	10.1	20.4

[a] Compound was formulated as a solution in DMA/propylene glycol (20/80, v/v) and administered at 5 mg/kg. [b] n = 3.

Figure Legends

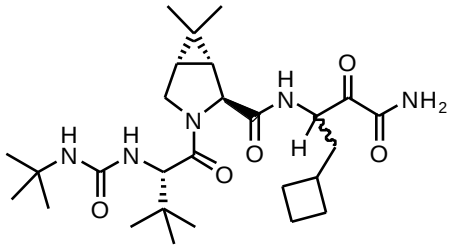
Figure 1. First generation direct-acting antivirals: Boceprevir and Telaprevir.

Figure 2. Second generation direct-acting antivirals: Simeprevir, Paritaprevir, Ledipasvir, Ombitasvir, Daclatasvir, Sofosbuvir and Dasabuvir

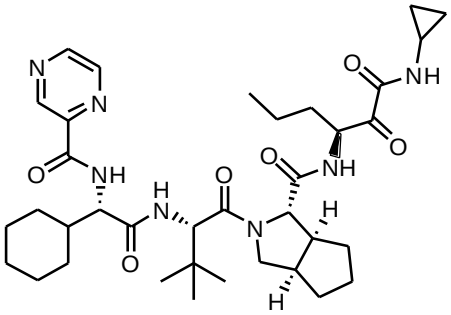
Figure 3. HCV NS5A inhibitors: compound **6**, **12**, **18**, and **22**.

Figure 4. Lead Optimization.

Figure 1

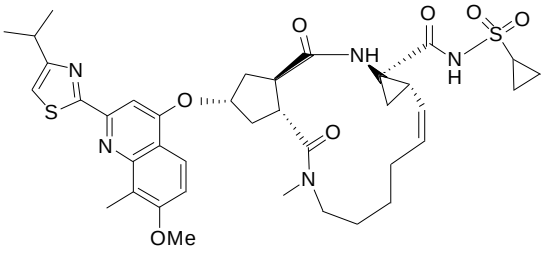


Boceprevir

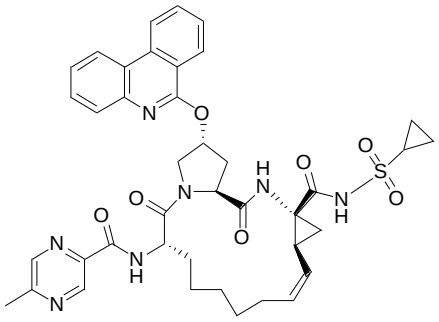


Telaprevir

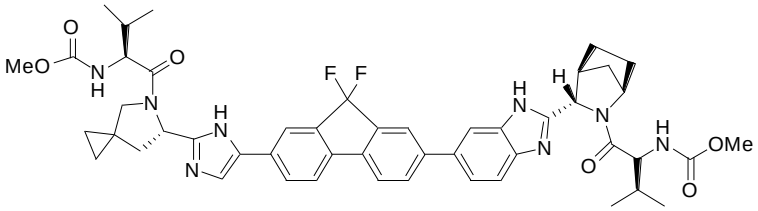
Figure 2



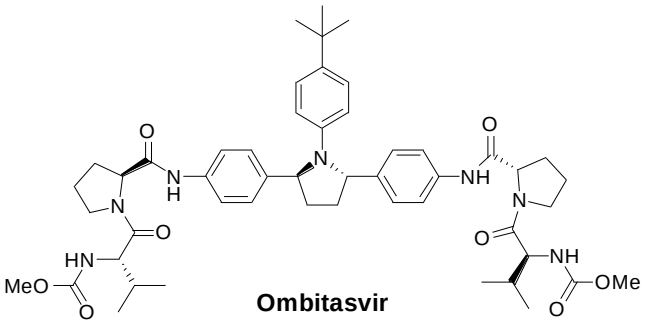
Simeprevir



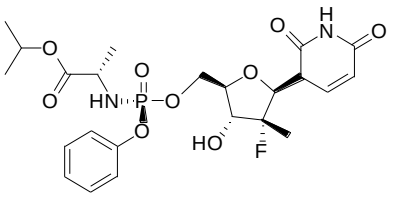
Paritaprevir



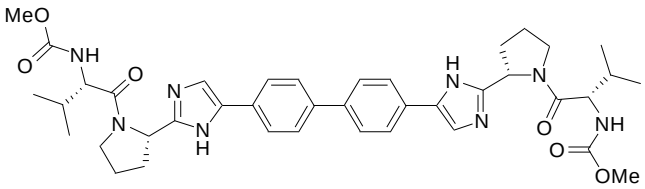
Ledipasvir



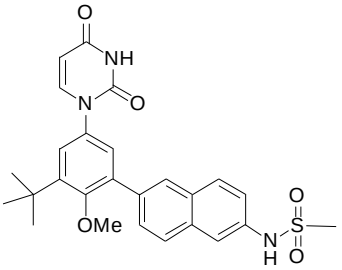
Ombitasvir



Sofosbuvir

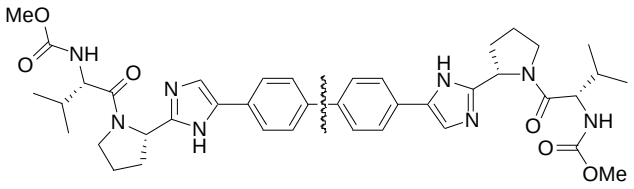


Daclatasvir

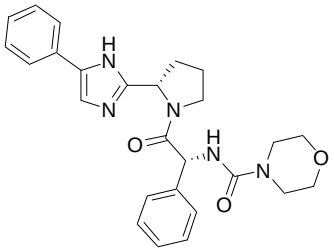


Dasabuvir

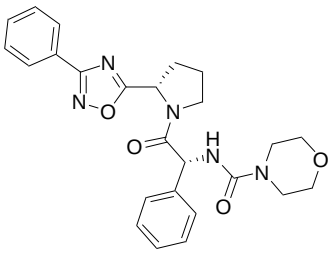
Figure 3



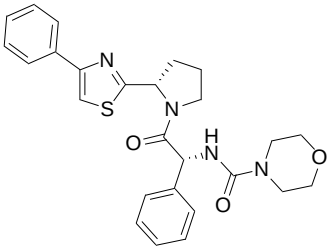
Daclatasvir



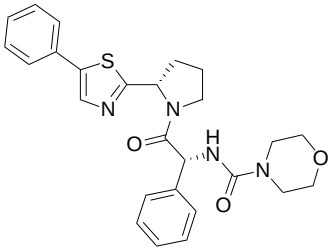
6
EC_{50} = 98 nM



12
EC_{50} > 50000 nM

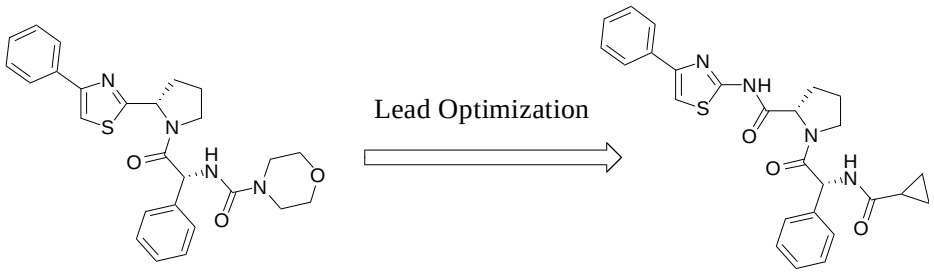


18
EC_{50} = 9440 nM



22
EC_{50} = 2970 nM

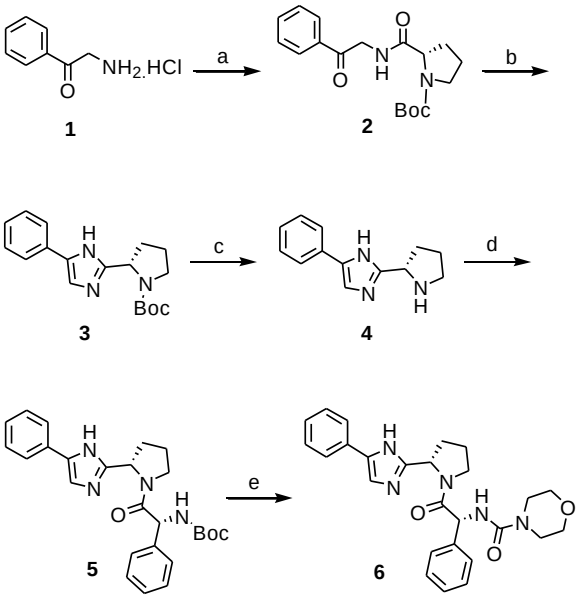
Figure 4



18
EC_{50} = 9440 nM

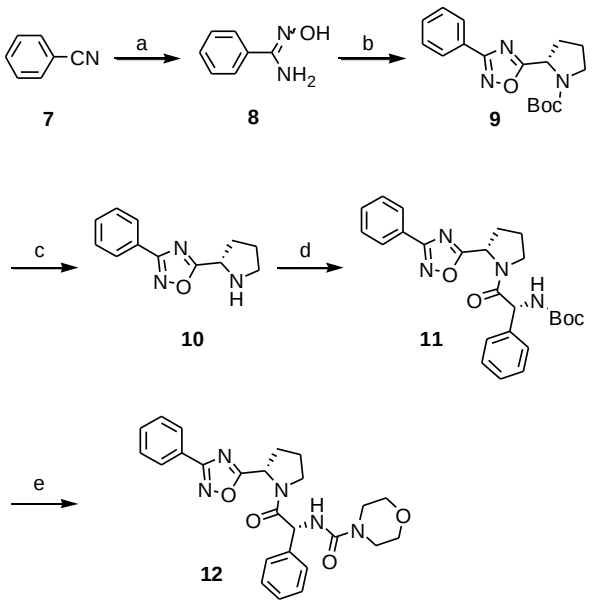
57
EC_{50} = 4.6 nM

Scheme 1[a]



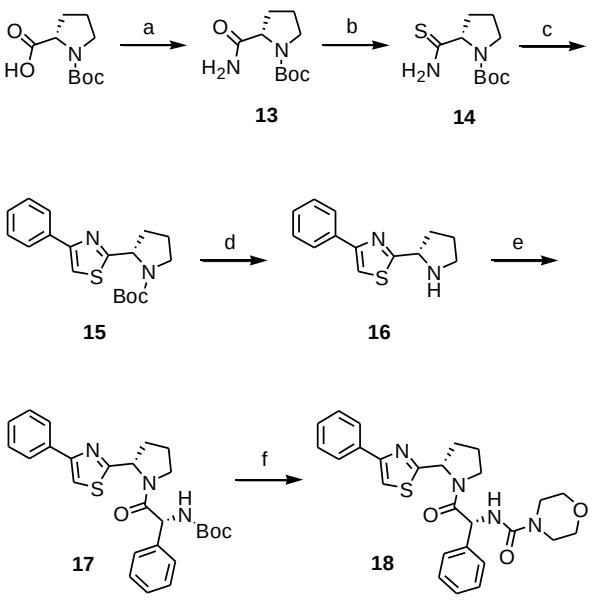
[a] Reagents and conditions: (a) N-Boc-*L*-proline, HOBt·H_2O, EDC, Et_3N, CH_2Cl_2, RT, 18 h, 40%; (b) NH_4OAc, AcOH, xylene, 160 ℃, 3 h, 74%; (c) TFA, CH_2Cl_2, RT, 1 h, 82%; (d) N-Boc-*D*-phenylglycine, HOBt·H_2O, EDC, CH_2Cl_2, RT, 18 h, 67%; (e) (i) TFA, CH_2Cl_2, RT, 1 h; (ii) 4-morpholinecarbonyl chloride, Et_3N, CH_2Cl_2, 0 ℃, 10 min, 31%.

Scheme 2[a]



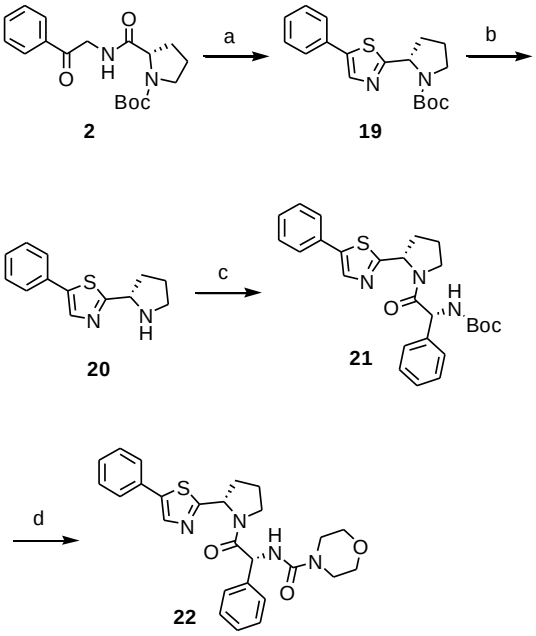
[a] Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, DIPEA, EtOH, 90 ℃, 5 h, 85%; (b) N-Boc-*L*-proline, TBTU, $HOBt{\cdot}H_2O$, DIPEA, DMF, 110 ℃, 3 h, 53%; (c) TFA, CH_2Cl_2, RT, 1 h, 90%; (d) N-Boc-*D*-phenylglycine, $HOBt{\cdot}H_2O$, EDC, CH_2Cl_2, RT, 18 h, 89%; (e) (i) TFA, CH_2Cl_2, RT, 1 h; (ii) 4-morpholinecarbonyl chloride, Et_3N, CH_2Cl_2, 0 ℃, 10 min, 77%.

Scheme 3[a]



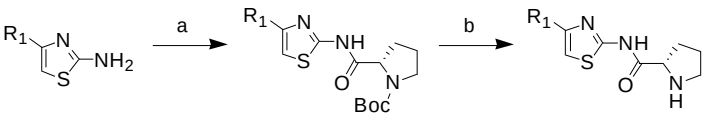
[a] Reagents and conditions: (a) $(Boc)_2O$, $(NH_4)_2CO_3$, pyridine, 1,4-dioxane, RT, 18 h, 95%; (b) Lawesson's reagent, THF, 70 ℃, 8 h, 89%; (c) phenacyl bromide, EtOH, reflux, 1 h, 74%; (d) TFA, CH_2Cl_2, RT, 1 h, 82%; (e) N-Boc-*D*-phenylglycine, $HOBt·H_2O$, EDC, CH_2Cl_2, RT, 18 h, 71%; (f) (i) TFA, CH_2Cl_2, RT, 1 h; (ii) 4-morpholinecarbonyl chloride, Et_3N, CH_2Cl_2, 0 ℃, 10 min, 58%.

Scheme 4[a]

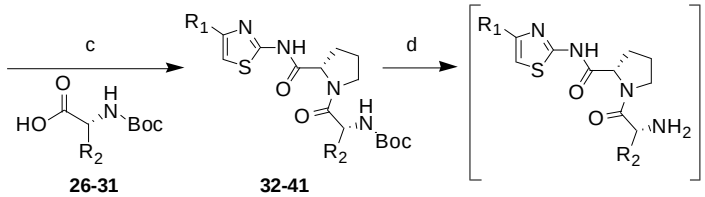


[a] Reagents and conditions: (a) Lawesson's reagent, THF, reflux, 6 h, 81%; (b) TFA, CH_2Cl_2, RT, 1 h, 88%; (c) N-Boc-*D*-phenylglycine, HOBt·H_2O, EDC, CH_2Cl_2, RT, 18 h, 72%; (d) (i) TFA, CH_2Cl_2, RT, 1 h; (ii) 4-morpholinecarbonyl chloride, Et_3N, CH_2Cl_2, 0 ℃, 10 min, 56%.

Scheme 5[a]



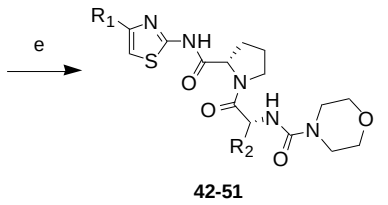
23a R_1=Ph **23d** R_1=*t*-Bu
23b R_1=H **23e** R_1=cyclohexy
23c R_1=CH_3



26. R_2=Ph
27. R_2=CH_3
28. R_2=Et
29. R_2=*n*-Pr
30. R_2=*i*-Pr
31. R_2=*t*-Bu

32. R_1=Ph, R_2=Ph
33. R_1=H, R_2=Ph
34. R_1=CH_3, R_2=Ph
35. R_1=*t*-Bu, R_2=Ph
36. R_1=Cyclohexyl, R_2=Ph

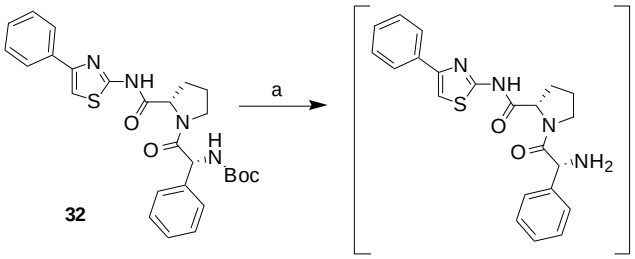
37. R_1=Ph, R_2=CH_3
38. R_1=Ph, R_2=Et
39. R_1=Ph, R_2=*n*-Pr
40. R_1=Ph, R_2=*i*-Pr
41. R_1=Ph, R_2=*t*-Bu



42. R_1=Ph, R_2=Ph
43. R_1=H, R_2=Ph
44. R_1=CH_3, R_2=Ph
45. R_1=*t*-Bu, R_2=Ph
46. R_1=Cyclohexyl, R_2=Ph
47. R_1=Ph, R_2=CH_3
48. R_1=Ph, R_2=Et
49. R_1=Ph, R_2=*n*-Pr
50. R_1=Ph, R_2=*i*-Pr
51. R_1=Ph, R_2=*t*-Bu

[a] Reagents and conditions: (a) N-Boc-*L*-proline, HATU, DIPEA, DMF, 50 ℃, 6 h; (b) TFA, CH_2Cl_2, RT, 1 h; (c) **26-31**, HOBt·H_2O, EDC, CH_2Cl_2, RT, 18 h, 76-93%; (d) TFA, CH_2Cl_2, RT, 1 h; (e) 4-morpholinecarbonyl chloride, Et_3N, CH_2Cl_2, 0 ℃→RT, 18 h, 41-89%.

Scheme 6[a]



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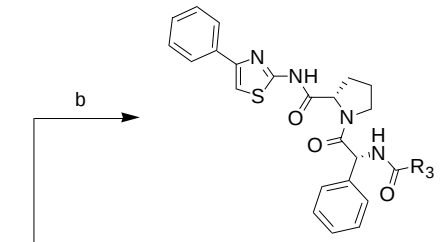
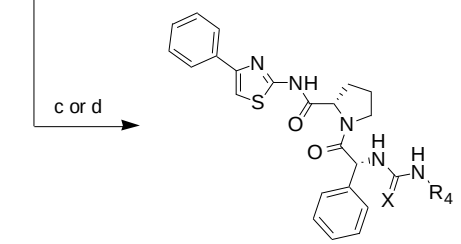


52. R_3=CH_3
53. R_3=Et
54. R_3=n-Pr
55. R_3=i-Pr
56. R_3=t-Bu
57. R_3=cyclopropyl
58. R_3=cyclopentyl
59. R_3=cyclohexyl
60. R_3=Ph
61. R_3=2-pyridyl
62. R_3=3-pyridyl
63. R_3=4-pyridyl
64. R_3=dimethylamine
65. R_3=diethylamine
66. R_3=pyrrolidine
67. R_3=piperidine
68. R_3=4-methyl-piperidine



69. X=O, R_4=cyclopropyl
70. X=O, R_4=cyclopentyl
71. X=O, R_4=cyclohexyl
72. X=O, R_4=cycloheptyl
73. X=O, R_4=Ph
74. X=O, R_4=2-pyridyl
75. X=O, R_4=3-pyridyl
76. X=O, R_4=4-pyridyl
77. X=S, R_4=cyclopropyl
78. X=S, R_4=cyclopentyl
79. X=S, R_4=cyclohexyl
80. X=S, R_4=Ph
81. X=S, R_4=3-pyridyl

[a] Reagents and conditions: (a) TFA, CH_2Cl_2, RT, 1 h; (b) R_3COCl, Et_3N, CH_2Cl_2, RT, 18 h, 21-90%; (c) R_4NCO or R_4NCS, Et_3N, CH_2Cl_2, RT, 18 h, 26-91%; (d) R_4NH_2, CDI or triphosgene, Et_3N, CH_2Cl_2, RT, 18 h, 26-68%.

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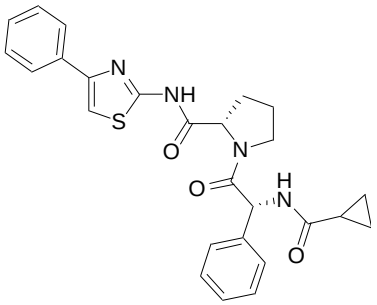

57

EC_{50} = 4.6 nM

A pyrrolidine amide analogue **57** was identified as a preclinical candidate for the treatment of HCV infection.