Comprehensive Synthesis of Amino Acid-Derived Thiazole Peptidomimetic Analogues to Understand Enigmatic Drug/Substrate-Binding Site of P-glycoprotein

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Abstract

A novel set of 64 analogues based on our lead compound **1** was designed and synthesized with an initial objective of understanding the structural requirements of ligands binding to a highly perplexing substrate-binding site of P-gp and their effect on modulating the ATPase function of the efflux pump. Compound **1**, a stimulator of P-gp ATPase activity, was transformed to ATPase inhibitory compounds **39**, **53** and **109**. The ATPase inhibition by these compounds was predominantly contributed by the presence of a cyclohexyl group in lieu of the 2-aminobenzophenone moiety of **1**. The 4,4-difluorocyclohexyl analogues, **53** and **109**, inhibited the photolabeling by [^{125}I]-IAAP, with IC$_{50}$ values of 0.1 and 0.76 μM, respectively. Selected compounds were shown to reverse paclitaxel resistance in HEK293 cells overexpressing P-gp and were selective toward P-gp over CYP3A4. Induced-fit docking highlighted a plausible binding pattern of inhibitory compounds in the putative-binding pocket of P-gp. The current study underscores the stringent requirement by P-gp to bind to chemically similar molecules.

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INTRODUCTION

Failure of chemotherapy to treat various types of cancer is often linked to the phenomenon of multidrug resistance (MDR), wherein the cancer becomes resistant to structurally and functionally diverse classes of drugs.[1] Amongst the several mechanisms responsible for MDR phenotype, such as decline in drug uptake, alterations in cell cycle progression and drug metabolism, augmented DNA damage repair, and decreased apoptosis, the increase in active efflux of a drug by ATP-binding cassette (ABC) transporters appears as a major contributing factor for acquired resistance.[2] Amongst the superfamily of ABC transporters which cause MDR, P-glycoprotein (P-gp/ABCB1/MDR1) has been considered the most prevalent.[3]

Human P-gp is a 1280 amino acid long membrane protein folded into two similar halves. Each half consist of a nucleotide-binding domain (NBD) and a transmembrane domain (TMD). Each TMD can further be divided into six transmembrane (TM) helices. P-gp, in normal tissues, functions to protect cells from xenobiotic insults. However, MDR cells develop an overexpression of P-gp, which leads to the efflux of amphipathic substrate chemotherapeutic drugs out of the cell, limiting the ability of these drugs to kill the cancer cells.[4] P-gp has a broad substrate selectivity ranging from small molecules to macrocycles with diverse chemical structures and high lipophilicity.[5] The substrate/drug-binding pocket, located in the TMDs, can accommodate two to three molecules simultaneously.[6, 7] Moreover, the same substrate can bind to multiple regions within the hydrophobic binding cavity of P-gp.[8] Powered with the energy from ATP binding/hydrolysis process at the NBDs, P-gp operates via an alternating access mechanism for the efflux of substrates. According to this model, a conformational change occurs upon binding of the drug to P-gp in an inward open state that brings the NBDs together forming an inward closed conformation. Drug translocation is induced during the closed conformation of P-gp, followed by the release of drug on the extracellular side where the efflux pump acquires an outward open conformation. Subsequently, ATP hydrolysis at the NBD facilitates P-gp to attain its original drug-binding competent inward open state conformation.[9] Recent cryo-EM analyses of the catalytic cycle of P-gp supports these open and closed conformational states of P-gp.[10]

Extensive research has been documented in the development of MDR reversal compounds, leading to three generations of P-gp modulators over the years;[11] however, these modulators have proved ineffective in clinical trials due to wide range of reasons. These failures were partly ascribed to insufficient bioavailability at tumor sites, non-specific toxicities, indiscriminate inhibition of multiple ABC transporters, including P-gp in the intestine, liver, kidney, and the blood-brain barrier as well as one of the major drug metabolizing enzymes, CYP3A4.[12–14] Moreover, certain concerns have been raised regarding the relevant selection of the patient population in clinical investigation of the P-gp modulators. The criteria for patient selection should consider the causal factor for MDR, including the specifically overexpressed ABC transporter for which the modulator was developed. Additionally, single nucleotide polymorphisms (SNPs) in these efflux pumps should be determined during patient selection process.[15] Accompanying conditions in patients such as inflammatory diseases may also have significant effect on the treatment outcomes.[16, 17] Despite these

issues, the key role played by these efflux transporters in MDR development warrants further research for ultimate clinical success.

Development of new classes of P-gp inhibitors, devoid of the limitations associated with previously disclosed compounds, is a significant need in oncologic settings for the treatment of resistant cancers. Consequently, new approaches are devised to develop MDR reversal compounds with high P-gp affinity and selectivity.[18] Using one such approach, we incorporated chemical moieties that are often present in P-gp modulators to develop a lead compound, (S)-N-(2-benzoylphenyl)-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carboxamide (TTT-28; **1**), which features 3,4,5-trimethoxybenzoyl and 2-aminobenzophenone moieties at the N- and C-termini, respectively, of the (S)-valine-derived thiazole scaffold.[19] Compound **1** increased intracellular paclitaxel concentration in SW620/Ad300 drug-resistant cell lines and demonstrated a significant reversal of resistance to paclitaxel, doxorubicin and vincristine at a concentration of 10 μM in vitro. It also had preferential selectivity toward P-gp than other tested ABC transporters. Moreover, it has shown desirable profile in vivo as evidenced from reduced tumor volume and tumor weight with no apparent side-effects in mice.[20] Additionally, biochemical assays such as [^{125}I]-IAAP and ATP hydrolysis indicated compound **1** to interact with the TMDs of P-gp at the drug-binding site with a potent stimulation of ATPase catalytic activity.[19]

One of the approaches to determine the direct interaction of test compounds with P-gp is to measure their effect on basal ATPase activity. P-gp utilizes ATP hydrolysis as a mechanism to energize its efflux function.[21, 22] The membrane vesicles prepared from cells expressing P-gp exhibit basal ATPase activity, which may have resulted from either endogenous substrates or uncoupled ATPase activity.[23, 24] Compounds which are P-gp substrates and likely to be transported usually are stimulators of basal P-gp ATPase activity. Alternatively, these compounds could competitively inhibit transport of other drug substrates. Inhibitors of basal and substrate-stimulated ATPase activity tend to be incompetent for transport by P-gp, yet capable of averting the transport of other substrate drugs. Moreover, inhibitors of ATP hydrolysis by P-gp are shown to act for a longer duration of time in vitro than their stimulatory counterparts to reverse the multi-drug resistance.[25–27] Compounds lacking interactions with P-gp are devoid of both stimulatory and inhibitory effects on basal ATPase activity. The affinity and/or the reversing ability of compounds, within the same class of ATPase modulators, can be better correlated with the concentrations required for half maximal stimulation (EC$_{50}$) or inhibition (IC$_{50}$) of the P-gp ATPase activity than their extent of stimulation or inhibition.[23] Most P-gp modulators stimulate ATPase activity, except for a few clinically tested inhibitors such as tariquidar, elacridar and zosuquidar.[28, 29] These clinical candidates potently inhibited ATPase activity, which is an indication of their high binding affinity toward P-gp. In addition, site-directed mutagenesis experiments have shown that the loss of ATPase inhibitory activity for these clinical candidates also resulted in loss of their ability to reverse efflux of a fluorescent substrate (NBD-cyclosporine A) by P-gp. This correlation implies the significance of ATPase inhibition for efficient reversal of efflux function.[29] The absence of high resolution human P-gp crystal structure and known promiscuity of the drug-binding site i.e., the presence of multiple ligand-binding pockets,

poses significant difficulties in elucidating common chemical pharmacophore/groups for efficient binding. With this backdrop, we pursued a two-pronged approach that involved: 1) transformation of an ATPase stimulator **1** into a compound with inhibitory action on ATPase activity and 2) probing the transport substrate-binding sites of P-gp through a multitude of analogue syntheses. Implementation of these approaches led to medicinal chemistry optimization program that entailed modifications at the: 1) *C*-terminus, 2) *N*-terminus and 3) central region (α-carbon and carboxamide groups) of the lead compound **1** (Figure 1).

Published data on P-gp modulators has firmly established how the substrate-binding region of P-gp distinguishes various structural classes of compounds on the basis of varied affinity. [5, 30] However, the present study reveals that moderate structural changes, including strictly classical and non-classical isosteric modifications in a particular series of compounds, can impact P-gp function to produce no effect, and induce stimulation or inhibition of ATPase activity. Therefore, structure-activity relationship (SAR) investigations pertaining to our previously published lead stimulator (**1**) of ATPase function of P-gp led to the comprehensive synthesis of 64 target compounds with varying impact on ATPase activity. This report highlights the rigid requirement of P-gp relevant to binding to chemical analogues belonging to the same chemical series. Moreover, this study also provides an insight for surmounting the P-gp efflux problem associated with the substrate behavior of many drugs and clinical development candidates by conducting subtle structural modifications in the lead candidate structures. According to February 2012 FDA guidance for industry, all investigational drugs should be tested in vitro to determine whether they are substrates of ABC transporters. Consequently, our SAR study may provide tools for converting a P-gp substrate structure into a compound with no affinity toward P-gp. Further induced-fit docking of representative ATPase inhibitory target compounds was conducted within the drug/substrate-binding pocket of homology modeled structure of human P-gp to analyze the observed SAR trends.

RESULTS AND DISCUSSION

Chemistry

Altogether, a set of 64 (*S*)-amino acid-derived thiazole target compounds were synthesized to assess their interactions with P-gp using ATPase activity assay. The SAR study was instigated based on our lead compound **1**, which is a (*S*)-valine thiazole-derived P-gp ATPase stimulator. Compounds **2**[31] and **6**[19] required for the synthesis of modifications of **1** were synthesized as per our previous reports. Scheme 1 shows the synthesis of target compounds **5** and **7**. The swapped analogue **5** was obtained over three steps: i) coupling of the acid **2** with the 3,4,5-trimethoxyaniline using HOBt, HCTU and DIEA; ii) Boc deprotection of the coupled product **3** using TFA and iii) subsequent coupling of generated free amine **4** with the 2-benzoylbenzoic acid. Compound **7** was synthesized by coupling the acid **6** with the 3-aminobenzophenone in the presence of HOBt, HCTU and DIEA.

Scheme 2 represents the synthesis of key intermediates required for coupling reactions to obtain various *N*- and *C*-terminal substitutions of (*S*)-valine-derived thiazole scaffold. Anthranilic acid was converted to an amide **8** by first activating the carboxyl group with the

thionyl chloride and subsequent treatment with aniline.[32] 2-Nitroaniline in pyridine was stirred with the benzoyl chloride and subsequent palladium-catalyzed hydrogenation of the nitro group gave the reverse amide **9**.[33] In the past, we observed low yields for coupling reaction with the 2-aminobenzophenone, which may have resulted from the electron withdrawing nature of the benzoyl group.[20] To solve this problem, we reduced the carbonyl group to produce corresponding benzhydrol intermediates, **10** and **11**, with desired nucleophilicity of the 2-amino group to facilitate amide bond formation during the coupling reactions, using previously reported procedure.[20] Next, 3-aminobenzoic acid was protected by reaction with Boc-anhydride in the presence of triethylamine to furnish **12**.[34] The amino substituted benzoic acids were converted to corresponding azido substituted benzoic acids (**13–15**) using diazotization reaction conditions followed by sodium azide treatment.[35] The 1-benzylpiperidin-4-amine, **17**, was prepared over two steps: i) benzylation of the piperidine ring nitrogen of a *tert*-butyl piperidin-4-yl-carbamate to provide **16** and ii) Boc deprotection.[36]

The synthesis of isosteric analogues of benzophenone is depicted in Scheme 3. The trimethoxybenzoyl-containing acid **6** was reacted with several synthesized as well as commercially available amines in the presence of HOBt, HCTU and DIEA to obtain final test compounds **18–21**.

The benzophenone analogues with *N*- and *C*-termini substitutions were synthesized according to Scheme 4. Acid **2** was reacted with amino benzhydrol derivatives **10** and **11** to provide coupled products **22** and **23**, respectively, which were then subjected to Dess-Martin periodinane oxidation to produce **24** and **25** and subsequent Boc removal provided **26** and **27**. Amine **27** was reacted with 3,4,5-trimethoxybenzoyl chloride furnishing the 4′-bromobenzophenone derivative **28**. The 4′-bromo was then converted to 4′-azido derivative **29** using sodium azide in the presence of proline and copper iodide.[37] Compound **31** was prepared by coupling **26** with acid intermediate **12** and subsequent Boc group removal. The *N*-terminal azide compounds **32–34** and **35** were synthesized starting from amines **26** and **27**, respectively, and reacting with appropriate azide intermediates **13–15**.

Scheme 5 illustrate the synthesis of alicyclic analogues **36–40**. The acid **6** was coupled with appropriate amines in the presence of HOBt, HCTU and DIEA coupling reagents.

The cyclohexyl and related analogues were synthesized according to Scheme 6. First, commercially available Boc-protected amines were coupled with the acid **6** to obtain compounds **41**, **45**, **47**, and **49** followed by Boc deprotection to yield free amine derivatives **42**, **46**, **48**, and **50**. Compounds **43**, **44**, and **51–67** were prepared by reacting the acid **6** with various synthesized and commercially available amines.

Variedly substituted *N*-terminus analogues were prepared according to Scheme 7. The acid **2** was coupled with the 4,4-difluorocyclohexylamine and then Boc-deprotected to the amine **77**. This amine was then coupled with commercially available carboxylic acids or acid chlorides providing compounds **69–76** and **78–80**.

The thioamide derivatives were synthesized by reacting **39** or **53** independently, with Lawesson's reagent as shown in Scheme 8. Both di- and mono-thioamide derivatives were obtained and characterized by ^{1}H-NMR and LCMS analyses. Compounds **82** and **84** were designated as thiazole-4-thioamides based on the 'NH' chemical shift of the thioamide. Comparison of ^{1}H-NMR spectrums of **53** and **77** allowed us to assign cyclohexyl amide peak as a doublet at ~8 ppm, and hence, the trimethoxybenzoyl amide 'NH' can be assigned as doublet at ~9 ppm (Supporting Information Figure S1). For mono-thioamide **84**, a doublet at 8.9 ppm suggests preservation of trimethoxybenzoyl amide oxygen and the absence of a doublet at ~8 ppm with appearance of a doublet at 10 ppm indicated occurrence of a thioamide conversion near the 4,4-difluorocyclohexyl ring. Such chemical shift trend is also applicable to compounds **39** and **82**. Moreover, ^{1}H-NMR spectrums of **81** and **83** showed chemical shifts of both the doublets at around ~9.8 and ~10.6 ppm.

Valine residue in **53** was replaced with several amino acids as shown in Scheme 9. The thiazole rings were constructed starting from the amino protected (*S*)-amino acids by first converting these to respective amides **85–89**, further to thioamides **90–94**[38] and subsequently, to *bis*-protected thiazoles **95–99**.[39] The thiazoles **95–99** were synthesized employing a procedure where calcium carbonate was used to neutralize in situ generated hydrobromic acid.[39] This procedure was opted against cumbersome three step method used previously for (*S*)-valine-derived thiazole construction to improve yields, simplify purification process and achieve rapid reaction output. However, the calcium carbonate method led to partial racemization at the chiral center for thiazoles **96–99**. This was inferred based on the smaller optical rotation values for compounds **96** and **97** as compared to those reported by Bredenkamp and colleagues.[40] The racemization at the α-carbon may have occurred via acid catalyzed imine-enamine transition states during the final aromatization step.[41–43] Deprotection using TFA for thiazoles **95–98** or 5% piperidine in DMF[44] for Fmoc-proline derivative **99** yielded respective amino derivatives. Further commercially available ethyl 2-amino-thiazole-4-carboxylate and the synthesized amines were each reacted with 3,4,5-trimethoxybenzoyl chloride to furnish **100** and **102–106**. Respective ethyl esters were then hydrolyzed and coupled with the 4,4-difluorocyclohexylamine using amide coupling reagents (HCTU, HOBt and DIEA) to provide target compounds **101** and **107–111**.

Structure-ATPase Activity Relationship

A novel set of 64 target compounds was synthesized and tested in an ATPase assay (at different concentrations: 0.05 μM, 0.5 μM, and 2.5 μM) using native membranes (High Five insect cells) that express human P-gp to determine their affinity toward P-gp. The biological readouts for these compounds are reported as percentage (stimulation/inhibition) of P-gp basal ATPase activity. In the following SAR discussion, percentage of P-gp's ATPase activity was reported as stimulation of the inherent/basal activity unless otherwise specified. The ATPase activity can be interpreted as: 1) compounds stimulating basal ATPase activity are considered as substrates; 2) no effect on basal ATPase activity means potentially no interaction with P-gp and 3) inhibition of basal ATPase activity indicates inhibition of P-gp catalytic activity and subsequent inhibition of its efflux function.

To analyze the SAR trends, we categorized target compounds into three clusters: 1) modifications at the *C*-terminus and 2) the *N*-terminus of the (*S*)-valine-derived thiazole scaffold, and 3) the α-carbon of the valine amino acid, including bioisosteric replacement of the *C*- and *N*-terminus carboxamide with the thiocarboxamide group. A majority of the synthesized compounds showed stimulation of ATPase activity, which suggests these compounds to be potential P-gp substrates. The comparisons between ATPase stimulatory compounds were described with respect to binding affinity. This description is based on the percentage stimulation at the lowest identical concentration tested for the analogues being discussed *i.e.* higher the stimulation (at the same concentration), higher the binding affinity. Some of the target compounds inhibited ATPase activity, which indicated improved affinity toward P-gp and inhibition of its efflux function. Certain analogues exhibited neither stimulation nor inhibition of ATPase activity suggesting the loss of their affinity toward P-gp. In this work, we used high-affinity P-gp inhibitor, zosuquidar, as a positive control.

Based on our previous molecular modeling studies,[19] we sought to improve the binding efficiency of our lead compound **1** through a complementary fit in the proposed P-gp substrate/drug-binding pocket. Consequently, our initial efforts were mainly focused on performing structural modifications on the existing *N*- and *C*-terminal substituents of compound **1** and subsequently, to analyze their activity based on modulation of the basal ATPase activity of P-gp (Table 1). Firstly, compound **5** was synthesized with the same 3,4,5-trimethoxyphenyl and benzophenone groups, as in lead compound **1**, except their positions are switched from the *N*- to the *C*-terminus and *vice-versa*. This switch led to the loss of affinity to P-gp as evidenced from no activity at 0.05 μM compared to that of compound **1**, which had high stimulation (54% at 0.05 μM, 83% at 0.5 μM and 88% at 2.5 μM) of basal ATPase activity. However, compound **5** displayed a moderate (46%) stimulation of basal ATPase activity at 0.5 μM concentration, which indicated its substrate nature toward P-gp. Based on this result, the next round of SAR efforts retained the positions of the aryl moieties similar to those seen in compound **1**. Prior to varying the aryl substitutions, we decided to find an optimal positional isomer of the benzophenone attachment to the *C*-terminus. Since the 2- and 4-aminobenzophenone modifications at the *C*-terminus were previously assessed,[19] we synthesized the only remaining 3-aminobenzophenone regioisomer **7**, which showed a weak stimulation of ATPase activity (13% at 0.05 μM). This data suggests the key contribution of an optimally positioned *C*-terminal 2-benzoyl moiety for affinity toward P-gp. Therefore, we retained an *ortho*-substituted arrangement for the benzophenone moiety in the next array of structural modifications on the *C*-terminus. Subsequently, we explored the influence of classical and non-classical isosteric replacements of the carbonyl group present in *C*-terminal 2-aminobenzophenone moiety. The replacement of the carbonyl bridge of the benzophenone moiety with an amide (**18**, 21% at 0.05 μM), reverse amide (**19**, NA at 0.05 μM), ether (**20**, NA at 0.05 μM) and methylene (**21**, 15% at 0.05 μM) resulted in loss of affinity toward P-gp compared to **1**. The stimulation at concentration of 0.05 μM for these compounds indicated substrate type interactions, albeit with low binding affinity as compared to compound **1**. Thereafter, a set of seven compounds was prepared representing modifications at the aryl moieties of both *N*- and *C*-termini of compound **1**. These compounds (**28**, 149% at 0.5 μM; **29**, 148% at 0.5 μM; **31**, 308% at 0.5 μM; **32**, 142% at 0.5 μM; **33**, 122% at 0.5 μM; **34**, 135% at 0.5 μM and **35**, 99% at 0.5 μM) showed high

stimulation of ATPase activity. Collectively, this data indicated the importance of the carbonyl group, in addition to the sharp angular shape produced by an *ortho*-benzophenone moiety, for potent ATPase stimulation. The azido analogues, **29** and **32–35** were primarily synthesized as potential photoprobes to map the residues in the binding pocket at the TMDs of P-gp. To our disappointment, we could not obtain any covalently bound P-gp adduct for compounds **1** (contains benzophenone as a photoprobe), and **29** and **32–35** (contains azide as a photoprobe). This may have resulted from a significant reduction in the affinity of these compounds for P-gp and/or degradation of the compounds upon exposure to UV light, which is required for photo crosslinking. All the compounds synthesized up to this point were either regioisomers, isosteres or modifications of the *N*- and *C*-terminal aryl substituents and showed ATPase stimulatory effect. Therefore, we may consider these analogues as potential P-gp substrates and, hence, by a mechanism similar to the resistant cancer drugs, might eventually undergo expulsion by the efflux pump. Based on the above data, we hypothesize that the respective aryl moieties at both *N*- and *C*-termini are involved in interactions within the hydrophobic substrate-binding site of P-gp, which causes adaptation of the P-gp conformation that results in stimulation of basal ATPase activity (vide infra). This observation prompted us to explore non-aromatic groups with a goal to obtain P-gp ATPase inhibitors. Toward this goal, first we decided to determine the impact of replacing the *C*-terminal benzophenone moiety with various alicyclic ring systems. Compounds **36–40** with saturated aliphatic cycles of increasing steric bulk and lipophilicity were synthesized. The ATPase assay showed either no activity or low stimulation of basal ATPase activity for four of the five compounds (**36**, NA at 0.5 μM; **37**, 25% at 0.5 μM and NA at 2.5 μM; **38**, NA at 0.5 μM; and **40**, NA at 0.5 μM), an indication of a complete loss of affinity toward P-gp. Encouragingly, compound **39**, a *C*-terminal cyclohexyl analogue, showed 20% inhibition of basal ATPase activity at 0.05 μM concentration. Thus, the first P-gp ATPase inhibitor of the series was realized. It is interesting to find that an increase in the three-dimensionality with reduction in steric bulk in compound **39** as compared to lead **1** resulted in increased affinity toward P-gp with an inhibition of the catalytic process. P-gp ATPase inhibitory activity, although weak, of compound **39** with insertion of a cyclohexyl ring in lieu of the 2-aminobenzophenone moiety in compound **1** prompted us to investigate additional saturated ring systems at the *C*-terminus.

Since smaller (cyclopropyl, cyclobutyl, and cyclopentyl) or larger (adamantyl) ring size than the cyclohexyl ring proved detrimental, we focused our attention on inserting six membered rings at the *C*-terminus of the (*S*)-valine-derived thiazole scaffold. In our previous study, we found that hydrogen bonding interactions in the drug-interacting site of P-gp played a critical role in offering high affinity to clinical candidates.[29] With this notion, we synthesized hydrogen bond donor- and/or acceptor group-containing cyclohexyl analogues to maximize interaction probability in the drug/substrate-binding pocket of P-gp (Table 2). A *tert*-butyl carbamate-protected piperidine-4-yl analogue, **41**, exhibited a stimulatory ATPase activity as evidenced from 48% stimulation at 2.5 μM. Isosteric replacement of the cyclohexyl ring with the piperidine ring (**42**, 15% at 2.5 μM) and pyran ring (**43**, NA at 2.5 μM) showed complete loss of their ability to inhibit ATPase activity. Additional set of compounds, wherein we replaced the cyclohexyl amine moiety of **39** with various saturated six-membered ring systems such as 4-piperidone (**44**, NA at 2.5 μM), piperidin-4-

methylamine (**46**, NA at 2.5 μM), 4-aminopiperidine (**48**, NA at 2.5 μM), 4-aminocyclohexyl methylamine (**50**, NA at 2.5 μM) and 4-aminothiopyran-1,1-dioxide (**51**, NA at 2.5 μM), had no inhibitory effect on the ATPase activity. This data has prompted us to revisit the cyclohexyl ring pattern as in compound **39** with the intent of bringing in substitutions such as fluoro, methyl and keto at the 4-position of the cyclohexyl ring. These efforts led to synthesis of cyclohexan-4-one analogue **52** (NA at 2.5 μM), 4,4-difluorocyclohexyl derivative **53** (34% inhibition at 2.5 μM) and 4,4-dimethylcyclohexyl analogue **54** (34% at 2.5 μM). This piece of SAR resulted in identification of **53** with ATPase inhibitory potential that was higher than that of the first ATPase inhibitory compound, **39**, of the series. It is worth mentioning that P-gp can discriminate between the 4,4-difluorocyclohexyl (**53**) versus 4,4-dimethylcyclohexyl (**54**) isosteric analogues for binding preference and inhibition versus stimulation of the basal ATPase activity. The bioactivities displayed by the alicyclic ring analogues imply that the cyclohexane portion of the molecule may fit favorably in the substrate-binding pocket of P-gp with non-polar residues around it. To probe the steric requirements of the substrate-binding pocket of P-gp in a cyclohexyl interacting region, we prepared a small set of compounds with varying steric bulk such as dicyclohexyl amine (**55**, 134% at 2.5 μM), piperidinyl piperidine (**56**, 47% at 2.5 μM), *N*-benzyl piperidine-4-amine (**57**, 154% at 2.5 μM), pyrimidinyl piperidine-4-amine (**58**, 83% at 2.5 μM), and two enantiomers of tetralane amines (**59**, 123% at 2.5 μM and **60**, 31% at 2.5 μM). The difference in the activities of diastereoisomers, **59** and **60**, suggested chiral discrimination by the *C*-terminus fragment binding region of P-gp. Moderate to high stimulation of ATPase activity by these analogues suggested no tolerance for bulky substituents around the cyclohexyl binding site with respect to inhibition of ATPase activity, which reinforces the existence of a sterically forbidden region around the *C*-terminal cyclohexyl binding pocket (vide infra).

The 4,4-difluorocyclohexyl being established as the optimal moiety at the *C*-terminus for ATPase inhibitory effect, we proceeded to probe optimal binding conformation of the fluoro containing rings within the P-gp drug-binding pocket. Working toward this hypothesis, several analogues containing the fluoro groups on various ring systems were linked to the *C*-terminus of the (*S*)-valine-derived thiazole scaffold as shown in Table 3. Compounds (**61**, 56% at 2.5 μM and **62**, 76% at 2.5 μM) with the piperidine rings, had moderate to high stimulation of ATPase activity. This clearly explains the importance of the conformational disposition of the cyclohexyl moiety in **53** for inhibitory action on ATPase activity. Two analogues, representing a conformationally flexible extension at the cyclohexane ring, were synthesized. Compound **63** with the methylene insertion between the 4,4-difluorocyclohexyl ring and an amide 'NH' inhibited ATPase activity by 27% at 0.5 μM but stimulated by 21% at 2.5 μM. Conversely, the 4-trifluoromethyl cyclohexyl analogue (**64**, 22% inhibition at 2.5 μM) weakly inhibited ATPase activity. The inhibition efficiency of the compound decreases when the 4,4-difluorocyclohexyl or the fluoro groups are extended by one linker atom. To determine whether ATPase inhibition can be improved by retaining the fluoro groups while eliminating saturated ring structure, we synthesized two analogues containing fluorinated aromatic rings (**65**, 244% at 2.5 μM and **66**, 176% at 2.5 μM) and a flexible non-cyclic 4,4,4-trifluorobutyl analogue (**67**, 77% at 2.5 μM). These analogues have the same number of bonds between the fluorine and the amide group, as in compound **53**, but with different

orientations. All of these compounds stimulated basal ATPase activity. The vector for the fluorine atoms in various fluorinated analogs is clearly distinct from that present in **53**, which demonstrates the contribution of an optimal axial and equatorial arrangement of the fluorines in **53** for P-gp affinity. Compounds **61**, **62**, **65**, **66**, and **67** substantiates the importance of a cyclohexyl ring whereas compounds **63** and **64** suggest the requirement for precise positioning of the fluorine atoms in the substrate-binding site of P-gp for inhibition of basal ATPase activity. It is evident from the above SAR that any deviation from the 4,4-difluorocyclohexyl group results in a loss of ATPase inhibitory activity. Therefore, in the next round of SAR, we retained the 4,4-difluorocyclohexyl ring structure on the *C*-terminus and conducted structural modifications at the *N*-terminus.

Toward this goal, we have synthesized 12 compounds as shown in Table 4. At the outset, various unsaturated and saturated ring systems were installed in lieu of the trimethoxyphenyl moiety. The aromatic heterocycles such as 4-thiazolyl (**69**, 50% at 2.5 μM) and 3-quinolinyl (**71**, 84% at 2.5 μM) produced a switch in the ATPase activity from the inhibition to a stimulation. The 5-pyrimidinyl analogue, **70** (NA at 2.5 μM), failed to interact with P-gp. Amongst the saturated ring analogues, pyran **72** (NA at 2.5 μM) proved inactive and the 4,4-difluorocyclohexyl analogue, **73** (16% inhibition at 2.5 μM), showed a weak inhibition of ATPase activity. These analogues substantiated the importance of a 3,4,5-trimethoxybenzoyl moiety for its affinity enhancing interactions at the substrate-binding pocket of P-gp (vide infra). This result prompted us to probe further SAR around the 3,4,5-trimethoxybenzoyl moiety by synthesizing analogues that feature sequential removal of the methoxy groups in compound **53** to obtain analogues **74** (12% at 2.5 μM), **75** (36% at 2.5 μM), **76** (11% at 2.5 μM) and complete elimination of 3,4,5-trimethoxybenzoyl moiety as in **77** (NA at 2.5 μM). These compounds produced no appreciable effect on the ATPase activity. It can be concluded that a 3,4,5-trimethoxybenzoyl moiety at the *N*-terminus along with the 4,4,-difluorocyclohexyl substituent at the *C*-terminus are indispensable for ATPase inhibition. However, it remains to be determined whether methoxy groups can be replaced with other hydrogen bond acceptor groups. In pursuit of this investigation, we synthesized a 3,4,5-trifluorobenzoyl-containing compound **78** (23% inhibition at 2.5 μM), which expectedly retained the ATPase inhibitory effect; however, not to the extent produced by **53**. Hence, it is anticipated that the methoxy oxygen atoms may be involved in hydrogen bonding interactions within the drug-binding pocket of P-gp (vide infra). To probe the influence of an altered positioning of the hydrogen bond forming methoxy groups, we decided to separate the 3,4,5-trimethoxyphenyl ring and the carboxamide group by a flexible methylene linker to produce compound **79** (70% at 2.5 μM) and by a *trans*-favored rigid carbonyl linker (sterically restricted vicinal dicarbonyl functionality) to obtain **80** (176% at 2.5 μM); both had shown stimulation of the ATPase activity. It is anticipated that these extended analogues may have undergone steric clash within the substrate-binding pocket of P-gp, and thus, led to loss of the inhibitory interactions. Based on this data, we believe that the *N*-terminus aromatic ring in compound **53** provide requisite hydrophobicity for recognition by P-gp as well as proper orientation of the hydrogen bond forming methoxy groups for ATPase inhibitory effect. As a result, hereafter, we retained the 3,4,5-trimethoxybenzoyl and the 4,4-difluorocyclohexyl moieties at the *N*- and *C*-termini of the (*S*)-valine-derived thiazole scaffold, respectively.

Subsequent investigations sought to identify the impact of structural modifications at the central region of compound **53**, including the carboxamide to thiocarboxamide isosteric replacements (Table 5). In anticipation of obtaining additional ATPase inhibitory compounds, we replaced the carboxamide group at both *N*- and *C*-terminus with isosteric thiocarboxamide. This structural change would also provide analogues that are stable to hydrolytic cleavage. Similarly, replacement of hydrophobic valine side chain with analogous proteinogenic amino acids is anticipated to produce additional inhibitors of ATPase activity. Toward these objectives, we synthesized isosteric thioamide analogues **81**–**84**, which were anticipated to behave similar to the carboxamide analogues **39** and **53**. Unexpectedly, the dithioamide analogues (**81**, 92% at 2.5 μM and **83**, 64% at 2.5 μM) stimulated basal ATPase activity whereas monothioamide **82** (NA at 0.5 μM) lost the affinity. We postulate that change of oxygen to sulfur atom may have resulted in loss or weakening of hydrogen bonding ability of the compounds (**81**–**83**) indicating their contribution in the interactions in the substrate-binding pocket of P-gp. Nevertheless, **84** (26% inhibition at 2.5 μM) with one thioamide retained the inhibitory potency comparable to that of carboxamide **53** presumably due to favorable 4,4-difluoro substitution. Surprising activity profile of the thioamide compounds demonstrates how P-gp discriminates between molecular changes as small as amide to thioamide.

Subsequently, we determined the role of the α-carbon and its side chain toward ATPase activity. Compound **101**, lacking the α-carbon, stimulated basal ATPase activity by 60% at 2.5 μM and glycine analogue, **107** (13% at 2.5 μM), devoid of the isopropyl side chain, had a weak interaction with P-gp. Moreover, replacement of the isopropyl with an isobutyl group as in leucine analogue, **108** (68% at 2.5 μM), led to a switch from inhibition to the stimulation of ATPase activity. We presume that compound **101** may have lost some of the ATPase inhibitory interactions due to the truncation by one bond length and a lack of side chain. Further the valine side chain may be responsible for proper orientation of the molecule in the drug/substrate-binding pocket of P-gp as evidenced from the lack of affinity of **107** toward P-gp. To further probe the isopropyl interacting region topology of P-gp, we replaced the isopropyl side chain with the benzyl and weakly acidic 4-hydroxybenzyl side chains yielding ATPase inhibitory compounds **109** (**TTT-150**; 43% inhibition at 2.5 μM) and **110** (23% inhibition at 2.5 μM). The preference for the aromatic amino acid residues, as in **109** and **110**, may have resulted from favorable pi-pi interactions in the isopropyl binding region of P-gp for ATPase inhibitory activity (vide infra). Analogue **110** showed slightly less ATPase inhibition as compared to **109**, an observation hinted toward unfavorable contribution of the weakly acidic and polar 4-hydroxyl group. To explore the impact of replacing flexible valine by a rigid amino acid such as proline, we prepared (*S*)-proline-derived thiazole analogue, **111** (21% at 2.5 μM), which lost the affinity toward P-gp. It may be noted that these α-carbon modified analogues (**108**–**111**) were partially racemized. In general, the side chain fragment of these compounds play an important role in interactions at the drug-binding region of P-gp.

Herein, we summarize the key SAR trends. Despite large, flexible and tolerant substrate/drug-binding region of P-gp, the SAR data on the (*S*)-amino acid-derived thiazole compounds in this report suggests a unique ability of P-gp to distinguish amongst essentially

considered as isosteric structures in medicinal chemistry. Alicyclic analogues **39**, **42**, and **43** showed preference for the cyclohexyl ring over isosteric piperidine and pyran rings. Only the difluoro substitution at the 4-position of the cyclohexyl ring (**53**), amongst different hydrogen bonding groups (**42**, **51** and **52**), improves inhibitory efficiency. Moreover, structural changes with either removal or addition of one atom resulted in a switch from ATPase inhibition to stimulation as depicted by analogues **53** versus **61**, **79**, and **108**. Unanticipated effects of the thioamides (**81**, **82** and **83**) demonstrated subtle ability of P-gp to recognize specific groups. Moreover, glycine analogue **107** indicates the significance of hydrophobic contacts in the substrate-binding site of P-gp. Additionally, positioning of the trimethoxybenzoyl moiety, commonly present in several P-gp modulators, is critical at the *N*-terminus and is involved in key hydrogen bonding interactions (vide infra). Introduction of an aryl ring at the *C*-terminus of the thiazole scaffold resulted in stimulation of ATPase activity and only all carbon six membered saturated ring resulted in inhibitory activity.

Furthermore, a variety of molecular descriptors, reviewed by Didziapetris et. al.,[45] were calculated based on 2D and 3D structures of the compounds (Table S1). Albeit weak correlation, atom based logP (AlogP/octanol-water partition coefficient) analysis indicates that compounds with AlogP 3 exhibits higher affinity (moderate stimulation or inhibition of ATPase) with few exceptions (**38**, **40**, **42**, **48**, **58**, **73**, **74**, **76**, **82**, and **101**) paralleling similar reports by others and our group.[19, 46–49] All compounds except **77** have molecular weight >400 Da. Majority of the target compounds are well within the acceptable range of Lipinski's Rule-of-Five. The values for the calculated descriptors spans to a similar range for ATPase stimulatory, inhibitory and no affinity compounds. No apparent discrimination was found for these properties against the biological behavior of the compounds. It is intriguing that such similar compounds render different effects on interaction with P-gp. Finally, we examined, in silico, our target compounds against a reference set to identify any substructure commonly found in Pan Assay Interference Compounds (PAINS).[50] None of the target compounds, with the exception of five azide-containing compounds **29**, and **32**–**35**, were identified as potential PAINS in our analysis. These azide-containing compounds were primarily synthesized for photoaffinity labeling experiments.

[^{125}I]-IAAP Photolabeling Assay

To determine whether the ATPase inhibitors are interacting with P-gp at its substrate-binding site, we conducted binding competition assay of compounds **53** and **109** against [^{125}I]-IAAP. The UV-light-induced covalent labeling of [^{125}I]-IAAP to P-gp was inhibited by both compounds **53** and **109** in a concentration-dependent fashion (Figure 2). Compound **53** inhibited a maximum of 90% labeling at >5 μM concentrations (Figure 2A). The [^{125}I]-IAAP labeling IC$_{50}$ for compound **53** was 0.1 μM, which is improved compared to lead compound **1** (0.72 μM).[19] However, the IC$_{50}$ for compound **109** was 0.76 μM, which is close to that of compound **1** (Figure 2B). These results and ATPase activity data show that compound **53** inhibited basal ATPase activity by binding to TMDs of human P-gp with higher affinity than compound **1**.

MDR Reversal Assay

MDR reversal assay was carried out using representative analogues (**1**, **31**, **39**, **53**, **60**, and **109**) to determine the translation from biochemical assay results (ATPase and [^{125}I]-IAAP) to clinical implication. Compounds were selected based on their effect on ATPase activity: potent stimulators (**1** and **31**), inhibitors (**39**, **53** and **109**), and moderate stimulator (**60**). We determined the effect on cytotoxicity to the parental cell line (HEK 293-pcDNA 3.1) and ABCB1 (P-gp) transfected cell line (HEK-ABCB1) upon treatment of each compound in combination with paclitaxel. Paclitaxel was used as the ABCB1 substrate chemotherapy drug. Verapamil (10 μM) and zosuquidar (0.25 μM) were used as positive reversal compounds against the HEK-ABCB1 cell line (Table 6). The non-toxic test concentration of 10 μM for reversal compounds was selected because the preliminary cytotoxicity data showed at least 80% cell survival in both parental and resistant cell lines when treated alone (data not shown).

The cytotoxicity results demonstrate that the ABCB1-expressing cells exhibit 28.9-fold resistance (FR) to paclitaxel, with an IC$_{50}$ of 2.054 μM, as compared to the parental HEK 293-pcDNA 3.1 cells, which showed an IC$_{50}$ of 0.071 μM when treated with paclitaxel alone. Pre-treatment with test compounds significantly reduced the IC$_{50}$ value of ABCB1 substrate, paclitaxel, in the HEK-ABCB1 resistant cells. While the effect of the reversal compounds in combination with paclitaxel showed a negligible toxic effect in the parental HEK 293-pcDNA 3.1 cells, they proved to diminish the resistance significantly in the resistant, P-gp overexpressing cells. The relative resistance of the HEK-ABCB1 cells reduced from 28.9-fold to 0.6, 0.6, 0.6, 2.9 and 1.7-fold in the presence of compounds **1**, **39**, **53**, **60**, and **109** (10 μM), respectively. These compounds performed comparably to our positive controls at their respective concentrations: verapamil, a first-generation P-gp inhibitor and known ATPase stimulator (10 μM); and zosuquidar, a third generation potent P-gp ATPase inhibitor (0.25 μM). The lowered test concentration of zosuquidar was necessary due to the potency of the compound and because it was cytotoxic at 5 μM concentration. Competitive inhibition of the P-gp can be reversible or irreversible throughout the 72 h incubation period. We envision the ATPase stimulators may reverse MDR through competition with the substrate paclitaxel while the ATPase inhibitors prevent the paclitaxel efflux by non-competitive inhibition. Compound **31** has a weak reversal effect at 10 μM (fold-resistance of 17.8) suggesting it might be effluxed out at a rapid rate. Overall, compounds **1**, **39**, **53**, **60**, and **109** were found to overcome P-gp-mediated resistance to paclitaxel.

One of the drawbacks, with previous generations of P-gp modulators, including our lead compound **1** (CYP3A4 IC$_{50}$ = 8.2 μM),[20] was concomitant inhibition of CYP3A4. Therefore, we conducted in vitro CYP3A4 inhibition assay with representative compounds as per our prior report (Table 7).[20] P-gp ATPase inhibitors (**39**, **53**, and **109**) did not inhibit CYP3A4 up to 25 μM whereas compound **31**, which is an ATPase stimulator and benzophenone-containing analogue has CYP3A4 inhibition of 44% at 25 μM and 83% at 50 μM. Hence, replacement of the *C*-terminus benzophenone moiety of compound **1** with a cyclohexyl group offered selectivity toward P-gp over CYP3A4.

Molecular Modeling

Based on the SAR on P-gp ATPase activity of the synthesized analogues, we hypothesize that inhibitory compounds occupy a sterically demanding cavity in the drug-binding site and are involved in hydrogen bonding interactions with specific residues. To rationalize this hypothesis, we carried out induced-fit docking (a method that allow both protein side chains and ligand flexibility) of compounds **53** and **109** (Figure 3) within the transmembrane domain of homology modeled structure of human P-gp derived from the crystal structure of mouse P-gp (PDB ID: 4Q9H).[51] We chose mouse P-gp because it is the only available mammalian crystal structure with high resolution and has 87% identical sequence to that of human P-gp. Figures 3A and 3B illustrates docking pose for compound **53**. The docking data showed hydrogen bonding interaction of the 4-methoxy oxygen atom with the side chain of Gln990 (H_3CO----H_2N-Gln990). The amide group, connecting the trimethoxyphenyl and the Cα of the valine, entered into a hydrogen bonding interaction with the phenolic hydroxyl of Tyr307 (C=O----HO-Tyr307). The 4,4-difluorocyclohexyl moiety occupies a space that is closely surrounded by residues Phe335, Phe336, Leu339, Ile340, and Phe343. Thus, any modification of the 4,4-difluorocyclohexyl moiety at the *C*-terminus will result in steric hindrance and a loss of inhibitory activity. The isopropyl group of a valine residue of the compound may be involved in hydrophobic interactions with the residues, Phe728 and Ala729, in the transmembrane region of P-gp.

Binding model of (*S*)-isomer of **109** within the substrate-binding region of P-gp indicates the formation of three hydrogen bonds (4-H_3CO----H_2N-Gln838; 3-H_3CO----H_2N-Gln990 and the *N*-terminus-NH----OH-Tyr307) as shown in Figures 3C and 3D. Moreover, the side chain benzyl is stabilized by an edge-to-face (T-shape) interaction with the side chain of Tyr310. The 4,4-difluorocyclohexyl moiety is surrounded by residues Phe335, Phe336, Leu339, Ile340, and Phe343 similar to that observed for compound **53**. The surface representation of bound (*S*)-isomer of **109** also shows its binding at the sterically demanding binding site especially at the cyclohexyl region (Figure 3E).

Since, both *cis*- and *trans*-forms for (*S*)-proline are reported to exist in small peptides, we performed ligand alignment using both isomers.[52] Superposition of docked conformations of compound **53** and conformationally restricted *cis*- and *trans*-proline isomers (**111**) indicated a substantial movement of the proline analogue as compared to the valine analogue **53** (Figure 3F and 3G). The trimethoxybenzoyl portion of the molecule shifts away from the position that was discerned from the valine-derived analogue **53**. Therefore, it can be concluded that the conformationally restricted proline-derived analogue, **111**, presented interactions in the manner that were detrimental to its binding affinity toward P-gp. This may have resulted from the loss of key hydrogen bonding interaction with the side chains of Tyr307 and Asn842 and/or Gln990 in case of proline analogue.

It may be noted that docking experiments cannot necessarily identify the correct binding pattern of the ligand and particularly in this case due to a large and flexible binding pocket of P-gp. However, predicted binding models may facilitate future optimization efforts. The interaction pattern based on the induced-fit docking poses as well as the SAR data together suggests that the trimethoxybenzoyl group is surrounded by polar side chains of Gln725,

Asn842, Gln838, and Gln990, which may facilitate proper orientation of the molecule in this region. Since the side chain benzyl group of **109** is involved in T-shape aromatic-aromatic interaction, it could be replaced with heteroaromatic ring-containing unnatural amino acids to strengthen an edge-to-face interaction with the side chain of Tyr310. Furthermore, the two amide nitrogen atoms can be linked together to form macrocyclic analogues by ring closing metathesis. Such macrocyclic analogues would bind within the substrate-binding region of P-gp in an entropically favorable manner compared to their non-cyclic counterparts. This will form the basis for our future SAR studies.

CONCLUSIONS

A series of substituted (*S*)-amino acid-derived thiazole analogues were synthesized to probe the substrate-binding site within the TMDs of P-gp and their effect on basal ATPase activity was determined. Starting from the lead compound **1**, a potent stimulator of ATPase activity, few inhibitors (**39**, **53**, **78**, **84**, **109** and **110**) of ATPase activity were identified. The inhibitory activity toward ATPase was largely contributed by the cyclohexane ring. Moreover, replacement of the cyclohexane moiety with piperidine (**61** and **62**), aromatic (**65** and **66**) or open chain alkyl (**67**) groups resulted in a switch from inhibition to stimulation of ATPase activity. The trimethoxy group at the *N*-terminus was critical for preserving inhibitory function and is involved in hydrogen bonding interactions as evidenced from **74**, **76** and **78**. Compounds **53**, **61**, **79**, and **108** show that variation by one bond length results in significant shift in affinity toward P-gp. Moreover, photolabeling competition data with [^{125}I]-IAAP and compounds **53** and **109** provided evidence for interaction of these compounds with the TMDs of P-gp. MDR reversal assay data showed that ATPase stimulatory compound **31** as well as ATPase inhibitors **39**, **53** and **109** act as MDR reversal compounds, presumably with different mechanisms. To our delight, these ATPase inhibitors did not inhibit CYP3A4 unlike starting lead compound **1** and previous generations of P-gp modulators. The substrate-binding pocket of P-gp is thought to be very flexible in nature and recognizes various size and type of chemical structures. The effect of the synthesized series, in this report, reinforces this fact; however, it is intriguing to discover that an extension or truncation by only one methylene group switches the inhibition to the stimulation of ATPase activity.

EXPERIMENTAL

Chemical Synthesis

Materials and Instrumentation—Chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI), AK scientific (Union City, CA), A2Z chemicals (Irvine, CA), Oakwood Products (West Columbia, SC), TCI America (Portland, OR) and Alfa Aesar (Ward Hill, MA), and were used as received. All chemicals were confirmed for uniformity by thin layer chromatography (TLC) with silica gel as the adsorbent layer (250 microns) on aluminum backed plates (Agela Technologies). Reactions were monitored by TLC, and visualized using Ultraviolet (UV) light at 254 nm. Melting points were recorded on a Stuart Melting Point apparatus (model - SMP20) and are uncorrected. Bruker 400 Ultrashield™ spectrometer (^{1}H at 400 MHz and ^{13}C at 100 MHz) equipped with a *z*-axis gradient probe

was used to record NMR experiments. ^{1}H NMR and ^{13}C NMR chemical shifts were reported downfield from tetramethylsilane (TMS, internal standard) in parts per million (δ ppm). The ^{1}H NMR data are presented as follows: chemical shift (multiplicity {s (singlet), bs (broad singlet), d (doublet), t (triplet), dd (doublet of doublets), m (multiplet) and oct (octet)}, number of protons, coupling constant). The ^{13}C NMR (proton decoupled, fluorine coupled) data are presented as follows: chemical shift (multiplicity {d (doublet), t (triplet)}). Column chromatography purifications were performed with silica gel (40–63 μm) obtained from Silicycle Inc. (Quebec City, Canada) and flash chromatography was performed using Reveleris® X2 flash chromatography system (BÜCHI Corporation, New Castle, DE). Preparative TLC was performed using Silica Gel GF 1000 μm 20×20cm glass backed plates from Analtech (Miles Scientific, Newark, DE). Target compounds purity analysis was accomplished utilizing Agilent 1100 HPLC system with an autosampler (Agilent, Santa Clara, CA) eluting a C-18 reverse phase column (Agilent Eclipse plus C18, 3.5 μm, 4.6 × 100 mm) with an isocratic mobile phase flow (1.0 mL/min), and samples monitored under UV light at 254 nm. All target compounds were established to be 95% pure (major peak area/total combined area of peaks). Mass analyses were carried out on Agilent 1260 infinity series liquid chromatography (LC) system (C18 column, Agilent InfinityLab poroshell 120, EC-C18, 2.7 μm, 4.6 × 50 mm) connected with Agilent 6120 quadrupole mass spectrometer (MS). The elemental analyses (C, H, and N) were performed by Atlantic Microlabs, Inc., (Norcross, GA) and the observed values were within ±0.4% of calculated values.

Synthesis

Compounds **2**,[31] **6**[19] and **10**[20] were synthesized according to the procedures as described in our previous reports.

Method A: Typical Procedure for Peptide Coupling Reactions

A homogenous solution/suspension of carboxylic acid (1 eq) in either dry *N*, *N*-dimethylacetamide (DMA; 10 mL) or dry dichloromethane (DCM; 15 mL), under nitrogen atmosphere, was brought to 0°C and then added with diisopropylethylamine (DIEA; 1.5 eq), HCTU (1.5 eq), and HOBt (1.5 eq). The resulting mixture was stirred for 10–15 min at 0°C before the addition of cold solution/suspension of amine (1.2 eq) in appropriate solvent (DMA or DCM; ~1–2 mL). It was then stirred overnight at rt, after which the resulting solution was evaporated. Ethyl acetate was added to a crude product mass and washed with 1N aqueous potassium bisulfate (KHSO$_4$) solution. The acidic aqueous layer was then extracted with 2X ethyl acetate. Combined ethyl acetate extracts were partitioned with saturated aqueous solution of sodium carbonate (Na$_2$CO$_3$). Ethyl acetate extract was collected, dried over anhydrous magnesium sulfate (MgSO$_4$) and evaporated. The remaining mass was purified by chromatography (preparative TLC or flash chromatography) using *n*-hexanes/ethyl acetate as mobile phase to obtain coupled product as a colorless oil. This oil was triturated with ethyl acetate and *n*-hexanes to provide a pure solid.

Method B: General Procedure for the *N*-Boc Deprotection

N-Boc protected amine (1 eq) dissolved in DCM (20 mL) was slowly added to trifluoroacetic acid (TFA; 10 eq) at 0°C. A resulting reaction mixture was kept at 0°C for 10

min and then it was stirred at rt for an appropriate period (4–12 h). The volatiles were evaporated to leave viscous mass, which was dissolved in water. It was partitioned with ethyl acetate and the acidic aqueous layer was collected. The water layer was carefully neutralized using saturated aqueous solution of Na_2CO_3 and then extracted with ethyl acetate. The ethyl acetate extract was passed over anhydrous $MgSO_4$ and evaporated to obtain free amine, which was used without further purification.

Method C: Procedure for the Synthesis of Carboxamides by Mixed-Anhydride Method

Appropriate carboxylic acid (1 eq) in anhydrous THF (50 mL) under nitrogen was cooled to −20°C. Then, isobutyl chloroformate (1.2 eq) and *N*-methylmorpholine (NMM; 1.2 eq) were added to the reaction mixture and stirred at −20°C for 1–2 h. To this suspension was added excess (25 mL) of aqueous ammonia (30%), which produced a clear biphasic solution that was allowed to stir for an additional 30 min – 2 h at rt. After concentrating the reaction mixture, it was extracted with 2X ethyl acetate. The combined ethyl acetate extract was partitioned with aqueous 1N $KHSO_4$, collected, dried over anhydrous $MgSO_4$ and evaporated to provide desired carboxamide as a solid.

Method D: General Procedure for the Synthesis of Thioamides

To a solution of carboxamide (1 eq) in anhydrous THF (40 mL) was added Lawesson's reagent (0.57 eq) under nitrogen atmosphere. The suspension was stirred for 8 h at rt. At this time, entire solid material in the reaction mixture dissolved to produce a clear yellow solution. Subsequently, saturated aqueous $NaHCO_3$ solution was slowly added to the reaction flask until effervescence ceases. Ethyl acetate (15 mL) was added to the quenched mixture and stirred for an additional hour. The TLC analysis showed two spots with near R_f values of which the upper faint spot disappeared from the organic layer after extraction workup. The ethyl acetate extract was dried (anhydrous $MgSO_4$), concentrated and then purified through column chromatography using *n*-hexanes/ethyl acetate as eluent giving pure thioamide as a thick oil.

Method E: General Procedure for the Construction of Thiazole Scaffold

The thioamide (1 eq) in 40 mL anhydrous ethanol under inert condition was cooled using a sodium chloride/ice-bath (−20°C). To it, was added calcium carbonate ($CaCO_3$; 2.5 eq) and the suspension was stirred at −20°C for 15 min and subsequently ethyl bromopyruvate (1.1 eq) was added. The dark mixture was further stirred at rt for 8 h. Afterwards, the solid was filtered off and the filtrates were concentrated to furnish crude product, which was purified using column chromatography with *n*-hexanes/ethyl acetate as eluent.

Method F: General Procedure for Peptide Coupling with Acyl Chlorides

Diisopropylethylamine (DIEA, 2 eq) and appropriate acyl chloride (1.2 eq) were sequentially added to a solution of amine (1 eq) in anhydrous THF (20 mL) at 0°C and stirred overnight at rt under nitrogen atmosphere. Ethyl acetate was added to the reaction mixture and it was then successively washed with 2N HCl and saturated aqueous Na_2CO_3 solution. The organic layers were collected, dried (anhydrous $MgSO_4$) and concentrated to a crude residue, which was purified by flash chromatography providing the coupled product.

Method G: General Procedure for the Thiazole Ester Hydrolysis

The thiazole ester in a 20 mL of THF/methanol/water (10/2/3) solvent mixture at 0°C was added sodium hydroxide (10 eq). The reaction mixture was brought to rt and allowed to stir overnight after which it was concentrated using rotary vacuum evaporation. The residual liquid was added with more water and partitioned with ethyl acetate. The aqueous layer was collected, acidified using 2N HCl and partitioned with ethyl acetate (2X). The organic extracts were collected, dried over anhydrous $MgSO_4$ and evaporated to provide crude acid that was used without further purification.

Tert-butyl (*S*)-(2-methyl-1-(4-((3,4,5-trimethoxyphenyl)carbamoyl)thiazol-2-yl)propyl) carbamate (3)

Compound **3** was obtained from acid **2** (475 mg, 1.58 mmol) and 3,4,5-trimethoxyaniline (350 mg, 1.91 mmol) using method A as a yellow solid (544 mg, 74%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 9.96 (s, 1H), 8.29 (s, 1H), 7.75 (d, 1H, J = 8.2 Hz), 7.30 (s, 2H), 4.77-4.74 (m, 1H), 3.77 (s, 6H), 3.64 (s, 3H), 2.37-2.29 (m, 1H), 1.42 (s, 9H), 0.90 (d, 6H, J = 6.5 Hz).

(*S*)-2-(1-Amino-2-methylpropyl)-*N*-(3,4,5-trimethoxyphenyl)thiazole-4-carboxamide (4)

Amine **4** was obtained from compound **3** using method B (12 h) as a yellow oil. ^{1}H NMR (400 MHz; CDCl₃; TMS) δ 9.08 (s, 1H), 8.09 (s, 1H), 7.02 (s, 2H), 4.21-4.18 (m, 1H), 3.87 (s, 6H), 3.81 (s, 3H), 2.31-2.23 (m, 1H), 1.01 (d, 3H, J = 6.5 Hz), 0.92 (d, 3H, J = 6.5 Hz).

(*S*)-2-(1-(2-Benzoylbenzamido)-2-methylpropyl)-*N*-(3,4,5-trimethoxyphenyl)thiazole-4-carboxamide (5)

Compound **5** was obtained from 2-benzoyl benzoic acid (50 mg, 0.22 mmol) and amine **4** (97 mg, 0.27 mmol) using method A as a white solid (42 mg, 33%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.79 (s, 1H), 8.16 (s, 1H), 7.79 (d, 1H, J = 6.8 Hz), 7.62-7.55 (m, 2H), 7.48 (s, 1H), 7.26 (d, 1H, J = 7.0 Hz), 7.13 (s, 2H), 7.06-6.93 (m, 5H), 4.13 (d, 1H, J = 10.9 Hz), 3.81 (s, 6H), 3.65 (s, 3H), 3.19-3.11 (m, 1H), 1.06 (d, 3H, J = 6.5), 0.71 (d, 3H, J = 6.5 Hz). m/z (ESI-MS) 574.3 ($C_{31}H_{31}N_3O_6S$ requires 574.19, [M + H]⁺). HPLC t_R (Acetonitrile/water 60/40) = 3.2 min, purity 98%.

(*S*)-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carboxylic acid (6)

The synthesis and ^{1}H NMR details for compound **6** are reported elsewhere.[19] ^{13}C NMR (100 MHz; DMSO-D₆; TMS) δ 172.98, 166.27, 162.58, 153.03, 147.12, 140.71, 129.41, 129.05, 105.64, 60.55, 57.93, 56.51, 32.19, 20.31, 19.74.

(*S*)-*N*-(3-Benzoylphenyl)-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carboxamide (7)

Compound **7** was obtained from acid **6** (50 mg, 0.13 mmol) and 3-aminobenzophenone (30 mg, 0.15 mmol) using method A as a white solid (30 mg, 41%). ^{1}H NMR (400 MHz; CDCl₃; TMS) δ 9.22 (s, 1H), 8.17-8.15 (m, 2H), 7.94-7.93 (m, 1H), 7.84-7.82 (m, 2H), 7.61 (d, 1H, J = 7.3 Hz), 7.55-7.47 (m, 4H), 7.05 (s, 2H), 6.67 (d, 1H, J = 8.6 Hz), 5.39 (dd, 1H, J = 8.8 Hz, 6.7 Hz), 3.90 (s, 6H), 3.88 (s, 3H), 2.56 (oct, 1H, J = 6.7 Hz), 1.10 (d, 3H, J = 6.8

Hz), 1.06 (d, 3H, $J = 6.8$ Hz). m/z (ESI-MS) 574.2 ($C_{31}H_{31}N_3O_6S$ requires 574.19, [M + H]$^+$). HPLC t_R (Acetonitrile/water 60/40) = 5.4 min, purity 99%.

2-Amino-*N*-phenylbenzamide (8).[32]

A diethyl ether solution (70 mL) of anthranilic acid (500 mg, 3.65 mmol) was added dropwise to thionyl chloride (5 mL) and refluxed for 2 h. The reaction mixture was then co-evaporated with DCM for three times. The remaining mass was taken up in ether and added to a stirring solution containing aniline (0.4 mL, 4.38 mmol) in pyridine (15 mL) and ether (40 mL), and heated at refluxing temperatures for 3 h. The dark solution was evaporated and the crude product was purified using flash chromatography (*n*-hexanes/ethyl acetate: 100/0% to 0/100%; gradient) as an off-white solid (**8**; 250 mg, 32%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 9.98 (s, 1H), 7.70 (d, 2H, $J = 8.2$ Hz), 7.61 (d, 1H, $J = 7.9$ Hz), 7.32 (t, 2H, $J = 7.5$ Hz), 7.19 (t, 1H, $J = 7.5$ Hz), 7.07 (t, 1H, $J = 7.3$ Hz), 6.75 (d, 1H, $J = 8.0$ Hz), 6.58 (t, 1H, $J = 7.4$ Hz), 6.31 (bs, 2H).

N-(2-Aminophenyl)benzamide (9).[33]

2-nitroaniline (300 mg, 2.17 mmol) in anhydrous THF (20 mL) was stirred overnight with pyridine (0.26 mL, 3.25 mmol) and benzoyl chloride (0.24 mL, 2.06 mmol) after which the mixture was concentrated. The crude product was dissolved in ethyl acetate and washed sequentially with 2N HCl and saturated aqueous solution of $NaHCO_3$. The organic layer was dried (anhydrous $MgSO_4$) and evaporated to obtain the coupled product as a yellow oil, which was dissolved in methanol and subjected to palladium catalyzed hydrogenation using pressure vessel (60 psi) at rt for overnight. The solution was then passed through celite and evaporated to furnish **9** (330 mg, 75%) as a light brown solid. ^{1}H NMR (400 MHz; CDCl₃; TMS) δ 11.39 (s, 1H), 9.04 (d, 1H, $J = 8.5$ Hz), 8.31 (d, 1H, $J = 8.5$ Hz), 8.03 (d, 2H, $J = 7.9$ Hz), 7.75 (t, 1H, $J = 7.8$ Hz), 7.65-7.62 (m, 1H), 7.59-7.55 (m, 2H), 7.25 (t, 1H, $J = 7.8$ Hz).

(2-Aminophenyl)(4-bromophenyl)methanol (11)

2-Amino-4′-bromobenzophenone (300 mg, 1.09 mmol) in combination with sodium borohydride (41 mg, 1.09 mmol) was refluxed in ethanol (15 mL) for 2 h and then the solution was concentrated. The crude remaining was dissolved in ethyl acetate and partitioned with water. The organic extract was dried (anhydrous $MgSO_4$) and concentrated to yield a brown solid (**11**, 236 mg, 78%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 7.48 (d, 2H, $J = 8.2$ Hz), 7.31 (d, 2H, $J = 7.9$ Hz), 7.03 (d, 1H, $J = 7.6$ Hz), 6.95 (t, 1H, $J = 7.2$ Hz), 6.59 (d, 1H, $J = 8.0$ Hz), 6.52 (t, 1H, $J = 7.3$ Hz), 5.96 (d, 1H, $J = 3.8$ Hz), 5.71 (d, 1H, 3.8 Hz), 4.97 (s, 2H).

3-((*Tert*-butoxycarbonyl)amino)benzoic acid (12).[34]

Compound **12** was obtained by reacting 3-aminobenzoic acid (1.20 g, 8.75 mmol) with di-*tert*-butyl dicarbonate (2.86 g, 13.12 mmol) and triethylamine (1.83 mL, 13.12 mmol) in a solvent mixture of dioxane (20 mL) and water (20 mL) at rt for 24 h. The reaction mixture was concentrated, acidified with 3N HCl and extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$ and evaporated to produce a white solid (1.91 g, 92%). [1]H

NMR (400 MHz; DMSO-d6; TMS) δ 9.38 (s, 1H), 8.15 (s, 1H), 7.63 (d, 1H, *J* = 6.3 Hz), 7.47 (d, 1H, *J* = 7.8 Hz), 7.21 (t, 1H, *J* = 7.8 Hz), 1.47 (s, 9H).

2-Azidobenzoic acid (13)

To anthranilic acid (500 mg, 3.65 mmol) dissolved in a cooled (0°C) aq. solution of 10% HCl was slowly added a 1.5 mL of aqueous sodium nitrite (302 mg, 4.38 mmol) solution and allowed to stir for 15 min at rt. Then, 1 mL of aqueous sodium azide (285 mg, 4.38 mmol) solution was added carefully resulting in vigorous precipitation. The precipitates were filtered and washed with water to obtain **13** as an orange solid in quantitative yield. ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 13.21 (s, 1H), 7.77-7.26 (m, 4H).

3-Azidobenzoic acid (14).[35]

Compound **14** was prepared from 3-aminobenzoic acid using the same procedure as that for azide **13** as a cream colored solid. ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 13.28 (s, 1H), 7.73-7.33 (m, 4H).

4-Azidobenzoic acid (15).[35]

Compound **15** was prepared from 4-aminobenzoic acid using the same procedure as that for azide **13** as a cream colored solid. ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 13.03 (bs, 1H), 7.95 (d, 2H, *J* = 7.4 Hz), 7.18 (d, 2H, *J* = 7.4 Hz).

Tert-butyl (1-benzylpiperidin-4-yl)carbamate (16).[36]

Tert-butyl piperidin-4-ylcarbamate (1.00 g, 4.99 mmol) was alkylated by heating for overnight with benzyl chloride (0.86 mL, 7.49 mmol) and triethylamine (1.39 mL, 9.98 mmol) at refluxing temperature in THF (20 mL). Afterwards, ethyl acetate was used to dilute the reaction mixture and then washed with aqueous 1N KHSO$_4$. The ethyl acetate layer was passed over anhydrous MgSO$_4$ and evaporated. The resulting crude mass was purified using flash chromatography to furnish **16** (1.11 g, 76%) as a yellow solid. ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 7.75 (d, 1H, *J* = 7.4 Hz), 7.42-7.31 (m, 5H), 3.49 (s, 2H), 3.07-2.93 (m, 1H), 2.82-2.80 (m, 2H), 2.12-2.07 (m, 2H), 1.93-1.81 (m, 4H), 1.45 (s, 9H).

1-Benzylpiperidin-4-amine (17)

Compound **17** was obtained from *N*-Boc protected **16** using method B in a period of 4 h as a colorless oil. ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 7.33-7.23 (m, 5H), 3.51 (s, 2H), 2.85 (d, 1H, *J* = 11.7 Hz), 2.71-2.64 (m, 2H), 2.03 (t, 2H, *J* = 11.3 Hz), 1.82-1.74 (m, 4H), 1.46-1.37 (m, 2H).

(*S*)-2-(2-Methyl-1-(3,4,5-trimethoxybenzamido)propyl)-*N*-(2-(phenylcarbamoyl)phenyl)thiazole-4-carboxamide (18)

Compound **18** was obtained from acid **6** (100 mg, 0.25 mmol) and aniline **8** (65 mg, 0.30) using method A as a white solid (10 mg, 7%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 12.06 (s, 1H), 10.55 (s, 1H), 9.02 (d, 1H, *J* = 8.4 Hz), 8.61 (d, 1H, *J* = 8.4 Hz), 8.39 (s, 1H), 7.90 (d, 1H, *J* = 7.8 Hz), 7.85-7.80 (m, 2H), 7.62 (t, 1H, *J* = 7.8 Hz), 7.37 (t, 2H, *J* = 7.8 Hz), 7.31-7.23 (m, 3H), 7.14 (t, 1H, 7.4 Hz), 5.21-5.17 (m, 1H), 3.83 (s, 6H), 3.71 (s, 3H),

2.69-2.60 (m, 1H), 1.09-1.05 (m, 6H). m/z (ESI-MS) 589.3 ($C_{31}H_{32}N_4O_6S$ requires 589.20, [M + H]⁺). HPLC t_R (acetonitrile/water 50/50) = 13.8 min, purity 95%.

(S)-N-(2-Benzamidophenyl)-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carboxamide (19)

Compound **19** was obtained from acid **6** (100 mg, 0.25 mmol) and aniline **9** (65 mg, 0.30) using method A as a white solid (10 mg, 7%). ¹H NMR (400 MHz; DMSO-d6; TMS) δ 10.45 (s, 1H), 9.89 (s, 1H), 8.90 (d, 1H, J = 8.5 Hz), 8.36 (s, 1H), 8.13-8.07 (m, 3H), 7.64 (t, 1H, J = 6.9 Hz), 7.57 (t, 2H, J = 6.9 Hz), 7.42-7.35 (m, 2H), 7.27-7.22 (m, 3H), 5.07-5.03 (m, 1H), 3.83 (s, 6H), 3.71 (s, 3H), 2.34-2.24 (m, 1H), 0.81 (t, 3H, J = 6.5 Hz), 0.66 (d, 3H, J = 6.5 Hz). m/z (ESI-MS) 589.3 ($C_{31}H_{32}N_4O_6S$ requires 589.20, [M + H]⁺). HPLC t_R (acetonitrile/water 50/50) = 7.3 min, purity 95%.

(S)-2-(2-Methyl-1-(3,4,5-trimethoxybenzamido)propyl)-N-(2-phenoxyphenyl)thiazole-4-carboxamide (20)

Compound **20** was obtained from acid **6** (100 mg, 0.25 mmol) and 2-phenoxyaniline (56 mg, 0.30) using method A as a white solid (30 mg, 21%). ¹H NMR (400 MHz; DMSO-d6; TMS) δ 9.79 (s, 1H), 8.97 (d, 1H, J = 8.2 Hz), 8.42 (d, 1H, J = 8.0 Hz), 8.39 (s, 1H), 7.39 (t, 2H, J = 7.3 Hz), 7.28-7.12 (m, 5H), 7.09-7.04 (m, 3H), 5.11-5.07 (m, 1H), 3.83 (s, 6H), 3.71 (s, 3H), 2.40-2.33 (m, 1H), 0.99 (d, 3H, J = 6.6 Hz), 0.87 (d, 3H, J = 6.6 Hz). m/z (ESI-MS) 562.20 ($C_{30}H_{31}N_3O_6S$ requires 562.19, [M + H]⁺). HPLC t_R (acetonitrile/water 60/40) = 9.6 min, purity 100%.

(S)-N-(2-Benzylphenyl)-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carboxamide (21)

Compound **21** was obtained from acid **6** (100 mg, 0.25 mmol) and 2-benzylamine (56 mg, 0.30) using method A as a white solid (22 mg, 16%). ¹H NMR (400 MHz; DMSO-d6; TMS) δ 9.53 (s, 1H), 8.95 (d, 1H, J = 8.2 Hz), 8.33 (s, 1H), 7.84 (d, 1H, J = 7.6 Hz), 7.35-7.17 (m, 10H), 5.23-5.19 (m, 1H), 4.09-4.00 (m, 2H), 3.84 (s, 6H), 3.71 (s, 3H), 2.47-2.41 (m, 1H), 1.06 (d, 3H, J = 6.3 Hz), 0.95 (d, 3H, J = 6.3 Hz). m/z (ESI-MS) 560.3 ($C_{31}H_{33}N_3O_5S$ requires 560.21, [M + H]⁺). HPLC t_R (acetonitrile/water 60/40) = 7.6 min, purity 99%.

Tert-butyl ((1S)-1-(4-((2-(hydroxy(phenyl)methyl)phenyl)carbamoyl)thiazol-2-yl)-2-methylpropyl)carbamate (22)

Compound **22** was obtained from acid **2** (500 mg, 1.66 mmol) and 2-aminobenzhydrol **10**[20] (398 mg, 2.00 mmol) using method A as a white solid (552 mg, 69%). ¹H NMR (400 MHz; CDCl₃; TMS) δ 10.47 (s, 1H), 8.30 (d, 1H, J = 8.2 Hz), 8.11 (s, 1H), 8.04 (d, 1H, J = 3.4 Hz), 7.44-7.03 (m, 8H), 6.02-6.01 (m, 1H), 5.42-5.40 (m, 1H), 4.94-4.91 (m, 1H), 2.40-2.31 (m, 1H), 1.47 (s, 9H), 1.00-0.98 (m, 6H).

Tert-butyl ((1S)-1-(4-((2-((4-bromophenyl)(hydroxy)methyl)phenyl)carbamoyl)thiazol-2-yl)-2-methylpropyl)carbamate (23)

Compound **23** was obtained from acid **2** and benzhydrol **11**, using method A as a dark brown oil. ¹H NMR (400 MHz; CDCl₃; TMS) δ 8.10-6.38 (m, 11H), 5.44-5.39 (m, 1H),

4.91-4.88 (m, 1H), 2.40-2.32 (m, 1H), 1.45 (s, 9H), 0.98 (d, 3H, J = 6.7 Hz), 0.92 (d, 3H, J = 6.7 Hz).

Tert-butyl (*S*)-(1-(4-((2-benzoylphenyl)carbamoyl)thiazol-2-yl)-2-methylpropyl) carbamate (24)

To a solution of benzhydrol derivative **22** (500 mg, 1.04 mmol) in dry THF (15 mL), Dess–Martin periodinane (662 mg, 1.56 mmol) was added and stirred at rt until the TLC indicated reaction completion (~30 min). Subsequently saturated $NaHCO_3$ and sodium thiosulfate ($Na_2S_2O_3$) aqueous solutions were added for quenching the reaction. The mixture was partitioned with ethyl acetate and the organic layer was collected, dried (anhydrous $MgSO_4$) and evaporated to give a crude mass, which was purified by column chromatography (*n*-hexane/ethyl acetate 80/20) to obtain compound **24** (478 mg, 96%) as a white solid. ^{13}C NMR (100 MHz; CDCl3; TMS) δ 198.47, 171.96, 159.82, 155.55, 150.22, 139.76, 138.69, 133.81, 133.26, 132.33, 129.98, 128.23, 124.48, 123.85, 122.41, 121.57, 79.91, 57.83, 33.38, 28.32, 19.25, 17.60. ^{1}H NMR (400 MHz; CDCl3; TMS) δ 12.25 (s, 1H), 8.81 (d, 1H, J = 8.4 Hz), 8.14 (s, 1H), 7.78 (d, 2H, J = 7.8 Hz), 7.65-7.59 (m, 3H), 7.52-7.48 (m, 2H), 7.15 (t, 1H, J = 7.4 Hz), 5.53-5.51 (m, 1H), 5.00-4.96 (m, 1H), 2.49 (oct, 1H, J = 6.5 Hz), 1.46 (s, 9H), 1.05 (d, 3H, J = 6.7 Hz), 1.00 (d, 3H, J = 6.7 Hz). m/z (ESI-MS) 480.2 ($C_{26}H_{29}N_3O_4S$ requires 480.19, [M + H]$^+$).

Tert-butyl (*S*)-(1-(4-((2-(4-bromobenzoyl)phenyl)carbamoyl)thiazol-2-yl)-2-methylpropyl) carbamate (25)

Compound **25** was prepared following the same procedure used for the synthesis of **24** except compound **23** was used as starting material. ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.06-8.03 (m, 1H), 7.99-7.96 (m, 1H), 7.90-7.88 (m, 1H,), 7.79-7.58 (m, 3H), 7.42-7.38 (m, 2H), 7.17-7.12 (m, 2H), 5.71-5.50 (m, 1H), 2.37-2.34 (m, 1H), 1.45 (s, 9H), 0.98-0.92 (m, 6H).

(*S*)-2-(1-Amino-2-methylpropyl)-*N*-(2-benzoylphenyl)thiazole-4-carboxamide (26)

Amine **26** was obtained from *N*-Boc protected **24** using method B (12 h) as a colorless oil and it was used without additional purification.

(*S*)-2-(1-Amino-2-methylpropyl)-*N*-(2-(4-bromobenzoyl)phenyl)thiazole-4-carboxamide (27)

Amine **27** was obtained from *N*-Boc protected **25** using method B (12 h) as a colorless oil, which was carried forward without additional purification.

(*S*)-*N*-(2-(4-Bromobenzoyl)phenyl)-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl) thiazole-4-carboxamide (28)

Compound **28** was obtained from 3,4,5-trimethoxybenzoyl chloride (122 mg, 0.53 mmol) and amine **27** (200 mg, 0.44 mmol) using method F as a white solid (159 mg, 56%). ^{1}H NMR (400 MHz; CDCl3; TMS) δ 12.39 (s, 1H), 8.83 (d, 1H, J = 8.4 Hz), 8.19 (s, 1H), 7.68-7.62 (m, 3H), 7.55 (d, 1H, J = 7.8 Hz), 7.51-7.49 (m, 2H), 7.34 (d, 1H, J = 8.4 Hz), 7.19-7.15 (m, 3H), 5.54 (dd, 1H, J = 8.7 Hz, 5.7 Hz), 3.92 (s, 3H), 3.85 (s, 6H), 2.54–2.46 (m, 1H), 1.10 (d, 3H, J = 6.8 Hz), 1.06 (d, 3H, J = 6.8 Hz). m/z (ESI-MS) 652.2

$(C_{31}H_{30}BrN_3O_6S$ requires 652.11, [M + H]$^+$). HPLC t_R (acetonitrile/water 60/40) = 14.1 min, purity 98%.

(*S*)-*N*-(2-(4-Azidobenzoyl)phenyl)-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl) thiazole-4-carboxamide (29)

The 4′-bromo-derivative **28** (120 mg, 0.18 mmol) was suspended in a 30 mL mixture of ethanol:water (7:3) under nitrogen atmosphere. Sodium hydroxide (2 mg, 0.05 mmol), copper iodide (4 mg, 0.02 mmol), (*S*)-proline (6 mg, 0.05 mmol) and sodium azide (23 mg, 0.36 mmol) were added to the solution and refluxed for 24 h. Afterwards, the reaction solution was cooled, concentrated and partitioned with ethyl acetate. The ethyl acetate layer was dried over anhydrous MgSO$_4$ and concentrated to give crude mass, which was purified by preparative TLC (*n*-hexane/ethyl acetate - 70/30) to yield compound **29** (30 mg, 27%) as a white solid. ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 12.35 (d, 1H, *J* = 33.9 Hz), 8.81 (dd, 1H, *J* = 12.8 Hz, 8.4 Hz), 8.19 (d, 1H, *J* = 2.6 Hz), 7.68-7.61 (m, 3H), 7.55 (dd, 1H, *J* = 7.8 Hz, 4.4 Hz), 7.50 (d, 1H, *J* = 8.3 Hz), 7.37-7.32 (m, 1H), 7.19 (d, 2H, *J* = 4.1 Hz), 7.17-7.14 (m, 1H), 7.10 (d, 1H, *J* = 8.6 Hz), 5.56-5.52 (m, 1H), 3.92 (s, 3H), 3.85-3.84 (m, 6H), 2.49 (oct, 1H, *J* = 6.4 Hz), 1.10 (d, 3H, *J* = 6.8 Hz), 1.06 (d, 3H, *J* = 6.8 Hz). m/z (ESI-MS) 615.3 $(C_{31}H_{30}N_6O_6S$ requires 615.19, [M + H]$^+$). HPLC t_R (acetonitrile/water 60/40) = 18.0 min, purity 98%.

Tert-butyl (*S*)-(3-((1-(4-((2-benzoylphenyl)carbamoyl)thiazol-2-yl)-2-methylpropyl) carbamoyl)phenyl)carbamate (30)

Compound **30** was obtained from acid **12** (100 mg, 0.42 mmol) and amine **26** (190 mg, 0.50 mmol) using method A as a white solid (110 mg, 44%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 12.44 (s, 1H), 8.82 (d, 1H, *J* = 8.2 Hz), 8.19 (s, 1H), 7.81 (bs, 1H), 7.75-7.70 (m, 5H), 7.60-7.57 (m, 3H), 7.38-7.34 (m, 2H), 7.14 (t, 1H, *J* = 7.5 Hz), 7.03 (t, 1H, *J* = 8.0 Hz), 6.98 (d, 1H, *J* = 7.4 Hz), 5.53 (dd, 1H, *J* = 8.5 Hz, 5.3 Hz), 2.44 (oct, 1H, *J* = 6.5 Hz), 1.44 (s, 9H), 1.04 (d, 3H, *J* = 6.8 Hz), 1.01 (d, 3H, *J* = 6.8 Hz).

(*S*)-2-(1-(3-Aminobenzamido)-2-methylpropyl)-*N*-(2-benzoylphenyl)thiazole-4-carboxamide (31)

Compound **31** was obtained from *N*-Boc compound **30** (40 mg, 0.07 mmol) in 4 h using method B as a white solid (30 mg, 90%). mp 146–150 °C. ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 12.60 (s, 1H), 8.89 (d, 1H, *J* = 8.6 Hz), 8.18 (s, 1H), 7.76 (d, 2H, *J* = 7.3 Hz), 7.68-7.62 (m, 3H), 7.54-7.50 (m, 3H), 7.38-7.34 (m, 2H), 7.16 (t, 1H, *J* = 7.7 Hz), 6.95 (t, 1H, *J* = 7.8 Hz), 6.75 (dd, 1H, *J* = 7.9 Hz, 2.1 Hz), 5.56 (dd, 1H, *J* = 8.6 Hz, 5.3 Hz), 3.69-3.66 (m, 2H), 2.45 (oct, 1H, *J* = 6.3 Hz), 1.08 (d, 3H, *J* = 6.8 Hz), 1.05 (d, 3H, *J* = 6.8 Hz). ^{13}C NMR (100 MHz; CDCl$_3$; TMS) δ 199.28, 169.50, 166.87, 159.68, 149.89, 140.14, 138.94, 134.92, 134.29, 133.75, 132.32, 129.93, 129.43, 128.36, 124.05, 123.90, 122.41, 121.38, 118.24, 117.15, 116.09, 114.21, 56.22, 34.38, 18.91, 18.19. m/z (ESI-MS) 499.2 $(C_{28}H_{26}N_4O_3S$ requires 499.18, [M + H]$^+$). HPLC t_R (acetonitrile/water 60/40) = 5.6 min, purity 99%. Anal. Calcd for $C_{28}H_{26}N_4O_3S \cdot 0.3$ H$_2$O: C, 66.73; H, 5.32; N, 11.12. Found: C, 67.02; H, 4.99; N, 10.75.

(S)-2-(1-(2-Azidobenzamido)-2-methylpropyl)-N-(2-benzoylphenyl)thiazole-4-carboxamide (32)

Compound **32** was obtained from acid **13** (50 mg, 0.31 mmol) and amine **26** (139 mg, 0.37) using method A as a white solid (5 mg, 3%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 12.32 (s, 1H), 8.80 (d, 1H, J = 8.4 Hz), 8.48 (d, 1H, J = 8.4 Hz), 8.17-8.15 (m, 2H), 7.67-7.44 (m, 9H), 7.14-7.10 (m, 2H), 5.55 (dd, 1H, J = 8.6 Hz, 5.3 Hz), 2.61-2.54 (m, 1H), 1.09 (d, 6H, J = 6.6 Hz). m/z (ESI-MS) 525.2 (C$_{28}$H$_{24}$N$_6$O$_3$S requires 525.16, [M + H]$^+$). HPLC t_R (acetonitrile/water 60/40) = 15.8 min, purity 98%.

(S)-2-(1-(3-Azidobenzamido)-2-methylpropyl)-N-(2-benzoylphenyl)thiazole-4-carboxamide (33)

Compound **33** was obtained from acid **14** (50 mg, 0.31 mmol) and amine **26** (139 mg, 0.37) using method A as a white solid (15 mg, 9%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 12.79 (s, 1H), 8.90 (d, 1H, J = 8.4 Hz), 8.20-8.18 (m, 1H), 7.78 (d, 1H, J = 7.4 Hz), 7.72-7.62 (m, 7H), 7.52 (t, 2H, J = 7.4 Hz), 7.16 (t, 1H, J = 7.6 Hz), 7.07 (d, 1H, J = 7.8 Hz), 7.00 (d, 1H, J = 7.6 Hz), 5.57 (dd, 1H, J = 8.6 Hz, 5.1 Hz), 2.44 (oct, 1H, J = 6.3 Hz), 1.09 (d, 3H, J = 6.8 Hz), 1.05 (d, 3H, J = 6.8 Hz). m/z (ESI-MS) 525.2 (C$_{28}$H$_{24}$N$_6$O$_3$S requires 525.16, [M + H]$^+$). HPLC t_R (acetonitrile/water 60/40) = 16.9 min, purity 98%.

(S)-2-(1-(4-Azidobenzamido)-2-methylpropyl)-N-(2-benzoylphenyl)thiazole-4-carboxamide (34)

Compound **34** was obtained from acid **15** (50 mg, 0.31 mmol) and amine **26** (139 mg, 0.37) using method A as a white solid (50 mg, 31%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 12.96 (s, 1H), 8.94 (d, 1H, J = 8.3 Hz), 8.19 (s, 1H), 8.03 (d, 2H, J = 8.6 Hz), 7.75-7.64 (m, 6H), 7.57 (t, 2H, J = 7.5 Hz), 7.15 (t, 1H, J = 7.6 Hz), 6.69 (d, 2H, J = 8.6 Hz), 5.59 (dd, 1H, J = 8.5 Hz, 5.1 Hz), 2.41 (oct, 1H, J = 6.8 Hz), 1.10 (d, 3H, J = 6.8 Hz), 1.06 (d, 3H, J = 6.8 Hz). m/z (ESI-MS) 525.2 (C$_{28}$H$_{24}$N$_6$O$_3$S requires 525.16, [M + H]$^+$). HPLC t_R (acetonitrile/water 60/40) = 15.1 min, purity 98%.

(S)-2-(1-(4-Azidobenzamido)-2-methylpropyl)-N-(2-(4-bromobenzoyl)phenyl)thiazole-4-carboxamide (35)

Compound **35** was obtained from acid **15** (50 mg, 0.31 mmol) and amine **27** (170 mg, 0.37) using method A as a white solid (40 mg, 22%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 12.71 (s, 1H), 8.89 (d, 1H, J = 8.4 Hz), 8.18 (s, 1H), 8.00 (d, 2H, J = 8.6 Hz), 7.68-7.63 (m, 4H), 7.58-7.55 (m, 3H), 7.14 (t, 1H, J = 7.6 Hz), 6.80 (d, 2H, J = 8.5 Hz), 5.55 (dd, 1H, J = 8.4 Hz, 5.1 Hz), 2.41 (oct, 1H, J = 6.2 Hz), 1.07 (d, 3H, J = 6.8 Hz), 1.03 (d, 3H, J = 6.8 Hz). m/z (ESI-MS) 603.1 (C$_{28}$H$_{23}$BrN$_6$O$_3$S requires 603.07, [M + H]$^+$). HPLC t_R (acetonitrile/water 70/30) = 12.0 min, purity 98%.

(S)-N-Cyclopropyl-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carboxamide (36)

Compound **36** was obtained from acid **6** (100 mg, 0.25 mmol) and cyclopropanamine (17 mg, 0.30) using method A as a white solid (6 mg, 5%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.90 (d, 1H, J = 7.9 Hz), 8.22 (d, 1H, J = 3.9 Hz), 8.16 (s, 1H), 7.21 (s, 2H),

5.15-5.11 (m, 1H), 3.84 (s, 6H), 3.71 (s, 3H), 2.47-2.41 (m, 1H), 1.28-1.22 (m, 1H), 1.03 (d, 3H, $J = 6.5$ Hz), 0.91 (d, 3H, $J = 6.5$ Hz), 0.70-0.60 (m, 4H). m/z (ESI-MS) 434.2 ($C_{21}H_{27}N_3O_5S$ requires 434.17, $[M + H]^+$). HPLC t_R (acetonitrile/water 50/50) = 2.3 min, purity 96%.

(S)-N-Cyclobutyl-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carboxamide (37)

Compound **37** was obtained from acid **6** (100 mg, 0.25 mmol) and cyclobutanamine (22 mg, 0.30 mmol) using method A as a white solid (15 mg, 13%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.04 (s, 1H), 7.35 (d, 1H, $J = 7.7$ Hz), 7.06 (s, 2H), 6.61 (d, 1H, $J = 8.7$ Hz), 5.40 (dd, 1H, $J = 8.8$ Hz, 6.3 Hz), 4.59 (oct, 1H, $J = 8.6$ Hz), 3.96 (s, 6H), 3.92 (s, 3H), 2.55-2.40 (m, 3H), 2.09-1.98 (m, 2H), 1.83-1.77 (m, 2H), 1.09-1.06 (m, 6H). m/z (ESI-MS) 448.3 ($C_{22}H_{29}N_3O_5S$ requires 448.19, $[M + H]^+$). HPLC t_R (acetonitrile/water 50/50) = 3.4 min, purity 100%.

(S)-N-Cyclopentyl-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carboxamide (38)

Compound **38** was obtained from acid **6** (100 mg, 0.25 mmol) and cyclopentanamine (26 mg, 0.30 mmol) using method A as a white solid (22 mg, 19%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.04 (s, 1H), 7.17 (d, 1H, $J = 7.6$ Hz), 7.05 (s, 2H), 6.60 (d, 1H, $J = 8.8$ Hz), 5.40 (dd, 1H, $J = 8.8$ Hz, 6.5 Hz), 4.40 (oct, 1H, $J = 7.2$ Hz), 3.95 (s, 6H), 3.92 (s, 3H), 2.56-2.44 (m, 1H), 2.15-2.06 (m, 2H), 1.79-1.64 (m, 4H), 1.57-1.50 (m, 2H), 1.08-1.05 (m, 6H). m/z (ESI-MS) 462.2 ($C_{23}H_{31}N_3O_5S$ requires 462.20, $[M + H]^+$). HPLC t_R (acetonitrile/water 50/50) = 4.7 min, purity 96%.

(S)-N-Cyclohexyl-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carboxamide (39)

Compound **39** was obtained from acid **6** (100 mg, 0.25 mmol) and cyclohexanamine (30 mg, 0.30 mmol) using method A as a white solid (30 mg, 25%). mp 87–89 °C. ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.94 (d, 1H, $J = 8.3$ Hz), 8.17 (s, 1H), 7.88 (d, 1H, $J = 8.4$ Hz), 7.22 (s, 2H), 5.13 (t, 1H, $J = 8.4$ Hz), 3.85 (s, 6H), 3.77 (bs, 1H), 3.71 (s, 3H), 2.50–2.40 (m, 1H), 1.81-1.78 (m, 2H), 1.73-1.70 (m, 2H), 1.61-1.58 (m, 1H), 1.42-1.25 (m, 4H), 1.19-1.09 (m, 1H), 1.04 (d, 3H, $J = 6.8$ Hz), 0.92 (d, 3H, $J = 6.8$ Hz). ^{13}C NMR (100 MHz; CDCl$_3$; TMS) δ 171.49, 167.02, 159.84, 153.33, 149.65, 141.46, 129.25, 123.04, 104.75, 60.94, 56.89, 56.45, 48.40, 33.29, 33.04, 25.50, 24.87, 19.60, 18.31. m/z (ESI-MS) 476.3 ($C_{24}H_{33}N_3O_5S$ requires 476.21, $[M + H]^+$). HPLC t_R (acetonitrile/water 60/40) = 3.0 min, purity 99%. Anal. Calcd for $C_{24}H_{33}N_3O_5S \cdot 0.45$ H_2O: C, 59.59; H, 7.06; N, 8.69. Found: C, 59.75; H, 7.04; N, 8.48.

(S)-N-(Adamantan-1-yl)-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carboxamide (40)

Compound **40** was obtained from acid **6** (100 mg, 0.25 mmol) and adamantan-1-amine (46 mg, 0.30 mmol) using method A as a white solid (40 mg, 30%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.92 (d, 1H, $J = 8.2$ Hz), 8.11 (s, 1H),7.21 (s, 3H), 5.11-5.07 (m, 1H),

3.83 (s, 6H), 3.70 (s, 3H), 2.45-2.38 (m, 1H), 2.05 (s, 9H), 1.66 (s, 6H), 1.04 (d, 3H, J = 6.5 Hz), 0.92 (d, 3H, J = 6.5 Hz). m/z (ESI-MS) 528.3 ($C_{28}H_{37}N_3O_5S$ requires 528.25, [M + H]$^+$). HPLC t_R (acetonitrile/water 60/40) = 8.4 min, purity 99%.

Tert-butyl (*S*)-4-(2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carboxamido)piperidine-1-carboxylate (41)

Compound **41** was obtained from acid **6** (200 mg, 0.51 mmol) and *tert*-butyl 4-aminopiperidine-1-carboxylate (122 mg, 0.61 mmol) using method A as a white solid (50 mg, 34%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 7.78 (s, 1H), 7.06 (s, 2H), 6.79 (d, 1H, J = 8.8 Hz), 5.43 (dd, 1H, J = 8.8 Hz, 6.2 Hz), 4.63-4.52 (m, 2H), 4.30-4.23 (m, 1H), 3.94 (s, 6H), 3.91 (s, 3H), 3.76-3.66 (m, 2H), 3.21-3.17 (m, 1H), 3.00-2.95 (m, 1H), 2.50-2.46 (m, 1H), 2.06-1.93 (m, 3H), 1.46 (s, 9H), 1.06-1.03 (m, 6H). m/z (ESI-MS) 577.3 ($C_{28}H_{40}N_4O_7S$ requires 577.26, [M + H]$^+$). HPLC t_R (acetonitrile/water 60/40) = 3.1 min, purity 95%.

(*S*)-2-(2-Methyl-1-(3,4,5-trimethoxybenzamido)propyl)-*N*-(piperidin-4-yl)thiazole-4-carboxamide (42)

Compound **42** was obtained from *N*-Boc compound **41** (30 mg, 0.05 mmol) using method B (12 h) as a white solid (24 mg, 97%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.04 (s, 1H), 7.16 (d, 1H, J = 8.4 Hz), 7.06 (s, 2H), 6.65 (d, 1H, J = 8.7 Hz), 5.39 (dd, 1H, J = 8.7 Hz, 6.6 Hz), 4.12-4.00 (m, 1H), 3.95 (s, 6H), 3.92 (s, 3H), 3.13 (d, 2H, J = 11.8 Hz), 2.77 (t, 2H, J = 11.8 Hz), 2.57-2.47 (m, 1H), 2.07-2.06 (m, 2H), 1.53-1.41 (m, 2H), 1.08 (d, 3H, J = 6.8 Hz), 1.06 (d, 3H, J = 6.8 Hz). m/z (ESI-MS) 477.3 ($C_{23}H_{32}N_4O_5S$ requires 477.21, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 3.3 min, purity 99%.

(*S*)-2-(2-Methyl-1-(3,4,5-trimethoxybenzamido)propyl)-*N*-(tetrahydro-2*H*-pyran-4-yl) thiazole-4-carboxamide (43)

Compound **43** was obtained from acid **6** (200 mg, 0.51 mmol) and tetrahydro-2*H*-pyran-4-amine (62 mg, 0.61 mmol) using method A as a white solid (100 mg, 41%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.05 (s, 1H), 7.16 (d, 1H, J = 8.2 Hz), 7.05 (s, 2H), 6.61 (d, 1H, J = 8.8 Hz), 5.39 (dd, 1H, J = 8.8 Hz, 6.5 Hz), 4.24-4.14 (m, 1H), 4.02 (d, 2H, J = 11.9 Hz), 3.95 (s, 6H), 3.92 (s, 3H), 3.56 (t, 2H, J = 11.9 Hz), 2.56-2.46 (m, 1H), 2.02-1.99 (m, 2H), 1.64-1.57 (m, 2H), 1.08 (d, 3H, J = 6.8 Hz), 1.06 (d, 3H, J = 6.8 Hz). m/z (ESI-MS) 478.3 ($C_{23}H_{31}N_3O_6S$ requires 478.19, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 1.9 min, purity 99%.

(*S*)-3,4,5-Trimethoxy-*N*-(2-methyl-1-(4-(4-oxopiperidine-1-carbonyl)thiazol-2-yl)propyl) benzamide (44)

Compound **44** was obtained from acid **6** (100 mg, 0.25 mmol) and 4-piperidone monohydrate hydrochloride (47 mg, 0.30 mmol) using method A as a white solid (40 mg, 33%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 7.93 (s, 1H), 7.02 (s, 2H), 6.65 (d, 1H, J = 8.7 Hz), 5.42 (dd, 1H, J = 8.6 Hz, 6.1 Hz), 4.09-4.03 (m, 4H), 3.92 (s, 6H), 3.89 (s, 3H), 2.57-2.45 (m, 5H), 1.05 (dd, 6H, J = 6.6 Hz, 1.3 Hz). m/z (ESI-MS) 476.2 ($C_{23}H_{29}N_3O_6S$ requires 476.18, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 1.8 min, purity 99%.

Tert-butyl (*S*)-4-((2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carboxamido)methyl)piperidine-1-carboxylate (45)

Compound **45** was obtained from acid **6** (200 mg, 0.51 mmol) and *tert*-butyl 4-(aminomethyl)piperidine-1-carboxylate (130 mg, 0.61 mmol) using method A as a white solid (70 mg, 23%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.05 (s, 1H), 7.38-7.35 (m, 1H), 7.05 (s, 2H), 6.56 (d, 1H, *J* = 8.8 Hz), 5.40 (dd, 1H, *J* = 8.8 Hz, 6.4 Hz), 3.95 (s, 3H), 3.93 (s, 6H), 3.37-3.33 (m, 4H), 2.74-2.67 (m, 2H), 2.54-2.51 (m, 1H), 1.77-1.74 (m, 3H), 1.61 (s, 9H), 1.27-1.17 (m, 2H), 1.08-1.05 (m, 6H).

(*S*)-2-(2-Methyl-1-(3,4,5-trimethoxybenzamido)propyl)-*N*-(piperidin-4-ylmethyl)thiazole-4-carboxamide (46)

Compound **46** was obtained from *N*-Boc compound **45** (70 mg, 0.12 mmol) using method B (12 h) as a white solid (55 mg, 95%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.04 (s, 1H), 7.37-7.34 (m, 1H), 7.05 (s, 2H), 6.65 (d, 1H, *J* = 8.8 Hz), 5.39 (dd, 1H, *J* = 8.8 Hz, 6.4 Hz), 3.94 (s, 6H), 3.91 (s, 3H), 3.37-3.33 (m, 2H), 3.11 (d, 2H, *J* = 11.9 Hz), 2.61 (t, 2H, *J* = 11.9 Hz), 2.51 (oct, 1H, *J* = 6.7 Hz), 1.78-1.74 (m, 3H), 1.29-1.19 (m, 2H), 1.08-1.05 (m, 6H). m/z (ESI-MS) 491.3 (C$_{24}$H$_{34}$N$_4$O$_5$S requires 491.22, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 3.8 min, purity 99%.

Tert-butyl (*S*)-(1-(2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carbonyl)piperidin-4-yl)carbamate (47)

Compound **47** was obtained from acid **6** (200 mg, 0.51 mmol) and *tert*-butyl piperidin-4-ylcarbamate (122 mg, 0.61 mmol) using method A as a white solid (30 mg, 10%). The coupled product was carried forward without characterization.

(*S*)-*N*-(1-(4-(4-Aminopiperidine-1-carbonyl)thiazol-2-yl)-2-methylpropyl)-3,4,5-trimethoxybenzamide (48)

Compound **48** was obtained from *N*-Boc compound **47** (20 mg, 0.04 mmol) using method B (12 h) as a white solid (16 mg, 97%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 7.76 (s, 1H), 7.06 (s, 2H), 6.84 (d, 1H, *J* = 8.7 Hz), 5.43 (dd, 1H, *J* = 8.8 Hz, 6.2 Hz), 4.61-4.57 (m, 1H), 4.24-4.20 (m, 1H), 3.94 (s, 6H), 3.91 (s, 3H), 3.18-3.11 (m, 1H), 3.03-2.94 (m, 2H), 2.49 (oct, 1H, *J* = 6.7 Hz), 1.98-1.77 (m, 2H), 1.46-1.36 (m, 2H), 1.06-1.03 (m, 6H). m/z (ESI-MS) 477.3 (C$_{23}$H$_{32}$N$_4$O$_5$S requires 477.21, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 2.3 min, purity 99%.

Tert-butyl ((1*S*,4*R*)-4-((2-((*S*)-2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carboxamido)methyl)cyclohexyl)carbamate (49)

Compound **49** was obtained from acid **6** (200 mg, 0.51 mmol) and *trans*-*tert*-butyl 4-(aminomethyl)cyclohexyl)carbamate (139 mg, 0.61 mmol) using method A as a white solid (25 mg, 8%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.04 (s, 1H), 7.36-7.33 (m, 1H), 7.04 (s, 2H), 6.57 (d, 1H, *J* = 8.8 Hz), 5.40 (dd, 1H, *J* = 8.8 Hz, 6.4 Hz), 3.95 (s, 6H), 3.93 (s, 3H), 3.41-3.28 (m, 5H), 2.55-2.45 (m, 1H), 2.06-2.03 (m, 4H), 1.59 (s, 9H), 1.30-1.26 (m, 4H), 1.09-1.05 (m, 6H).

N-(((1*R*,4*S*)-4-Aminocyclohexyl)methyl)-2-((*S*)-2-methyl-1-(3,4,5-trimethoxybenzamido) propyl)thiazole-4-carboxamide (50)

Compound **50** was obtained from *N*-Boc compound **49** (25 mg, 0.04 mmol) using method B (12 h) as a white solid (20 mg, 96%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.97 (d, 1H, J = 8.3 Hz), 8.32 (t, 1H, J = 6.3 Hz), 8.16 (s, 1H), 7.82-7.72 (m, 2H), 7.22 (s, 2H), 5.15-5.11 (m, 1H), 3.84 (s, 6H), 3.71 (s, 3H), 3.21-3.04 (m, 2H), 2.97-2.90 (m, 1H), 2.46-2.39 (m, 1H), 1.93 (d, 2H, J = 11.6 Hz), 1.75 (d, 2H, J = 11.6 Hz), 1.55-1.47 (m, 2H), 1.30-1.20 (m, 3H), 1.05 (d, 3H, J = 6.7 Hz), 0.92 (d, 3H, J = 6.7 Hz). m/z (ESI-MS) 505.3 (C$_{25}$H$_{36}$N$_4$O$_5$S requires 505.24, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 2.5 min, purity 99%.

(*S*)-*N*-(1,1-Dioxidotetrahydro-2*H*-thiopyran-4-yl)-2-(2-methyl-1-(3,4,5-trimethoxy benzamido)propyl)thiazole-4-carboxamide (51)

Compound **51** was obtained from acid **6** (100 mg, 0.25 mmol) and 4-aminotetrahydro-2*H*-thiopyran-1,1-dioxide hydrochloride (56 mg, 0.30 mmol) using method A as a white solid (12 mg, 9%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.93 (d, 1H, J = 8.3 Hz), 8.39 (d, 1H, J = 8.3 Hz), 8.20 (s, 1H), 7.21 (s, 2H), 5.16-5.12 (m, 1H), 4.23-4.15 (m, 1H), 3.84 (s, 6H), 3.70 (s, 3H), 3.40-3.27 (m, 2H), 3.09-3.05 (m, 2H), 2.47-2.44 (m, 1H), 2.23-2.04 (m, 4H), 1.03 (d, 3H, J = 6.7 Hz), 0.93 (d, 3H, J = 6.7 Hz). m/z (ESI-MS) 526.2 (C$_{23}$H$_{31}$N$_3$O$_7$S$_2$ requires 526.15, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 1.7 min, purity 96%.

(*S*)-2-(2-Methyl-1-(3,4,5-trimethoxybenzamido)propyl)-*N*-(4-oxocyclohexyl)thiazole-4-carboxamide (52)

Compound **52** was obtained from acid **6** (100 mg, 0.25 mmol) and 4-aminocyclohexan-1-one (34 mg, 0.30 mmol) using method A as a white solid (15 mg, 12%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.94 (d, 1H, J = 8.3 Hz), 8.21 (s, 1H), 8.15 (d, 1H, J = 8.3 Hz), 7.21 (s, 2H), 5.15-5.11 (m, 1H), 4.36-4.26 (m, 1H), 3.83 (s, 6H), 3.70 (s, 3H), 2.60-2.53 (m, 2H), 2.47-2.40 (m, 1H), 2.24-2.21 (m, 2H), 2.08-2.03 (m, 2H), 1.92-1.82 (m, 2H), 1.04 (d, 3H, J = 6.7 Hz), 0.91 (d, 3H, J = 6.7 Hz). m/z (ESI-MS) 490.3 (C$_{24}$H$_{31}$N$_3$O$_6$S requires 490.19, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 2.0 min, purity 96%.

(*S*)-*N*-(4,4-Difluorocyclohexyl)-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl) thiazole-4-carboxamide (53)

Compound **53** was obtained from acid **6** (150 mg, 0.38 mmol) and 4,4-difluorocyclohexan-1-amine (62 mg, 0.46 mmol) using method A as a white solid (85 mg, 44%). mp 183–185 °C. ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.06 (s, 1H), 7.16 (d, 1H, J = 8.2 Hz), 7.04 (s, 2H), 6.59 (d, 1H, J = 8.8 Hz), 5.38 (dd, 1H, J = 8.8 Hz, 6.4 Hz), 4.13-4.05 (m, 1H), 3.94 (s, 6H), 3.91 (s, 3H), 2.51 (oct, 1H, J = 6.6 Hz), 2.18-2.09 (m, 4H), 2.02-1.85 (m, 2H), 1.74-1.62 (m, 2H), 1.08 (t, 3H, J = 6.8 Hz), 1.06 (d, 3H, J = 6.8 Hz). ^{13}C NMR (100 MHz; CDCl$_3$; TMS) δ 171.56, 166.96, 160.24, 153.39, 149.77, 141.60, 129.30, 123.19, 104.75, 60.97, 56.84, 56.52, 46.19, 33.36, 32.25 (t, J = 24.7 Hz), 28.70 (d, J = 8.5 Hz), 19.61, 18.18. m/z (ESI-MS) 512.2 (C$_{24}$H$_{31}$F$_2$N$_3$O$_5$S requires 512.19, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 4.6 min, purity 99%. Anal. Calcd for C$_{24}$H$_{31}$F$_2$N$_3$O$_5$S: C, 56.35; H, 6.11; N, 8.21. Found: C, 56.18; H, 5.95; N, 7.95.

(*S*)-*N*-(4,4-Dimethylcyclohexyl)-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl) thiazole-4-carboxamide (54)

Compound **54** was obtained from acid **6** (100 mg, 0.25 mmol) and 4,4-dimethylcyclohexan-1-amine (39 mg, 0.30 mmol) using method A as a white solid (5 mg, 4%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.02 (s, 1H), 7.11 (d, 1H, J = 8.2 Hz), 7.03 (s, 2H), 6.58 (d, 1H, J = 8.9 Hz), 5.38 (dd, 1H, J = 8.8 Hz, 6.4 Hz), 3.93 (s, 6H), 3.89 (s, 3H), 2.52-2.42 (m, 1H), 1.89-1.83 (m, 2H), 1.45-1.35 (m, 7H), 1.06-1.03 (m, 6H), 0.94 (s, 6H). m/z (ESI-MS) 504.3 (C$_{26}$H$_{37}$N$_3$O$_5$S requires 504.25, [M + H]$^+$). HPLC t_R (acetonitrile/water 60/40) = 6.3 min, purity 99%.

(*S*)-*N*, *N*-Dicyclohexyl-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carboxamide (55)

Compound **55** was obtained from acid **6** (100 mg, 0.25 mmol) and dicyclohexylamine (55 mg, 0.30 mmol) using method A as a white solid (84 mg, 59%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 7.59 (s, 1H), 7.05 (s, 2H), 7.00 (d, 1H, J = 8.6 Hz), 5.40 (dd, 1H, J = 8.9 Hz, 6.3 Hz), 3.90 (s, 6H), 3.88 (s, 3H), 3.83-3.69 (m, 1H), 3.08-2.99 (m, 1H), 2.56-2.40 (m, 3H), 1.80-1.51 (m, 18H), 1.04 (d, 3H, J = 6.8 Hz), 1.01 (d, 3H, J = 6.8 Hz). m/z (ESI-MS) 558.3 (C$_{30}$H$_{43}$N$_3$O$_5$S requires 558.29, [M + H]$^+$). HPLC t_R (acetonitrile/water 60/40) = 14.5 min, purity 99%.

(*S*)-*N*-(1-(4-([1,4′-Bipiperidine]-1′-carbonyl)thiazol-2-yl)-2-methylpropyl)-3,4,5-trimethoxybenzamide (56)

Compound **56** was obtained from acid **6** (100 mg, 0.25 mmol) and 1,4′-bipiperidine (51 mg, 0.30 mmol) using method A as a white solid (13 mg, 9%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.95 (d, 1H, J = 8.3 Hz), 7.96 (s, 1H), 7.23 (s, 2H), 5.11-5.07 (m, 1H), 4.48-4.45 (m, 1H), 4.12-4.03 (m, 1H), 3.83 (s, 6H), 3.70 (s, 3H), 3.05-2.97 (m, 1H), 2.73-2.67 (m, 1H), 2.46-2.40 (m, 6H), 1.81-1.76 (m, 1H), 1.70-1.59 (m, 1H), 1.46-1.36 (m, 8H), 1.04 (d, 3H, J = 6.7 Hz), 0.93 (d, 3H, J = 6.7 Hz). m/z (ESI-MS) 545.3 (C$_{28}$H$_{40}$N$_4$O$_5$S requires 545.27, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 2.7 min, purity 99%.

(*S*)-*N*-(1-Benzylpiperidin-4-yl)-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl) thiazole-4-carboxamide (57)

Compound **57** was obtained from acid **6** (200 mg, 0.51 mmol) and 1-benzylpiperidin-4-amine (**17**, 116 mg, 0.61 mmol) using method A as a white solid (142 mg, 49%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.02 (s, 1H), 7.34-7.33 (m, 5H), 7.14 (d, 1H, J = 8.4 Hz), 7.05 (s, 2H), 6.67 (d, 1H, J = 8.8 Hz), 5.38 (dd, 1H, J = 8.8 Hz, 6.4 Hz), 4.03-3.96 (m, 1H), 3.94 (s, 6H), 3.91 (s, 3H), 3.53 (s, 2H), 2.90-2.86 (m, 2H), 2.49 (oct, 1H, J = 6.6 Hz), 2.18 (t, 2H, J = 11.09 Hz), 2.04-1.99 (m, 2H), 1.66-1.55 (m, 2H), 1.07 (d, 3H, J = 6.8 Hz), 1.05 (d, 3H, J = 6.8 Hz). m/z (ESI-MS) 567.3 (C$_{30}$H$_{38}$N$_4$O$_5$S requires 567.26, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 1.8 min, purity 99%.

(S)-2-(2-Methyl-1-(3,4,5-trimethoxybenzamido)propyl)-N-(1-(pyrimidin-2-yl)piperidin-4-yl)thiazole-4-carboxamide (58)

Compound **58** was obtained from acid **6** (100 mg, 0.25 mmol) and 1-(pyrimidin-2-yl)piperidin-4-amine (54 mg, 0.30 mmol) using method A as a white solid (55 mg, 39%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.92 (d, 1H, J = 8.3), 8.36 (d, 2H, J = 4.7 Hz), 8.18 (s, 1H), 8.12 (d, 1H, J = 8.4 Hz), 7.20 (s, 2H), 6.61 (t, 1H, J = 4.5 Hz), 5.15-5.10 (m, 1H), 4.67 (d, 2H, J = 12.8 Hz), 4.14-4.06 (m, 1H), 3.83 (s, 6H), 3.70 (s, 3H), 2.99 (t, 2H, J = 12.9 Hz), 2.47-2.40 (m, 1H), 1.84-1.81 (m, 2H), 1.61-1.52 (m, 2H), 1.02 (d, 3H, J = 6.7 Hz), 0.91 (d, 3H, J = 6.7 Hz). m/z (ESI-MS) 555.3 ($C_{27}H_{34}N_6O_5S$ requires 555.23, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 3.1min, purity 98%.

2-((S)-2-Methyl-1-(3,4,5-trimethoxybenzamido)propyl)-N-((S)-1,2,3,4-tetrahydronaphthalen-1-yl)thiazole-4-carboxamide (59)

Compound **59** was obtained from acid **6** (100 mg, 0.25 mmol) and (S)-1,2,3,4-tetrahydronaphthalen-1-amine (45 mg, 0.30 mmol) using method A as a white solid (8 mg, 6%). ^{1}H NMR (400 MHz; CDCl₃; TMS) δ 8.10 (s, 1H), 7.47 (d, 1H, J = 8.8 Hz), 7.33-7.32 (m, 1H), 7.22-7.12 (m, 3H), 6.96 (s, 2H), 6.55 (d, 1H, J = 8.8 Hz), 5.42-5.36 (m, 1H), 5.33 (dd, 1H, J = 8.8 Hz, 6.6 Hz), 3.87 (s, 3H), 3.84 (s, 6H), 2.89-2.78 (m, 2H), 2.42 (oct, 1H, J = 6.6 Hz), 2.21-2.14 (m, 1H), 1.94-1.88 (m, 3H), 1.02 (d, 3H, J = 6.7 Hz), 0.99 (d, 3H, J = 6.7 Hz). m/z (ESI-MS) 524.3 ($C_{28}H_{33}N_3O_5S$ requires 524.21, [M + H]$^+$). HPLC t_R (acetonitrile/water 60/40) = 4.9 min, purity 99%.

2-((S)-2-Methyl-1-(3,4,5-trimethoxybenzamido)propyl)-N-((R)-1,2,3,4-tetrahydronaphthalen-1-yl)thiazole-4-carboxamide (60)

Compound **60** was obtained from acid **6** (100 mg, 0.25 mmol) and (R)-1,2,3,4-tetrahydronaphthalen-1-amine (45 mg, 0.30 mmol) using method A as a white solid (22 mg, 17%). mp 97–100 °C. ^{1}H NMR (400 MHz; CDCl₃; TMS) δ 8.10 (s, 1H), 7.47 (d, 1H, J = 8.8 Hz), 7.37-7.34 (m, 1H), 7.22-7.19 (m, 2H), 7.15-7.13 (m, 1H), 6.99 (m, 2H), 6.56 (d, 1H, J = 8.8 Hz), 5.40-5.32 (m, 2H), 3.88 (bs, 9H), 2.85-2.81 (m, 2H), 2.42 (oct, 1H, J = 6.8 Hz), 2.20-2.14 (m, 1H), 1.91-1.84 (m, 3H), 1.02-0.99 (m, 6H). ^{13}C NMR (100 MHz; CDCl₃; TMS) δ 170.96, 166.88, 160.39, 153.35, 150.16, 141.41, 137.69, 136.63, 129.26, 128.72, 127.38, 126.39, 125.30, 123.15, 104.56, 60.96, 56.68, 56.40, 47.52, 33.50, 30.44, 29.32, 20.33, 19.56, 18.08. m/z (ESI-MS) 524.3 ($C_{28}H_{33}N_3O_5S$ requires 524.21, [M + H]$^+$). HPLC t_R (acetonitrile/water 60/40) = 5.1 min, purity 99%. Anal. Calcd for $C_{28}H_{33}N_3O_5S \cdot 0.7\ H_2O$: C, 62.71; H, 6.47; N, 7.84. Found: C, 62.78; H, 6.07; N, 7.44.

(S)-N-(1-(4-(4,4-Difluoropiperidine-1-carbonyl)thiazol-2-yl)-2-methylpropyl)-3,4,5-trimethoxybenzamide (61)

Compound **61** was obtained from acid **6** (100 mg, 0.25 mmol) and 4,4-difluoropiperidine hydrochloride (48 mg, 0.30 mmol) using method A as a white solid (30 mg, 24%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.96 (d, 1H, J = 8.3 Hz), 8.08 (s, 1H), 7.22 (s, 2H), 5.13-5.09 (m, 1H), 3.83 (s, 6H), 3.77-3.73 (m, 4H), 3.70 (s, 3H), 2.47-2.40 (m, 1H), 2.09-1.99 (m, 4H), 1.04 (d, 3H, J = 6.7 Hz), 0.93 (d, 3H, J = 6.7 Hz). m/z (ESI-MS) 498.3

$(C_{23}H_{29}F_2N_3O_5S$ requires 498.18, $[M + H]^+$). HPLC t_R (acetonitrile/water 50/50) = 4.0 min, purity 98%.

(S)-3,4,5-Trimethoxy-N-(2-methyl-1-(4-(4-(trifluoromethyl)piperidine-1-carbonyl)thiazol-2-yl)propyl)benzamide (62)

Compound **62** was obtained from acid **6** (100 mg, 0.25 mmol) and 4-(trifluoromethyl)piperidine hydrochloride (58 mg, 0.30 mmol) using method A as a white solid (30 mg, 22%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.96 (d, 1H, J = 8.3 Hz), 8.02 (s, 1H), 7.23 (s, 2H), 5.13-5.08 (m, 1H), 4.57-4.44 (m, 1H), 4.25-4.15 (m, 1H), 3.84 (s, 6H), 3.70 (s, 3H), 3.13-3.02 (m, 1H), 2.84-2.65 (m, 2H), 2.47-2.42 (m, 1H), 1.92-1.74 (m, 2H), 1.47-1.36 (m, 2H), 1.05 (d, 3H, J = 6.6 Hz), 0.94 (d, 3H, J = 6.6 Hz). m/z (ESI-MS) 530.2 $(C_{24}H_{30}F_3N_3O_5S$ requires 530.19, $[M + H]^+$). HPLC t_R (acetonitrile/water 50/50) = 3.1 min, purity 99%.

(S)-N-((4,4-Difluorocyclohexyl)methyl)-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carboxamide (63)

Compound **63** was obtained from acid **6** (100 mg, 0.25 mmol) and (4,4-difluorocyclohexyl)methanamine hydrochloride (56 mg, 0.30 mmol) using method A as a white solid (25 mg, 19%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.05 (s, 1H), 7.40-7.37 (m, 1H), 7.04 (s, 2H), 6.55 (d, 1H, J = 8.8 Hz), 5.41 (dd, 1H, J = 8.8 Hz, 6.4 Hz), 3.95 (s, 6H), 3.92 (s, 3H), 3.44-3.31 (m, 2H), 2.51 (oct, 1H, J = 6.7 Hz), 2.18-2.09 (m, 2H), 1.89-1.65 (m, 5H), 1.44-1.34 (m, 2H), 1.09-1.06 (m, 6H). m/z (ESI-MS) 526.3 $(C_{25}H_{33}F_2N_3O_5S$ requires 526.21, $[M + H]^+$). HPLC t_R (acetonitrile/water 50/50) = 6.1 min, purity 99%.

2-((S)-2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)-N-((1R,4S)-4-(trifluoromethyl)cyclohexyl)thiazole-4-carboxamide (64)

Compound **64** was obtained from acid **6** (100 mg, 0.25 mmol) and 4-(trifluoromethyl)cyclohexan-1-amine (51 mg, 0.30 mmol) using method A as a white solid (20 mg, 15%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.94 (d, 1H, J = 8.3 Hz), 8.17 (s, 1H), 7.99 (d, 1H, J = 8.3 Hz), 7.21 (s, 2H), 5.15-5.10 (m, 1H), 3.83 (s, 6H), 3.70 (s, 3H), 2.50-2.40 (m, 1H), 2.23-2.20 (m, 1H), 1.91 (d, 4H, J = 10.9 Hz), 1.53-1.33 (m, 4H), 1.26-1.23 (m, 1H), 1.03 (d, 3H, J = 6.7 Hz), 0.91 (d, 3H, J = 6.7 Hz). m/z (ESI-MS) 544.3 $(C_{25}H_{32}F_3N_3O_5S$ requires 544.20, $[M + H]^+$). HPLC t_R (acetonitrile/water 60/40) = 4.1 min, purity 100%.

(S)-N-(4-Fluorophenyl)-2-(2-methyl-1-(3,4,5-trimethoxybenzamido)propyl)thiazole-4-carboxamide (65)

Compound **65** was obtained from acid **6** (100 mg, 0.25 mmol) and 4-fluoroaniline (34 mg, 0.30 mmol) using method A as a white solid (118 mg, 95%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 10.2 (s, 1H), 8.97 (d, 1H, J = 7.7 Hz), 8.38 (s, 1H), 7.85 (s, 2H), 7.24-7.19 (m, 4H), 5.25-5.21 (m, 1H), 3.85 (s, 6H), 3.71 (s, 3H), 2.51-2.48 (m, 1H), 1.06 (d, 3H, J = 5.8 Hz), 0.96 (d, 3H, J = 5.8 Hz). m/z (ESI-MS) 488.2 $(C_{24}H_{26}FN_3O_5S$ requires 488.16, $[M + H]^+$). HPLC t_R (acetonitrile/water 60/40) = 3.7 min, purity 99%.

(*S*)-2-(2-Methyl-1-(3,4,5-trimethoxybenzamido)propyl)-*N*-(3,4,5-trifluorophenyl)thiazole-4-carboxamide (66)

Compound **66** was obtained from acid **6** (100 mg, 0.25 mmol) and 3,4,5-trifluoroaniline (45 mg, 0.30 mmol) using method A as a white solid (100 mg, 75%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 10.56 (s, 1H), 8.97 (d, 1H, *J* = 8.3 Hz), 8.44 (s, 1H), 7.88 (dd, 2H, *J* = 10.5 Hz, 6.7 Hz), 7.23 (s, 2H), 5.25-5.21 (m, 1H), 3.85 (s, 6H), 3.71 (s, 3H), 2.49-2.44 (m, 1H), 1.06 (d, 3H, *J* = 6.7 Hz), 0.95 (d, 3H, *J* = 6.7 Hz). m/z (ESI-MS) 524.2 ($C_{24}H_{24}F_3N_3O_5S$ requires 524.14, [M + H]$^+$). HPLC t_R (acetonitrile/water 60/40) = 6.6 min, purity 100%.

(*S*)-2-(2-Methyl-1-(3,4,5-trimethoxybenzamido)propyl)-*N*-(4,4,4-trifluorobutyl)thiazole-4-carboxamide (67)

Compound **67** was obtained from acid **6** (200 mg, 0.61 mmol) and 4,4,4-trifluorobutan-1-amine hydrochloride (100 mg, 0.46 mmol) using method A as a white solid (145 mg, 57%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.04 (s, 1H), 7.37 (t, 1H, *J* = 5.7 Hz), 7.05 (s, 2H), 6.65 (d, 1H, *J* = 8.7 Hz), 5.39 (dd, 1H, *J* = 8.8 Hz, 6.7 Hz), 3.93 (s, 6H), 3.91 (s, 3H), 3.53 (q, 2H, *J* = 6.7 Hz), 2.49 (oct, 1H, *J* = 6.7 Hz), 2.27-2.15 (m, 2H), 1.91 (quin, 2H, *J* = 7.5 Hz), 1.08-1.04 (m, 6H). m/z (ESI-MS) 504.2 ($C_{22}H_{28}F_3N_3O_5S$ requires 504.17, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 4.8 min, purity 95%.

Tert-butyl (*S*)-(1-(4-((4,4-difluorocyclohexyl)carbamoyl)thiazol-2-yl)-2-methylpropyl)carbamate (68)

Compound **68** was obtained from acid **2** (500 mg, 1.66 mmol) and 4,4-difluorocyclohexan-1-amine (270 mg, 2.00 mmol) using method A as a white solid (464 mg, 67%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.17 (d, 1H, *J* = 8.6 Hz), 8.14 (s, 1H), 7.69 (d, 1H, *J* = 8.6 Hz), 4.69-4.66 (m, 1H), 3.99-3.94 (m, 1H), 2.29-2.20 (m, 1H), 2.04-2.01 (m, 2H), 1.91-1.70 (m, 6H), 1.40 (s, 9H), 0.89-0.86 (m, 6H).

(*S*)-*N*-(4,4-Difluorocyclohexyl)-2-(2-methyl-1-(thiazole-4-carboxamido)propyl)thiazole-4-carboxamide (69)

Compound **69** was obtained from thiazole-4-carboxylic acid (100 mg, 0.77 mmol) and amine **77** (295 mg, 0.93 mmol) using method A as a yellow solid (20 mg, 6%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 9.24-9.23 (m, 1H), 8.89 (d, 1H, *J* = 8.9 Hz), 8.40-8.39 (m, 1H), 8.23 (d, 1H, *J* = 8.4 Hz), 8.17 (s, 1H), 5.16-5.12 (m, 1H), 4.00-3.93 (m, 1H), 2.49-2.42 (m, 1H), 2.02-1.67 (m, 8H), 0.97 (d, 3H, *J* = 6.7 Hz), 0.87 (d, 3H, *J* = 6.7 Hz). m/z (ESI-MS) 429.2 ($C_{18}H_{22}F_2N_4O_2S_2$ requires 429.12, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 3.5 min, purity 97%.

(*S*)-*N*-(4,4-Difluorocyclohexyl)-2-(2-methyl-1-(pyrimidine-5-carboxamido)propyl) thiazole-4-carboxamide (70)

Compound **70** was obtained from pyrimidine-5-carboxylic acid (100 mg, 0.81 mmol) and amine **77** (307 mg, 0.97 mmol) using method A as a gray solid (10 mg, 3%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 9.39 (d, 1H, *J* = 8.1 Hz), 9.35 (s, 1H), 9.19 (s, 2H), 8.21-8.19 (m, 2H), 5.22-5.18 (m, 1H), 3.98-3.96 (m, 1H), 2.47-2.39 (m, 1H), 2.03-1.67 (m, 8H), 1.03 (d,

3H, J = 6.7 Hz), 0.93 (d, 3H, J = 6.7 Hz). m/z (ESI-MS) 424.2 ($C_{19}H_{23}F_2N_5O_2S$ requires 424.15, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 1.9 min, purity 98%.

(S)-N-(4,4-Difluorocyclohexyl)-2-(2-methyl-1-(quinoline-3-carboxamido)propyl)thiazole-4-carboxamide (71)

Compound **71** was obtained from quinolone-3-carboxylic acid (100 mg, 0.58 mmol) and amine **77** (220 mg, 0.69 mmol) using method A as a white solid (60 mg, 22%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 9.38 (d, 1H, J = 8.2 Hz), 9.28 (d, 1H, J = 2.1 Hz), 8.89 (s, 1H), 8.23-8.21 (m, 2H), 8.14 (d, 1H, J = 8.0 Hz), 8.11 (d, 1H, J = 8.3 Hz), 7.89 (t, 1H, J = 7.5 Hz), 7.72 (t, 1H, J = 7.5 Hz), 5.28-5.24 (m, 1H), 4.02-3.95 (m, 1H), 2.49-2.44 (m, 1H), 2.06-1.69 (m, 8H), 1.07 (d, 3H, J = 6.7 Hz), 0.96 (d, 3H, J = 6.7 Hz). m/z (ESI-MS) 473.3 ($C_{24}H_{26}F_2N_4O_2S$ requires 473.17, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 4.3 min, purity 97%.

(S)-N-(4,4-Difluorocyclohexyl)-2-(2-methyl-1-(tetrahydro-2H-pyran-4-carboxamido)propyl)thiazole-4-carboxamide (72)

Compound **72** was obtained from tetrahydro-2H-pyran-4-carboxylic acid (100 mg, 0.77 mmol) and amine **77** (293 mg, 0.92 mmol) using method A as a yellow solid (35 mg, 11%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.45 (d, 1H, J = 8.3 Hz), 8.17 (d, 1H, J = 8.2 Hz), 8.14 (s, 1H), 4.99 (dd, 1H, J = 8.8 Hz, 6.5 Hz), 3.97-3.86 (m, 3H), 3.31-3.28 (m, 1H), 2.64-2.56 (m, 1H), 2.33 (oct, 1H, J = 6.8 Hz), 2.03-1.55 (m, 13H), 0.89 (dd, 6H, J =6.9 Hz, 2.0 Hz). m/z (ESI-MS) 430.2 ($C_{20}H_{29}F_2N_3O_3S$ requires 430.19, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 2.2 min, purity 97%.

(S)-2-(1-(4,4-Difluorocyclohexane-1-carboxamido)-2-methylpropyl)-N-(4,4-difluorocyclohexyl)thiazole-4-carboxamide (73)

Compound **73** was obtained from 4,4-difluorocyclohexane carboxylic acid (100 mg, 0.61 mmol) and amine **77** (232 mg, 0.73 mmol) using method A as a white solid (100 mg, 30%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.03 (s, 1H), 7.12 (d, 1H, J = 8.2 Hz), 6.02 (d, 1H, J = 8.8 Hz), 5.21 (dd, 1H, J = 8.8 Hz, 6.1 Hz), 4.13-4.06 (m, 1H), 2.42-2.30 (m, 2H), 2.26-2.10 (m, 6H), 2.01-1.65 (m, 10H), 0.98 (dd, 6H, J = 6.8 Hz, 5.3 Hz). m/z (ESI-MS) 464.2 ($C_{21}H_{29}F_4N_3O_2S$ requires 464.19, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 5.3 min, purity 95%.

(S)-N-(4,4-Difluorocyclohexyl)-2-(1-(3,4-dimethoxybenzamido)-2-methylpropyl)thiazole-4-carboxamide (74)

Compound **74** was obtained from 3,4-dimethoxybenzoyl chloride (76 mg, 0.38 mmol) and amine **77** (100 mg, 0.32 mmol) using method F as a white solid (16 mg, 11%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.84 (d, 1H, J = 8.3 Hz), 8.19-8.17 (m, 2H), 7.56 (d, 1H, J = 8.6 Hz), 7.45 (s, 1H), 7.04 (d, 1H, J = 8.6 Hz), 5.16-5.12 (m, 1H), 4.02-3.93 (m, 1H), 3.82 (s, 3H, 3.81 (s, 3H), 2.47-2.40 (m, 1H), 2.04-1.68 (m, 8H), 1.02 (d, 3H, J = 6.6 Hz), 0.91 (d, 3H, J = 6.6 Hz). m/z (ESI-MS) 482.2 ($C_{23}H_{29}F_2N_3O_4S$ requires 482.18, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 3.9 min, purity 99%.

(*S*)-*N*-(4,4-Difluorocyclohexyl)-2-(1-(4-methoxybenzamido)-2-methylpropyl)thiazole-4-carboxamide (75)

Compound **75** was obtained from 4-methoxybenzoyl chloride (0.05 mL, 0.38 mmol) and amine **77** (100 mg, 0.32 mmol) using method F as a white solid (30 mg, 21%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.84 (d, 1H, *J* = 8.3 Hz), 8.19-8.16 (m, 2H), 7.88 (d, 2H, *J* = 8.7 Hz), 7.02 (d, 2H, *J* = 8.7 Hz), 5.16-5.12 (m, 1H), 4.01-3.92 (m, 1H), 3.82 (s, 3H), 2.45-2.38 (m, 1H), 2.03-1.67 (m, 8H), 1.01 (d, 3H, *J* = 6.5 Hz), 0.91 (d, 3H, *J* = 6.5 Hz). m/z (ESI-MS) 452.2 ($C_{22}H_{27}F_2N_3O_3S$ requires 452.17, $[M + H]^+$). HPLC t_R (acetonitrile/water 50/50) = 5.3 min, purity 98%.

(*S*)-2-(1-Benzamido-2-methylpropyl)-*N*-(4,4-difluorocyclohexyl)thiazole-4-carboxamide (76)

Compound **76** was obtained from benzoyl chloride (0.04 mL, 0.38 mmol) and amine **77** (100 mg, 0.32 mmol) using method F as a white solid (30 mg, 23%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 9.01 (d, 1H, *J* = 8.3 Hz), 8.20-8.18 (m, 2H), 7.88 (d, 2H, *J* = 7.3), 7.58-7.48 (m, 3H), 5.18-5.14 (m, 1H), 3.99-3.96 (m, 1H), 2.43 (oct, 1H, *J* = 6.8 Hz), 2.03-1.68 (m, 8H), 1.02 (d, 3H, *J* = 6.5 Hz), 0.92 (d, 3H, *J* = 6.5 Hz). m/z (ESI-MS) 422.2 ($C_{21}H_{25}F_2N_3O_2S$ requires 422.16, $[M + H]^+$). HPLC t_R (acetonitrile/water 50/50) = 5.2 min, purity 98%.

(*S*)-2-(1-Amino-2-methylpropyl)-*N*-(4,4-difluorocyclohexyl)thiazole-4-carboxamide (77)

Compound **77** was obtained from *N*-Boc compound **68** (240 mg, 0.57 mmol) using method B (12 h) as a white solid (174 mg, 95%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.13 (d, 1H, *J* = 8.3 Hz), 8.09 (s, 1H), 4.00-3.92 (m, 2H), 2.26-2.15 (m, 3H), 2.02-1.66 (m, 8H), 0.94 (d, 3H, *J* = 6.9 Hz), 0.78 (d, 3H, *J* = 6.9 Hz). ^{13}C NMR (100 MHz; DMSO-d$_6$; TMS) δ 179.81, 160.72, 150.12, 123.63, 58.88, 46.05, 34.31, 32.39 (t, *J* = 24.0 Hz), 28.36 (d, *J* = 9.5 Hz), 19.80, 16.87. m/z (ESI-MS) 318.2 ($C_{14}H_{21}F_2N_3OS$ requires 318.14, $[M + H]^+$). HPLC t_R (acetonitrile/water 50/50) = 3.3 min, purity 99%.

(*S*)-*N*-(4,4-Difluorocyclohexyl)-2-(2-methyl-1-(3,4,5-trifluorobenzamido)propyl)thiazole-4-carboxamide (78)

Compound **78** was obtained from 3,4,5-trifluorobenzoyl chloride (0.05 mL, 0.38 mmol) and amine **77** (100 mg, 0.32 mmol) using method F as a white solid (14 mg, 9%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 9.17 (d, 1H, *J* = 8.3 Hz), 8.20-8.18 (m, 2H), 7.88 (t, 2H, *J* = 7.6 Hz), 5.16-5.12 (m, 1H), 4.02-3.94 (m, 1H), 2.45-2.39 (m, 1H), 2.04-1.67 (m, 8H), 1.02 (d, 3H, *J* = 6.7 Hz), 0.91 (d, 3H, *J* = 6.7 Hz). m/z (ESI-MS) 476.3 ($C_{21}H_{22}F_5N_3O_2S$ requires 476.14, $[M + H]^+$). HPLC t_R (acetonitrile/water 50/50) = 12.1 min, purity 95%.

(*S*)-*N*-(4,4-Difluorocyclohexyl)-2-(2-methyl-1-(2-(3,4,5-trimethoxyphenyl)acetamido)propyl)thiazole-4-carboxamide (79)

Compound **79** was obtained from 2-(3,4,5-trimethoxyphenyl)acetic acid (100 mg, 0.44 mmol) and amine **77** (168 mg, 0.53 mmol) using method A as a white solid (100 mg, 43%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.73 (d, 1H, *J* = 8.7 Hz), 8.19-8.16 (m, 2H), 6.59 (s, 2H), 4.99 (dd, 1H, *J* = 8.7 Hz, 6.5 Hz), 4.00-3.91 (m, 1H), 3.73 (s, 6H), 3.62 (s, 3H), 3.49 (s, 2H), 2.35 (oct, 1H, *J* = 6.7 Hz), 2.02-1.66 (m, 8H), 0.92 (d, 3H, *J* = 6.7 Hz), 0.87 (d, 3H, *J* =

6.7 Hz). m/z (ESI-MS) 526.3 ($C_{25}H_{33}F_2N_3O_5S$ requires 526.21, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 3.5 min, purity 97%.

(*S*)-*N*-(4,4-Difluorocyclohexyl)-2-(2-methyl-1-(2-oxo-2-(3,4,5-trimethoxyphenyl) acetamido)propyl)thiazole-4-carboxamide (80)

Compound **80** was obtained from 2-oxo-2-(3,4,5-trimethoxyphenyl)acetic acid (100 mg, 0.42 mmol) and amine **77** (159 mg, 0.50 mmol) using method A as a light orange solid (34 mg, 15%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 9.68 (d, 1H, *J* = 8.6 Hz), 8.22 (s, 1H), 8.20 (d, 1H, *J* = 8.5 Hz), 7.16 (s, 2H), 5.19 (dd, 1H, *J* = 8.8 Hz, 7.1 Hz), 4.01-3.92 (m, 1H), 3.79-3.78 (s, 9H), 2.44 (oct, 1H, *J* = 6.7 Hz), 2.03-1.65 (m, 8H), 0.96 (d, 6H, *J* = 6.6 Hz). m/z (ESI-MS) 540.2 ($C_{25}H_{31}F_2N_3O_6S$ requires 540.19, [M + H]$^+$). HPLC t_R (acetonitrile/ water 60/40) = 3.4 min, purity 100%.

(*S*)-*N*-Cyclohexyl-2-(2-methyl-1-(3,4,5-trimethoxyphenylthioamido)propyl)thiazole-4-carbothioamide (81) and (*S*)-*N*-(1-(4-(cyclohexylcarbamothioyl)thiazol-2-yl)-2-methyl propyl)-3,4,5-trimethoxybenzamide (82)

Compounds **81** and **82** were obtained from carboxamide **39** (200 mg, 0.42 mmol) using method D (except using 1.1 eq of Lawesson's reagent and preparative TLC; *n*-hexane/ethyl acetate – 50/50; for purification) as yellow solids.

Dithioamide **81**: Yield - 20 mg, 9%; ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 10.6 (d, 1H, *J* = 8.1 Hz), 9.74 (d, 1H, *J* = 8.3 Hz), 8.34 (s, 1H), 7.05 (s, 2H), 5.88-5.83 (m, 1H), 4.48-4.40 (m, 1H), 3.84 (s, 6H), 3.71 (s, 3H), 2.62 (oct, 1H, *J* = 6.8 Hz), 1.94 (t, 2H, *J* = 13.2 Hz), 1.75 (d, 2H, *J* = 12.2 Hz), 1.63 (d, 1H, *J* = 12.2 Hz), 1.49 (q, 2H, *J* = 11.5 Hz), 1.33 (q, 2H, *J* = 11.5 Hz), 1.20 (t, 1H, *J* = 12.2 Hz), 1.09 (d, 3H, *J* = 6.7 Hz), 0.97 (d, 3H, *J* = 6.7 Hz). m/z (ESI-MS) 508.2 ($C_{24}H_{33}N_3O_3S_3$ requires 508.17, [M + H]$^+$). HPLC t_R (acetonitrile/water 70/30) = 9.4 min, purity 99%.

Monothioamide **82**: Yield - 8 mg, 4%; ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 9.79 (d, 1H, *J* = 8.3 Hz), 8.92 (d, 1H, *J* = 8.3), 8.32 (s, 1H), 7.21 (s, 2H), 5.17-5.13 (m, 1H), 4.48-4.39 (m, 1H), 3.84 (s, 6H), 3.71 (s, 3H), 2.45 (oct, 1H, *J* = 6.8 Hz), 1.93 (t, 2H, *J* = 11.2 Hz), 1.75 (d, 2H, *J* = 12.3 Hz), 1.63 (d, 1H, *J* = 12.3 Hz), 1.56-1.46 (m, 2H), 1.33 (q, 2H, *J* = 12.3 Hz), 1.20 (t, 1H, *J* = 12.3 Hz), 1.04 (d, 3H, *J* = 6.7 Hz, 3.7 Hz), 0.94 (d, 3H, *J* = 6.7 Hz, 3.7 Hz). m/z (ESI-MS) 492.2 ($C_{24}H_{33}N_3O_4S_2$ requires 492.19, [M + H]$^+$). HPLC t_R (acetonitrile/water 70/30) = 4.7 min, purity 99%.

(*S*)-*N*-(4,4-Difluorocyclohexyl)-2-(2-methyl-1-(3,4,5-trimethoxyphenylthioamido)propyl) thiazole-4-carbothioamide (83) and (*S*)-*N*-(1-(4-((4,4-difluorocyclohexyl)carbamothioyl) thiazol-2-yl)-2-methylpropyl)-3,4,5-trimethoxybenzamide (84)

Compounds **83** and **84** were obtained from carboxamide **53** (100 mg, 0.20 mmol) using method D (except using 1.1 eq of Lawesson's reagent and preparative TLC; *n*-hexane/ethyl acetate - 50/50; for purification) as yellow solids,

Dithioamide **83**: Yield - 3 mg, 3%; ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 10.65 (d, 1H, *J* = 8.3 Hz), 9.96 (d, 1H, *J* = 8.3 Hz), 8.35 (s, 1H), 7.05 (s, 2H), 5.89-5.84 (m, 1H), 4.66-4.59

(m, 1H), 3.84 (s, 6H), 3.71 (s, 3H), 2.67-2.59 (m, 1H), 2.12-1.81 (m, 8H), 1.09 (d, 3H, $J =$ 6.7 Hz), 0.98 (d, 3H, $J =$ 6.7 Hz). m/z (ESI-MS) 544.2 ($C_{24}H_{31}F_2N_3O_3S_3$ requires 544.15, $[M + H]^+$). HPLC t_R (acetonitrile/water 70/30) = 5.9 min, purity 99%.

Monothioamide **84**: Yield - 3 mg, 3%; ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 10.02 (d, 1H, $J =$ 8.3 Hz), 8.93 (d, 1H, $J =$ 8.3 Hz), 8.34 (s, 1H), 7.21 (s, 2H), 5.18-5.14 (m, 1H), 4.67-4.60 (m, 1H), 3.84 (s, 6H), 3.71 (s, 3H), 2.47-2.43 (m, 1H), 2.10-1.82 (m, 8H), 1.04 (d, 3H, $J =$ 6.7 Hz), 0.94 (d, 3H, $J =$ 6.7 Hz). m/z (ESI-MS) 528.2 ($C_{24}H_{31}F_2N_3O_4S_2$ requires 528.17, $[M + H]^+$). HPLC t_R (acetonitrile/water 60/40) = 6.9 min, purity 97%.

Tert-butyl (2-amino-2-oxoethyl)carbamate (85)

Compound **85** was obtained from *N*-Boc glycine (5 g, 28.54 mmol) using method C as a light yellow solid (4.62 g, 93%). ^{1}H NMR (400 MHz; CDCl₃; TMS) δ 6.64-6.50 (m, 2H), 5.70-5.65 (m, 1H), 3.80-3.76 (m, 2H), 1.40 (s, 9H).

Tert-butyl (*S*)-(1-amino-4-methyl-1-oxopentan-2-yl)carbamate (86)

Compound **86** was obtained from *N*-Boc (*S*)-leucine (7 g, 30.27 mmol) using method C as a white solid (6.94 g, 99%). ^{1}H NMR (400 MHz; CDCl₃; TMS) δ 6.47 (s, 1H), 5.91 (s, 1H), 5.09 (s, 1H), 4.18 (s, 1H), 1.74-1.60 (m, 2H), 1.54-1.49 (m, 1H), 1.44 (s, 9H), 0.96-0.93 (m, 6H).

Tert-butyl (*S*)-(1-amino-1-oxo-3-phenylpropan-2-yl)carbamate (87)

Compound **87** was obtained from *N*-Boc (*S*)-phenylalanine (10 g, 37.69 mmol) using method C as a white solid (9.74 g, 98%). ^{1}H NMR (400 MHz; CDCl₃; TMS) δ 7.32-7.28 (m, 2H), 7.26-7.22 (m, 3H), 6.24-6.14 (m, 1H), 5.95-5.84 (m, 1H), 5.27-5.22 (m, 1H), 4.44-4.40 (m, 1H), 3.06 (s, 2H), 1.40 (s, 9H).

Tert-butyl (*S*)-(1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamate (88)

Compound **88** was obtained from *N*-Boc (*S*)-tyrosine (10 g, 35.54 mmol) using method C as a white solid (9.45 g, 95%). ^{1}H NMR (400 MHz; CDCl₃; TMS) δ 8.00 (s, 1H), 7.01 (d, 2H, $J =$ 8.0 Hz), 6.74 (d, 2H, $J =$ 8.0 Hz), 6.30 (s, 1H), 6.02 (s, 1H), 5.38 (d, 1H, $J =$ 8.0 Hz), 4.36-4.30 (m, 1H), 2.88 (s, 2H), 1.39 (s, 9H).

(*S*)-(9*H*-Fluoren-9-yl)methyl 2-carbamoylpyrrolidine-1-carboxylate (89)

Compound **89** was obtained from Fmoc (*S*)-proline (10 g, 29.64 mmol) using method C as a white solid (9.21 g, 92%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 7.89 (t, 2H, $J =$ 6.8 Hz), 7.68 (t, 2H, $J =$ 6.8 Hz), 7.44-7.33 (m, 4H), 7.14-6.97 (m, 2H), 4.31-4.07 (m, 4H), 3.51-3.45 (m, 1H), 3.35-3.31 (m, 1H), 2.26-1.79 (m, 4H).

Tert-butyl (2-amino-2-thioxoethyl)carbamate (90)

Compound **90** was obtained from carboxamide **85** (4.5 g, 25.83 mmol) using method D as a colorless oil (3.44 g, 70%). ^{1}H NMR (400 MHz; CDCl₃; TMS) δ 8.06 (s, 2H), 5.54 (s, 1H), 3.91-3.82 (m, 2H), 1.45 (s, 9H).

Tert-butyl (*S*)-(1-amino-4-methyl-1-thioxopentan-2-yl)carbamate (91)

Compound **91** was obtained from carboxamide **86** (6 g, 26.05 mmol) using method D as a yellow oil (5.25 g, 82%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.39 (s, 1H), 7.81 (s, 1H), 5.25 (s, 1H), 4.55-4.52 (m, 1H), 1.76-1.63 (m, 3H), 1.44 (s, 9H), 0.97-0.94 (m, 6H).

Tert-butyl (*S*)-(1-amino-3-phenyl-1-thioxopropan-2-yl)carbamate (92)

Compound **92** was obtained from carboxamide **87** (9 g, 34.05 mmol) using method D as a yellow oil (7.44 g, 78%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.23 (s, 1H), 8.05 (s, 1H), 7.25-7.19 (m, 5H), 5.61 (s, 1H), 4.77-4.75 (m, 1H), 3.11-2.99 (m, 2H), 1.33 (s, 9H).

Tert-butyl (*S*)-(1-amino-3-(4-hydroxyphenyl)-1-thioxopropan-2-yl)carbamate (93)

Compound **93** was obtained from carboxamide **88** (9 g, 32.10 mmol) using method D as a yellow oil (8.17 g, 86%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 7.83 (s, 1H), 7.75 (s, 1H), 7.04 (d, 2H, J = 8.0 Hz), 6.72 (d, 2H, J = 8.0 Hz), 5.59 (s, 1H), 4.58 (q, 1H, J = 7.2 Hz), 2.99 (s, 2H), 1.40 (s, 9H).

(*S*)-(9*H*-Fluoren-9-yl)methyl-2-carbamothioylpyrrolidine-1-carboxylate (94)

Compound **94** was obtained from carboxamide **89** (9 g, 26.75 mmol) using method D as a yellow oil (6.78 g, 72%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 9.78-9.60 (m, 1H), 9.33-9.18 (m, 1H), 7.90 (t, 2H, J = 7.6 Hz), 7.73-7.68 (m, 2H), 7.44-7.40 (m, 2H), 7.37-7.31 (m, 2H), 4.73-4.47 (m, 1H), 4.27-4.15 (m, 3H), 3.62-3.55 (m, 1H), 3.43-3.39 (m, 1H), 2.36-2.15 (m, 1H),1.97-1.80 (m, 3H).

Ethyl 2-(((*tert*-butoxycarbonyl)amino)methyl)thiazole-4-carboxylate (95)

Compound **95** was obtained from thioamide **90** (3 g, 15.77 mmol) using method E as a dark red oil (1.89 g, 42%). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.12 (s, 1H), 5.61 (s, 1H), 4.65 (d, 2H, J = 6.2 Hz), 4.41 (q, 2H, J = 6.9 Hz), 1.46 (s, 9H), 1.40 (t, 3H, J = 7.1 Hz).

Ethyl-2-(1-((*tert*-butoxycarbonyl)amino)-3-methylbutyl)thiazole-4-carboxylate (96)

Compound **96** was obtained from thioamide **91** (5 g, 20.29 mmol) using method E as a light brown solid (3.96 g, 57%). $[a]_D^{25}$= −11.84° (*c* 0.1, CHCl$_3$) (literature, $[a]_D^{25}$ = −44.0°);[40] ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.08 (s, 1H), 5.17-5.13 (m, 1H), 4.44-4.38 (m, 3H), 1.96-1.68 (m, 3H), 1.44 (s, 9H), 1.32-1.28 (m, 3H), 0.98-0.95 (m, 6H).

Ethyl-2-(1-((*tert*-butoxycarbonyl)amino)-2-phenylethyl)thiazole-4-carboxylate (97)

Compound **97** was obtained from thioamide **92** (7.2 g, 25.68 mmol) using method E as a yellow solid (5.99 g, 62%). $[a]_D^{25}$= −1.34° (*c* 0.1, CHCl$_3$) (literature, $[a]_D^{25}$= −20.1°);[40] ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.05 (s, 1H), 7.28-7.21 (m, 3H), 7.12 (d, 2H, J = 6.9 Hz), 5.47-5.46 (m, 1H), 5.31-5.28 (m, 1H), 4.45-4.40 (m, 2H), 3.35-3.39 (m, 2H), 1.39 (s, 9H), 1.27-1.23 (m, 3H).

Ethyl-2-(1-((*tert*-butoxycarbonyl)amino)-2-(4-hydroxyphenyl)ethyl)thiazole-4-carboxylate (98)

Compound **98** was obtained from thioamide **93** (7.5 g, 25.30 mmol) using method E as a yellow solid (5.36 g, 54%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.42 (s, 1H), 7.79 (d, 1H, J = 8.3 Hz), 7.07 (d, 2H, J = 8.0 Hz), 6.66 (d, 2H, J = 8.0 Hz), 4.90-4.85 (m, 1H), 4.34-4.28 (m, 3H), 3.18-3.14 (m, 2H), 1.32 (s, 9H), 1.23-1.20 (m, 3H).

Ethyl-2-(1-(((9*H*-fluoren-9-yl)methoxy)carbonyl)pyrrolidin-2-yl)thiazole-4-carboxylate (99)

Compound **99** was obtained from thioamide **94** (6.5 g, 18.44 mmol) using method E as a reddish brown oil (3.89 g, 47%). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.37 (d, 1H, J = 8.0 Hz), 7.88 (d, 1H, J = 7.4 Hz), 7.76 (d, 1H, J = 6.4 Hz), 7.66 (d, 1H, J = 6.4 Hz), 7.40-7.11 (m, 5H), 5.12 (s, 1H), 4.40-4.27 (m, 5H), 3.52-3.40 (m, 2H), 2.34-2.26 (m, 1H), 2.02-1.82 (m, 3H), 1.33-1.26 (m, 3H).

Ethyl 2-(3,4,5-trimethoxybenzamido)thiazole-4-carboxylate (100)

Compound **100** was obtained from 3,4,5-trimethoxybenzoyl chloride (1.61 g, 6.97 mmol) and ethyl 2-amino thiazole-4-carboxylate (1 g, 5.81 mmol) using method F as a white solid (1.48 g, 70%). ^{1}H NMR (400 MHz; CDCl₃; TMS) δ 11.43 (s, 1H), 7.78 (s, 1H), 7.14 (s, 2H), 4.08 (q, 2H, J = 7.0 Hz), 3.80 (s, 3H), 3.72 (s, 6H), 1.18-1.16 (m, 3H).

N-(4,4-Difluorocyclohexyl)-2-(3,4,5-trimethoxybenzamido)thiazole-4-carboxamide (101)

Ester **100** (120 mg, 0.33 mmol) was hydrolyzed using method G providing corresponding acid, which was reacted further with 4,4-difluorocyclohexylamine (54 mg, 0.40 mmol) using method A to obtain compound **101** (80 mg, 54% over two steps) as a white solid. ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 12.68 (bs, 1H), 7.85 (s, 1H), 7.82 (d, 1H, J = 8.0 Hz), 7.48 (s, 2H), 3.98-3.91 (m, 1H), 3.87 (s, 6H), 3.74 (s, 3H), 2.05-1.89 (m, 6H), 1.65-1.55 (m, 2H). m/z (ESI-MS) 456.2 ($C_{20}H_{23}F_2N_3O_5S$ requires 456.13, [M + H]$^+$). HPLC t_R (acetonitrile/ water 50/50) = 3.7 min, purity 98%.

Ethyl 2-((3,4,5-trimethoxybenzamido)methyl)thiazole-4-carboxylate (102)

Compound **95** (1 g, 3.49 mmol) was hydrolyzed using method B to obtain free amine as an oil, which was reacted with 3,4,5-trimethoxybenzoyl chloride (0.97 g, 4.19 mmol) using method F to furnish compound **102** as a brown solid (0.62 g, 47% over two steps). ^{1}H NMR (400 MHz; CDCl₃; TMS) δ 8.41 (t, 1H, J = 5.8 Hz), 8.03 (s, 1H), 7.08 (s, 2H), 4.79 (d, 2H, J = 5.9 Hz), 4.27 (q, 2H, J = 7.1 Hz), 3.76 (s, 3H), 3.71 (s, 6H), 1.26 (t, 3H, J = 7.0 Hz).

Ethyl-2-(3-methyl-1-(3,4,5-trimethoxybenzamido)butyl)thiazole-4-carboxylate (103)

Compound **96** (1 g, 2.92 mmol) was hydrolyzed using method B to obtain free amine as an oil, which was reacted with 3,4,5-trimethoxybenzoyl chloride (0.79 g, 3.40 mmol) using method F to give compound **103** as a light yellow solid (0.86 g, 68% over two steps). ^{1}H NMR (400 MHz; CDCl₃; TMS) δ 8.09 (s, 1H), 7.04 (s, 2H), 6.87 (d, 1H, J = 8.5 Hz), 5.62 (q, 1H, J = 7.9 Hz), 4.41 (q, 2H, J = 7.1 Hz), 3.91 (s, 6H), 3.87 (s, 3H), 2.03-2.00 (m, 2H), 1.75-1.65 (m, 1H), 1.39 (t, 3H, J = 6.9 Hz), 1.00 (dd, 6H, J = 6.7 Hz, 2.1 Hz).

Ethyl-2-(2-phenyl-1-(3,4,5-trimethoxybenzamido)ethyl)thiazole-4-carboxylate (104)

Compound **97** (1.5 g, 3.98 mmol) was hydrolyzed using method B to obtain free amine as an oil, which was reacted with 3,4,5-trimethoxybenzoyl chloride (1.10 g, 4.78 mmol) using method F to yield compound **104** as a light yellow solid (1.53 g, 82% over two steps). ^{1}H NMR (400 MHz; CDCl$_3$; TMS) δ 8.05 (s, 1H), 7.29-7.21 (m, 3H), 7.16 (d, 2H, J = 7.1 Hz), 7.03-7.00 (m, 1H), 6.93 (s, 2H), 5.77 (q, 1H, J = 7.3 Hz), 4.42 (q, 2H, 7.1 Hz), 3.92 (s, 3H), 3.87 (m, 6H), 3.52-3.40 (m, 2H), 1.41 (t, 3H, J = 7.1 Hz). ^{13}C NMR (100 MHz; CDCl$_3$; TMS) δ 171.29, 166.62, 161.17, 153.19, 146.98, 141.17, 136.24, 129.41, 128.98, 128.75, 127.49, 127.21, 104.42, 61.59, 60.92, 56.29, 52.73, 41.26, 14.37.

Ethyl-2-(2-(4-hydroxyphenyl)-1-(3,4,5-trimethoxybenzamido)ethyl)thiazole-4-carboxylate (105)

Compound **98** (1.5 g, 3.82 mmol) was hydrolyzed using method B to obtain free amine as an oil, which was reacted with 3,4,5-trimethoxybenzoyl chloride (1.06 g, 4.59 mmol) using method F to obtain compound **105** as a white solid (1.37 g, 74% over two steps). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 9.22 (s, 1H), 9.13 (d, 1H, J = 8.1 Hz), 8.45 (s, 1H), 7.50 (d, 1H, J = 7.8 Hz), 7.20-7.12 (m, 4H), 6.65 (d, 1H, J = 8.0 Hz), 5.63-5.42 (m, 1H), 4.35-4.29 (m, 2H), 3.85 (m, 2H), 3.82 (s, 6H), 3.69 (s, 3H), 1.34-1.29 (m, 3H).

Ethyl-2-(1-(3,4,5-trimethoxybenzoyl)pyrrolidin-2-yl)thiazole-4-carboxylate (106)

Compound **99** (1.5 g, 3.34 mmol) was stirred with a solution of 5% piperidine in DMF (10 mL) for 2 h. It was further diluted with ethyl acetate and partitioned with 2N HCl solution. Aqueous layer was collected and basified with aqueous saturated solution of Na$_2$CO$_3$. It was then extracted 2× with ethyl acetate. The combined organic layer was dried (anhydrous MgSO$_4$) and evaporated to obtain free amine as an oil, which was reacted further with 3,4,5-trimethoxybenzoyl chloride (1.00 g, 4.34 mmol) using method F to prepare compound **106** as a white solid (0.75 g, 53% over two steps). ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.45 (s, 1H), 6,83 (s, 2H), 5.47-5.43 (m, 1H), 4.32-4.27 (m, 2H), 3.82 (s, 6H), 3.70 (s, 3H), 3.63-3.58 (m, 2H), 2.44-2.37 (m, 1H), 2.17-2.11 (m, 1H), 1.98-1.89 (m, 2H), 1.32-1.28 (m, 3H).

N-(4,4-Difluorocyclohexyl)-2-((3,4,5-trimethoxybenzamido)methyl)thiazole-4-carboxamide (107)

Ester **102** (130 mg, 0.34 mmol) was hydrolyzed using method G providing acid, which was further reacted with 4,4-difluorocyclohexan-1-amine (56 mg, 0.41 mmol) using method A to obtain compound **107** (11 mg, 7% over two steps) as a cream colored solid. ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 9.43 (t, 1H, J = 5.9 Hz), 8.30 (d, 1H, J = 8.3 Hz), 8.17 (s, 1H), 7.25 (s, 2H), 4.77 (d, 2H, J = 5.8 Hz), 4.01-3.92 (m, 1H), 3.83 (s, 6H), 3.71 (s, 3H), 2.04-1.69 (m, 8H). m/z (ESI-MS) 470.2 (C$_{21}$H$_{25}$F$_2$N$_3$O$_5$S requires 470.15, [M + H]$^+$). HPLC t_R (acetonitrile/water 50/50) = 2.2 min, purity 95%.

N-(4,4-Difluorocyclohexyl)-2-(3-methyl-1-(3,4,5-trimethoxybenzamido)butyl)thiazole-4-carboxamide (108)

Ester **103** (123 mg, 0.28 mmol) was hydrolyzed using method G providing acid, which was further reacted with 4,4-difluorocyclohexan-1-amine (46 mg, 0.34 mmol) using method A to furnish compound **108** (115 mg, 78% over two steps) as a white solid. ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 9.07 (d, 1H, J = 8.2 Hz), 8.21 (d, 1H, J = 8.3 Hz), 8.15 (s, 1H), 7.27 (s, 2H), 5.48-5.42 (m, 1H), 4.02-3.94 (m, 1H), 3.84 (s, 6H), 3.71 (s, 3H), 2.04-1.70 (m, 11H), 0.98 (d, 3H, J = 6.5 Hz), 0.94 (d, 3H, J = 6.5 Hz). m/z (ESI-MS) 526.3 ($C_{25}H_{33}F_2N_3O_5S$ requires 526.21, $[M + H]^+$). HPLC t_R (acetonitrile/water 60/40) = 3.6 min, purity 97%.

N-(4,4-Difluorocyclohexyl)-2-(2-phenyl-1-(3,4,5-trimethoxybenzamido)ethyl)thiazole-4-carboxamide (109)

Ester **104** (125 mg, 0.27 mmol) was hydrolyzed using method G providing acid, which was further reacted with 4,4-difluorocyclohexan-1-amine (43 mg, 0.32 mmol) using method A to provide compound **109** (100 mg, 67% over two steps) as a white solid. mp 188-191 °C. ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 9.15 (d, 1H, J = 8.3 Hz), 8.23 (d, 1H, J = 8.4 Hz), 8.19 (s, 1H), 7.40 (d, 2H, J = 7.5 Hz), 7.30 (t, 2H, J = 7.5 Hz), 7.20 (t, 1H, J = 7.3 Hz), 7.11 (s, 2H), 5.60-5.54 (m, 1H), 4.05-3.96 (m, 1H), 3.81 (s, 6H), 3.69 (s, 3H), 3.61 (dd, 1H, J = 13.8 Hz, 4.0 Hz), 3.29 (dd, 1H, J = 13.8 Hz, 11.0 Hz), 2.06-1.72 (m, 8H). ^{13}C NMR (100 MHz; CDCl3; TMS) δ 172.08, 166.79, 159.98, 153.31, 149.29, 141.43, 135.81, 129.38, 128.87, 127.49, 123.73, 104.48, 60.95, 56.35, 52.42, 46.28, 41.08, 32.18 (t, J = 24.7 Hz), 28.63 (d, J = 6.4 Hz). m/z (ESI-MS) 560.3 ($C_{28}H_{31}F_2N_3O_5S$ requires 560.20, $[M + H]^+$). HPLC t_R (acetonitrile/water 50/50) = 7.3 min, purity 95%. Anal. Calcd for $C_{28}H_{31}F_2N_3O_5S$: C, 60.09; H, 5.58; N, 7.51. Found: C, 59.89; H, 5.58; N, 7.51.

N-(4,4-Difluorocyclohexyl)-2-(2-(4-hydroxyphenyl)-1-(3,4,5-trimethoxybenzamido)ethyl) thiazole-4-carboxamide (110)

Ester **105** (120 mg, 0.25 mmol) was hydrolyzed using method G providing acid, which was reacted further with 4,4-difluorocyclohexan-1-amine (40 mg, 0.30 mmol) using method A to obtain compound **110** (50 mg, 35% over two steps) as a white solid. ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 9.23 (s, 1H), 9.07 (d, 1H, J = 8.3 Hz), 8.23 (d, 1H, J = 8.3 Hz), 8.17 (s, 1H), 7.17 (d, 2H, J = 8.4 Hz), 7.11 (s, 2H), 6.66 (d, 2H, J = 8.4 Hz), 5.49-5.44 (m, 1H), 4.04-3.95 (m, 1H), 3.82 (s, 6H), 3.69 (s, 3H), 3.47 (dd, 1H, J = 13,9 Hz, 4.3 Hz), 3.15 (dd, 1H, J = 13.9 Hz, 10.8 Hz), 2.06-1.69 (m, 8H). m/z (ESI-MS) 576.2 ($C_{28}H_{31}F_2N_3O_6S$ requires 576.19, $[M + H]^+$). HPLC t_R (acetonitrile/water 50/50) = 2.8 min, purity 97%.

N-(4,4-Difluorocyclohexyl)-2-(1-(3,4,5-trimethoxybenzoyl)pyrrolidin-2-yl)thiazole-4-carboxamide (111)

Ester **106** (120 mg, 0.29 mmol) was hydrolyzed using method G providing acid, which was further reacted with 4,4-difluorocyclohexan-1-amine (47 mg, 0.35 mmol) using method A to furnish compound **111** (56 mg, 39% over two steps) as a white solid. ^{1}H NMR (400 MHz; DMSO-d6; TMS) δ 8.27 (d, 1H, J = 8.0 Hz), 8.16 (s, 1H), 6.82 (s, 2H), 5.48-5.44 (m, 1H), 4.01-3.94 (m, 1H), 3.82 (s, 6H), 3.70 (s, 3H), 3.62-3.55 (m, 2H), 2.45-2.36 (m, 1H),

2.22-2.16 (m, 1H), 2.03-1.69 (m, 10H). m/z (ESI-MS) 510.2 ($C_{24}H_{29}F_2N_3O_5S$ requires 510.18, $[M + H]^+$). HPLC t_R (acetonitrile/water 50/50) = 3.0 min, purity 99%.

Bioassay Procedures

Chemicals—Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin, and Trypsin 0.25% EDTA were products of Hyclone (GE Healthcare Bio-Sciences, Pittsburgh, PA), Thermo Fisher Scientific (Waltham, MA). Phosphate buffered saline (PBS) 20× concentrate (pH 7.5) was purchased from AMRESCO (Solon, OH). Paclitaxel, verapamil, zosuquidar, G418, 3-(4,5-dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). The lyophilized compounds were solubilized in DMSO at 10 mM stock concentrations and kept at −20°C until experimentation. Those stocks were further diluted in media on the same day, prior to experimentation to the respective working concentrations for reversal assay. Microplates were read and analyzed with Multiskan™ GO Microplate Spectrophotometer using SkanIt™ Software Research Addition purchased from Thermo Scientific (Waltham, MA). Vivid CYP3A4 green screening kit was purchased from Thermo Fisher Scientific (Waltham, MA).

Cell Lines and Cell Culture

HEK 293-pcDNA 3.1 and HEK-ABCB1 cells were generated by transfecting the HEK293 cells respectively with the control pcDNA3.1 vector and ABCB1 expression vector. All transfected cells were cultured in DMEM medium with 2 mg/mL of G418. These cell lines were maintained in T-75 culture flasks, using DMEM media, supplemented with 10% Fetal Bovine Serum and 1% penicillin/streptomycin and incubated in 5% CO_2 at 37°C. The cells were frequently examined under a microscope and only used for assay when both cell lines were growing well. The cells were washed with PBS and grown as adherent monolayers, maintained on drug free media at least 14 days prior to experimentation. Cells were checked on under the microscope throughout the assay to ensure even plate seeding and growth. The untreated row of cells in each 96-well plate confirmed the growth over the course of the experiment.

ATPase Activity of P-gp

High Five insect cell membrane vesicles prepared from P-gp expressing insect cells were used to measure the vanadate-sensitive ATPase activity of P-gp in the presence of indicated concentrations of synthesized compounds. All compounds were dissolved in dimethyl sulfoxide (DMSO) and 100× concentrated working stocks of compounds were prepared. In the ATPase assay, the final concentration of DMSO was kept at 1% (v/v). The basal ATPase activity was measured in the absence of synthesized compounds but, in the presence of same percentage volume of DMSO solvent. The drug-modulated activity was measured in the presence of indicated compounds. Total membrane protein (10 μg/100 μL), was pre-incubated with the synthesized compound or DMSO control at 37 °C for 5 min in a 50 mM MES-Tris buffer (pH 6.8) containing 50 mM KCl, 10 mM $MgCl_2$, 5 mM NaN_3, 1 mM EGTA, 1 mM ouabain, and 2 mM DTT in the presence and absence of sodium orthovanadate (0.3 mM). The reaction was initiated by the addition of 5 mM ATP and

terminated with addition of 0.1 mL of 5% (w/v) sodium dodecyl sulfate solution (final concentration, 2.5%); the extent of inorganic phosphate liberated over 20 minutes at 37 °C was measured using a colorimetric method as described previously.[53] The results reported as mean ± SD are obtained from three independent experiments performed in duplicates. For some compounds, mean values from two independent experiments in triplicates are reported.

[^{125}I]-Iodoarylazidoprazosin (IAAP) photoaffinity Labeling of human P-gp

Increasing concentrations of compound **53** (0–5 μM) or compound **109** (0–10 μM) were incubated with P-gp expressing High Five insect cell membrane vesicles (65–75 μg protein/100 μL) in 50 mM MES-Tris buffer (pH 6.8) and 150 mM sodium chloride at 37 °C for 10 min and then the samples were displaced to 4 °C bath. At that time, 4–5 nM [^{125}I]-IAAP (2200 Ci/mmol; PerkinElmer Lifesciences Corp.) was added under subdued light. Photo-cross-linking with [^{125}I]-IAAP was performed by exposing the samples to 366 nm UV light for 10 min and the incorporation of IAAP in P-gp after SDS-PAGE was quantified as described previously.[54] [^{125}I]-IAAP incorporation into P-gp band in the absence of compound (only DMSO) was taken as 100%.

MDR Reversal (Cell Killing) Cytotoxicity Assay[55]

The chemo-sensitivity was determined using cell viability colorimetric assay, which was designed and performed using 96-well plates in triplicate. Parental HEK 293-pcDNA 3.1 cells and P-gp overexpressing (ABCB1 transfected) cells evenly seeded at 5000 cells per well in 160 μL of culture medium were incubated under 5% CO_2 at 37°C. After 24 h incubation, the cells were dosed with designated treatments. Combination treatments received 20 μL of the compounds at a non-toxic concentration of 10 μM and incubated for 1 hour prior to the chemotherapy. Verapamil and zosuquidar were used as positive control reversal agents at 10 μM and 0.25 μM, respectively. ABCB1 substrate chemotherapy drug paclitaxel was serially diluted and dosed to respective cell lines. HEK 293-pcDNA 3.1 and HEK-ABCB1 received paclitaxel to up to 10 μM. The cells under treatment were then incubated under 5% CO_2 at 37°C for 72 h. Following the 3 days, each well received 20 μL of MTT solution (4 mg/mL) followed by incubation of the plates for 4 h at 37°C to ensure the complete formation of the formazan crystallization. After aspirating the culture media MTT solution, the resulting formazan was dissolved in 100 μL of dimethyl sulfoxide. Finally, plates were shaken for 10 min and absorbances were measured at 570 nm, using a microplate reader, and analyzed.

Cytochrome P4503A4 inhibition assay

The CYP3A4 inhibition assay was conducted by following the manufacturer's guidelines (vivid® CYP450 green screening kit; Thermofisher Scientific, Waltham, MA) and as reported in Wang and colleagues.[20]

Molecular descriptors calculation

The molecular descriptors were calculated using the calculate property functionality in Maestro v10.1 (AlogP, HBA, HBD, Polar SA and Number of rotatable bonds; Schrödinger, LLC, New York, NY, 2015), Qikprop (Mol. Wt. and No. of N and O; QikProp, Schrödinger,

LLC, New York, NY, 2015), ACD/Chemsketch (Molar refractivity and Molar volume; ACD/ Labs 2017.1.2, Advanced Chemistry Development Inc. Toronto, Ontario, Canada) and Open Notebook Science Challenge (A and B; AbrahamModel001; http:// showme.physics.drexel.edu/onsc/models/AbrahamDescriptorsModel001.php).

In silico PAINS analysis

A program KNIME (v3.4.1, KNIME GmbH, Konstanz, Germany) was used to run the workflow (http://myexperiment.org/workflows/1841) for PAINS analysis.[56, 57] Target compounds were manually input into the workflow in the form of SMILES. The output file for the run listed the predicted PAINS.

Molecular Modeling

All docking experiments were performed on Mac Pro 6-core Intel Xenon X5 processor with Macintosh Operating System (OS X El Capitan) using Schrodinger 2015-1 (Schrödinger, LLC, New York, NY, 2015) software. Ligand preparations of the thiazole compounds, homology modeling (except using template as PDB: 4Q9H),[51] and protein preparation of the homology model were essentially performed following our reported protocols.[19] Homology model validation was performed based on Ramachandran plot and root-mean-square deviation (RMSD) of Cα atoms of the residues of the generated model and experimental structure. The Ramachandran plot analysis revealed >94% residues in the favored region, ~4% residues in the allowed regions, and ~1.2% residues (all glycine and Ala82) in the disallowed regions. The RMSD was found to be 0.061 Å suggesting good alignment. The grid was generated by selecting residues at 4 Å from bound inhibitors in homology modeled proteins (template PDBs: 4Q9I, 4Q9J, 4Q9K, 4Q9L). These residues are as follows: 61, 64, 65, 68, 69, 72, 118, 125, 222, 299, 303, 306, 307, 310, 336, 339, 340, 342, 343, 721, 725, 728, 729, 732, 770, 841, 842, 870, 871, 872, 942, 945, 949, 953, 957, 975, 978, 979, 982, 983, 984, 985, 986, 987, 990, and 991. Default protocol was used to perform induced-fit docking except the number of poses were reduced to 10. The surface representation of the bound ligand is generated by using 'Create binding site surfaces' tool in Maestro with default parameters and residue-type color scheme.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

ABBREVIATIONS

Cryo-EM	Cryo-Electron Microscopy
DTT	Dithiothreitol
EGTA	Ethylene glycol-bis(2-aminoethylether)-*N,N,N,N*-tetraacetic acid

HCTU	O-(1*H*-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOBt	1-Hydroxybenzotriazole
IAAP	Iodoarylazidoprazocin
MES	2-(*N*-morpholino)ethanesulfonic acid
PAINS	Pan assay interference compounds
P-gp	P-glycoprotein.

Authors will release the atomic coordinates and experimental data upon article publication.

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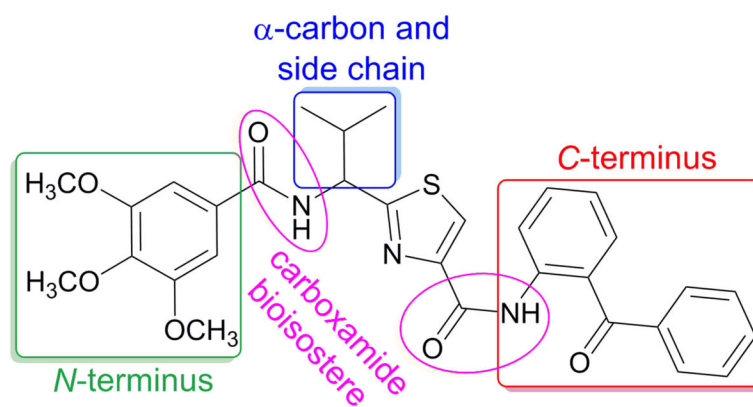
Figure 1.
Modification of various structural elements of a lead compound 1.
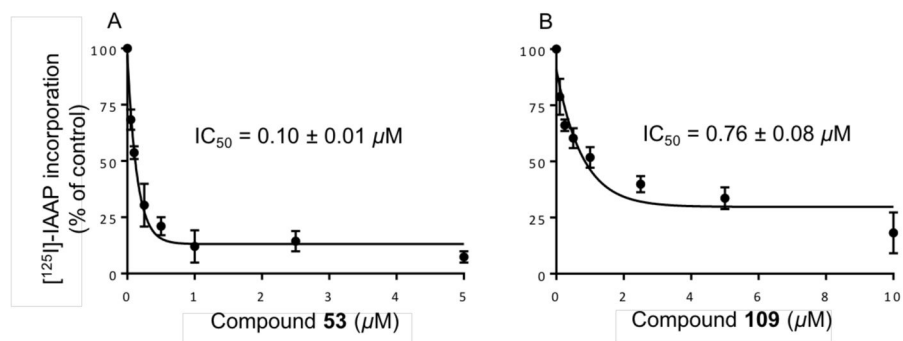
Figure 2.
Photoaffinity labeling competition assay with compounds **53** (A) and **109** (B) inhibiting the incorporation of [^{125}I]-IAAP to human P-gp. [^{125}I]-IAAP incorporation into P-gp band in the absence of compounds (only DMSO) was taken as 100%. The data, in the graphs, were fitted for (A) $R^2 = 0.94$ and (B) $R^2 = 0.92$ with a one-phase decay equation using GraphPad Prism 7 with points representing the average of three independent experiments and error bars denote SD.
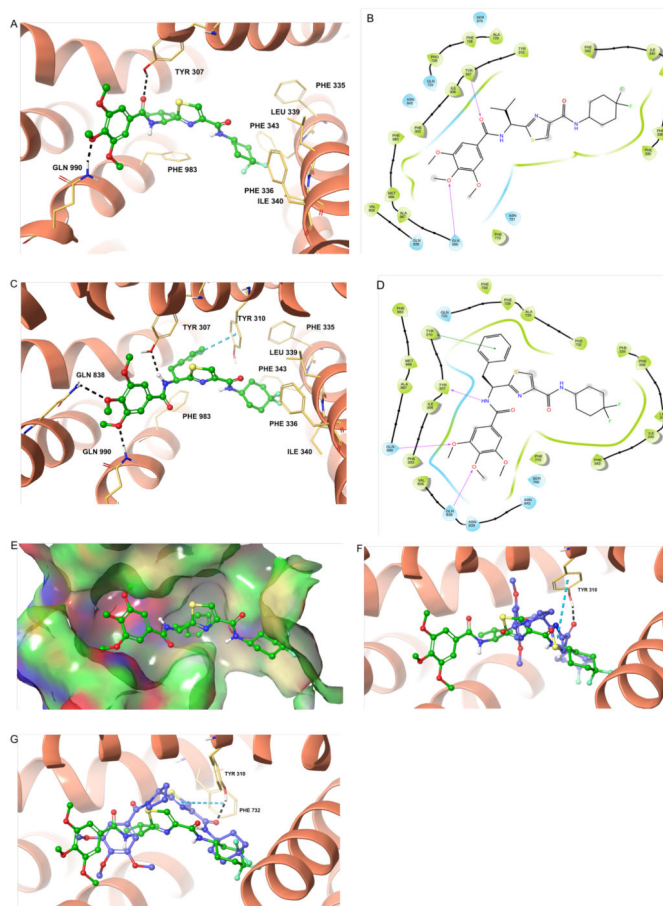
Figure 3. Induced-fit docking model of inhibitors within transmembrane domains of homology modeled human P-gp

(A) Docking pose of compound **53**. Amino acid residues are illustrated as thin tubes with the color representations as carbon in faded orange; hydrogen in white; nitrogen in blue; oxygen in red and sulfur in yellow. The protein is represented in faded red-orange colored ribbon form. The ball and stick model of inhibitor with the identical color scheme as above except carbon atoms represented in green and fluorine atoms in faded-green is shown. Black dashes represent protein-ligand intermolecular hydrogen bonds. (B) 2-D representation of docking pose of compound **53**. The colored drops (cyan, polar and green, hydrophobic) indicate amino acid residues within 5 Å of the ligand and arrows (magenta) show hydrogen bonds. (C) Docking pose of (*S*)-isomer of compound **109**. Representation is same as in A and aromatic interaction is shown as light blue dash. (D) 2-D representation of docking pose of (*S*)-isomer of compound **109**. Representation is same as in B and aromatic interaction is shown by a green line. (E) A surface representation of the docked pose is shown. The ball and stick model represents (*S*)-isomer of inhibitor **109** and the surrounding is drug binding site surface in residue-type color scheme. (F) Superposition of docked structures of compound **53** and *cis*- isomer of (*S*)-proline analogue **111**. Only residues which are interacting with compound **111** are shown. Carbon atoms are represented using faded blue color for **111** and other atoms follow the same color representation as indicated above. (G)

Superposition of docked structures of compound **53** and *trans*-(*S*)-proline analogue **111**. The representation is same as in F.
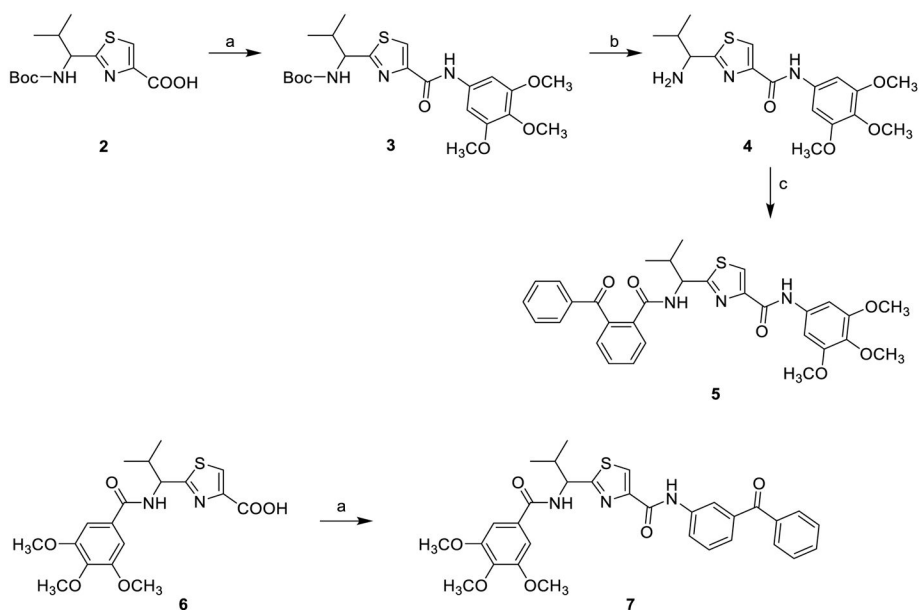
Scheme 1. Synthesis of Target Compounds 5 and 7[a]

[a]Reagents and conditions: (a) Appropriate aniline, HCTU, HOBt, DIEA, DMA, 0°C to rt, overnight; (b) TFA, CH₂Cl₂, 0°C to rt, 12 h; (c) 2-Benzoyl benzoic acid, HCTU, HOBt, DIEA, DMA, 0°C to rt, overnight.
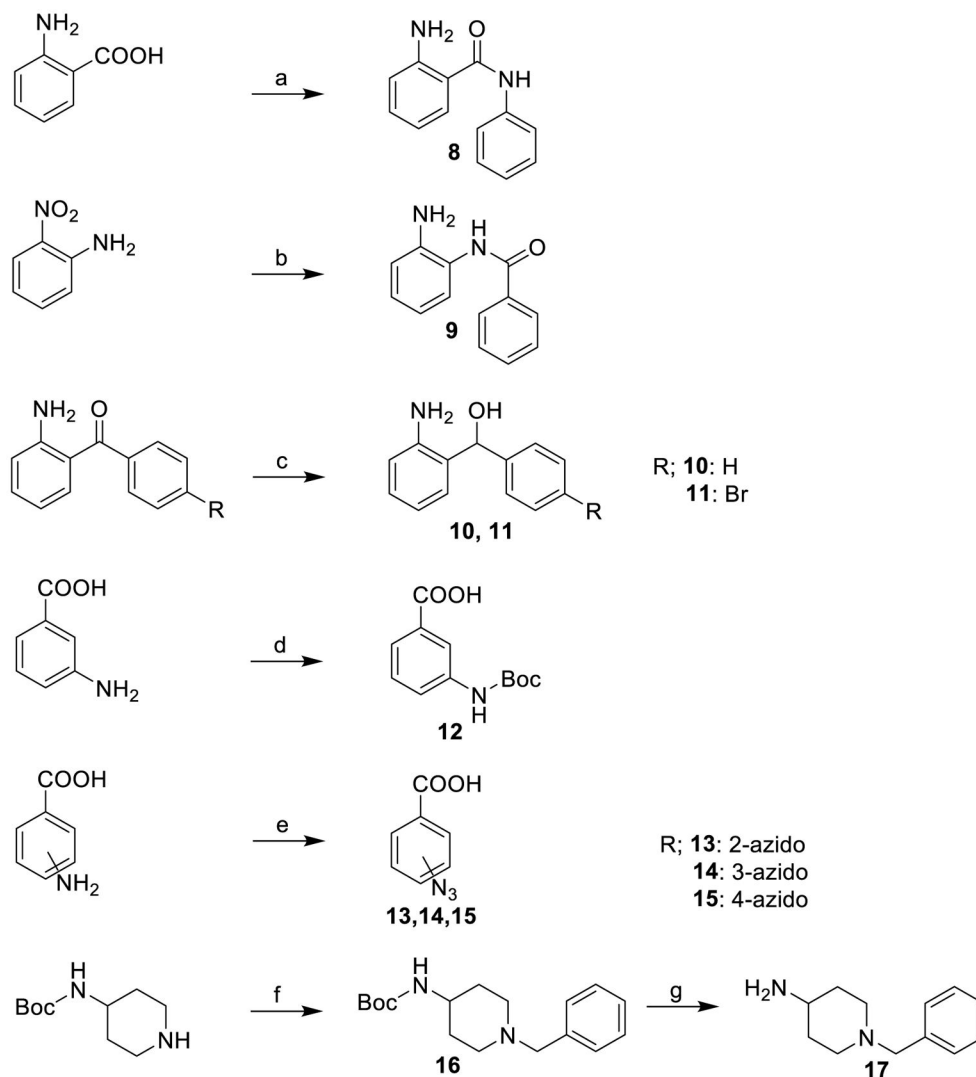
Scheme 2. Synthesis of Key Intermediates Required for the Coupling Reactions to Obtain Various *N*- and *C*-terminal Substitutions on (*S*)-Valine Thiazole Amino Acid[a]

[a]Reagents and conditions: (a) (i) SOCl$_2$, Et$_2$O, reflux, 2 h; (ii) Aniline, pyridine, Et$_2$O, reflux, 3 h; (b) (i) Benzoyl chloride, pyridine, THF, rt. Overnight; (ii) H$_2$, Pd/C, MeOH, rt, overnight; (c) NaBH$_4$, EtOH, reflux, 2 h; (d) Di-*tert*-butyl dicarbonate, Et$_3$N, dioxane, H$_2$O, rt, 24 h; (e) (i) HCl, H$_2$O, 0°C; (ii) NaNO$_2$, H$_2$O, rt, 15 min; (iii) NaN$_3$, H$_2$O, rt; (f) Benzyl chloride, Et$_3$N, THF, reflux, overnight; (g) TFA, CH$_2$Cl$_2$, 0°C to rt, 4 h.
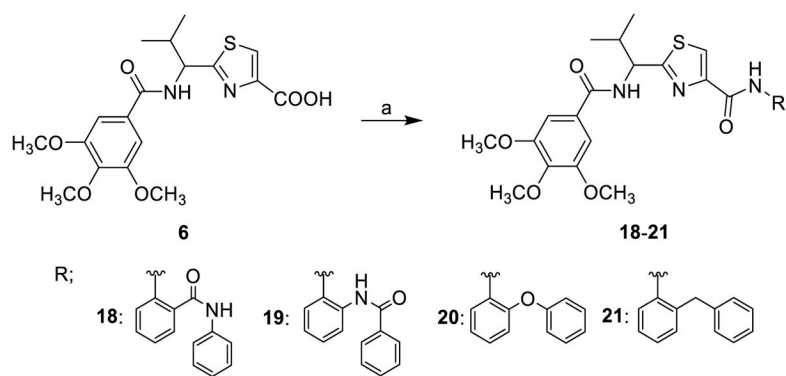
Scheme 3. Synthesis of Target Compounds 18–21[a]

[a]Reagents and conditions: (a) Appropriate amine, HCTU, HOBt, DIEA, DMA, 0°C to rt, overnight.
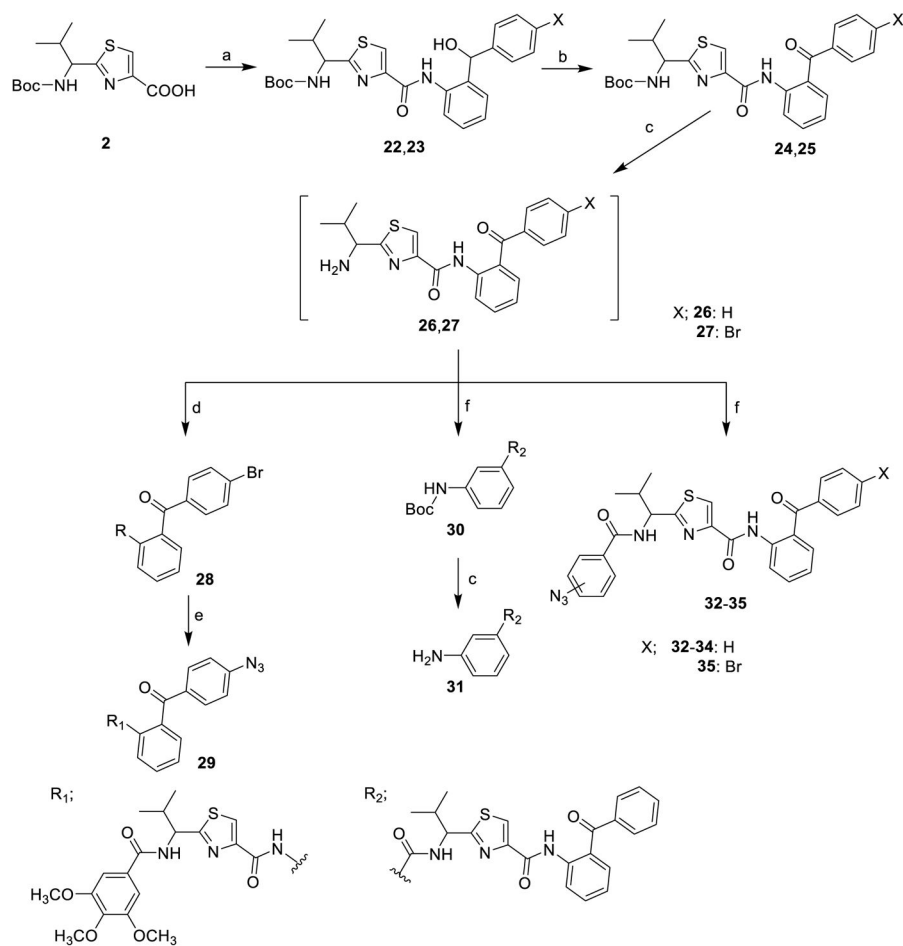
Scheme 4. Synthesis of Target Compounds 28–29 and 31–35[a]

[a]Reagents and conditions: (a) Appropriate amine, HCTU, HOBt, DIEA, DMA, 0°C to rt, overnight; (b) Dess-Martin periodinane, THF, rt, 30 min; (c) TFA, CH$_2$Cl$_2$, 0°C to rt, 4–12 h; (d) 3,4,5-Trimethoxybenzoyl chloride, DIEA, THF, 0°C to rt, overnight; (e) NaOH, NaN$_3$, (S)-proline, CuI, H$_2$O, EtOH, reflux, 24 h; (f) Appropriate acid, HCTU, HOBt, DIEA, DMA, 0°C to rt, overnight.
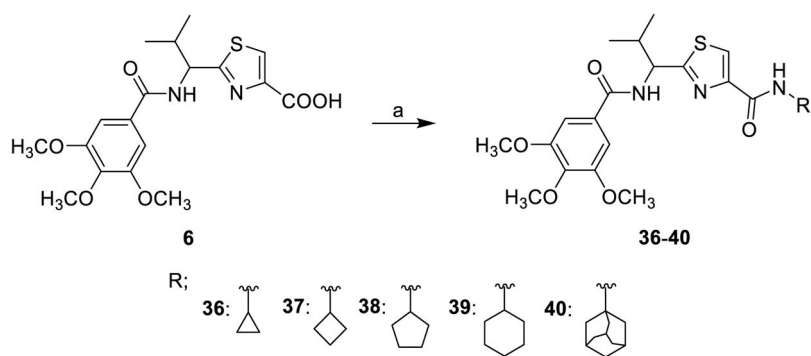
Scheme 5. Synthesis of Target Compounds 36–40*[a]*
*[a]*Reagents and conditions: (a) Appropriate amine, HCTU, HOBt, DIEA, DMA, 0°C to rt, overnight.
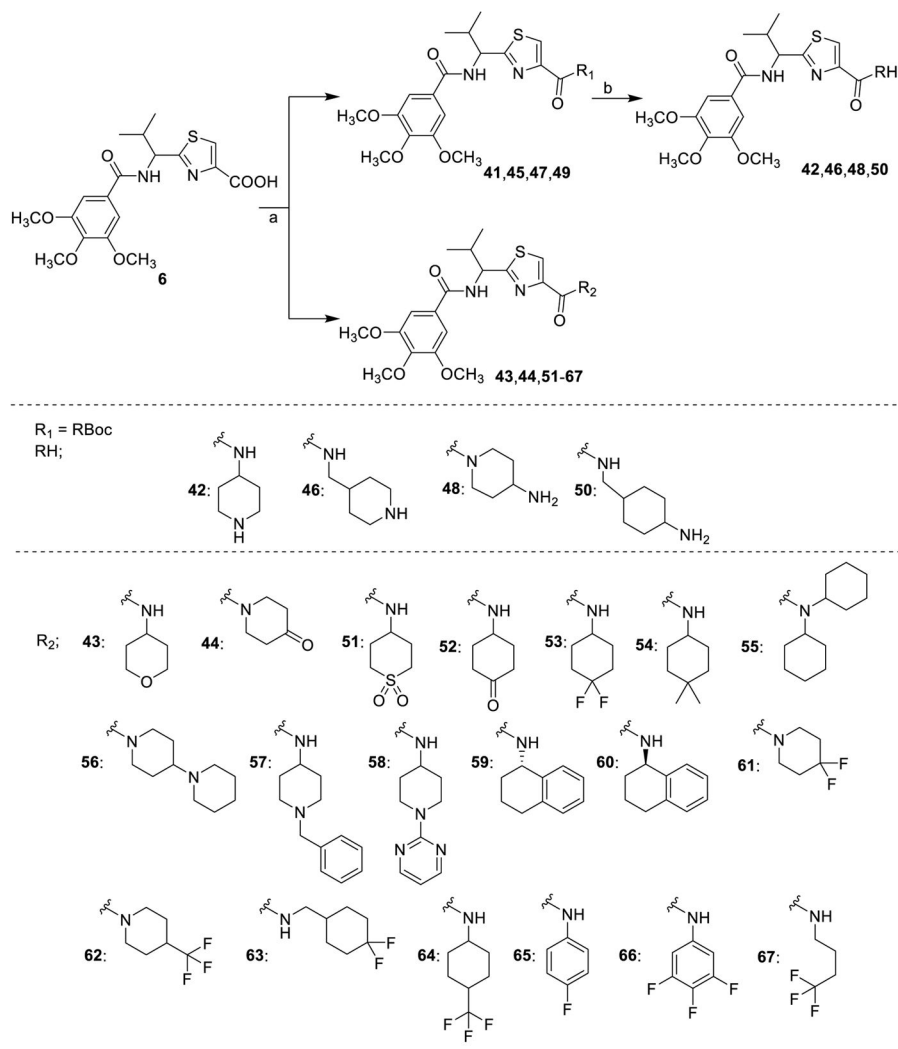
Scheme 6. Synthesis of Target Compounds 41–44, 46, 48, and 50–67[a]

[a]Reagents and conditions: (a) Appropriate amine, HCTU, HOBt, DIEA, CH₂Cl₂, 0°C to rt, overnight; (b) TFA, CH₂Cl₂, 0°C to rt, 12 h.
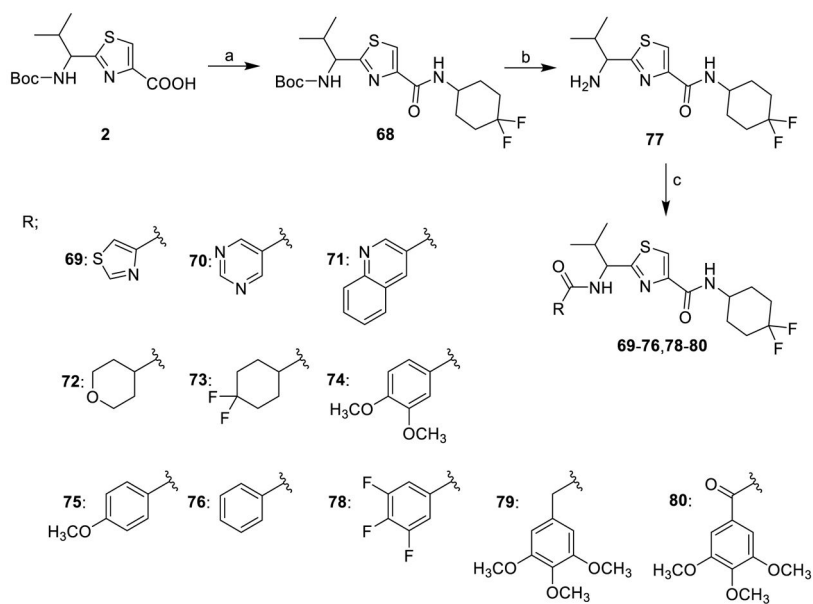
Scheme 7. Synthesis of Target Compounds 69–76 and 78–80 with Various Substitutions on the *N*-terminus of (*S*)-Valine Thiazole Amino Acid while Retaining the 4,4-Difluorocyclohexyl Substitution on the *C*-terminus[a]

[a]Reagents and conditions: (a) 4,4-Difluorocyclohexylamine, HCTU, HOBt, DIEA, CH$_2$Cl$_2$, 0°C to rt, overnight; (b) TFA, CH$_2$Cl$_2$, 0°C to rt, 12 h; (c) Appropriate acid, HCTU, HOBt, DIEA, CH$_2$Cl$_2$, 0°C to rt, overnight or appropriate acid chloride, DIEA, THF, 0°C to rt, overnight.
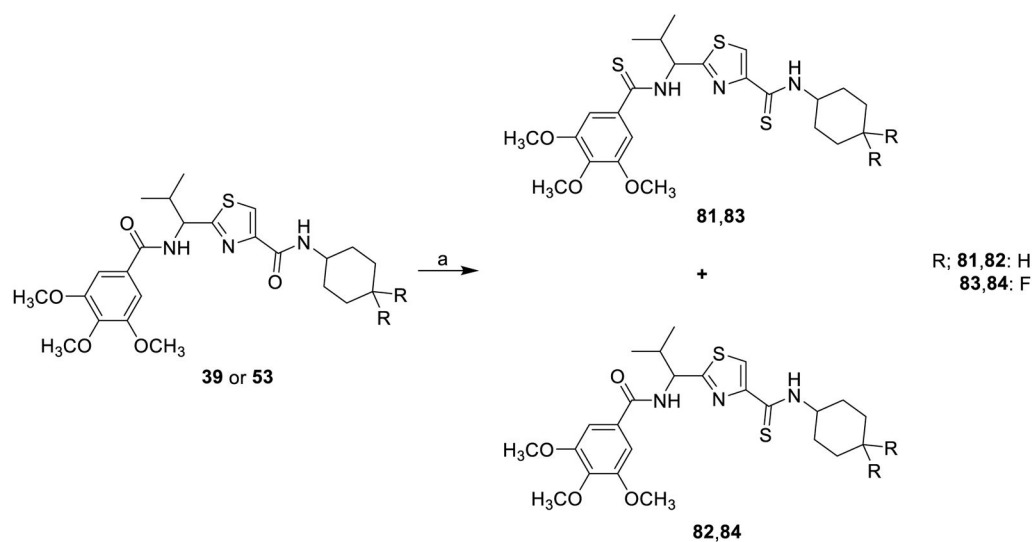
Scheme 8. Synthesis of the Thioamide Analogues 81–82 and 83–84 Starting from carboxamides 39 and 53, Respectively[a]

[a]Reagents and conditions: (a) Lawesson's reagent, THF, rt, 8 h.
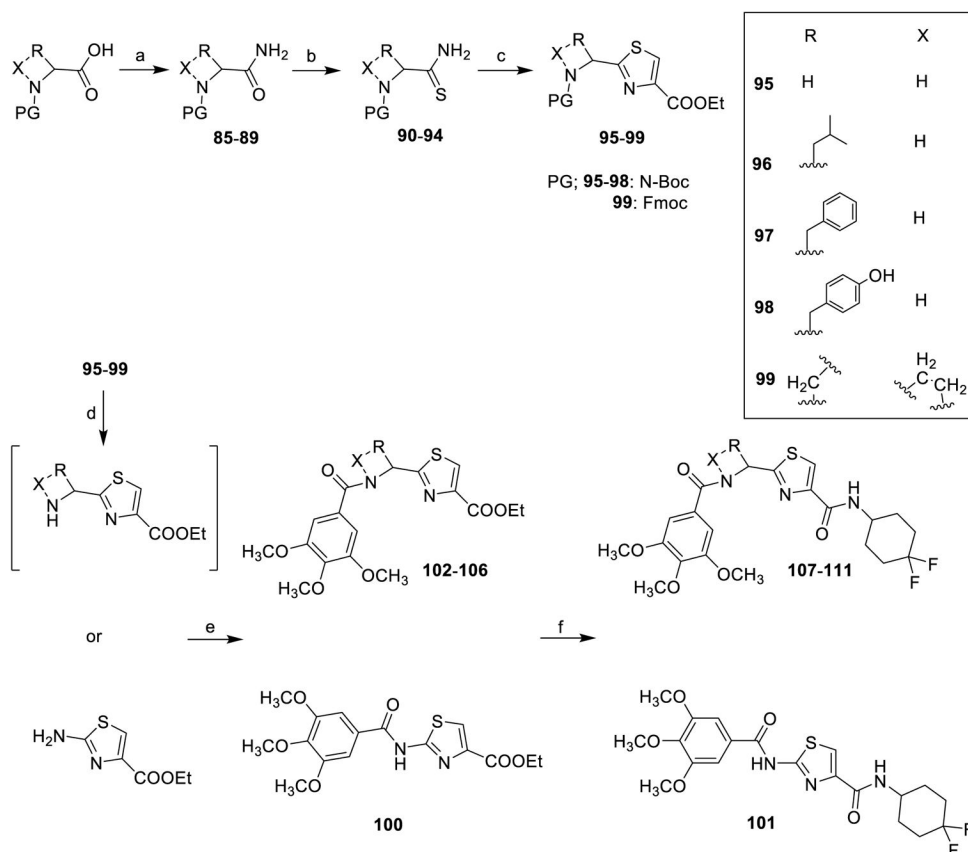
Scheme 9. Synthesis of the a-Carbon Modified Target Compounds 101 and 107–111[a]

[a]Reagents and conditions: (a) (i) Isobutyl chloroformate, *N*-methyl morpholine, THF, −20°C, 45 min-2 h; (ii) 30% NH$_4$OH in excess, −20°C to rt, 30 min-2 h; (b) Lawesson's reagent, THF, rt, 8 h; (c) Ethyl bromopyruvate, CaCO$_3$, EtOH, −20°C to rt, 8 h; (d) TFA, CH$_2$Cl$_2$, 0°C to rt, 12 h or 5% piperidine in DMF, rt, 2 h; (e) 3,4,5-Trimethoxybenzoyl chloride, DIEA, THF, 0°C to rt, overnight; (f) (i) NaOH, THF:Methanol:H$_2$O (10:2:3), 0°C to rt, overnight; (ii) 4,4-Difluorocyclohexylamine, HCTU, HOBt, DIEA, CH$_2$Cl$_2$, 0°C to rt, overnight.

Table 1

Effect of Positional Isomers, Isosteres, and Alicyclic Analogues of Compound 1 on the ATPase Activity of Human P-glycoprotein

Compd	R₁	R₂	ATPase activity[a] % Stimulation or [b] Inhibition			AlogP[c]
			0.05 µM	0.5 µM	2.5 µM	
1[d]	-		54	83	88	4.98
5			NA[e]	46	ND[f]	4.98
7	-		13	143	ND	4.98
18	-		21	142	ND	4.36

Compd	R₁	R₂	ATPase activity[a] % Stimulation or [b] Inhibition			AlogP[c]
			0.05 μM	0.5 μM	2.5 μM	
31			376	308	ND	4.29
32			ND	142.0±8.3	182.0±29.2	3.86
33			ND	121.7±15.8	194.5±24.5	3.86
34			ND	134.9±10.3	164.2±40.5	3.86
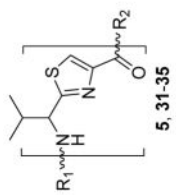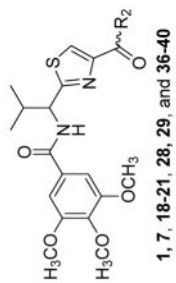
Compd	R$_1$	R$_2$	ATPase activity[a] % Stimulation or [b] Inhibition			AlogP[c]
			0.05 μM	0.5 μM	2.5 μM	
35			ND	99.4±3.5	84.7±8.6	4.61
36	-		NA	NA	ND	2.49
37	-		ND	25.3±13.2	NA	2.95
38	-		ND	NA	NA	3.41

Compd	R₁	R₂	ATPase activity[a] % Stimulation or [b] Inhibition			AlogP[c]
			0.05 μM	0.5 μM	2.5 μM	
39	-		20[b]	18[b]	ND	3.86
40	-		NA	NA	ND	4.15

[a] ATPase activity measured using High Five insect cells membranes expressing P-gp. Mean ± SD values shown are obtained from three independent experiments performed in duplicates. For some compounds, average values from two independent experiments in triplicates are given. Basal ATPase activity is considered as '0'. The values in the ATPase activity column indicates stimulation or

[b] inhibition of basal ATPase activity. Zosuquidar was used as a positive control, which showed inhibition of ATPase activity as 40% at 0.05 μM and 58% at 0.5 μM.

[c] Calculated using calculate property functionality in Maestro v10.1 (Schrödinger, LLC, New York, NY, 2015).

[d] Compound reported in our previous published work.[19]

ATPase values below or equal to 10% are indicated as

[e] NA and

[f] ND means Not Determined.

Table 2

Effect of the Cyclohexyl Analogues on ATP Hydrolysis by P-gp

Compd	R	ATPase activity[a] % Stimulation or [b] Inhibition		AlogP[c]
		0.5 μM	2.5 μM	
39	—NH (cyclohexyl)	18[b]	ND[f]	3.86
41	—NH (N-Boc piperidinyl)	52.7±11.2	48.0±13.9	3.47
42	—NH (piperidinyl NH)	46.3±4.9	14.5±5.3	1.58
43	—NH (tetrahydropyranyl)	26.9±18.9	NA[e]	1.84
44	—N (piperidinone)	NA	NA	1.83
46	—NH—CH2—(piperidinyl NH)	23.0±3.6	NA	2.16

Compd	R	ATPase activity[a] % Stimulation or [b] Inhibition		AlogP[c]
		0.5 μM	2.5 μM	
48		45.4±13.2	NA	1.35
50		NA	NA	2.67
51		NA	NA	1.57
52		13.1±0.3	NA	2.30
53		30.7±4.9[b]	33.9±2.9[b]	3.11
54		NA	34.3±3.6	4.32
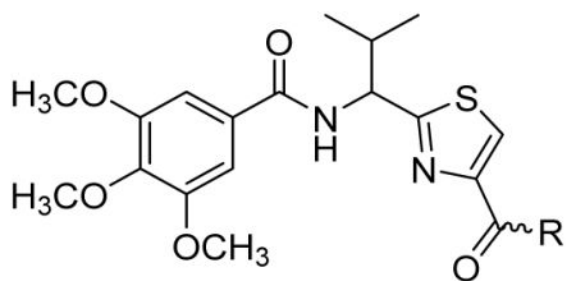
Compd	R	ATPase activity[a] % Stimulation or [b] Inhibition		AlogP[c]
		0.5 μM	2.5 μM	
55		109	134	5.93
56		37.2±1.6	47.2±20.7	3.24
57		169.1±16.3	153.5±21.2	3.70
58		17.1±6.6	83.0±18.2	2.53
59		108.5±17.3	122.8±24.3	4.60
60		33.9±4.3	31.3±19.1	4.60

a,b,c,e,f same as Table 1 footnotes.

Table 3

Modulation of ATPase Activity of P-gp by Difluorocyclohexyl Analogues
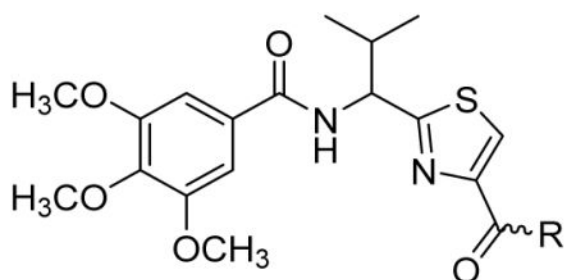
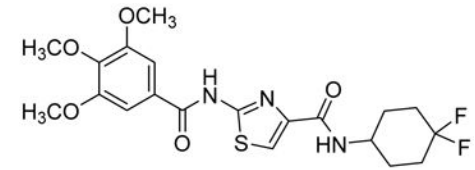| Compd	R	ATPase activity[a] % Stimulation or [b] Inhibition		AlogP[c]
		0.5 μM	2.5 μM	
53		30.7±4.9[b]	33.9±2.9[b]	3.11
61		40.9±3.2	55.9±16.9	3.03
62		13.3±2.2	75.6±2.5	3.73
63		27.0±3.5[b]	20.7±10.3	3.44
64		NA[e]	21.7±9.0[b]	4.47
65		59.5±3.5	244.4±3.4	3.79
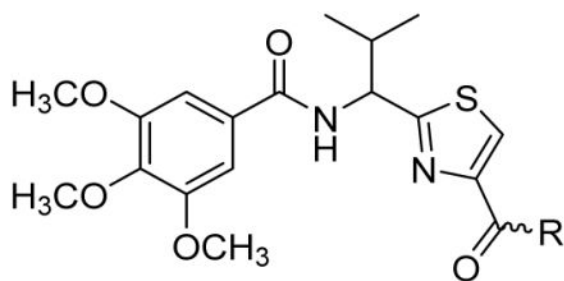
Compd	R	ATPase activity[a] % Stimulation or [b] Inhibition		AlogP[c]
		0.5 μM	2.5 μM	
66		57.0±0.4	176.3±6.3	4.20
67		17.1±0.4	77.3±1.1	3.59

[a,b,c,e] same as Table 1 footnotes.

Table 4

Effect of Amino Terminus Modification of Compound 53 on the ATPase Activity of P-gp
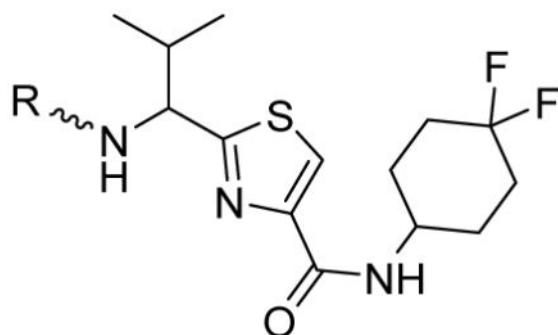
Compd	R	ATPase activity[a] % Stimulation or [b] Inhibition		AlogP[c]
		0.5 μM	2.5 μM	
53		30.7±4.9[b]	33.9±2.9[b]	3.11
69		ND[f]	49.7±27.18	1.86
70		ND	NA[e]	1.38
71		ND	83.9±27.0	3.34
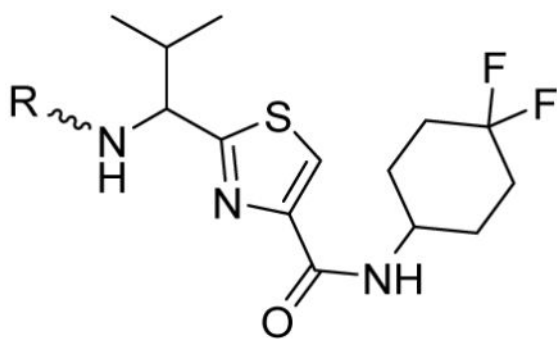
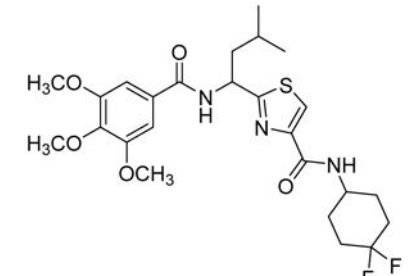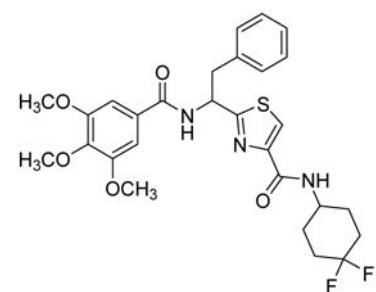| Compd	R	ATPase activity[a] % Stimulation or [b] Inhibition		AlogP[c]
		0.5 μM	2.5 μM	
72		ND	NA	1.85
73		NA	16.4±13.7[b]	2.87
74		ND	11.9±8.03	3.13
75		ND	35.8±14.2	3.14
76		ND	10.5±11.12	3.16
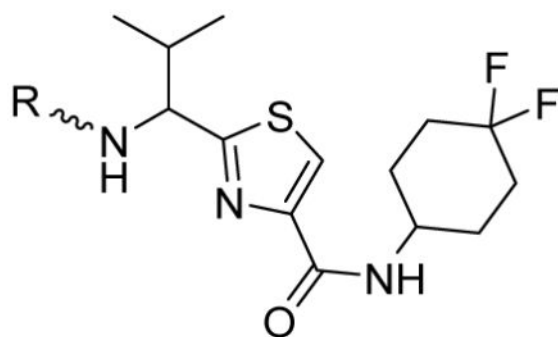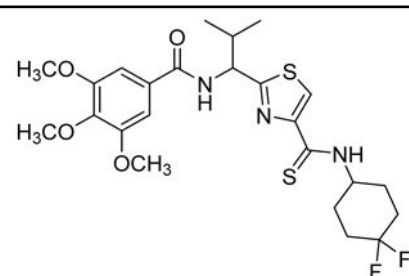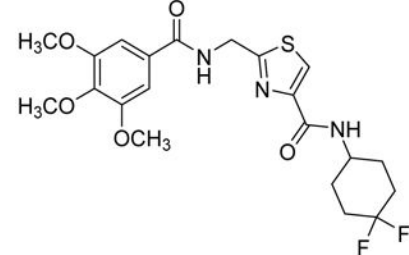
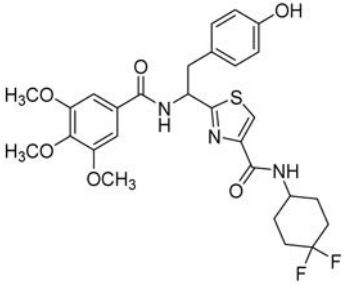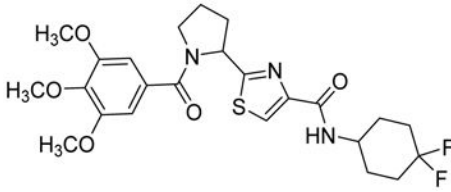| Compd	R	ATPase activity[a] % Stimulation or [b] Inhibition		AlogP[c]
		0.5 μM	2.5 μM	
77		NA	NA	1.47
78		16.6±1.3[b]	23.3±0.1[b]	3.77
79		ND	70.2±19.7	3.14
80		ND	177.5±26.7	3.00

a,b,c,e,f same as Table 1 footnotes.

Table 5

Effect of Bioisosteres and Amino Acid Analogues of compound 53 on P-gp ATPase Activity

Compd	Structure	ATPase activity[a] % Stimulation or [b] Inhibition		AlogP[c]
		0.5 μM	2.5 μM	
53		30.7±4.9[b]	33.9±2.9[b]	3.11
81		65	92	6.05
82		NA[e]	27	4.96
83		25	64	5.30

Compd	Structure	ATPase activity[a] % Stimulation or [b] Inhibition		AlogP[c]
		0.5 μM	2.5 μM	
84		37.4±5.5[b]	25.9±2.1[b]	4.20
101		53.9±0.6	60.2±2.7	2.12
107		NA	13	1.78
108		16	68	3.50
109		39.6±4.7[b]	42.5±6.3[b]	3.82

Compd	Structure	ATPase activity[a] % Stimulation or [b] Inhibition		AlogP[c]
		0.5 μM	2.5 μM	
110		22.4±4.9[b]	23.0±6.4[b]	3.55
111		NA	21	2.58

[a,b,c,e] same as Table 1 footnotes.

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Table 6

Reversal Effect of the Representative Compounds on the Cytotoxicity of Paclitaxel to Parental HEK 293-pcDNA 3.1 and P-gp transfected HEK-ABCB1 Cell Lines

Treatment	Conc.	HEK 293-pcDNA 3.1		HEK-ABCB1	
		$IC_{50} \pm SD$ (μM)[a]	FR[b]	$IC_{50} \pm SD$ (μM)[a]	FR[b]
Paclitaxel		0.071 ± 0.015	1	2.054 ± 0.087	28.9
+ **1**	10 μM	0.072 ± 0.021	1	0.046 ± 0.017	0.6
+ **31**	10 μM	0.037 ± 0.013	0.5	1.272 ± 0.190	17.9
+ **39**	10 μM	0.068 ± 0.014	1	0.042 ± 0.020	0.6
+ **53**	10 μM	0.041 ± 0.020	0.6	0.042 ± 0.016	0.6
+ **60**	10 μM	0.099 ± 0.031	1.4	0.207 ± 0.097	2.9
+ **109**	10 μM	0.037 ± 0.019	0.5	0.118 ± 0.015	1.7
+ Verapamil	10 μM	0.066 ± 0.012	0.9	0.052 ± 0.018	0.7
+ Zosuquidar	0.25 μM	0.013 ± 0.008	0.2	0.143 ± 0.010	2.0

[a] Half maximal inhibitory concentration (IC50) values calculated for paclitaxel concentration, with shown treatment, required for 50% inhibition of cell survival. Standard Deviation (±SD) values calculated from four independent experiments each performed in triplicates.

[b] Fold Resistance (FR) values calculated by dividing the IC50 value of paclitaxel in the presence of indicated compound, with shown treatment, in the ABCB1 expressing HEK-ABCB1 cells by the IC50 value in the presence of paclitaxel alone in HEK293 cells with control plasmid.

Table 7

In Vitro CYP3A4 Enzyme Inhibition Assay with Selected Compounds

Compound	CYP3A4 Inhibition (%)[a]	
	25 μM	50 μM
31	43.61 ± 3.20	83.33 ± 1.19
39	7.03 ± 5.33	51.95 ± 7.63
53	9.32 ± 1.32	28.66 ± 19.13
109	NI[b]	32.90 ± 5.36
1	IC_{50} = 8.23 ± 0.37 μM[c]	

[a] Mean values ± SD obtained using Vivid CYP3A4 green screening kit from two independent experiments each performed in triplicates. Ketoconazole was used as a positive control for CYP3A4 inhibition (IC_{50} = 0.04 μM).[58]

[b] No inhibition

[c] IC_{50} value for compound **1** as reported in Wang et. al.[20]