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Design, synthesis and biological evaluation of novel FXIa inhibitors with 2-phenyl-1*H*-imidazole-5-carboxamide moiety as P1 fragment

Yu Lei [a, 1], Bing Zhang [a, 1], Yan Zhang [b], Xiwen Dai [a], Yulin Duan [a], Qing Mao [a], Jun Gao [a], Yuwei Yang [a], Ziyang Bao [a], Xuefeng Fu [a], Kunqi Ping [a], Chengda Yan [c], Yanhua Mou [b, **], Shaojie Wang [a, *]

[a] *Key Laboratory of Structure-Based Drugs Design & Discovery of Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, 103 Culture Road, Shenhe District, Shenyang, 110016, China*
[b] *Department of Pharmacology, Shenyang Pharmaceutical University, 103 Culture Road, Shenhe District, Shenyang, 110016, China*
[c] *Department of Pharmacy, The First Hospital of China Medical University, 155 Nanjing North Street, Shenyang, 110001, Liaoning, China*

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ABSTRACT

Factor XIa, as a blood coagulation enzyme, amplifies the generation of the last enzyme thrombin in the blood coagulation cascade. It was proved that direct inhibition of factor XIa could reduce pathologic thrombus formation without an enhanced risk of bleeding. **WSJ-557**, a nonpurine imidazole-based xanthine oxidase inhibitor in our previous reports, could delay blood coagulation during its animal experiments, which prompted us to investigate its action mechanism. Subsequently, during the exploration of the action mechanism, it was found that **WSJ-557** exhibited weak *in vitro* factor XIa binding affinity. Under the guide of molecular modeling, we adopted molecular hybridization strategy to develop novel factor XIa inhibitors with **WSJ-557** as an initial compound. This led to the identification of the most potent compound **44g** with a Ki value of 0.009 μM, which was close to that of **BMS-724296** (Ki = 0.0015 μM). Additionally, serine protease selectivity study indicated that compound **44g** display a desired selectivity, more 400-fold than those of thrombin, factor VIIa and factor Xa in coagulation cascade. Moreover, enzyme kinetics studies suggested that the representative compound **44g** acted as a competitive-type inhibitor for FXIa, and molecular modeling revealed that it could tightly bind to the S1, S1′ and S2′ pockets of factor XIa. Furthermore, *in vivo* efficacy in the rabbit arteriovenous shunt model suggested that compound **44g** demonstrated dose-dependent antithrombotic efficacy. Therefore, these results supported that compound **44g** could be a potential and efficacious agent for the treatment of thrombotic diseases.

1. Introduction

Thrombotic diseases which include stroke, myocardial infarction and deep vein thrombosis, continue to be major causes of death worldwide [1–3]. For decades, warfarin and heparin have been the mainstay in the treatment and prevention of these thrombotic diseases [4–7]. However, they both cause enhanced risk of bleeding and have a narrow therapeutic index [7–9]. As a result, novel anticoagulants targeting two key serine proteases in the blood coagulation cascade: thrombin and factor Xa (FXa), are popular in treating thrombotic diseases [1,10,11]. The approved drugs such as direct FXa inhibitors (rivaroxaban [11], apixaban [12], and edoxaban [13]) and direct thrombin inhibitor (dabigatran [14]) are highly effective for the prevention and treatment of these diseases. Although they have offered patients an alternative to warfarin and heparin [4,15,16], there is still a risk of major bleeding as high as 2–3% per year in patients with thrombotic diseases which limit their widespread use [1,4,15,16]. Consequently, novel anticoagulants that can effectively reduce thrombosis without enhanced risk of bleeding are urgently needed [1,4,5,15,16].

Factor XIa (FXIa) as a potential feedback activator of FXI [4] amplifies the generation of the last enzyme thrombin in the blood

* Corresponding author.
** Corresponding author.
E-mail addresses: mu_hua_jj@sina.com (Y. Mou), wangshaojie@syphu.edu.cn (S. Wang).
[1] These two authors contributed equally to this work.

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coagulation cascade [17,18], which further leads to fibrin clot formation [17,18]. Several preclinical studies have demonstrated that inhibition of FXI (antisense oligonucleotides or neutralizing antibodies [19–21]) or FXIa (small-molecule inhibitors) could provide antithrombotic efficacy with a low risk of bleeding in animal thrombosis models [17,19–21]. In addition, it is shown that FXI gene disruption in mice does not lead to an increase in bleeding times [10,22], and human subjects with FXI deficiency such as hemophilia C [1,4] do not suffer spontaneous bleeding [1,4]. Furthermore, a recent phase II clinical trial with an antisense oligonucleotide targeting FXI proved efficacious in preventing venous thrombosis in patients undergoing total knee arthroplasty, and also appeared to be safe with respect to bleeding [17,20]. Taken together, these cases suggest that FXIa may serve as a powerful route to achieve an antithrombotic effect safer than that of FXa or thrombin [1,17–21].

Recently, several monoclonal antibodies targeting FXI (*e.g.,* **BAY 1213790** [23], **Xisomab 3G3** [24] and **MAA-868** [25]) and small-molecule FXIa inhibitors including **1** (**Milvexian** [26], phase II), **2** (**BMS-962212** [21], phase I), and **3** (**EP-7041** [27], phase I) in Fig. 1 have entered clinical trials. In addition, other FXIa inhibitors, such as **4** (**BMS-724296**) [2,28], **5** [17], **6** [29], **7** [30], **8** [20], **9** [4], **10** [16] and **11** [17] shown in Fig. 1, are in preclinical research. These cases inspire us to explore novel FXIa inhibitors to fulfill the urgent needs in clinical practice [1,17–21].

Human FXIa is a serine protease disulfide homodimer belonging to the intrinsic coagulation pathway [5,6]. The active site of FXIa contains several subsites with characteristic features for substrate selectivity. Substrate amino acid nomenclatures are P4–P3–P2–P1–P1′–P2′–P3′–P4′, respectively, and the substrate binding pockets are labeled in a corresponding manner to the substrate: S4, S3, S2, S1, S1′, S2′, S3′ and S4′, respectively [5,6]. Among them, P1–P1′ denotes the cleaved peptide bond, which implies that the corresponding S1 and S1′ pockets are crucial for catalyzing reaction of substrates [5,6]. It is reported that binding in multiple pockets in FXIa can lead to improve affinity and selectivity, and the active site of FXIa contains a deep S1 pocket accompanied by open and shallow prime binding regions S1′ and S2′ (Fig. 2) [1,5,6]. Therefore, For the S1 pocket that catalyzes the cleavage of the substrate peptide, S1–S1′–S2′ mode in FXIa is often used to

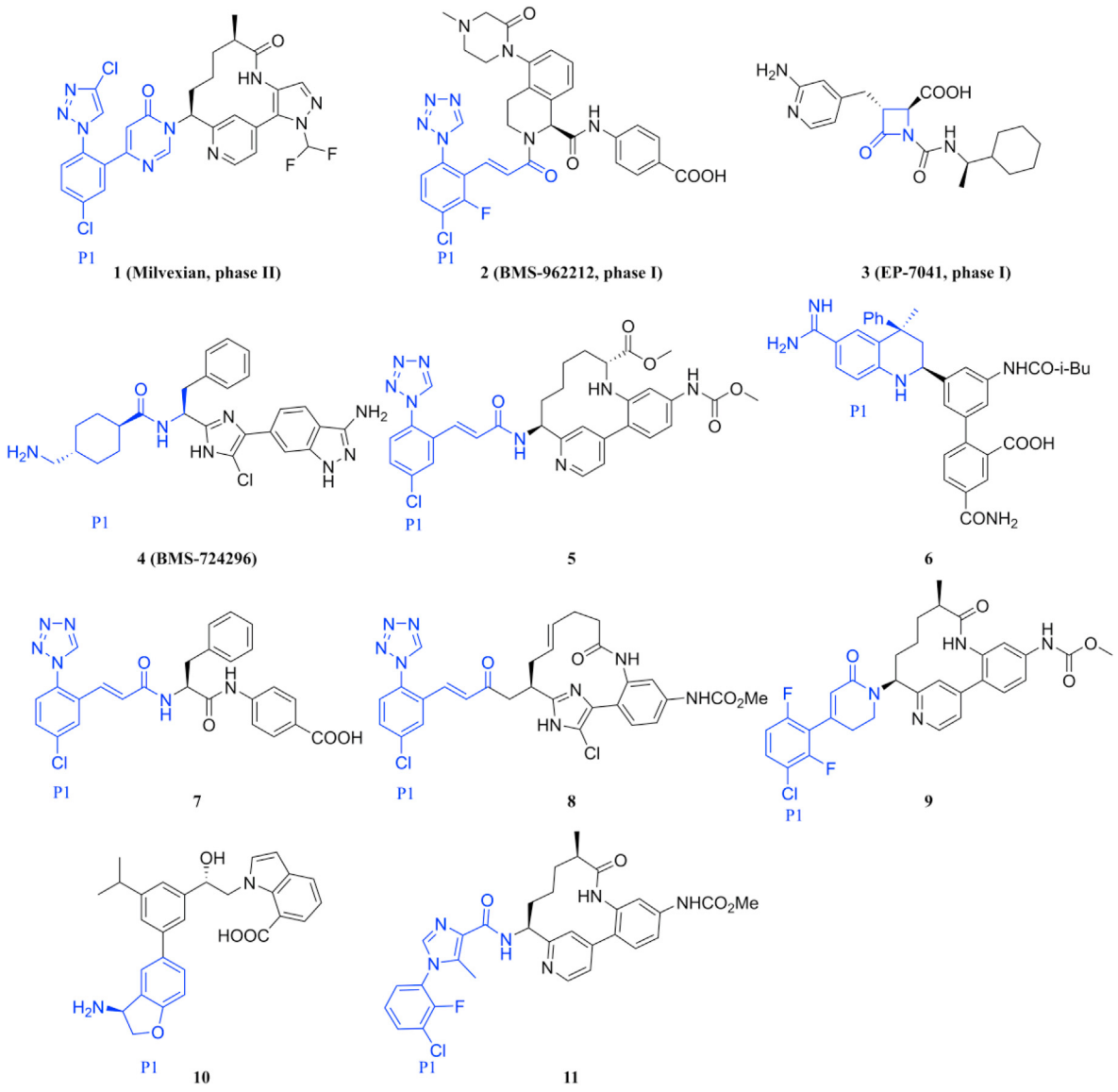


Fig. 1. Structures of the representative FXIa inhibitors: **1** (**Milvexian**, phase II), **2** (**BMS-962212**, phase I), **3** (**EP-7041**, phase I),**4** (**BMS-724296**), **5**, **6**, **7**, **8**, **9**, **10** and **11**.

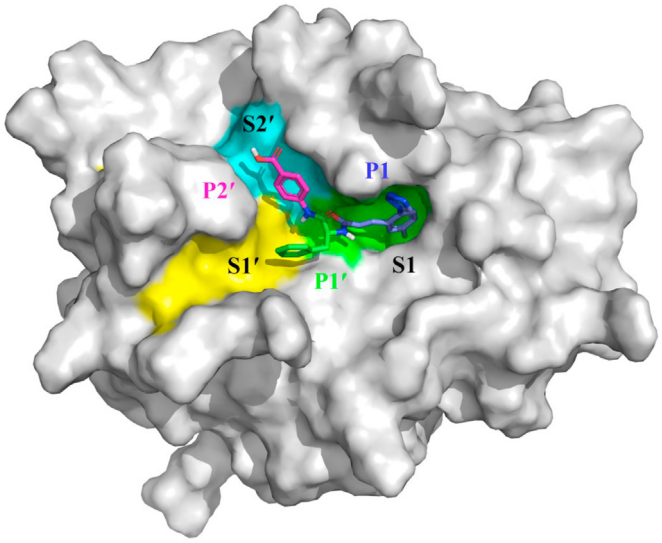


Fig. 2. Crystal structure of inhibitor **7** with FXIa (PDB: 5E2O) [30], and the surfaces of S1, S1′ and S2′ pockets are shown as green, yellow and cyan, respectively.

design novel FXIa inhibitors [1,5,6]. In addition, the oxyanion hole completes the perimeter of the S1 pocket and is formed by the backbone nitrogen atoms of residues Gly193, Asp194 and Ser195, which stabilizes the negative charge formed during proteolysis, and it is indicated that Gly193, Asp194 and Ser195 of oxyanion hole may be key amino acid residues for catalyzing the substrate peptide [1,5,6]. Moreover, the above reported compounds **1**–**11** (Fig. 1) all could contact with Ser195 of oxyanion hole through hydrogen bonds or covalent bonds, displaying excellent FXIa binding affinity, which further suggests that the interaction between P1 group and oxyanion hole is essential for design of FXIa inhibitors [16,17,20,21].

In our previous reports, **WSJ-557** (2-(3-cyano-4-isobutoxyphenyl)-1-hydroxy-4-methyl-1*H*-imidazole-5-carboxylic acid, a nonpurine XO inhibitor, Fig. 3) demonstrated stronger XO inhibitory potency than that of febuxostat (**WSJ-557** vs febuxostat, IC_{50} = 0.003 and 0.01 μM for XO) [31–33]. Interestingly, during its animal experiments, it was found that **WSJ-557** could delay blood coagulation [33], which encouraged us to investigate its action mechanism. Subsequently, molecular modeling study suggested that **WSJ-557** was accommodated in the FXIa S1 pocket, which implied that **WSJ-557** could be used as a P1 fragment to design novel FXIa inhibitors, and it was hoped that hydroxyl and carbonyl groups on imidazole moiety could interact with key amino acid residues (Gly193, Asp194 and Ser195) of oxyanion hole. Therefore, we attempted to introduce P1′ and P2′ fragments of FXIa inhibitor **7** into the carboxyl group of **WSJ-557** to design a series of novel FXIa inhibitors to investigate the possibility of **WSJ-557** as a P1 fragment (Fig. 3).

In this paper, we describe the procedure in discovery of a series of novel FXIa inhibitors. These FXIa inhibitors were evaluated on FXIa binding affinity *in vitro*, and the structure-activity relationships (SAR) were further explored. Moreover, serine protease selectivity profile, including thrombin, FVIIa, FXa and plasma kallikrein, was performed to investigate the selectivity of the most potent compound **44g**. Additionally, to determine the inhibitory behavior of these target compounds, the representative compound **44g** was further evaluated by molecular modeling studies and steady-state kinetic analysis. Furthermore, *in vivo* efficacy in the rabbit arteriovenous shunt model was carried out to explore the antithrombotic effect of **44g**.

2. Results and discussion

2.1. Discovery of lead compound *28a*

Molecular modeling study was employed to guide the structural optimization of imidazole derivatives. The result of molecular modeling showed that isobutoxy group of **WSJ-557** was inserted into the deep part of FXIa S1 pocket (Fig. 4), and its carboxyl group formed two electrostatic interactions with Arg39 in the FXIa active site. However, this bulky isobutoxy group pushed the hydroxyl group of imidazole moiety so far away from key residue Cys191 that it could not form hydrogen bond interaction, which reminded of us that decreasing the size of 4′-substitutions on phenyl moiety could induce phenyl moiety into the S1 pocket tightly in the hope of improving the FXIa affinity. Subsequently, the isobutoxy group on phenyl moiety was changed into methoxy, hydroxy groups or removed to give the imidazole derivatives **16a**, **17** and **16e** (Table 1), which all improved the FXIa affinity by more than 2-fold (**WSJ-557** < **16a** < **17** < **16e**, Ki = >500, 243, 228 and 120 μM, respectively, Table 1), thus verifying the above envisages. Among them, compound **16e** displayed more potent than those of others. In addition, the results of molecular modeling suggested that imidazole

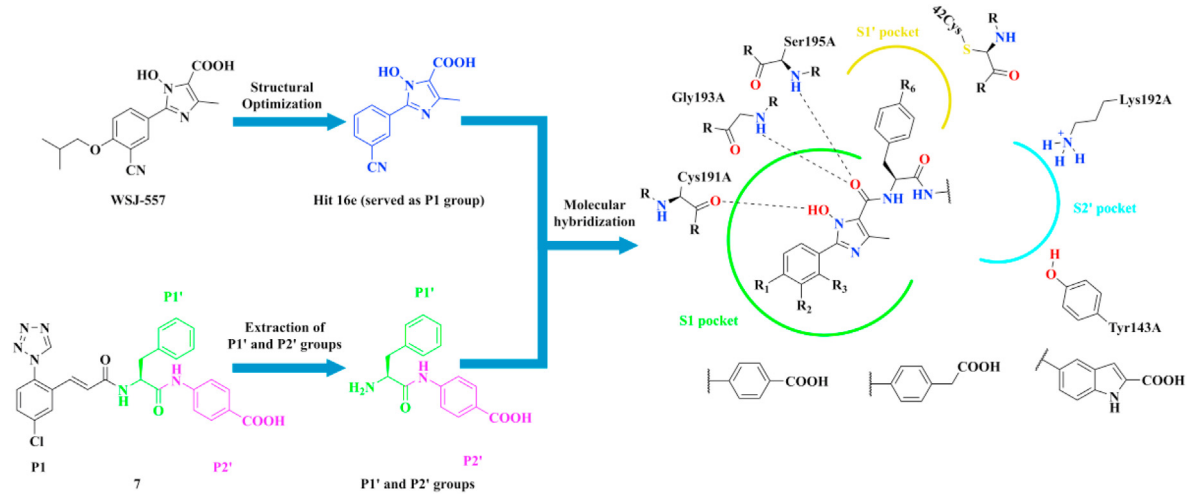


Fig. 3. Design of FXIa inhibitors based on **WSJ-557**.

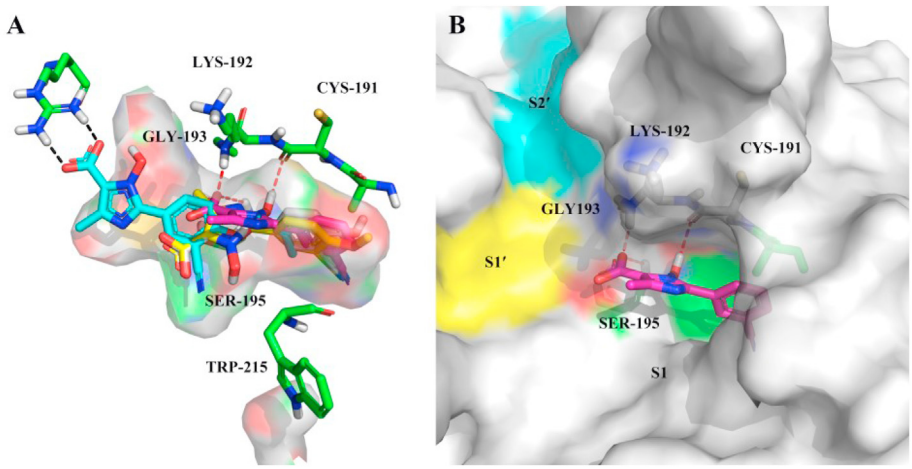
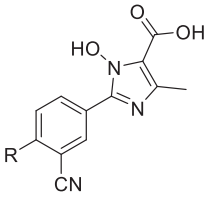
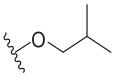


Fig. 4. Binding modes of compounds **16a**, **16e**, **17** and **WSJ-557** within the FXIa binding pocket. (A) The surface of the protein (PDB code 4TY7) [2] is shown in rainbow, and small molecules as well as residues (green) are shown as sticks. **16a** (yellow), **16e** (magenta), **17** (grey) and **WSJ-557** (cyan) occupy the same S1 binding pocket in FXIa. Electrostatic interactions of **WSJ-557** are shown as black dashed lines, and hydrogen bonds of **16e** are shown as red dashed lines, respectively. (B) Docking pose of **16e** (magenta) with FXIa.

Table 1
Structural modification of imidazole derivatives.



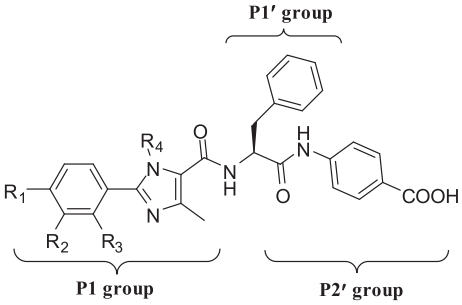
Compounds	R	Ki (μM)[a]
WSJ-557		>500[b]
16a	-OCH_3	243
16e	–	120
17	-OH	228

[a] Ki values were derived from IC_{50} values using Cheng Prussof transformation [37,38].
[b] Inhibition in 500 μM: 29.83%.

derivative **16e** could fit more tightly into S1 pocket than others. Specifically, carboxyl group of **16e** pointed into the oxyanion hole and formed two hydrogen bonds with key residues Gly193 and Ser195, in addition, its hydroxyl group could engage an extra hydrogen bond with Cys191 (Fig. 4B). Meanwhile, this optimization also made imidazole derivative **16e** almost closer to comply with the "rule of three" of the hit (see Table S1) [34–36]. Therefore, compound **16e** was selected as a hit for further optimization of novel FXIa inhibitors.

It was reported that binding in multiple pockets in FXIa could improve affinity and selectivity [5,6]. As a result, with using 2-phenyl-1*H*-imidazole fragment of **16e** as a P1 group, we introduced P1′ phenylalanine and P2′ *p*-aminobenzoic acid fragments into the carbonyl group on imidazole ring to afford the lead compound **28a**, which displayed a 5.03-fold increase for FXIa affinity in comparison to **16e** (Table 2, **28a** vs **16e**, Ki = 23.88 and 120 μM, respectively). This suggested that linking P1′ and P2′ fragments to fit into active pockets (S1′ and S2′) of FXIa was necessary for improving FXIa affinity. To investigate the effect of cyano group positions for P1 groups binding to S1 pocket, the corresponding

Table 2
The effect of substituents around the 2-phenyl-imidazole moiety on FXIa binding affinity.

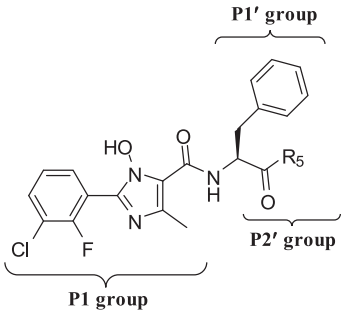


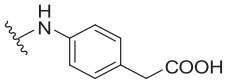
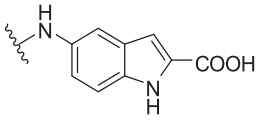
Compounds	R_1	R_2	R_3	R_4	Ki (μM)[a]
28a	-H	-CN	-H	-OH	23.88
28b	-H	-H	-CN	-OH	18.86
28c	-CN	-H	-H	-OH	>100[b]
28d	-H	-H	-H	-OH	>100[b]
28e	-Cl	-H	-H	-OH	>100[b]
28f	-H	-Cl	-H	-OH	14.50
28g	-H	-H	-Cl	-OH	>100[b]
28h	-H	-Cl	-F	-OH	11.76
28i	-H	-Cl	-F	-H	26.38
28j	-H	-Cl	-F	-CH_3	22.37
28k	-H	-Cl	-F	-OCH_3	>100[b]

[a] Ki values were derived from IC_{50} values using Cheng Prussof transformation [37,38].
[b] Inhibition rates of compounds **28b**, **28c**, **28e**, **28g** and **28k** at concentration of 100 μM were 16.4%, 0%, 22.5%, 34.1% and 0%, respectively.

ortho-, *para*- and no cyano substituted derivatives **28b-d** were prepared. Among them, the *ortho*-cyano substituted derivative **28b** retained a similar FXIa binding affinity as that of compound **28a** (**28b** vs **28a**, Ki = 18.86 and 23.88 μM, respectively), whereas *para*- and no cyano substituted derivatives **28c** and **28d** led to the loss of the FXIa affinity (Ki values of **28c** and **28d** were both more than 100 μM). According to the current case, the introduction of halogen atoms on P1 phenyl moiety could enhance the affinity [4,17,21]. Then, substituting the cyano groups on phenyl moiety with halogen atoms gave compounds **28e-h**. The compounds **28f**, **h** exhibited

Table 3
The FXIa binding affinity of 2-phenyl-1*H*-imidazole derivatives with various R_5 moieties.



Compounds	R_5	Ki (μM)[a]
34a		6.86
34b		2.82

[a] Ki values were derived from IC_{50} values using Cheng Prussof transformation [37,38].

slightly stronger than that of **28a** (**28f** > **28h** > **28a**, Ki = 11.76, 14.50 and 23.88 μM, respectively), while there was no improvement in FXIa binding affinity through inserting chlorine atom to *ortho*- and *para*-positions of phenyl moiety (Ki values of **28e** and **28g** were both more than 100 μM). As we continued to explore modifications of P1 group, compounds **28i** and **28k** were prepared through changing hydroxyl group on imidazole moiety into hydrogen, methyl or methoxy group, and they showed no increase in FXIa binding affinity (**28i**, **28j** and **28k** vs **28h**, Ki = 26.38, 22.37, >100 and 11.76 μM, respectively). These results indicated that the *meta*-chlorine and hydroxyl groups on 2-phenyl-1*H*-imidazole moiety were critical for improving FXIa binding affinity. Hence, compound **28h** was selected for further optimization by exploring structural modifications of P1′ and P2′ groups.

2.2. Discovery of compound *44g*

We next explored the modification of P2′ groups based on compound **28h**. Inserting a carbon atom between the carboxyl group and phenyl ring gave compound **34a**, which was 1.71-fold more potent than **28h** against FXIa (**34a** > **28h**, Ki = 6.86 and 11.76 μM, respectively, Table 3), suggesting that carboxyl group closer to the S2′ active pocket might be beneficial for FXIa binding affinity. Subsequently, in order to decrease the distance between the carboxyl group and key residues of S2′ pocket, the phenyl ring of P2′ group was replaced with an indole ring to afford compounds **34b** (Table 3), and results revealed that the addition of a carboxyl group to 2-position on the indole ring provided a 4.17-fold improvement in FXIa binding affinity in comparison with compound **28h** (**34b** > **28h**, Ki = 2.82 and 11.76 μM, respectively). Therefore, compound **34b** was selected for further optimization to investigate the FXIa binding affinity.

Based on the crystal structure of inhibitor **7**, it was found that there was a large space in P1′ prime region to accommodate substitutions at para-position of P1′ phenyl moiety. Therefore, introduction of various substitutions at the *para*-position of P1′ phenyl moiety was performed to occupy this space to investigate the FXIa binding affinity. Initially, the addition of halogen atoms (fluorine, chlorine and bromine) at *para*-position of P1′ phenyl moiety of compound **34b** offered compounds **39a-c** (Table 4) with Ki values of 0.486, 0.356 and 0.962 μM, respectively, which showed a 2.93−7.92-fold improvement for FXIa binding affinity compared with compound **34b**. But changing these halogen atoms to a nitro or amine group could damage the FXIa binding affinity (**39e** < **39d** < **34b**, Ki = 7.32, 3.08 and 2.82 μM, respectively).

Then, with carboxamide as a linker, insertion of various substitutions at the carbonyl carbon atom of the carboxamide moiety to fill the S1′ active pocket was further explored. Specifically, increasing the size of the substituent at carbonyl carbon of the carboxamide moiety from a methyl to an isobutyl group could steadily strengthen the FXIa binding potency (**44a** < **44b** < **44c**, Ki = 0.392, 0.156 and 0.026 μM, respectively). Moreover, the replacement of saturated alkyl groups with an ethynyl or prop-1-yn-1-yl group at carbonyl carbon of the carboxamide moiety provided compounds **44d-e** with Ki values of 0.053 and 0.031 μM, retaining desired FXIa binding potency, nevertheless, they did not show apparent increase on FXIa binding potency compared with that of **44c**. This finding indicated that increasing the size of saturated alkyl and alkynyl groups at carbonyl carbon of the carboxamide moiety could maintain a satisfactory FXIa binding potency, which might be due to the improved hydrophobic interactions with residues at the S1′ active pocket.

To continue to investigate the size effect of the R_6 substituents on binding potency, the conversion of saturated alkyl groups into various cycloalkyl substitutions led to compounds **44f-j** (Table 4). Interestingly, compound **44g** showed the most potent FXIa binding affinity with a Ki value of 0.009 μM, which was close to that of **BMS-724296** (Ki = 0.0015 μM). Presumably, the 2-methylcyclopropane-1-carboxamido substitution on P1′ phenyl moiety kept the P1′ group in a more favorable position so that it could fill better in FXIa S1′ active pocket. Following this discovery, cycloalkyl substitutions were changed into various aromatic rings, and compounds **44k-m** were prepared, yet they resulted in a 9.22−40.67-fold decrease in FXIa binding affinity in comparison to **44g** (**44k**, **44l**, **44m** < **44g**, Ki = 0.366, 0.155, 0.083 and 0.009 μM, respectively). This implied unsubstituted aromatic groups on P1′ phenylalanine linker was not fit to fill in S1′ active pocket, likely owing to the steric hindrance of these aromatic substituents with the amino acids at the entrance of the S1′active pocket.

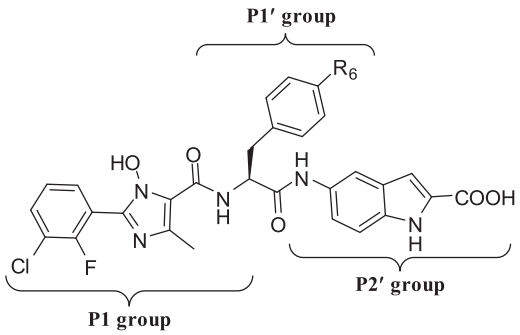
2.3. Serine protease selectivity

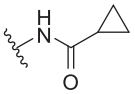
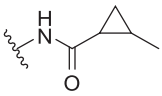
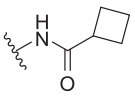
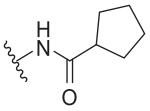
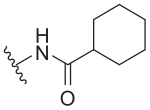
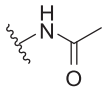
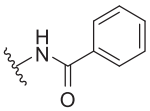
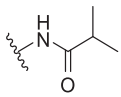
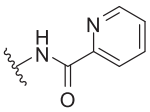
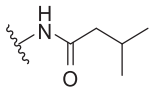
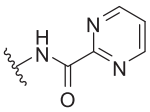
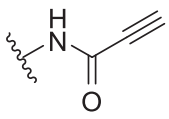
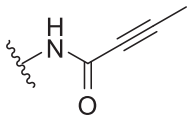
To investigate the selectivity of the most potent compound **44g**, serine protease selectivity was further evaluated against other serine proteases including factor VIIa (FVIIa), factor Xa (FXa), thrombin and plasma kallikrein in the coagulation cascade. In Table 5, compound **44g** exhibited >400-fold selectivity against FVIIa, FXa and thrombin, except for plasma kallikrein (21.3-fold).

2.4. Molecular modeling

To explore a probable binding model of inhibitors and FXIa active site, molecular docking of the representative compound **44g** in the substrate binding pocket of FXIa was performed using the Glide XP docking protocol (2016, Schrödinger Suite) [39], and **BMS-724296** was used as a reference [2]. Co-crystallized ligand **BMS-724296** was redocked using the Glide XP docking protocol. The results suggested that the key interactions between ligand and key residues of active pocket were consistent with that of crystallographic bound ligand (Fig. 5A and B), which verified that the docking model was reliable.

Table 4
The effect of *para*-substituents at P1′ phenyl moiety on FXIa binding affinity.



Compounds	R_6	Ki (μM) [a]	Compounds	R_6	Ki (μM) [a]
39a	-F	0.486	**44f**		0.259
39b	-Cl	0.356	**44g**		0.009
39c	-Br	0.962	**44h**		0.0225
39d	$-NO_2$	7.32	**44i**		0.0339
39e	$-NH_2$	3.08	**44j**		0.0563
44a		0.392	**44k**		0.366
44b		0.156	**44l**		0.155
44c		0.026	**44m**		0.0813
44d		0.053	**BMS-724296**	-	0.0015
44e		0.031			

[a] Ki values were derived from IC_{50} values using Cheng Prussof transformation [37,38].

Table 5
Human serine protease selectivity profile for compound **44g**.

Human enzyme (Ki)	**44g** (nM)[a]
FXIa	9.0
FVIIa	4193
FXa	>8000
Thrombin	>12,500
Plasma kallikrein	191.8

[a] Ki values were derived from IC_{50} values using Cheng Prussof transformation [37,38].

In the P1 prime region (Fig. 5A, C), 2-phenyl-1*H*-imidazole scaffold as a P1 group could bind deep into the S1 active pocket. Specifically, the carbonyl oxygen of 2-phenyl-1*H*-imidazole-5-amide moiety was positioned over the oxyanion hole formed by Gly193, Asp194 and Ser195 to participate in two key hydrogen bonds with the backbone amino groups of Gly193 and Ser195, which was consistent with that of the carbonyl oxygen atom in **BMS-724296** [2]. Moreover, the hydroxyl group of imidazole moiety could engage in a hydrogen bond with backbone carbonyl of Cys191. Interestingly, two halogen interactions were observed between the fluorine atom of P1 phenyl moiety and Ser214 and Trp215 [40]. Consequently, the above results suggested that 2-phenyl-1*H*-imidazole scaffold of **WSJ-557** could be used as P1 fragment to design novel FXIa inhibitor. In the P1′ prime region (Fig. 5C), phenyl ring of P1′ phenylalanine linker occupied on the entrance of S1′ active pocket and interacted with Cys42 through a π-sulfur interaction. In the deepest part of the P1′ active pocket, the amide carbonyl group on the phenyl ring of P1′ formed an additional hydrogen bond with the guanidine group of Arg39. In the P2′ prime region (Fig. 5C), the nitrogen atom of the indole moiety formed a hydrogen bond with the side chain of Tyr143. Furthermore, carboxyl group interacted with the side chain of Lys192 via a strong electrostatic interaction.

2.5. Steady-state kinetic analysis

To investigate the action mode of the target compounds with FXIa, enzyme kinetics studies of the representative compound **44g** were performed. As shown in Fig. 6, the analysis on the data of compound **44g** indicated that V_{max} remained unchanged with changing slope (K_m/V_{max}) in the presence of increasing concentrations of the inhibitor, where the K_m values in the presence of

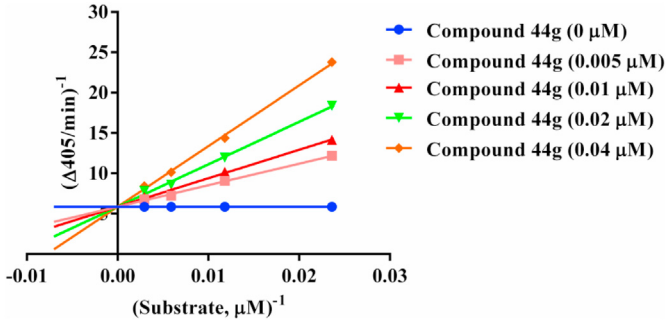


Fig. 6. Lineweaver-Burk plots of FXIa inhibition by compound **44g**.

0.005−0.04 μM of compound 44 g were 45.54, 60.28, 89.85 and 128.25 μM, respectively, and V_{max} was a constant value of 0.17 μM/min. This behavior revealed that **44g** acted as a competitive-type inhibitor with respect to FXIa. In addition, dose-dependent inhibition of FXIa by **44g** was exhibited (Fig. 7) [41].

2.6. In vivo efficacy in the rabbit arteriovenous shunt model

To determine its antithrombotic effect, compound **44g** was next performed in the rabbit arteriovenous shunt (AV shunt) thrombosis

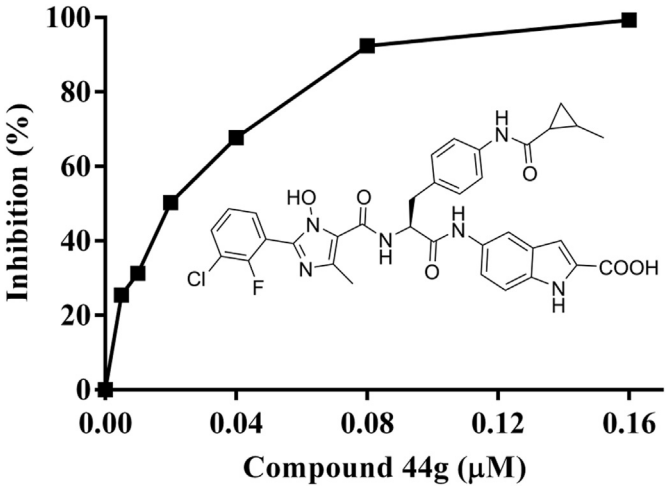


Fig. 7. The inhibition of FXIa by compound **44g**. Values are means ± SDs, n = 3.

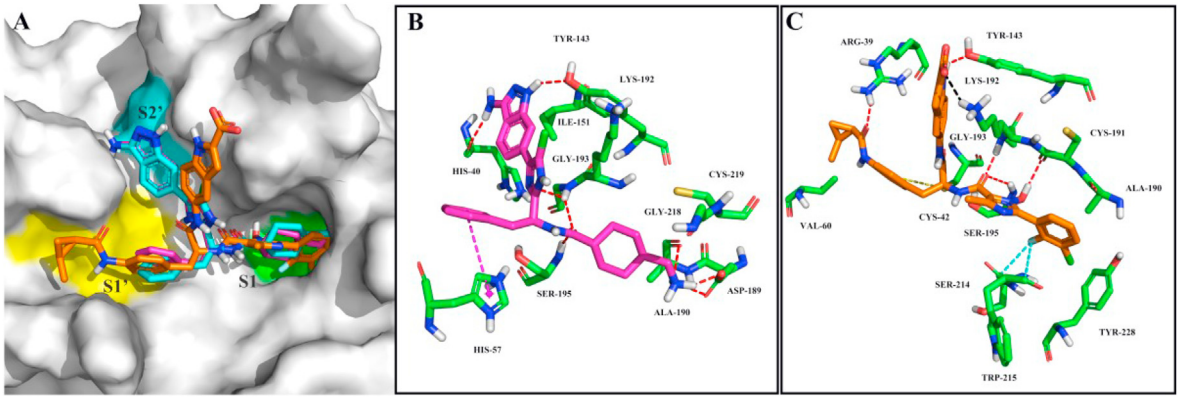


Fig. 5. Binding modes of compounds **44g** and **BMS-724296** within the FXIa binding pocket. (A) Superposition of compound **44g** (orange), re-docked **BMS-724296** (magenta) and crystallographic ligand **BMS-724296** (cyan) occupy the same binding site in FXIa. The surface of the protein (PDB code 4TY7) is shown in grey, and small molecules are shown as a line. (B) FXIa residues interacting with re-docked BMS-724296 are depicted by green lines. Hydrogen bonds and π-π stacking interactions are shown as red and magentas dashed lines, respectively. (C) FXIa residues interacting with compound **44g** are depicted by green lines. Hydrogen bonds, π-π stacking interactions, electrostatic interactions and halogen interactions are shown as red, magenta, black and cyan dashed lines, respectively.

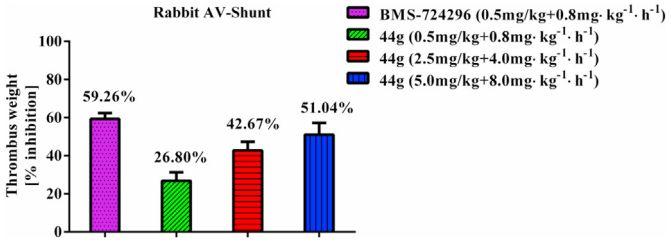
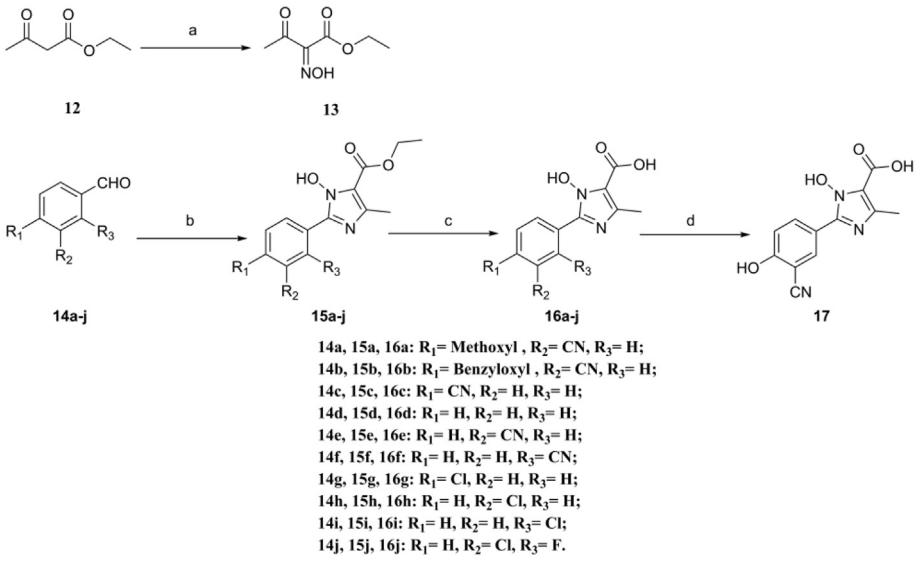


Fig. 8. Antithrombotic effect of compounds **44g** and **BMS-724296** in the rabbit AV-shunt model. Data are mean values ± SD (n = 3 per group).

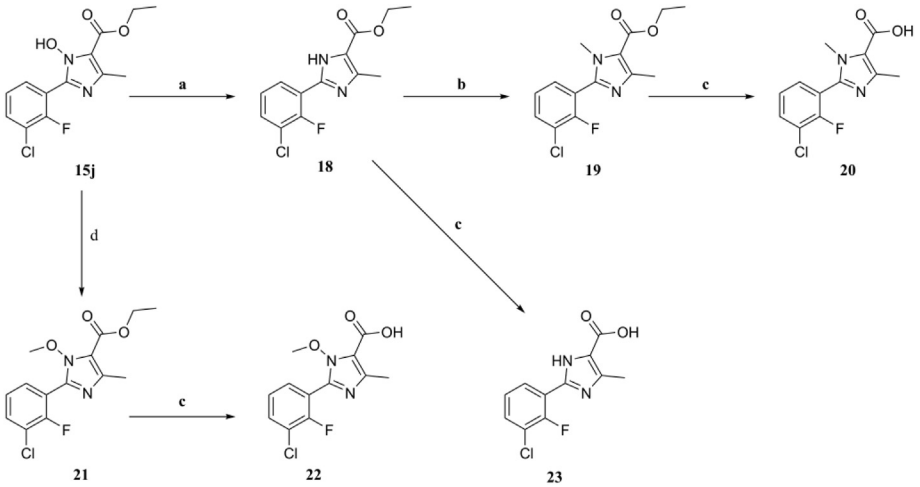
model (Fig. 8), and **BMS-724296** was used as a positive control. At a single intravenous (IV) dose of 0.5 mg/kg + 0.8 mg $kg^{-1}\cdot h^{-1}$, positive control **BMS-724296** could markedly reduce thrombus weight, suggesting that the model was successfully established. Moreover, compound **44g** was given as a single loading dose followed by continuous IV infusion at three doses: 0.5 mg/kg + 0.8 mg $kg^{-1}\cdot h^{-1}$, 2.5 mg/kg + 4.0 mg $kg^{-1}\cdot h^{-1}$, 5.0 mg/kg + 8.0 mg $kg^{-1}\cdot h^{-1}$, and inhibitions of thrombus weight were 26.80%, 42.67% and 51.04%, respectively, which suggested that compound **44g** caused a dose-dependent reduction of thrombus weight with an ED_{50} of 4.7 mg/kg + 7.5 mg $kg^{-1}\cdot h^{-1}$. Specifically, at the highest dose (5.0 mg/kg + 8.0 mg $kg^{-1}\cdot h^{-1}$), antithrombotic effect of compound **44g** was compared to that of **BMS-724296** (0.5 mg/kg +0.8 mg $kg^{-1}\cdot h^{-1}$). The results of *in vivo* efficacy in the rabbit arteriovenous shunt model suggested that compound 44 g was a potential and efficacious agent in the treatment of thrombotic diseases.

2.7. Chemistry

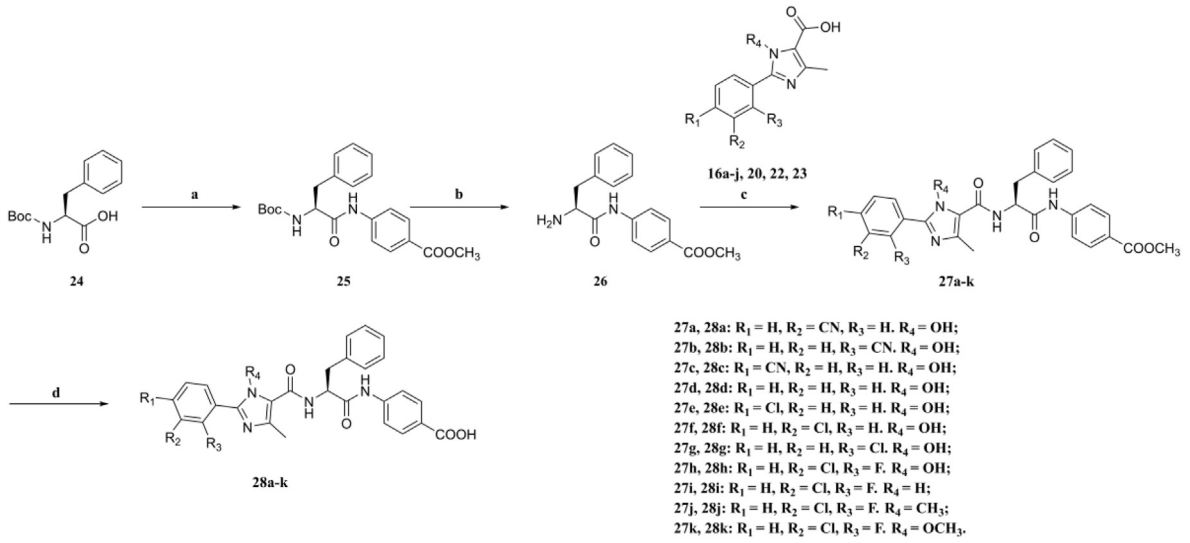
The syntheses of compounds **16a-j** and **17** were shown in Scheme 1. The key intermediate ethyl 2-hydroxyimino-3-oxobutanoate **13** was obtained by nitrosation of the commercially available ethyl 3-oxobutanoate **12** with sodium nitrite in acetic acid [31], which was cyclized with the corresponding benzaldehyde derivatives 14a-j to give the intermediates **15a-j** [31]. Then, compounds **16a-j** were prepared through hydrolysis of intermediates



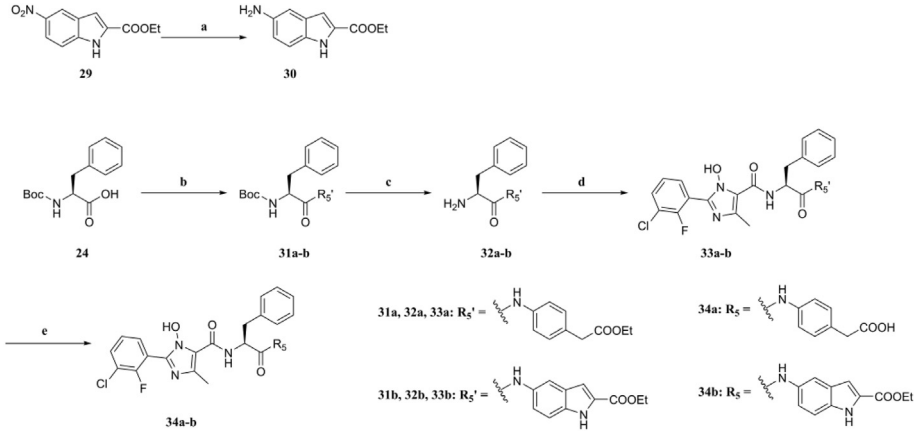
Scheme 1. Reagents and conditions: (a) $NaNO_2$, AcOH, 0°C; (b) **13**, CH_3COOH/CH_3COONH_4, 50°C; (c) LiOH, THF, H_2O, 50°C; (d) H_2, Pd/C, Methanol, 25°C.



Scheme 2. Reagents and conditions: (a) Me_3SiCl, NaI, CH_3CN, reflux; (b) MeI, K_2CO_3, N_2, DMF, 25°C; (c) LiOH, THF, H_2O, 50°C; (d) Me_2SO_4, K_2CO_3, DMF, 0°C.



Scheme 3. Reagents and conditions: (a) HATU, DMF, DIPEA, methyl 4-aminobenzoate, 25°C; (b) 4 M HCl in ethyl acetate; (c) HATU, DMF, DIPEA, **16a-j**, **20**, **22** or **23**, 25°C; (d) LiOH, THF, H_2O, 50°C.



Scheme 4. Reagents and conditions: (a) THF, 10% Pd/C, $HCOONH_4$, 60°C; (b) HATU, DMF, DIPEA, amines, 25°C; (c) 4 M HCl in ethyl acetate; (d) HATU, DMF, DIPEA, **16j**, 25°C; (e) LiOH, THF, H_2O, 50°C.

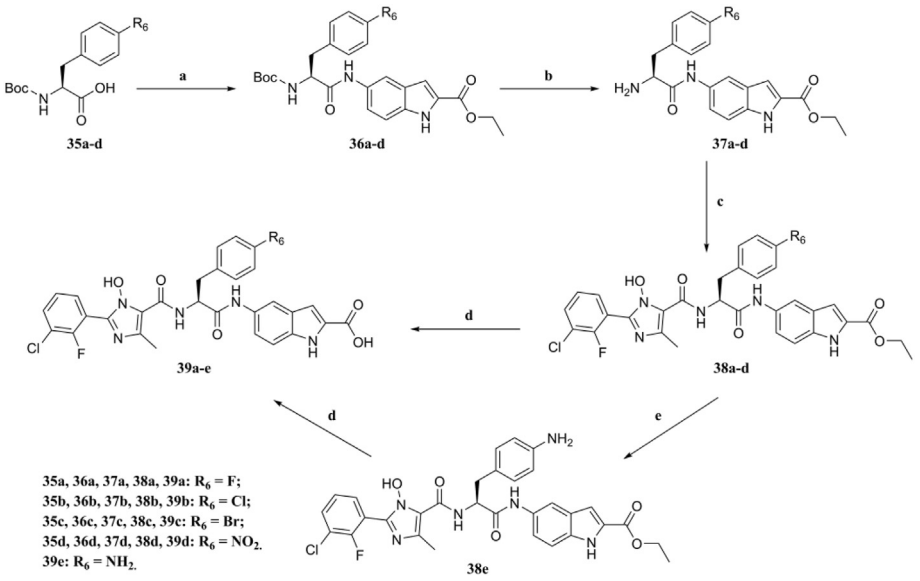
15a-j using an aqueous solution of lithium hydroxide, among them, the benzyl group of compound **16b** was removed in H_2 and Pd/C to provide compound **17**.

The syntheses of compounds **20**, **22** and **23** were shown in Scheme 2. With compound **15j** as a starting material, removal of a hydroxyl group in chlorotrimethylsilane and sodium iodide afforded intermediate **18** [31], and insertion of a methyl group offered intermediate **21** by alkylation reaction with Me_2SO_4 in K_2CO_3 and DMF. Then, intermediate **19** was prepared through the alkylation reaction of the resulting intermediate 18 with iodomethane in DMF in the presence of K_2CO_3. Finally, compounds **20**, **22** and **23** were obtained by hydrolyzing intermediates **18**, **19** and **21** in an aqueous solution of lithium hydroxide.

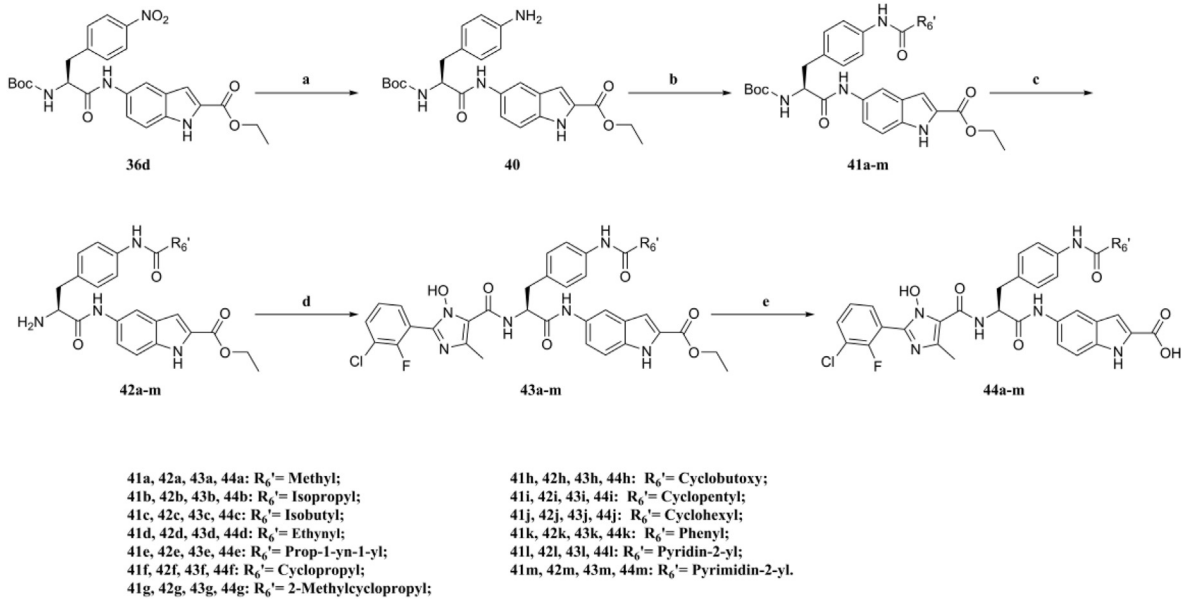
Syntheses of compounds **28a-k** were shown in Scheme 3. Commercially available starting material **24** was activated with HATU as a coupling reagent and then reacted with methyl 4-aminobenzoate to yield intermediate **25** [42], which further provided intermediate **26** through removing the Boc protected group [2]. Then, the resulting intermediate **26** was treated with carboxylic acids **16a-j**, **20**, **22** and **23** to give intermediates **27a-k**. Subsequent hydrolysis of the esters **27a-k** offered target compounds **28a-k** in an aqueous solution of lithium hydroxide with a good yield.

The syntheses of compounds **34a-b** were described in Scheme 4. Ethyl 5-amino-1*H*-indole-2-carboxylate **30** was prepared by reducing the nitro group of ethyl 5-nitro-1*H*-indole-2-carboxylate **29** catalyzed by Pd/C in $HCOONH_4$ and THF. Following the same procedure as described in Scheme 3, condensation of (*tert*-butoxycarbonyl)-L-phenylalanine **24** with **30**, ethyl 2-(4-aminophenyl) acetate or methyl 5-amino-1*H*-indole-3-carboxylate gave intermediates **31a-b** in the presence of HATU and DIPEA. Then, removal of Boc protected groups in aqueous hydrochloric acid afforded **32a-b**, and amino groups of **32a-b** followed by condensation with intermediate **16j** provided intermediates **33a-b**, which were followed by hydrolysis reactions using an aqueous solution of lithium hydroxide to give compounds **34a-b**.

The compounds **39a-e** were prepared as shown in Scheme 5. Reaction of commercially available starting materials **35a-d** with **30** afforded compounds **36a-d** in the same manner as Schemes 3 and 4. Then, deprotection of Boc group in aqueous 4 M HCl in ethyl acetate yielded compounds **37a-d**, which further reacted with



Scheme 5. Reagents and conditions: (a) HATU, DMF, DIPEA, **30**, 25°C; (b) 4 M HCl in ethyl acetate; (c) HATU, DMF, DIPEA, **16j**, 25°C; (d) LiOH, THF, H_2O, 50°C; (e) 5% Pt/C, Ethanol, H_2, 25°C.



Scheme 6. Reagents and conditions: (a) 5% Pt/C, EtOH, H_2, 25°C; (b) HATU, DMF, DIPEA, carboxylic acid derivatives, 25°C; (c) HCl in ethyl acetate; (d) HATU, DMF, DIPEA, **16j**, 25°C; (e) LiOH, THF, H_2O, 50°C.

intermediate **16j** by condensation reaction to give compounds **38a-d**. Among them, compound **38d** was converted into **38e** by reducing the nitro group under a hydrogen atmosphere at room temperature catalyzed by Pd/C in ethanol. The resulting compounds **38a-e** were subsequently hydrolyzed to provide compounds **39a-e**.

The compounds **44a-m** were prepared in five steps from intermediate **36d** as shown in Scheme 6. Reduction of intermediate **36d** gave compound **40**, followed by condensation with carboxylic acid derivatives which afforded compounds **41a-m** in the presence of HATU and DIPEA. Deprotection of compounds **41a-m** with an aqueous 4 M HCl provided compounds **42a-m**, followed by condensation with intermediate **16j** to offer compounds **43a-m**. Subsequent hydrolysis of the resulting compounds **43a-m** afforded compounds **44a-m** in the same method as Schemes 1–5.

3. Conclusion

Based on our previously reported nonpurine XO inhibitor **WSJ-557** with an apparent anticoagulation potency, structural modification was employed, which resulted in the discovery of hit **16e** (Ki = 120 μM) under the guide of molecular modeling. Subsequently, we adopted molecular hybridization strategy to introduce P1′ phenylalanine and P2′ *p*-aminobenzoic acid fragments into the carbonyl group of hit **16e** to afford lead compound **28a** (Ki = 23.88 μM), which was further structurally optimized to lead to

identification of the most potent compound **44g** (Ki = 0.009 μM), close to that of **BMS-724296** (Ki = 0.0015 μM). Moreover, molecular modeling provided the molecular basis for rationalizing the binding affinity of the designed compounds and suggested that 2-phenyl-1*H*-imidazole-5-carboxamide moiety could be used as a P1 fragment. In addition, the result of serine protease selectivity indicated that the representative compound **44g** displayed a desired selectivity against thrombin, FVIIa, FXa and plasma kallikrein in the coagulation cascade. Furthermore, *in vivo* efficacy in the rabbit AV shunt model suggested that compound **44g** demonstrated dose-dependent antithrombotic efficacy. Therefore, these results supported that compound **44g** could be a potential and efficacious agent for treatment of thrombotic diseases. The investigations performed in this research confirmed the possibility of nonpurine imidazole XO inhibitor **WSJ-557** as a P1 fragment to design novel FXIa inhibitors.

4. Experimental protocols

4.1. Chemistry

Compounds **WSJ-557**, **13**, **15a-f**, **16a-b** and **17** were prepared from our previous report [31]. In addition, positive control **BMS-724296** was synthesized by the reported literature [2], and compounds **12**, **14a-j**, **24**, **29** and **35a** were commercially available. Reagents and solvents were purchased from commercial sources and used without further purification. All reactions were monitored by TLC using silica gel aluminum cards (0.2 mm thickness) with 254 nm and 365 nm fluorescent indicator. Melting points were obtained using a YRT-3 melting apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Bruker 400 or 600 MHz spectrometer, and ^{13}C NMR spectra were recorded on a Bruker 400 or 600 MHz spectrometer. Chemical shifts were expressed in parts per million using tetramethylsilane as an internal reference and DMSO-d_6 as the solvent. ESI-MS data were gathered using an Agilent 1100 instrument and ESI-HRMS data were recorded in the Agilent 6540 Series Q-TOF-MS system.

4.1.1. Synthesis of ethyl 2-(4-chlorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylate (**15g**)

A mixture of 4-chlorobenzaldehyde **14g** (32.1 g, 0.229 mol), ethyl 2-hydroxyimino-3-oxobutanoate **13** (43.7 g, 0.285 mol), ammonium acetate (176.3 g, 2.29 mol), and acetic acid (600 mL) was stirred at 50 °C under nitrogen atmosphere for 24 h. The reaction mixture was cooled to room temperature and then slowly poured into cold water (2000 mL). The resulting precipitate was filtered, dried, and washed with ethyl acetate to obtain the compound **15g** as a white solid, yield: 87.1%. Mp 147.5–148.7 °C. ESI-MS (m/z) = 281.11 [M +H] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35–7.89 (m, 2H), 7.72–7.23 (m, 2H), 4.31–4.26 (m, 2H), 2.37 (s, 3H), 1.31 (t, J = 7.1 Hz, 3H).

4.1.2. Synthesis of ethyl 2-(3-chlorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylate (**15h**)

Compound **15h** was prepared from the compound **14h** in the same manner as described for **15g**. A wihte solid, yield: 81.8%. Mp 144.2–145.4°C. ESI-MS (m/z) = 281.07 [M+H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 8.08 (s, 1H), 8.05–7.98 (m, 1H), 7.51 (dd, J = 3.7, 2.0 Hz, 2H), 4.28 (q, J = 7.1 Hz, 2H), 2.21 (s, 3H), 1.31 (t, J = 7.1 Hz, 3H).

4.1.3. Synthesis of ethyl 2-(2-chlorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylate (**15i**)

Compound **15i** was prepared from the compound **14i** in the same manner as described for **15g**. A yellow solid, yield: 77.6%. Mp 99.8–101.2°C. ESI-MS (m/z) = 281.07 [M +H] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.75 (s, 1H), 7.60 (dt, J = 9.2, 1.3 Hz, 1H), 7.57–7.49 (m, 2H), 7.46 (dd, J = 8.0, 1.6 Hz, 1H), 4.29 (q, J = 7.1 Hz, 2H), 2.39 (s, 3H), 1.32 (t, J = 7.1 Hz, 3H).

4.1.4. Synthesis of ethyl 2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylate (**15j**)

Compound **15j** was prepared from the compound **14j** in the same manner as described for **15g**. A white solid, yield: 80.6%. Mp 137.8–139.1 °C. ESI-MS (m/z) = 299.09 [M + H] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.01 (s, 1H), 7.79–7.69 (m, 1H), 7.62 (dd, J = 10.2, 3.8 Hz, 1H), 7.36 (td, J = 8.0, 0.7 Hz, 1H), 4.30 (q, J = 7.1 Hz, 2H), 2.39 (s, 3H), 1.32 (t, J = 7.1 Hz, 3H).

4.1.5. Synthesis of 2-(4-cyanophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid (**16c**)

A mixture of ethyl 2-(4-cyanophenyl)-1-hydroxy-4-methyl-1*H*-imidazole-5-carboxylate **15c** (2.9 mmol), 1 M LiOH aqueous (11 mL), THF (5 mL), and ethanol (5 mL) was stirred at 50 °C for 6 h. The solvent was concentrated in a vacuum, and the residue was acidified with dilute hydrochloric acid to pH 1. The resulting precipitate was filtered, dried, and recrystallized with a mixture of methanol and ethyl acetate (2:1) to yield the corresponding 2-(4-cyanophenyl)-1-hydroxy-4-methyl-1*H*-imidazole-5-carboxylic acid **16c** as a white solid, yield: 82.3%. Mp 221.3–222.8°C. ESI-MS (m/z) = 244.06 [M + H] $^+$;^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (d, J = 8.6 Hz, 2H), 7.95 (d, J = 8.5 Hz, 2H), 2.39 (s, 3H).

4.1.6. Synthesis of 1-hydroxy-4-methyl-2-phenyl-1H-imidazole-5-carboxylic acid (**16d**)

Compound **16d** was prepared from the compound **15d** in the same manner as described for **16c**. A white solid, yield: 89.2%. Mp 196.6–198.1°C. ESI-MS (m/z) = 217.11 [M – H] $^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30–8.24 (m, 2H), 7.62–7.56 (m, 2H), 7.54 (dt, J = 5.2, 2.1 Hz, 1H), 2.52 (s, 3H).

4.1.7. Synthesis of 2-(3-cyanophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid(**16e**)

Compound **16e** was prepared from the compound **15e** in the same manner as described for **16c**. A white solid, yield: 69.7%. Mp 227.5–228.8°C. ESI-MS (m/z) = 244.07 [M +H] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (s, 1H), 8.57 (d, J = 8.1 Hz, 1H), 7.97 (t, J = 7.8 Hz, 1H), 7.78 (t, J = 7.9 Hz, 1H), 2.53 (s, 3H).

4.1.8. Synthesis of 2-(2-cyanophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid(**16f**)

Compound **16f** was prepared from the compound **15f** in the same manner as described for **16c**. A white solid, yield: 77.8%. Mp 181.2–182.7°C. ESI-MS (m/z) = 244.05 [M +H] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77 (dd, J = 7.6, 1.4 Hz, 1H), 7.70 (dd, J = 8.0, 0.9 Hz, 1H), 7.64 (td, J = 7.8, 1.7 Hz, 1H), 7.57–7.52 (m, 1H), 2.52 (s, 3H).

4.1.9. Synthesis of 2-(4-chlorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid (**16g**)

Compound **16g** was prepared from the compound **15g** in the same manner as described for **16c**. A white solid, yield: 88.3%. Mp 219.7–221.2°C. ESI-MS (m/z) = 253.05 [M +H] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (d, J = 8.6 Hz, 2H), 7.66 (d, J = 8.6 Hz, 2H), 2.52 (s, 3H).

4.1.10. Synthesis of 2-(3-chlorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid (**16h**)

Compound **16h** was prepared from the compound **15h** in the same manner as described for **16c**. A white solid, yield: 72.4%. Mp

229.4–231.3°C. ESI-MS (m/z) = 253.05 [M +H] $^{+}$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (d, J = 1.8 Hz, 1H), 8.26 (dt, J = 7.3, 1.7 Hz, 1H), 7.76–7.50 (m, 2H), 2.53 (s, 3H).

*4.1.11. Synthesis of 2-(2-chlorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid (**16i**)*

Compound **16i** was prepared from the compound **15i** in the same manner as described for **16c**. A white solid, yield: 73.6%. Mp 243.2–244.6°C. ESI-MS (m/z) = 251.12 [M − H] $^{+}$;^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77 (dd, J = 7.6, 1.5 Hz, 1H), 7.70 (dd, J = 8.0, 1.0 Hz, 1H), 7.64 (td, J = 7.8, 1.7 Hz, 1H), 7.55 (td, J = 7.5, 1.1 Hz, 1H), 2.52 (s, 3H).

*4.1.12. Synthesis of 2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid (**16j**)*

Compound **16j** was prepared from the compound **15j** in the same manner as described for **16c**. A white solid, yield: 87.6%. Mp 233.0–234.8°C. ESI-MS (m/z) = 271.04 [M +H] $^{+}$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (t, J = 6.8 Hz, 1H), 7.92–7.66 (m, 1H), 7.45 (t, J = 8.0 Hz, 1H), 2.53 (s, 3H).

*4.1.13. Synthesis of ethyl 2-(3-chloro-2-fluorophenyl)-4-methyl-1H-imidazole-5-carboxylate (**18**)*

A suspension of compound **15j** (2.98 g, 10 mmol), potassium iodide (1.16 g, 10 mmol), chlorotrimethylsilane (1.63 g, 15 mmol) and acetonitrile (30 mL) was stirred at 60°C for 6 h. The reaction mixture was poured into a solution of 1 M sodium hydroxide aqueous and was stirred for 1 h. The precipitate was filtered, washed with ethyl acetate to obtain compound **18** as a white solid. Yield: 67.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 (t, J = 6.8 Hz, 1H), 7.57 (t, J = 6.9 Hz, 1H), 7.29 (t, J = 7.9 Hz, 1H), 4.24 (q, J = 7.1 Hz, 2H), 2.49 (s, 3H), 1.29 (t, J = 7.1 Hz, 3H). ESI-MS (m/z) = 281.11[M − H]-.

*4.1.14. Synthesis of ethyl 2-(3-chloro-2-fluorophenyl)-1,4-dimethyl-1H-imidazole-5-carboxylate (**19**)*

A solution of compound **18** (0.56 g, 2.0 mmol), anhydrous potassium carbonate (0.33 g, 2.40 mmol) and iodomethane (0.33 g, 2.4 mmol) in DMF (5.4 mL) was stirred at 35 °C under nitrogen atmosphere for 1 h. After the completion of the reaction, the reaction mixture was poured into 11 mL water and stirred for 10 min. The precipitate was filtered and washed with water, then ethyl acetate (5.0 mL) was added to wash residue to yield compound **19** as a white solid, which was used for the next reaction without further purification.

*4.1.15. Synthesis of 2-(3-chloro-2-fluorophenyl)-1,4-dimethyl-1H-imidazole-5-carboxylic acid (**20**)*

Compound **20** was prepared from the compound **19** in the same manner as described for **16c**, and it was used for the next reaction without further purification.

*4.1.16. Synthesis of ethyl 2-(3-chloro-2-fluorophenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylate (**21**)*

Compound **21** was prepared from the compound **15j** in the same manner as described for **19**, and it was used for the next reaction without further purification.

*4.1.17. Synthesis of 2-(3-chloro-2-fluorophenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylic acid (**22**)*

Compound **22** was prepared from the compound **21** in the same manner as described for **16c**. Yield: 91.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (t, J = 6.8 Hz, 1H), 7.92–7.66 (m, 1H), 7.45 (t, J = 8.0 Hz, 1H), 2.53 (s, 3H). ESI-MS (m/z) = 285.09[M + H]$^{+}$.

*4.1.18. Synthesis of 2-(3-chloro-2-fluorophenyl)-4-methyl-1H-imidazole-5-carboxylic acid (**23**)*

Compound **23** was prepared from the compound **18** in the same manner as described for **16c**, and it was used for the next reaction without further purification.

*4.1.19. Synthesis of methyl (S)-4-{2-[(tert-butoxycarbonyl)amino]-3-phenylpropanamido}benzoate (**25**)*

A solution of (tert-butoxycarbonyl)-L-phenylalanine **24** (10.0 g, 37.7 mmol), methyl 4-aminobenzoate (5.7 g, 37.7 mmol), DIPEA (5.85 g, 45.24 mmol) and HATU (17.2 g, 45.24 mmol) in DMF (100 mL) was stirred at 25°C for 4 h. The reaction mixture was poured into 150 mL ethyl acetate and 100 mL water. The organics were washed with 1 M NaOH (100 mL), 1 M HCl (100 mL) and brine (100 mL), dried (Na_2SO_4), and concentrated to afford **25** as a yellow solid, yield: 77.3%. Mp 114.6–116.3°C. ESI-MS (m/z) = 421.29 [M + Na] $^{+}$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (s, 1H), 7.93 (d, J = 8.6 Hz, 2H), 7.74 (d, J = 8.7 Hz, 2H), 7.45–7.07 (m, 6H), 4.36 (d, J = 9.4 Hz, 1H), 3.83 (s, 3H), 2.98 (d, J = 4.2 Hz, 1H), 2.87 (d, J = 10.2 Hz, 1H), 1.32 (s, 9H).

*4.1.20. Synthesis of methyl (S)-4-(2-amino-3-phenylpropanamido) benzoate (**26**)*

Methyl (S)-4-{2-[(tert-butoxycarbonyl)amino]-3-phenylpropanamido}benzoate **25** was added into a solution of 4 M HCl in ethyl acetate, and stirred in 25°C. The resulting mixture was filtered and precipitate was washed with 20 mL ethyl acetate, dried to afford **26** as a white solid, yield: 84.3%. Mp 127.4–128.8°C. ESI-MS (m/z) = 299.20 [M + H]$^{+}$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.27 (s, 1H), 8.45 (s, 3H), 7.93–7.91 (d, J = 8.4 Hz, 2H), 7.73–7.71 (d, J = 8.8 Hz, 2H), 7.32–7.23 (m, 5H), 4.34 (m, 1H), 3.81 (s, 3H), 3.24–3.12 (m, 2H).

*4.1.21. General procedure for synthesis of methyl (S)-4-[2-(2-phenyl-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido)-3-phenylpropanamido]benzoate derivatives (**27a-27k**)*

A 100 mL flask was charged with 5 mL of DMF, 10 mmol of the compounds **16a-j**, **20**, **22** and **23**, DIPEA (0.15 g, 12 mmol) and HATU (0.46 g, 12 mmol). The complex was stirred for 24 h, and the reaction mixture was poured into 20 mL ethyl acetate and 20 mL water. Then, the organics were washed with 1 M NaOH (20 mL), 1 M HCl (20 mL) and brine (20 mL), dried (Na_2SO_4), and concentrated to give the compounds **27a-27k**, which were used for the next reaction without further purification.

*4.1.22. General procedure for synthesis of (S)-4-[2-(2-phenyl-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido)-3-phenylpropanamido]benzoic acids derivatives (**28a-28k**)*

A 100 mL flask was charged with 5 mL of DMF, 10 mmol of the compounds **16a-j**, **20**, **22** and **23**, DIPEA (0.15 g, 12 mmol) and HATU (0.46 g, 12 mmol). The complex was stirred for 24 h, and the reaction mixture was poured into 20 mL ethyl acetate and 20 mL water. Then, the organics were washed with 1 M NaOH (20 mL), 1 M HCl (20 mL) and brine (20 mL), dried (Na_2SO_4), and concentrated to give the compounds **27a-27k**, which were used for the next reaction without further purification. A mixture of the crude materials **27a-27k**, 1 M LiOH aqueous (10 mL) and methanol (10 mL) was stirred at 40 °C until the material spot disappeared by TLC. The mixture was washed with *tert*-butyl methyl ether (10 mL), and water phase was acidified with dilute hydrochloric acid to pH 3–4, filtered dried and purified by silica gel chromatography with dichloromethane/methanol (10: 1) to afford the corresponding 2-[4-alkoxy-3-(1*H*-tetrazol-1-yl) phenyl]-6-oxo-1, 6-dihydropyrimidine-5-carboxylic acids (S)-4-[2-(2-phenyl-1-hydroxy-4-methyl-1*H*-imidazole-5-carboxamido)-3-phenyl propanamido] benzoic acids derivatives **28a-28k**.

*4.1.23. Synthesis of (S)-4-{2-[2-(3-cyanophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-phenylpropanamido} benzoic acid (**28a**)*

A white solid, yield: 15.45%. Mp 152.1–153.8 °C. ESI-HRMS calcd for $C_{28}H_{23}N_5O_5$ [M − OH]$^-$ 492.1677 found: 492.1671; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.68 (s, 1H), 8.45 (d, J = 7.7 Hz, 1H), 8.06 (dd, J = 3.5, 1.8 Hz, 2H), 7.96 (d, J = 8.7 Hz, 2H), 7.87 (d, J = 7.8 Hz, 1H), 7.79 (d, J = 8.8 Hz, 2H), 7.64 (t, J = 8.1 Hz, 1H), 7.29 (dd, J = 13.5, 7.2 Hz, 3H), 7.16 (t, J = 7.6 Hz, 2H), 4.94 (dd, J = 14.6, 9.8 Hz, 1H), 3.24 (d, J = 4.5 Hz, 1H), 3.11 (dd, J = 13.8, 9.9 Hz, 1H), 2.23 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.11, 166.28, 159.21, 143.67, 138.40, 137.84, 133.00, 132.53, 132.10, 130.84, 130.81, 130.34, 129.97, 129.71, 129.47, 128.72, 127.08, 124.71, 121.03, 119.24, 112.17, 55.93, 37.55, 15.07.

*4.1.24. Synthesis of (S)-4-{2-[2-(2-cyanophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-phenylpropanamido} benzoic acid (**28b**)*

A white solid, yield: 18.18%. Mp 100.4–101.3 °C. ESI-HRMS calcd for $C_{28}H_{23}N_5O_5$ [M − OH]$^-$ 492.1677 found: 492.1685; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.70 (s, 1H), 8.21 (d, J = 3.9 Hz, 2H), 7.92 (d, J = 8.6 Hz, 2H), 7.77 (d, J = 8.7 Hz, 2H), 7.69–7.53 (m, 3H), 7.45 (d, J = 7.4 Hz, 2H), 7.30 (t, J = 7.5 Hz, 2H), 7.21 (t, J = 7.3 Hz, 1H), 4.97–4.83 (m, 1H), 3.24 (dd, J = 13.7, 4.8 Hz, 1H), 3.15 (dd, J = 13.6, 9.7 Hz, 1H), 2.49 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ170.82, 167.35, 164.09, 159.65, 143.45, 143.31, 137.93, 135.13, 134.78, 132.01, 131.93, 131.89, 130.85, 129.80, 129.76, 128.72, 128.67, 127.27, 127.02, 126.96, 125.89, 124.77, 119.15, 56.22, 37.65, 11.04.

*4.1.25. Synthesis of (S)-4-{2-[2-(4-cyanophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-phenylpropanamido} benzoic acid (**28c**)*

A white solid, yield: 21.86%. Mp 161.3–162.6°C. ESI-HRMS calcd for $C_{28}H_{23}N_5O_5$ [M − OH]$^-$ 492.1677 found: 492.1664; ^{1}H NMR (400 MHz, DMSO-d_6) δ11.25 (s, 1H), 8.58 (d, J = 7.8 Hz, 1H), 7.91 (ddd, J = 12.8, 8.6, 3.2 Hz, 8H), 7.28 (dd, J = 9.4, 5.4 Hz, 3H), 7.15 (d, J = 7.5 Hz, 2H), 5.04–4.96 (m, 1H), 3.31 (d, J = 4.2 Hz, 1H), 3.11 (dd, J = 13.5, 10.5 Hz, 1H), 2.20 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ170.12, 165.20, 158.07, 142.80, 138.73, 137.24, 136.88, 131.36, 129.74, 129.62, 128.70, 127.75, 127.36, 126.94, 125.91, 123.45, 120.47, 118.10, 110.57, 55.14, 36.31, 15.23.

*4.1.26. Synthesis of (S)-4-[2-(1-hydroxy-4-methyl-2-phenyl-1H-imidazole-5-carboxamido)-3-phenylpropanamido]benzoic acid (**28d**)*

A white solid, yield: 17.76%. Mp 136.8°C-137.6°C. ESI-HRMS calcd for $C_{27}H_{24}N_4O_5$ [M − OH]$^-$ 467.1725 found: 467.1738; ^{1}H NMR (400 MHz, DMSO-d_6) δ12.70 (s, 1H), 10.54 (s, 1H), 7.94 (dd, J = 7.8, 5.5 Hz, 4H), 7.84 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 8.7 Hz, 2H), 7.48 (t, J = 7.6 Hz, 2H), 7.39 (t, J = 7.4 Hz, 1H), 7.28 (q, J = 7.6 Hz, 4H), 7.20 (dt, J = 9.2, 4.2 Hz, 1H), 4.90 (dd, J = 14.0, 7.8 Hz, 1H), 3.24–3.05 (m, 2H), 2.48 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.15, 166.28, 159.30, 143.69, 141.43, 137.81, 132.74, 130.82, 130.62, 129.88, 129.69, 129.15, 128.77, 128.72, 127.09, 124.69, 120.53, 119.24, 55.97, 37.55, 15.00.

*4.1.27. Synthesis of (S)-4-{2-[2-(4-chlorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-phenylpropanamido} benzoic acid (**28e**)*

A white solid, yield: 22.36%. Mp 178.7–179.9°C. ESI-HRMS calcd for $C_{27}H_{23}ClN_4O_5$ [M − H]$^-$ 517.1284 found: 517.1265; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.66 (s, 1H), 8.38 (d, J = 7.6 Hz, 1H), 7.96 (d, J = 8.8 Hz, 2H), 7.89–7.81 (m, 2H), 7.79 (d, J = 8.8 Hz, 2H), 7.58–7.48 (m, 2H), 7.30 (q, J = 7.7 Hz, 3H), 7.22–7.19 (m, 2H), 5.17–4.54 (m, 1H), 3.24 (dd, J = 13.8, 4.8 Hz, 1H), 3.08 (dd, J = 13.8, 9.9 Hz, 1H), 2.21 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ171.15, 166.28, 159.33, 143.69, 140.50, 138.17, 137.83, 134.44, 132.66, 130.82, 130.74, 129.69, 129.18, 128.73, 127.08, 124.68, 120.73, 119.23, 55.98, 37.52, 15.10.

*4.1.28. Synthesis of (S)-4-{2-[2-(3-chlorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-phenylpropanamido} benzoic acid (**28f**)*

A white solid, yield: 24.46%. Mp135.8–136.9 °C. ESI-HRMS calcd for $C_{27}H_{23}ClN_4O_5$ [M + Na]$^+$ 541.1249 found: 541.1240; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H), 8.42 (d, J = 7.3 Hz, 1H), 7.96 (d, J = 8.8 Hz, 2H), 7.80 (dd, J = 8.2, 5.5 Hz, 4H), 7.50 (d, J = 4.5 Hz, 2H), 7.20 (t, J = 7.6 Hz, 3H), 7.38 (d, J = 7.2 Hz, 2H), 4.98 (d, J = 9.9 Hz, 1H), 3.26 (s, 1H), 3.10 (s, 1H), 2.22 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ171.12, 166.28, 159.27, 143.68, 140.00, 138.22, 137.83, 133.75, 132.61, 131.02, 130.76, 130.23, 129.93, 129.70, 128.71, 127.03, 126.15, 124.70, 120.92, 119.24, 55.96, 37.53, 15.08.

*4.1.29. Synthesis of (S)-4-{2-[2-(2-chlorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-phenylpropanamido} benzoic acid (**28g**)*

A white solid, yield: 17.44%. Mp.98.1–99.2 °C. ESI-HRMS calcd for $C_{27}H_{23}ClN_4O_5$ [M + Na]$^+$ 541.1249 found: 541.1253; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.66 (s, 1H), 8.29 (d, J = 7.6 Hz, 1H), 8.01–7.83 (m, 2H), 7.82–7.73 (m, 2H), 7.58 (dt, J = 6.6, 5.0 Hz,2H), 7.47–7.39 (m, 2H), 7.31–7.23 (m, 3H), 7.16 (t, J = 7.6 Hz, 2H), 5.03–4.92 (m, 1H), 3.28–3.16 (m, 1H), 3.09 (dd, J = 13.8, 9.5 Hz, 1H), 2.24 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ171.08, 166.27, 159.30, 143.65, 140.49, 137.95, 137.72, 133.60, 132.97, 132.77, 132.05, 130.83, 130.12, 129.75, 128.79, 127.59, 127.09, 124.70, 119.84, 119.24, 55.79, 37.56, 15.24.

*4.1.30. Synthesis of (S)-4-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-phenylpropanamido}benzoic acid (**28h**)*

A white solid, yield: 15.23%. Mp 181.3–183.1 °C. ESI-HRMS calcd for $C_{27}H_{22}ClFN_4O_5$ [M − H]$^-$ 535.1190 found: 535.1197; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.31 (s, 1H), 7.91 (d, J = 8.5 Hz, 2H), 7.78–7.66 (m, 3H), 7.42 (t, J = 8.0 Hz, 1H), 7.34–7.12 (m, 5H), 4.95 (dd, J = 13.6, 7.8 Hz, 1H), 3.19 (dd, J = 13.8, 5.3 Hz, 1H), 3.02 (dd, J = 13.7, 8.7 Hz, 1H), 2.49 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.63, 167.34, 159.45, 155.45, 152.94, 143.26, 137.49, 132.44, 130.85, 129.64, 129.42, 128.74, 127.05, 126.06, 126.02, 125.88, 121.14, 120.89, 120.72, 119.14, 54.88, 38.68, 12.16.

*4.1.31. Synthesis of (S)-4-{2-[2-(3-chloro-2-fluorophenyl)-4-methyl-1H-imidazole-5-carboxamido]-3-phenylpropanamido} benzoic acid (**28i**)*

A white solid, yield: 14.72%. Mp 181.3–183.0°C. ESI-HRMS calcd for $C_{27}H_{22}ClFN_4O_4$ [M − H]$^-$ 519.1241 found: 519.1244; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1H), 8.31 (t, J = 6.3 Hz, 1H), 7.92 (d, J = 8.6 Hz, 2H), 7.74 (dd, J = 16.1, 7.9 Hz, 3H), 7.42 (t, J = 8.0 Hz, 1H), 7.29 (q, J = 8.0 Hz, 4H), 7.20 (t, J = 6.7 Hz, 1H), 4.96 (dd, J = 13.6, 7.8 Hz, 1H), 3.20 (dd, J = 13.8, 5.3 Hz, 1H), 3.03 (dd, J = 13.7, 8.7 Hz, 1H), 2.49 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.52, 171.17, 167.50, 159.99, 158.34, 143.31, 137.75, 131.65, 130.77, 129.76, 128.65, 126.97, 126.09, 125.34, 119.12, 118.36, 118.28, 116.80, 116.65, 113.03, 54.61, 38.35, 21.61.

*4.1.32. Synthesis of (S)-4-{2-[2-(3-chloro-2-fluorophenyl)-1,4-dimethyl-1H-imidazole-5-carboxamido]-3-phenylpropanamido} benzoic acid (**28j**)*

A white solid, yield: 18.39%. Mp 162.3–163.7°C. ESI-HRMS calcd for $C_{28}H_{24}ClFN_4O_4$ [M − H]$^-$ 533.1397 found: 533.1422; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.15 (s, 1H), 11.24 (s, 1H), 10.56 (s, 1H), 8.39 (s, 1H), 7.92 (t, J = 8.3 Hz, 2H), 7.81–7.60 (m, 3H), 7.42 (t, J = 8.0 Hz,

1H), 7.34–7.24 (m, 4H), 7.20 (dt, J = 9.3, 5.0 Hz, 1H), 4.93 (dd, J = 13.5, 8.0 Hz, 1H), 3.83 (s, 3H), 3.18 (dd, J = 13.8, 5.4 Hz, 2H), 3.01 (dd, J = 13.7, 8.7 Hz, 1H), 2.49 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.74, 166.26, 159.55, 152.85, 143.62, 137.48, 132.36, 130.75, 129.62, 129.33, 128.75, 127.06, 126.05, 126.00, 124.68, 121.10, 120.87, 120.70, 119.24, 54.89, 52.36, 38.65, 11.87.

*4.1.33. Synthesis of (S)-4-{2-[2-(3-chloro-2-fluorophenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxamido]-3-phenylpropanamido}benzoic acid (**28k**)*

A white solid, yield: 38.11%. Mp 118.4–120.1 °C. ESI-HRMS calcd for $C_{28}H_{24}ClFN_4O_5$ [M − H]$^-$ 549.1341 found: 549.1372; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (s, 1H), 8.50 (s, 1H), 8.34–8.24 (m, 1H), 8.12 (d, J = 8.1 Hz, 1H), 7.95 (d, J = 8.7 Hz, 2H), 7.78 (d, J = 8.6 Hz, 2H), 7.63 (t, J = 7.8 Hz, 1H), 7.40 (d, J = 7.3 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2H), 7.23 (t, J = 7.3 Hz, 1H), 5.12–4.80 (m, 1H), 3.74 (s, 1H), 3.28 (dd, J = 13.6, 3.8 Hz, 1H), 3.18–3.02 (m, 1H), 2.26 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.91, 167.79, 167.36, 158.70, 158.60, 143.32, 139.91, 137.77, 135.57, 130.92, 130.86, 129.96, 129.73, 129.47, 129.13, 128.73, 127.07, 125.91, 120.01, 119.15, 67.90, 55.61, 37.65, 14.76.

*4.1.34. Synthesis of ethyl 5-amino-1H-indole-2-carboxylate (**30**)*

A mixture of ethyl 5-nitro-1*H*-indole-2-carboxylate **29** (17.0 g, 77.0 mmol) and 5% Pd/C (3.4 g) in ethanol was stirred at room temperature for 12 h under hydrogen atmosphere. After the completion of the reaction, the Pd/C was filtered out and the filtrate was evaporated to give a grey solid, yield: 87.9%. Mp 126.1–127.2°C. ESI-MS (m/z) = 205.09 [M +H] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H), 7.17 (d, J = 9.0 Hz, 1H), 6.78 (d, J = 53.9 Hz, 3H), 4.42 (t, J = 73.8 Hz, 4H), 1.32 (t, J = 7.1 Hz, 3H).

*4.1.35. Synthesis of ethyl (S)-2-{4-{2-[(tert-butoxycarbonyl) amino]-3-phenylpropanamido}phenyl}acetate (**31a**)*

Compound **31a** was prepared from the commercially available ethyl 2-(4-aminophenyl)acetate in the same manner as described for **25**, and it was used for the next reaction without further purification.

*4.1.36. Synthesis of ethyl (S)-5-{2-[(tert-butoxycarbonyl)amino]-3-phenylpropanamido}-1H-indole-2-carboxylate (**31b**)*

Compound **31b** was prepared from the commercially available ethyl 5-amino-1*H*-indole-2-carboxylate in the same manner as described for **25**. A grey solid, yield: 77.6%. Mp 192.5–193.7°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (s, 1H), 9.83 (s, 1H), 7.99 (s, 1H), 7.36 (dt, J = 9.0, 5.3 Hz, 2H), 7.11 (s, 1H), 6.95 (d, J = 8.0 Hz, 2H), 6.85 (d, J = 8.1 Hz, 1H), 6.47 (d, J = 8.1 Hz, 2H), 4.34 (q, J = 7.1 Hz, 2H), 4.22 (dd, J = 13.3, 8.3 Hz, 1H), 2.83 (dd, J = 13.7, 4.7 Hz, 1H), 2.69 (dd, J = 13.3, 9.7 Hz, 1H), 1.42–1.21 (m, 12H).

*4.1.37. Synthesis of ethyl (S)-2-[4-(2-amino-3-phenylpropanamido) phenyl]acetate (**32a**)*

Compound **32a** was prepared from **31a** in the same manner as described for **26**. A grey solid, yield: 85.4%. ESI-MS (m/z) = 325.29 [M − H]$^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (d, J = 8.5 Hz, 2H), 7.26 (t, J = 4.1 Hz, 5H), 7.18 (d, J = 8.5 Hz, 3H), 4.07 (q, J = 7.1 Hz, 2H), 3.64–3.53 (m, 4H), 3.00 (dd, J = 13.4, 5.5 Hz, 1H), 2.72 (dd, J = 13.4, 8.0 Hz, 1H), 1.18 (t, J = 7.1 Hz, 3H).

*4.1.38. Synthesis of ethyl (S)-5-(2-amino-3-phenylpropanamido)-1H-indole-2-carboxylate (**32b**)*

Compound **32b** was prepared from **31b** in the same manner as described for **26**. A white solid, yield: 87.7%. Mp 135.2–136.7°C. ESI-MS (m/z) = 352.10 [M + H] $^+$.

*4.1.39. Synthesis of ethyl (S)-2-{4-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-phenylpropanamido}phenyl}acetate (**33a**)*

Compound **33a** was prepared from **32a** in the same manner as described for **27a-27k**, and it was used for the next reaction without further purification.

*4.1.40. Synthesis of ethyl (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-phenylpropanamido}-1H-indole-2-carboxylate (**33b**)*

Compound **33b** was prepared from **32b** in the same manner as described for **27a-27k**, and it was used for the next reaction without further purification.

*4.1.41. Synthesis of (S)-2-{4-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-phenylpropanamido}phenyl}acetic acid (**34a**)*

Compound **34a** was prepared from **33a** in the same manner as described for **28a-28k**.A yellow solid, yield: 12.33%. Mp 187.1–189.0°C. ESI-HRMS calcd for $C_{28}H_{24}ClFN_4O_5$ [M + Na]$^+$ 573.1311 found: 573.1309; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.36 (s, 1H), 10.13 (d, J = 3.6 Hz, 1H), 8.34 (dd, J = 15.8, 7.8 Hz, 1H), 7.71–7.62 (m, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.50 (dd, J = 14.5, 8.3 Hz, 2H), 7.37 (t, J = 6.6 Hz, 2H), 7.19 (d, J = 8.0 Hz, 3H), 7.14–7.05 (m, 2H), 4.95 (dd, J = 13.5, 8.6 Hz, 1H), 3.62 (s, 2H), 3.30–3.18 (m, 1H), 3.11 (dd, J = 13.7, 9.8 Hz, 1H), 2.23 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.60, 169.51, 157.97, 154.66, 152.98, 137.55, 136.82, 131.62, 131.17, 129.28, 128.96, 128.62, 127.59, 127.56, 125.93, 124.60, 119.25, 118.77, 118.59, 54.47, 40.81, 36.71, 13.46.

*4.1.42. Synthesis of (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-phenylpropanamido}-1H-indole-2-carboxylic acid (**34b**)*

Compound **34b** was prepared from **33b** in the same manner as described for **28a-28k**. A white solid, yield: 21.23%. Mp 186.2–187.6 °C. ESI-HRMS calcd for $C_{29}H_{23}ClFN_6O_6$ [M − H]$^-$ 574.1299 found: 574.1276; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.54 (s, 1H), 10.08 (s, 1H), 8.26 (s, 1H), 7.97 (d, J = 17.6 Hz, 1H), 7.78–7.51 (m, 1H), 7.36 (dt, J = 4.9, 3.3 Hz, 3H), 7.29 (d, J = 6.3 Hz, 2H), 7.27–7.22 (m, 2H), 7.21–7.17 (m, 1H), 7.15 (d, J = 8.6 Hz, 1H), 6.80 (d, J = 8.6 Hz, 1H), 5.10–4.72 (m, 1H), 3.16 (dd, J = 13.5, 5.8 Hz, 1H), 3.06–2.95 (m, 1H), 2.45 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ174.26, 169.86, 163.46, 158.36, 155.62, 138.08, 134.47, 133.62, 132.09, 129.81, 129.72, 128.63, 127.48, 126.84, 125.54, 120.72, 120.60, 118.79, 118.13, 116.80, 112.81, 112.33, 109.83, 107.02, 54.41, 38.03, 15.19.

*4.1.43. Synthesis of ethyl (S)-5-{2-[(tert-butoxycarbonyl)amino]-3-(4-fluorophenyl)propanamido}-1H-indole-2-carboxylate (**36a**)*

Compound **36a** was prepared from commercially available (*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(4-fluorophenyl)propanoic acid **35a** in the same manner as described for **25**. A grey solid, yield: 71.9%. Mp 173.5–175.0°C. ESI-MS (m/z) = 492.30 [M + Na] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (s, 1H), 9.94 (s, 1H), 8.01 (s, 1H), 7.68–7.24 (m, 4H), 7.24–6.89 (m, 4H), 4.34 (q, J = 7.1 Hz, 3H), 3.26–2.88 (m, 1H), 2.94–2.68 (m, 1H), 1.71–0.94 (m, 12H).

*4.1.44. Synthesis of ethyl (S)-5-{2-[(tert-butoxycarbonyl)amino]-3-(4-chlorophenyl)propanamido}-1H-indole-2-carboxylate (**36b**)*

Compound **36b** was prepared from commercially available (*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(4-chlorophenyl)propanoic acid **35b** in the same manner as described for **25**. A grey solid, yield: 69.6%. Mp 213.7–215.2°C. ESI-MS (m/z) = 508.30 [M + Na] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.83 (s, 1H), 9.95 (s, 1H), 8.01 (s, 1H), 7.68–7.24 (m, 6H), 7.12 (t, J = 5.5 Hz, 2H), 4.38–4.03 (m, 3H), 3.02 (dd, J = 13.6, 4.5 Hz, 1H), 2.85 (dd, J = 13.6, 10.4 Hz, 1H), 1.45–1.16 (m, 12H).

*4.1.45. Synthesis of ethyl (S)-5-{3-[4-bromophenyl]-2-[(tert-butoxycarbonyl)amino]propanamido}-1H-indole-2-carboxylate (**36c**)*

Compound **36c** was prepared from commercially available (*S*)-3-(4-bromophenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoic acid **35c** in the same manner as described for **25**. A grey solid, yield: 66.3%. Mp 218.0–219.6°C. ESI-MS (*m*/*z*) = 552.28 [M + Na] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.83 (s, 1H), 9.97 (s, 1H), 8.01 (s, 1H), 7.49 (d, *J* = 8.2 Hz, 2H), 7.40 (d, *J* = 8.8 Hz, 1H), 7.39–7.28 (m, 3H), 7.11 (t, *J* = 5.0 Hz, 2H), 4.33 (t, *J* = 7.1 Hz, 3H), 2.98 (dt, *J* = 23.1, 11.6 Hz, 1H), 2.82 (dt, *J* = 25.1, 12.5 Hz, 1H), 1.58–0.83 (m, 12H).

*4.1.46. Synthesis of ethyl (S)-5-{2-[(tert-butoxycarbonyl)amino]-3-(4-nitrophenyl)propanamido}-1H-indole-2-carboxylate (**36d**)*

Compound **36d** was prepared from commercially available (S)-2-[(*tert*-butoxycarbonyl)amino]-3-(4-nitrophenyl)propanoic acid **35d** in the same manner as described for **25**. A grey solid, yield: 72.7%. Mp 200.3–201.8°C. ESI-MS (*m*/*z*) = 519.24 [M +Na] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.83 (s, 1H), 10.01 (s, 1H), 8.19 (d, *J* = 8.6 Hz, 2H), 8.01 (s, 1H), 7.63 (d, *J* = 8.5 Hz, 2H), 7.40 (s, 1H), 7.36 (d, *J* = 1.9 Hz, 1H), 7.21 (d, *J* = 8.5 Hz, 1H), 7.13 (d, *J* = 1.5 Hz, 1H), 4.34 (q, *J* = 7.1 Hz, 3H), 3.17 (dd, *J* = 13.4, 4.2 Hz, 1H), 3.00 (dd, *J* = 13.2, 10.7 Hz, 1H), 1.33 (dd, *J* = 14.5, 7.4 Hz, 12H).

*4.1.47. Synthesis of ethyl (S)-5-[2-amino-3-(4-fluorophenyl) propanamido]-1H-indole-2-carboxylate (**37a**)*

Compound **37a** was prepared from **36a** in the same manner as described for **26**. A white solid, yield: 88.2%. Mp 268.2–269.8°C. ESI-MS (*m*/*z*) = 370.28 [M + H] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (s, 1H), 7.41–7.34 (m, 2H), 7.29 (dd, *J* = 8.6, 5.7 Hz, 2H), 7.10 (dd, *J* = 10.5, 7.3 Hz, 3H), 4.34 (d, *J* = 7.1 Hz, 2H), 3.55 (dd, *J* = 7.8, 5.6 Hz, 1H), 3.00 (dd, *J* = 13.4, 5.4 Hz, 1H), 2.74 (dd, *J* = 13.5, 8.0 Hz, 1H), 1.34 (s, 3H).

*4.1.48. Synthesis of ethyl (S)-5-[2-amino-3-(4-chlorophenyl) propanamido]-1H-indole-2-carboxylate (**37b**)*

Compound **37b** was prepared from **36b** in the same manner as described for **26**. A white solid, yield: 78.4%. Mp 277.2–278.5°C. ESI-MS (*m*/*z*) = 386.27 [M + H] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.78 (s, 2H), 8.00 (s, 1H), 7.41–7.21 (m, 4H), 7.09 (dd, *J* = 10.2, 7.5 Hz, 3H), 4.33 (q, *J* = 7.1 Hz, 2H), 3.65–3.44 (m, 1H), 2.99 (dd, *J* = 13.4, 5.5 Hz, 1H), 2.79–2.67 (m, 1H), 1.34 (t, *J* = 7.1 Hz,3H).

*4.1.49. Synthesis of ethyl (S)-5-[2-amino-3-(4-bromophenyl) propanamido]-1H-indole-2-carboxylate (**37c**)*

Compound **37c** was prepared from **36c** in the same manner as described for **26**. A white solid, yield: 75.5%. Mp 257.4–259.1°C. ESI-MS (*m*/*z*) = 430.23 [M + H] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.85 (s, 1H), 10.34 (s, 1H), 7.97 (s, 1H), 7.51 (d, *J* = 8.1 Hz, 2H), 7.40 (s, 1H), 7.37–7.31 (m, 1H), 7.27 (d, *J* = 8.2 Hz, 2H), 7.13 (s, 1H), 4.34 (d, *J* = 7.1 Hz, 2H), 4.03 (d, *J* = 14.0 Hz, 1H), 3.44 (dt, *J* = 13.9, 6.9 Hz, 1H), 3.14 (dd, *J* = 13.5, 5.8 Hz, 1H), 2.99 (d, *J* = 7.3 Hz, 1H), 1.34 (s, 3H).

*4.1.50. Synthesis of ethyl (S)-5-[2-amino-3-(4-nitrophenyl) propanamido]-1H-indole-2-carboxylate (**37d**)*

Compound **37d** was prepared from **36d** in the same manner as described for **26**. A yellow solid, yield: 69.4%. Mp 168.0–169.4°C. ESI-MS (*m*/*z*) = 397.26 [M + H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (s, 1H), 9.82 (s, 1H), 8.15 (d, *J* = 7.4 Hz, 2H), 8.01 (s, 1H), 7.56 (d, *J* = 7.4 Hz, 2H), 7.36 (d, *J* = 6.6 Hz, 2H), 7.10 (s, 1H), 4.33 (d, *J* = 6.6 Hz, 2H), 3.64 (s, 1H), 3.14 (d, *J* = 9.2 Hz, 1H), 2.90 (d, *J* = 7.7 Hz, 1H), 1.34 (s, 3H).

*4.1.51. Synthesis of ethyl (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-(4-fluorophenyl)propanamido}-1H-indole-2-carboxylate (**38a**)*

Compound **38a** was prepared from **37a** in the same manner as described for **27a-27k**, and it was used for the next reaction without further purification.

*4.1.52. Synthesis of ethyl (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-(4-chlorophenyl)propanamido}-1H-indole-2-carboxylate (**38b**)*

Compound **38b** was prepared from **37b** in the same manner as described for **27a-27k**, and it was used for the next reaction without further purification.

*4.1.53. Synthesis of ethyl (S)-5-{3-(4-bromophenyl)-2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]propanamido}-1H-indole-2-carboxylate (**38c**)*

Compound **38c** was prepared from **37c** in the same manner as described for **27a-27k**, and it was used for the next reaction without further purification.

*4.1.54. Synthesis of ethyl (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-(4-nitrophenyl)propanamido}-1H-indole-2-carboxylate (**38d**)*

Compound **38d** was prepared from **37d** in the same manner as described for **27a-27k**, and it was used for the next reaction without further purification.

*4.1.55. Synthesis of ethyl (S)-5-{3-(4-aminophenyl)-2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]propanamido}-1H-indole-2-carboxylate (**38e**)*

Compound **38e** was prepared from **37d** in the same manner as described for **30**, and it was used for the next reaction without further purification.

*4.1.56. Synthesis of (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-(4-fluorophenyl)propanamido}-1H-indole-2-carboxylic acid (**39a**)*

Compound **39a** was prepared from **38a** in the same manner as described for **28a-28k**. A white solid, yield: 19.35%. Mp 148.6–150.1°C. ESI-HRMS calcd for $C_{29}H_{22}ClF_2N_5O_5$ [M − H]$^-$ 592.1205 found: 592.1210; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.83 (s, 1H), 10.23 (s, 1H), 8.34 (d, *J* = 7.9 Hz, 1H), 8.14–7.98 (m, 1H), 7.47–7.39 (m, 4H), 7.36–7.31 (m, 1H), 7.14–7.06 (m, 5H), 4.97 (td, *J* = 8.6, 5.1 Hz, 1H), 3.25 (dd, *J* = 13.9, 4.8 Hz, 1H), 3.12 (dd, *J* = 13.8, 9.4 Hz, 1H), 2.26 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.81, 167.36, 160.51, 159.45, 158.02, 143.41, 138.62, 137.95, 137.78, 134.91 134.60, 133.08, 132.24, 130.84, 130.39, 129.81, 129.76, 128.66, 127.37, 127.01, 125.89, 125.62, 119.16, 116.85, 56.31, 37.59, 11.02.

*4.1.57. Synthesis of (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-(4-chlorophenyl)propanamido}-1H-indole-2-carboxylic acid (**39b**)*

Compound **39b** was prepared from **38b** in the same manner as described for **28a-28k**. A white solid, yield: 18.47%. Mp 167.2–168.6°C. ESI-MS (*m*/*z*) = 610.31 [M + H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.83 (s, 1H), 10.24 (s, 1H), 8.34 (d, *J* = 7.9 Hz, 1H), 8.04 (s, 1H), 7.75–7.62 (m, 1H), 7.45–7.38 (m, 4H), 7.37–7.30 (m, 3H), 7.26 (d, *J* = 2.9 Hz, 1H), 7.13 (d, *J* = 2.2 Hz, 1H), 5.00–4.95 (m, 1H), 3.28–3.20 (m, 1H), 3.13 (dd, *J* = 13.9, 9.4 Hz, 1H), 2.26 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ169.69, 161.69, 159.03, 155.77, 154.09, 138.65, 137.17, 137.10, 134.85, 132.71, 132.28, 132.20, 131.73, 130.60, 130.35, 129.73, 128.65, 128.43, 127.07, 120.36, 119.46, 113.17, 112.52, 108.13, 55.34, 37.36, 14.78.

*4.1.58. Synthesis of (S)-5-{3-(4-bromophenyl)-2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido] propanamido}-1H-indole-2-carboxylic acid (**39c**)*

Compound **39c** was prepared from **38c** in the same manner as described for **28a-28k**. A white solid, yield: 21.05%. Mp 152.3–153.6°C. ESI-HRMS calcd for $C_{29}H_{22}BrClFN_5O_5$ [M – H]$^-$ 652.0404 found: 652.0438; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H), 8.40 (d, J = 7.6 Hz, 1H), 7.96 (d, J = 8.8 Hz, 2H), 7.79 (d, J = 8.8 Hz, 2H), 7.68 (t, J = 6.6 Hz, 1H), 7.39 (d, J = 7.1 Hz, 2H), 7.29–7.23 (m, 2H), 7.19 (t, J = 7.4 Hz, 1H), 7.11 (t, J = 7.7 Hz, 2H), 4.98 (s, 1H), 3.25 (d, J = 4.5 Hz, 1H), 3.18–2.90 (m, 1H), 2.23 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.29, 163.17, 161.38, 159.37, 155.42, 153.73, 137.22, 134.70, 132.73, 131.95, 131.56, 129.81, 129.46, 127.14, 126.07, 126.04, 121.05, 120.93, 119.13, 117.89, 115.68, 112.97, 107.78, 54.34, 38.41, 12.67.

*4.1.59. Synthesis of (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-(4-nitrophenyl)propanamido}-1H-indole-2-carboxylic acid (**39d**)*

Compound **39d** was prepared from **38d** in the same manner as described for **28a-28k**. A white solid, yield: 15.34%. Mp 178.2–179.6°C. ESI-HRMS calcd for $C_{29}H_{22}ClFN_6O_7$ [M – H]$^-$ 619.1150 found: 619.1165; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.90 (s, 1H), 11.81 (s, 1H), 11.45 (s, 1H), 8.32 (s, 1H), 7.64–7.45 (m, 2H), 7.40 (d, J = 8.6 Hz, 1H), 7.25 (t, J = 7.8 Hz, 1H), 7.13 (d, J = 8.5 Hz, 1H), 7.04 (s, 1H), 6.92 (d, J = 10.0 Hz, 1H), 6.72 (s, 1H), 6.33 (d, J = 9.9 Hz, 1H), 6.19 (d, J = 10.1 Hz, 1H), 6.04 (d, J = 9.4 Hz, 1H), 3.44 (dd, J = 13.8, 6.9 Hz, 2H), 3.17 (s, 1H), 2.43 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.52, 162.13, 159.13, 154.20, 152.51, 145.46, 135.56, 135.46, 131.38, 130.64, 128.77, 128.31, 127.76, 126.14, 125.32, 124.10, 123.96, 120.78, 119.53, 119.41, 118.52, 116.43, 111.93, 106.44, 55.39, 39.43, 17.93.

*4.1.60. Synthesis of (S)-5-{3-(4-aminophenyl)-2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido] propanamido}-1H-indole-2-carboxylic acid (**39e**)*

Compound **39e** was prepared from **38e** in the same manner as described for **28a-28k**. A white solid, yield: 13.22%. Mp 167.3–168.9°C. ESI-HRMS calcd for $C_{29}H_{24}ClFN_6O_5$ [M – H]$^-$ 589.1408 found: 589.1613; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.72 (d, J = 8.0 Hz, 1H), 8.25 (dd, J = 6.6, 2.8 Hz, 1H), 7.93 (d, J = 8.0 Hz, 2H), 7.78 (dd, J = 8.6, 7.6 Hz, 2H), 7.70–7.56 (m, 2H), 7.52–7.36 (m, 3H), 7.30 (td, J = 7.4, 3.4 Hz, 2H), 7.22 (dd, J = 10.5, 4.1 Hz, 1H), 5.03–4.81 (m, 1H), 3.29–3.20 (m, 1H), 3.19–3.09 (m, 1H), 2.50 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.06, 165.38, 164.03, 162.82, 156.45, 155.52, 153.84, 137.88, 134.14, 131.95, 131.39, 120.12, 129.92, 129.58, 127.31, 125.28, 125.25, 120.63, 120.51, 119.31, 117.41, 112.59, 112.24, 109.72, 54.81, 39.16, 15.20.

*4.1.61. Synthesis of ethyl (S)-5-{3-(4-aminophenyl)-2-[(tert-butoxycarbonyl)amino]propanamido}-1H-indole-2-carboxylate (**40**)*

Compound **40** was prepared from **36d** in the same manner as described for **30**. An off-white solid, yield: 82.6%. Mp 200.8–201.6°C. ESI-MS (m/z) = 465.45 [M – H] $^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (s, 1H), 9.83 (s, 1H), 7.99 (s, 1H), 7.36 (dt, J = 9.0, 5.3 Hz, 2H), 7.11 (s, 1H), 6.95 (d, J = 8.0 Hz, 2H), 6.85 (d, J = 8.1 Hz, 1H), 6.47 (d, J = 8.1 Hz, 2H), 4.34 (q, J = 7.1 Hz, 2H), 4.22 (dd, J = 13.3, 8.3 Hz, 1H), 2.83 (dd, J = 13.7, 4.7 Hz, 1H), 2.69 (dd, J = 13.3, 9.7 Hz, 1H), 1.73–0.70 (m, 12H).

*4.1.62. Synthesis of ethyl (S)-5-{3-[4-(acetylamino)phenyl]-2-[(tert-butoxycarbonyl)amino]propanamido}-1H-indole-2-carboxylate (**41a**)*

A solution of ethyl (S)-5-{3-(4-aminophenyl)-2-[(*tert*-butoxycarbonyl)amino]propanamido}-1*H*-indole-2-carboxylate **40** (0.47 g, 1.0 mmol), acetic acid (0.77 g, 1.28 mmol), DIPEA (0.17 g, 1.32 mmol) and HATU (0.49 g, 1.3 mmol) in DMF (10 mL) was stirred at 25°C for 4 h. The reaction mixture was poured into 15 mL ethyl acetate and 10 mL water. The organics were washed with 1 M NaOH (10 mL), 1 M HCl (10 mL) and brine (10 mL), dried (Na_2SO_4), and concentrated to afford **41a**, which was used for the next reaction without further purification.

*4.1.63. Synthesis of ethyl (S)-5-{2-[(tert-butoxycarbonyl)amino]-3-{4-[(isopropylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylate (**41b**)*

Compound **41b** was prepared from commercially available isobutyric acid in the same manner as described for **41a**. An off-white solid, yield: 69.7%. Mp 223.1–224.8°C. ESI-MS (m/z) = 559.49 [M +Na]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (s, 1H), 9.87 (s, 1H), 9.71 (s, 1H), 7.98 (s, 1H), 7.50 (d, J = 8.3 Hz, 2H), 7.35 (dt, J = 8.9, 5.3 Hz, 2H), 7.22 (d, J = 8.2 Hz, 2H), 7.11 (d, J = 1.5 Hz, 1H), 6.99 (d, J = 8.1 Hz, 1H), 4.34 (d, J = 7.1 Hz, 3H), 2.99–2.87 (m, 1H), 2.79 (dd, J = 26.6, 13.1 Hz, 1H), 2.56 (dt, J = 13.8, 6.9 Hz, 1H), 1.33 (s, 12H), 1.08 (d, J = 6.8 Hz, 6H).

*4.1.64. Synthesis of ethyl (S)-5-{2-[(tert-butoxycarbonyl)amino]-3-{4-[(isobutylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylate (**41c**)*

Compound **41c** was prepared from commercially available 3-methylbutanoic acid in the same manner as described for **41a**. An off-white solid, yield: 70.1%. Mp 163.2–164.9°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 9.95 (s, 1H), 9.81 (s, 1H), 7.99 (s, 1H), 7.50 (d, J = 8.2 Hz, 2H), 7.47–7.30 (m, 2H), 7.23 (d, J = 8.1 Hz,2H), 7.11 (d, J = 1.5 Hz, 1H), 6.99 (d, J = 8.2 Hz, 1H), 4.34 (q, J = 7.1 Hz, 3H), 2.97 (dd, J = 13.5, 4.4 Hz, 1H), 2.89–2.71 (m, 1H), 2.17 (d, J = 7.0 Hz, 2H), 2.06 (dt, J = 13.8, 6.7 Hz, 1H), 1.46–1.19 (m, 12H), 0.92 (d, J = 6.5 Hz, 6H).

*4.1.65. Synthesis of ethyl (S)-5-{2-[(tert-butoxycarbonyl)amino]-3-{4-[(ethynylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylate (**41d**)*

Compound **41d** was prepared from commercially available propiolic acid in the same manner as described for **41a**. An off-white solid, yield: 65.3%. Mp 162.4–163.9°C.

*4.1.66. Synthesis of ethyl (S)-5-{3-{4-{[(prop-1-yn-1-yl)carbonyl] amino}phenyl}-2-[(tert-butoxycarbonyl) amino]propanamido}-1H-indole-2-carboxylate (**41e**)*

Compound **41e** was prepared from commercially available but-2-ynoic acid in the same manner as described for **41a**. An off-white solid, yield: 66.4%. Mp 149.6–151.3°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 10.51 (s, 1H), 9.93 (s, 1H), 7.99 (s, 1H), 7.49 (d, J = 8.3 Hz, 2H), 7.36 (dt, J = 9.0, 5.3 Hz, 2H), 7.25 (d, J = 8.2 Hz, 2H), 7.11 (d, J = 1.6 Hz, 1H), 7.00 (d, J = 8.2 Hz, 1H), 4.79–3.94 (m, 3H), 2.97 (dd, J = 13.6, 4.4 Hz, 1H), 2.87–2.73 (m, 1H), 2.03 (s, 3H), 1.55–1.14 (m, 12H).

*4.1.67. Synthesis of ethyl (S)-5-{2-[(tert-butoxycarbonyl)amino]-3-{4-[(cyclopropylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylate (**41f**)*

Compound **41f** was prepared from commercially available cyclopropanecarboxylic acid in the same manner as described for **41a**. A white solid, yield: 77.0%. Mp 152.4–153.8°C. ESI-MS (m/z) = 557.25 [M +Na] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 10.08 (s, 1H), 9.88 (s, 1H), 7.99 (s, 1H), 7.48 (d, J = 8.1 Hz, 2H), 7.37 (dd, J = 14.4, 5.2 Hz, 2H), 7.23 (d, J = 8.1 Hz, 2H), 7.12 (d, J = 1.2 Hz, 1H), 6.97 (dd, J = 15.6, 7.2 Hz, 1H), 4.34 (d, J = 7.1 Hz, 3H), 2.96 (dd, J = 13.6, 4.4 Hz, 1H), 2.88–2.77 (m, 1H), 1.86–1.66 (m, 1H), 1.44–1.18

(m, 12H), 0.77 (t, J = 6.0 Hz, 4H).

*4.1.68. Synthesis of ethyl 5-{(2S)-2-[(tert-butoxycarbonyl)amino]-3-{4-[(2-methylcyclopropylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylate(**41g**)*

Compound **41g** was prepared from commercially available 2-methylcyclopropane-1-carboxylic acid in the same manner as described for **41a**. An off-white solid, yield: 69.7%. Mp 221.4–222.8°C. ESI-MS (m/z) = 547.55[M − H] $^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (s, 1H), 10.02 (s, 1H), 9.88 (s, 1H), 7.98 (s, 1H), 7.47 (d, J = 8.3 Hz, 2H), 7.35 (dt, J = 9.0, 5.2 Hz, 2H), 7.21 (d, J = 8.2 Hz, 2H), 7.11 (d, J = 1.4 Hz, 1H), 6.99 (d, J = 8.0 Hz, 1H), 4.45–4.21 (m, 3H), 3.02–2.87 (m, 1H), 2.87–2.70 (m, 1H), 1.49 (dt, J = 7.9, 4.0 Hz, 1H), 1.41–1.13 (m, 13H), 1.09 (t, J = 6.3 Hz, 3H), 1.02–0.91 (m, 1H), 0.67–0.47 (m, 1H).

*4.1.69. Synthesis of ethyl (S)-5-{2-[(tert-butoxycarbonyl)amino]-3-{4-[(cyclobutylcarbonyl)amino]phenyl}propanamido)-1H-indole-2-carboxylate (**41h**)*

Compound **41h** was prepared from commercially available cyclobutanecarboxylic acid in the same manner as described for **41a**. A yellow solid, yield: 73.7%. Mp 189.2–190.9°C. ESI-MS (m/z) = 571.44 [M + Na] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (s, 1H), 9.98 (d, J = 14.9 Hz, 1H), 9.68 (s, 1H), 8.08–7.89 (m, 1H), 7.60–7.44 (m, 2H), 7.44–7.31 (m, 2H), 7.23 (d, J = 8.4 Hz, 2H), 7.15–7.07 (m, 1H), 6.99 (t, J = 9.0 Hz, 1H), 4.40–4.23 (m, 3H), 3.31–3.10 (m, 1H), 3.04–2.92 (m, 1H), 2.86–2.76 (m, 1H), 2.28–1.71 (m, 6H), 1.50–1.13 (m, 12H).

*4.1.70. Synthesis of ethyl (S)-5-{2-[(tert-butoxycarbonyl)amino]-3-{4-[(cyclopentylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylate (**41i**)*

Compound **41i** was prepared from commercially available cyclopentanecarboxylic acid in the same manner as described for **41a**. An off-yellow solid, yield: 77.3%. Mp 237.9–239.2°C. ESI-MS (m/z) = 585.48[M + Na] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (d, J = 1.4 Hz, 1H), 9.88 (s, 1H), 9.76 (s, 1H), 7.99 (s, 1H), 7.50 (d, J = 8.4 Hz, 2H), 7.43–7.31 (m, 2H), 7.22 (d, J = 8.3 Hz, 2H), 7.12 (d, J = 1.5 Hz, 1H), 6.99 (d, J = 8.2 Hz, 1H), 4.34 (d, J = 7.1 Hz, 3H), 2.94 (d, J = 4.6 Hz, 1H), 2.86–2.67 (m, 2H), 1.83 (d, J = 8.0 Hz, 2H), 1.76–1.62 (m, 4H), 1.55 (d, J = 2.6 Hz, 2H), 1.35 (s, 12H).

*4.1.71. Synthesis of ethyl (S)-5-{2-[(tert-butoxycarbonyl)amino]-3-{4-[(cyclohexylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylate (**41j**)*

Compound **41j** was prepared from commercially available cyclohexanecarboxylic acid in the same manner as described for **41a**. An off-white solid, yield: 81.2%. Mp 252.3–253.7°C. ESI-MS (m/z) = 599.49[M + Na] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 9.87 (s, 1H), 9.69 (s, 1H), 7.98 (s, 1H), 7.50 (d, J = 8.3 Hz, 2H), 7.36 (dt, J = 10.3, 5.2 Hz, 2H), 7.22 (d, J = 8.2 Hz, 2H), 7.12 (s, 1H), 6.98 (d, J = 8.1 Hz, 1H), 4.34 (d, J = 7.1 Hz, 3H), 2.95 (dd, J = 13.5, 4.5 Hz, 1H), 2.87–2.71 (m, 1H), 2.30 (dd, J = 15.5, 7.3 Hz, 1H), 1.76 (t, J = 11.5 Hz, 4H), 1.65 (d, J = 10.6 Hz, 1H), 1.51–1.07 (m, 17H).

*4.1.72. Synthesis of ethyl (S)-5-{3-{4-[(phenylcarbonyl)amino]phenyl}-2-[(tert-butoxycarbonyl)amino]propanamido}-1H-indole-2-carboxylate (**41k**)*

Compound **41k** was prepared from commercially available benzoic acid in the same manner as described for **41a**. An off-white solid, yield: 72.7%. Mp 225.4–226.9°C. ESI-MS (m/z) = 593.47[M + Na] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 10.17 (s, 1H), 9.91 (s, 1H), 8.00 (s, 1H), 7.97–7.90 (m, 2H), 7.69 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 7.2 Hz, 1H), 7.53 (t, J = 7.3 Hz, 2H), 7.39 (s, 1H), 7.37 (d, J = 1.8 Hz, 1H), 7.30 (d, J = 8.3 Hz, 2H), 7.12 (d, J = 1.6 Hz, 1H), 7.03 (d, J = 8.3 Hz, 1H), 4.34 (d, J = 7.1 Hz, 3H), 3.00 (dd, J = 13.8, 4.6 Hz, 1H), 2.91–2.72 (m, 1H), 1.52–1.14 (m, 12H).

*4.1.73. Synthesis of ethyl (S)-5-{2-[(tert-butoxycarbonyl)amino]-3-{4-{[(pyridin-2-yl)carbonyl]amino}phenyl}propanamido}-1H-indole-2-carboxylate (**41l**)*

Compound **41l** was prepared from commercially available picolinic acid in the same manner as described for **41a**. An off-white solid, yield: 74.4%. Mp 147.4–148.7°C. ESI-MS (m/z) = 594.45[M + Na] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 10.55 (s, 1H), 9.92 (s, 1H), 8.73 (d, J = 4.5 Hz, 1H), 8.16 (d, J = 7.8 Hz, 1H), 8.07 (td, J = 7.7, 1.6 Hz, 1H), 8.01 (s, 1H), 7.82 (d, J = 8.3 Hz, 2H), 7.72–7.60 (m, 1H), 7.39 (dd, J = 14.6, 5.3 Hz, 2H), 7.33 (dd, J = 10.5, 5.0 Hz, 2H), 7.12 (d, J = 1.3 Hz, 1H), 7.04 (d, J = 8.2 Hz, 1H), 4.48–4.06 (m, 3H), 3.01 (dd, J = 13.6, 4.3 Hz, 1H), 2.92–2.77 (m, 1H), 1.65–1.13 (m, 12H).

*4.1.74. Synthesis of ethyl (S)-5-{2-[(tert-butoxycarbonyl) amino]-3-{4-{[(pyrimidine-2-yl)carbonyl]amino}phenyl}propanamido}-1H-indole-2-carboxylate (**41m**)*

Compound **41m** was prepared from commercially available pyrimidine-2-carboxylic acid in the same manner as described for **41a**. An off-white solid, yield: 65.8%. Mp 236.3–238.0°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 10.66 (s, 1H), 9.92 (s, 1H), 9.03 (d, J = 4.9 Hz, 2H), 8.01 (s, 1H), 7.79 (d, J = 8.3 Hz, 2H), 7.73 (t, J = 4.9 Hz, 1H), 7.45–7.27 (m, 4H), 7.12 (d, J = 1.5 Hz, 1H), 7.05 (d, J = 8.2 Hz, 1H), 4.34 (q, J = 7.0 Hz, 3H), 3.02 (dd, J = 13.6, 4.4 Hz, 1H), 2.91–2.81 (m, 1H), 1.45–1.08 (m, 12H).

*4.1.75. Synthesis of ethyl (S)-5-{3-[(4-acetylamino)phenyl]-2-aminopropanamido}-1H-indole-2-carboxylate (**42a**)*

Compound **42a** was prepared from **41a** in the same manner as described for **26**, and it was used for the next reaction without further purification.

*4.1.76. Synthesis of ethyl (S)-5-{2-amino-3-{4-[(isopropylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylate (**42b**)*

Compound **42b** was prepared from **41b** in the same manner as described for **26**. An off-white solid, yield: 82.4%. Mp 275.3–276.8°C. ESI-MS (m/z) = 459.33[M + Na] $^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (s, 1H), 9.76 (s, 2H), 8.01 (s, 1H), 7.50 (d, J = 7.8 Hz, 2H), 7.36 (s, 2H), 7.16 (d, J = 7.7 Hz, 2H), 7.09 (s, 1H), 4.85–4.02 (m, 2H), 3.54 (s, 1H), 2.96 (d, J = 8.9 Hz, 1H), 2.75–2.63 (m, 1H), 2.61–2.54 (m, 1H), 1.34 (t, J = 6.8 Hz, 3H), 1.08 (d, J = 6.5 Hz, 6H).

*4.1.77. Synthesis of ethyl (S)-5-{2-amino-3-{4-[(isobutylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylate (**42c**)*

Compound **42c** was prepared from **41c** in the same manner as described for **26**. An off-white solid, yield: 83.6%. Mp 237.1–238.6°C. ESI-MS (m/z) = 449.45[M − H] $^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (s, 1H), 9.75 (s, 2H), 8.00 (s, 1H), 7.49 (d, J = 7.7 Hz, 2H), 7.37 (t, J = 6.9 Hz, 2H), 7.16 (d, J = 7.6 Hz, 2H), 7.10 (s, 1H), 4.34 (d, J = 6.8 Hz, 2H), 3.58 (s, 1H), 2.97 (d, J = 8.3 Hz, 1H), 2.78–2.66 (m, 1H), 2.15 (d, J = 6.6 Hz, 2H), 2.04 (dd, J = 10.7, 8.0 Hz, 1H), 1.34 (t, J = 6.7 Hz, 3H), 0.92 (d, J = 6.0 Hz, 6H).

*4.1.78. Synthesis of ethyl (S)-5-{2-amino-3-{4-[(ethynylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylate (**42d**)*

Compound **42d** was prepared from **41d** in the same manner as described for **26**. An off-white solid, yield: 72.3%. Mp 217.6–219.3°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.90 (s, 1H), 10.73 (s, 1H), 7.99 (s, 1H), 7.64 (dd, J = 16.3, 9.1 Hz, 2H), 7.41 (dd, J = 14.2, 7.7 Hz, 3H), 7.32–7.23 (m, 1H), 7.13 (s, 1H), 4.56–4.08 (m, 3H), 3.87 (s, 1H), 3.20 (dd, J = 13.5, 6.2 Hz, 1H), 3.15–2.96 (m, 1H), 1.34 (t, J = 6.7 Hz, 3H).

*4.1.79. Synthesis of ethyl (S)-5-{2-amino-3-{4-{[(prop-1-yn-1-yl) carbonyl]amino}phenyl}propanamido}-1H-indole-2-carboxylate (**42e**)*

Compound **42e** was prepared from **41e** in the same manner as described for **26**. An off-white solid, yield: 78.3%. Mp 209.6–210.9°C. ESI-MS (m/z) = 455.31[M + Na]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.88 (s, 1H), 10.79 (s, 1H), 10.61 (s, 1H), 8.45 (s, 3H), 7.97 (s, 1H), 7.54 (d, J = 8.4 Hz, 2H), 7.40 (dt, J = 9.0, 5.3 Hz, 2H), 7.29 (d, J = 8.4 Hz, 2H), 7.13 (d, J = 1.5 Hz, 1H), 4.47–4.22 (m, 3H), 3.20 (dd, J = 13.8, 6.6 Hz, 1H), 3.12 (dd, J = 13.8, 7.0 Hz, 1H), 2.03 (s, 3H), 1.34 (t, J = 7.1 Hz, 3H).

*4.1.80. Synthesis of ethyl (S)-5-{2-amino-3-{4-[(cyclopropylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylate (**42f**)*

Compound **42f** was prepared from **41f** in the same manner as described for **26**. An off-white solid, yield: 81.9%. Mp 212.3–214.0°C. ESI-MS (m/z) = 435.34[M + H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (s, 1H), 10.24 (s, 1H), 8.01 (s, 1H), 7.50 (d, J = 8.3 Hz, 2H), 7.37 (s, 2H), 7.16 (d, J = 8.4 Hz, 2H), 7.09 (s, 1H), 4.34 (q, J = 7.1 Hz, 2H), 3.61–3.47 (m, 1H), 2.96 (dd, J = 13.4, 5.3 Hz, 1H), 2.68 (dd, J = 13.3, 8.1 Hz, 1H), 1.80 (m, 1H), 1.34 (t, J = 7.1 Hz, 4H), 0.76 (t, J = 6.8 Hz, 4H).

*4.1.81. Synthesis of ethyl 5-{(2S)-2-amino-3-{4-{[(2-methylcyclopropyl)carbonyl]amino}phenyl}propanamido}-1H-indole-2-carboxylate (**42g**)*

Compound **42g** was prepared from **41g** in the same manner as described for **26**. A white solid, yield: 76.1%. Mp 271.7–273.0°C. ESI-MS (m/z) = 449.38 [M + H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (s, 1H), 10.04 (s, 1H), 8.01 (s, 1H), 7.47 (d, J = 8.3 Hz, 2H), 7.41–7.27 (m, 2H), 7.18 (t, J = 14.6 Hz, 2H), 7.10 (s, 1H), 4.34 (q, J = 7.1 Hz, 2H), 3.69–3.44 (m, 1H), 2.96 (dd, J = 13.4, 5.3 Hz, 1H), 2.68 (dd, J = 13.5, 7.8 Hz, 1H), 1.50 (dt, J = 8.1, 4.1 Hz, 1H), 1.34 (t, J = 7.1 Hz, 3H), 1.10 (t, J = 6.2 Hz, 4H), 0.98 (dt, J = 8.1, 3.9 Hz, 1H), 0.60 (dd, J = 11.4, 5.8 Hz, 1H).

*4.1.82. Synthesis of ethyl (S)-5-{2-amino-3-{4-[(cyclobutylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylate (**42h**)*

Compound **42h** was prepared from **41h** in the same manner as described for **26**. A white solid, yield: 75.8%. Mp 251.3–252.7°C. ESI-MS (m/z) = 449.36 [M + H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.81 (s, 1H), 9.65 (s, 1H), 8.00 (t, J = 9.0 Hz, 1H), 7.49 (t, J = 8.1 Hz, 2H), 7.43–7.28 (m, 2H), 7.16 (d, J = 8.4 Hz, 1H), 7.10 (d, J = 6.1 Hz, 1H), 6.90 (d, J = 8.2 Hz, 1H), 6.47 (t, J = 7.5 Hz, 1H), 4.34 (q, J = 7.1 Hz, 2H), 3.25–3.16 (m, 1H), 2.96 (dd, J = 13.4, 5.5 Hz, 1H), 2.69 (dd, J = 13.5, 7.9 Hz, 1H), 2.60–2.54 (m, 1H), 2.29–1.69 (m, 6H), 1.34 (t, J = 7.1 Hz, 3H).

*4.1.83. Synthesis of ethyl (S)-5-{2-amino-3-{4-[(cyclopentylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylate (**42i**)*

Compound **42i** was prepared from **41i** in the same manner as described for **26**. A white solid, yield: 74.3%. Mp 277.2–278.9°C. ESI-MS (m/z) = 463.38 [M + H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.78 (s, 1H), 9.77 (s, 2H), 8.00 (s, 1H), 7.50 (d, J = 8.3 Hz, 2H), 7.41–7.28 (m, 2H), 7.16 (d, J = 8.4 Hz, 2H), 7.09 (s, 1H), 4.33 (q, J = 7.1 Hz, 2H), 3.54 (dd, J = 7.5, 5.7 Hz, 1H), 2.95 (dd, J = 13.4, 5.3 Hz, 1H), 2.83–2.54 (m, 2H), 1.95–1.76 (m, 2H), 1.69 (dd, J = 13.3, 5.8 Hz, 4H), 1.59–1.45 (m, 2H), 1.34 (t, J = 7.1 Hz, 3H).

*4.1.84. Synthesis of ethyl (S)-5-{2-amino-3-{4-[(cyclohexylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylate (**42j**)*

Compound **42j** was prepared from **41j** in the same manner as described for **26**. A white solid, yield: 82.5%. Mp > 280.0°C. ESI-MS (m/z) = 477.41 [M + H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.78 (s, 1H), 9.69 (s, 2H), 8.01 (d, J = 6.8 Hz, 1H), 7.49 (d, J = 8.4 Hz, 2H), 7.37 (t, J = 6.2 Hz, 2H), 7.21–7.04 (m, 3H), 4.34 (q, J = 7.1 Hz, 2H), 3.53 (s, 1H), 2.95 (dd, J = 13.5, 5.5 Hz, 1H), 2.79–2.60 (m, 1H), 2.31 (d, J = 14.9 Hz, 1H), 1.76 (s, 4H), 1.42–1.15 (m, 9H).

*4.1.85. Synthesis of ethyl (S)-5-{2-amino-3-{4-[(phenylcarbonyl) amino]phenyl}propanamido}-1H-indole-2-carboxylate (**42k**)*

Compound **42k** was prepared from **41k** in the same manner as described for **26**. An off-white solid, yield: 84.5%. Mp 278.4–280.1°C. ESI-MS (m/z) = 471.35 [M + H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.87 (s, 1H), 10.77 (s, 1H), 10.28 (s, 1H), 8.48 (s, 3H), 8.01–7.90 (m, 3H), 7.75 (d, J = 8.5 Hz, 2H), 7.58 (dd, J = 8.4, 6.0 Hz, 1H), 7.52 (t, J = 7.4 Hz, 2H), 7.46–7.35 (m, 2H), 7.32 (d, J = 8.5 Hz, 2H), 7.14 (d, J = 2.0 Hz, 1H), 4.34 (dd, J = 14.2, 7.1 Hz, 3H), 3.23 (dd, J = 13.8, 6.8 Hz, 1H), 3.15 (dd, J = 13.8, 7.0 Hz, 1H), 1.34 (t, J = 7.1 Hz, 3H).

*4.1.86. Synthesis of ethyl (S)-5-{2-amino-3-{4-{[(pyridin-2-yl) carbonyl]amino}phenyl}propanamido}-1H-indole-2-carboxylate (**42l**)*

Compound **42l** was prepared from **41l** in the same manner as described for **26**. An off-white solid, yield: 86.3%. Mp 200.9–202.2°C. ESI-MS (m/z) = 472.36 [M + H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (s, 1H), 10.54 (s, 1H), 9.87 (s, 1H), 8.73 (d, J = 4.4 Hz, 1H), 8.15 (d, J = 7.7 Hz, 1H), 8.06 (dd, J = 15.1, 6.2 Hz, 2H), 7.81 (d, J = 8.3 Hz, 2H), 7.73–7.60 (m, 1H), 7.38 (s, 2H), 7.26 (d, J = 8.3 Hz, 2H), 7.10 (s, 1H), 4.33 (q, J = 7.0 Hz, 2H), 3.59 (s, 1H), 3.01 (dd, J = 13.3, 5.3 Hz, 1H), 2.73 (dd, J = 13.3, 7.8 Hz, 1H), 1.34 (t, J = 7.1 Hz, 3H).

*4.1.87. Synthesis of ethyl (S)-5-{2-amino-3-{4-{[(pyrimidin-2-yl) carbonyl]amino}phenyl}propanamido}-1H-indole-2-carboxylate (**42m**)*

Compound **42m** was prepared from **41m** in the same manner as described for **26**. An off-white solid, yield: 81.3%. Mp > 280°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (s, 1H), 10.66 (s, 1H), 9.78 (s, 1H), 9.03 (d, J = 4.9 Hz, 2H), 8.03 (s, 1H), 7.90–7.59 (m, 3H), 7.37 (s, 2H), 7.26 (d, J = 8.3 Hz, 2H), 7.10 (s, 1H), 4.34 (q, J = 7.1 Hz, 2H), 3.66–3.50 (m, 1H), 3.02 (dd, J = 13.4, 5.4 Hz, 1H), 2.74 (dd, J = 13.4, 7.8 Hz, 1H), 1.34 (t, J = 7.1 Hz, 3H).

*4.1.88. Synthesis of ethyl (S)-5-{3-phenyl-2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido] propanamido}-1H-indole-2-carboxylate (**43a-m**)*

Compound **43a** was prepared from **42a** in the same manner as described for **27a-27k**, and they were used for the next reaction without further purification.

*4.1.89. Synthesis of (S)-5-{3-(4-acetylaminophenyl)-2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]propanamido}-1H-indole-2-carboxylic acid (**44a**)*

Compound **44a** was prepared from **43a** in the same manner as described for **28a-28k**. An off-white solid, yield: 17.33%. Mp 173.6–174.7°C. ESI-HRMS calcd for $C_{31}H_{27}ClFN_6O_6$ [M + H]$^+$ 633.1659 found: 633.1524; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.15 (s, 1H), 10.04 (d, J = 10.4 Hz, 2H), 8.33 (d, J = 8.0 Hz, 1H), 7.96 (s, 1H), 7.88 (s, 1H), 7.55 (s, 1H), 7.49 (d, J = 7.4 Hz, 2H), 7.38–7.09 (m, 5H), 6.73 (s, 1H), 4.62 (d, J = 4.3 Hz, 1H), 3.00 (d, J = 13.3 Hz, 1H), 2.86–2.74 (m, 1H), 2.32 (s, 3H), 2.02 (s, 3H). ^{13}C NMR (100 MHz,

DMSO-d_6) δ176.67, 170.37, 161.68, 155.26, 153.57, 138.24, 138.04, 136.05, 133.88, 133.11, 131.78, 130.03, 129.61, 129.38, 128.85, 127.50, 125.23, 124.68, 120.44, 120.37, 119.96, 117.82, 112.44, 112.32, 112.20, 112.11, 105.47, 54.89, 38.55, 15.88.

*4.1.90. Synthesis of (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-{4-[(isopropylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylic acid (**44b**)*

Compound **44b** was prepared from **43b** in the same manner as described for **28a-28k**. A white solid, yield: 18.14%. Mp173.1−174.5 °C. ESI-HRMS calcd for $C_{33}H_{30}ClFN_6O_6$ [M + Na]$^+$ 683.1792 found: 683.1797; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.93 (s, 1H), 9.96 (d, J = 8.6 Hz, 1H), 7.92 (d, J = 8.7 Hz, 2H), 7.84 (d, J = 8.7 Hz, 2H), 7.68 (dd, J = 14.4 Hz, 1H), 7.52 (t, J = 7.9 Hz, 1H), 7.39 (d, J = 7.5 Hz, 2H), 7.26 (t, J = 7.5 Hz, 3H), 7.19 (d, J = 7.8 Hz, 2H), 5.32 (t, J = 4.7 Hz, 1H), 4.37 (d, J = 11.5 Hz, 1H), 3.16 (d, J = 7.0 Hz, 1H), 3.06 (d, J = 9.1 Hz, 1H), 2.39 (s, 3H), 1.23 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 175.55, 169.98, 163.48, 160.23, 155.20, 153.52, 138.30, 134.48, 132.41, 132.03, 131.07, 130.30, 129.83, 129.51, 127.18, 125.50, 125.48, 120.70, 120.63, 120.58, 119.41, 118.92, 112.78, 112.41, 107.27, 54.86, 38.48, 35.30, 19.96, 14.41.

*4.1.91. Synthesis of (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-{4-[(isobutylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylic acid (**44c**)*

Compound **44c** was prepared from **43c** in the same manner as described for **28a-28k**. A white solid, yield: 10.69%. Mp 177.7−179.2°C. ESI-HRMS calcd for $C_{27}H_{23}ClN_4O_5$ [M − H]$^-$ 673.1983 found: 673.1973; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.68 (s, 1H), 10.10 (s, 1H), 8.16 (s, 1H), 8.11−7.92 (m, 2H), 7.77−7.68 (m, 2H), 7.48 (d, J = 8.6 Hz, 2H), 7.40−7.31 (m, 3H), 7.21 (d, J = 8.2 Hz, 2H), 7.04 (s, 1H), 4.88 (t, J = 5.7 Hz, 1H), 3.14 (dd, J = 11.0, 7.1 Hz, 1H), 2.98 (d, J = 10.9 Hz, 1H), 2.42 (s,3H), 1.68−1.55 (m, 4H), 0.91 (t, J = 7.4 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.61, 167.80, 163.15, 161.69, 157.53, 155.48, 153.80, 138.05, 134.73, 132.17, 131.67, 131.11, 129.76, 129.13, 127.11, 126.04, 125.74, 125.71, 120.96, 120.85, 119.38, 117.69, 113.37, 112.31, 118.11, 55.48, 46.06, 38.55, 27.02, 22.77, 14.78.

*4.1.92. Synthesis of (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-{4-[(ethynylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylic acid (**44d**)*

Compound **44d** was prepared from **43d** in the same manner as described for **28a-28k**. A white solid, yield: 14.01%. Mp 183.6−184.7°C. ESI-HRMS calcd for $C_{32}H_{24}ClFN_6O_6$ [M − H]$^-$ 641.1357 found: 641.5392; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 1H), 8.51 (d, J = 7.8 Hz, 1H), 8.28 (d, J = 11.6 Hz, 2H), 7.91 (dd, J = 13.7, 7.7 Hz, 3H), 7.74 (t, J = 7.8 Hz, 1H), 7.65 (d, J = 8.2 Hz, 2H), 7.40 (dd, J = 13.3, 8.3 Hz, 4H), 4.94 (dd, J = 12.4, 9.3 Hz, 1H), 3.91 (s, 1H), 3.26 (dd, J = 13.7, 3.8 Hz, 1H), 3.19−3.04 (m, 1H), 2.23 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ173.42, 170.33, 169.71, 166.25, 158.77, 140.89, 139.31, 138.91, 138.73, 138.35, 137.17, 133.36, 131.72, 131.67, 130.84, 130.49, 130.36, 129.33, 128.59, 127.97, 120.67, 120.22, 118.77, 118.66, 112.67, 68.02, 67.82, 55.68, 37.07, 15.14.

*4.1.93. Synthesis of (S)-5-{3-{4-{[(prop-1-yn-1-yl)carbonyl]amino}phenyl}-2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]propanamido}-1H-indole-2-carboxylic acid (**44e**)*

Compound **44e** was prepared from **43e** in the same manner as described for **28a-28k**. A white solid, yield: 12.38%. Mp 181.1−182.8°C. ESI-MS (m/z) = 655.36 [M − H]$^-$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.71 (s, 1H), 11.19 (s, 1H), 10.16 (s, 1H), 9.87 (s, 1H), 8.35 (s, 1H), 7.87 (s, 1H), 7.61−7.40 (m, 3H), 7.29 (d, J = 8.5 Hz, 1H), 7.20 (dd, J = 13.3, 8.0 Hz, 4H), 6.76 (s, 1H), 4.81 (d, J = 6.8 Hz, 1H), 3.10−2.99 (m, 1H), 2.94−2.85 (m, 1H), 2.31 (s, 3H), 1.76 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.42, 169.67, 165.58, 163.17, 161.18, 155.42, 153.73, 134.64, 132.93, 131.99, 129.94, 129.86, 129.39, 127.12, 126.15, 126.12, 121.06, 120.95, 119.30, 119.21, 117.93, 115.27, 112.89, 112.45, 107.78, 95.20, 63.47, 52.78, 37.89, 14.69, 12.50.

*4.1.94. Synthesis of (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-{4-[(cyclopropylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylic acid (**44f**)*

Compound **44f** was prepared from **43f** in the same manner as described for **28a-28k**. A yellow solid, yield: 14.83%. Mp 200.2−201.9°C. ESI-HRMS calcd for $C_{33}H_{28}ClFN_6O_6$ [M − H]$^-$ 657.1670 found: 657.1672; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.41 (s, 1H), 10.12−9.79 (m, 2H), 8.28 (s, 1H), 7.95 (d, J = 14.8 Hz, 2H), 7.60−7.45 (m, 2H), 7.27 (tdd, J = 15.0, 14.7, 8.1 Hz, 4H), 6.88 (s, 1H), 4.92−4.80 (m, 1H), 3.15−3.04 (m, 1H), 2.96 (dd, J = 15.5, 8.2 Hz, 1H), 2.35 (d, J = 14.5 Hz, 3H), 1.23 (s, 3H), 0.85 (t, J = 6.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.93, 169.68, 168.49, 167.44, 163.80, 162.80, 157.77, 138.34, 133.72, 133.57, 132.16, 132.01, 130.74, 129.87, 129.42, 129.13, 127.31, 125.92, 121.59, 120.80, 120.69, 119.26, 114.11, 112.86, 110.85, 55.40, 38.52, 14.91, 14.02, 7.49.

*4.1.95. Synthesis of (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-{4-[(2-methylcyclopropylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylic acid (**44g**)*

Compound **44g** was prepared from **43g** in the same manner as described for **28a-28k**. An off yellow solid, yield: 15.32%. Mp 203.9−205.4°C. ESI-HRMS calcd for $C_{34}H_{30}ClFN_6O_6$ [M + Na]$^+$ 695.1792 found: 695.1809; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.50 (s, 1H), 10.1 (d, J = 7.1 Hz, 1H) 9.99 (s, 1H), 8.36 (d, J = 7.1 Hz, 1H) 8.26 (s, 1H), 8.14 (d, J = 7.3 Hz, 1H) 7.95 (d, J = 7.6 Hz, 1H) 7.55 (t, J = 8.1 Hz, 1H) 7.32 (d, J = 8.3 Hz, 2H), 7.27−7.21 (m, 3H), 7.18 (t, J = 14.6 Hz, 3H), 6.92 (s, 1H), 4.83 (q, J = 7.1 Hz, 1H), 3.08 (m, 1H), 2.92 (dd, J = 13.4, 5.3 Hz, 1H), 2.37 (s, 1H), 1.50 (dt, J = 8.1, 4.1 Hz, 1H), 1.34 (d, J = 7.1 Hz, 1H), 1.10 (t, J = 6.2 Hz, 3H), 0.98 (dt, J = 8.1, 3.9 Hz, 1H), 0.60 (d, J = 11.4, 5.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.40, 169.02, 162.75, 161.70, 154.14, 152.46, 145.72, 138.70, 137.17, 133.78, 133.19, 131.38, 130.88, 128.78, 128.36, 126.44, 126.18, 124.06, 119.50, 119.39, 119.28, 118.04, 117.48, 111.56, 111.20, 53.73, 37.45, 22.33, 16.93, 14.74, 14.47, 11.19.

*4.1.96. Synthesis of (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-{4-[(cyclobutylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylic acid (**44h**)*

Compound **44h** was prepared from **43h** in the same manner as described for **28a-28k**. An off yellow solid, yield: 17.77%. Mp 192.2−194.0°C. ESI-HRMS calcd for $C_{34}H_{30}ClFN_6O_6$ [M + Na]$^+$ 695.1792 found: 695.1788; ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.15 (s, 1H), 11.70 (s, 1H), 10.07 (s, 1H), 9.63 (s, 1H), 8.40 (s, 1H), 8.02 (d, J = 16.6 Hz, 2H), 7.76 (s, 2H), 7.40 (m, 7H), 7.20 (s, 2H), 7.05 (s, 1H), 4.90 (s, 1H), 3.15 (d, J = 11.1 Hz, 4H), 2.96 (s, 2H), 2.19 (s, 2H), 2.07 (s, 2H), 1.92 (s, 1H), 1.79 (s, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ173.16, 169.58, 163.17, 161.13, 159.36, 155.41, 153.73, 138.29, 134.66, 132.97, 132.35, 132.14, 132.06, 129.85, 129.42, 127.12, 126.16, 126.03, 122.37, 120.83, 119.42, 119.15, 117.93, 112.91, 112.47, 107.77, 54.71, 38.54, 25.04, 18.18, 12.46, 11.92.

*4.1.97. Synthesis of (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-{4-[(isopentylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylic acid (**44i**)*

Compound **44i** was prepared from **43i** in the same manner as described for **28a-28k**. A white solid, yield: 16.21%. Mp 186.3–187.8°C. ESI-HRMS calcd for $C_{35}H_{32}ClFN_6O_6$ [M − H]$^-$ 685.1983 found: 685.2048; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.09 (s, 1H), 10.05 (s, 1H), 9.92 (s, 1H), 8.34 (d, J = 8.3 Hz, 1H), 7.92 (d, J = 10.9 Hz, 2H), 7.53 (d, J = 7.9 Hz, 3H), 7.38–7.07 (m, 4H), 6.73 (s, 1H), 4.62 (t, J = 14.3, 8.8 Hz, 1H), 3.01 (dd, J = 13.9, 4.1 Hz, 1H), 2.81 (dd, J = 14.6, 9.8 Hz, 1H), 2.47 (s, 3H), 2.41–2.24 (m, 1H), 1.76 (m, 4H), 1.22 (m, 4H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.52, 169.13, 168.45, 164.06, 161.64, 160.10, 137.14, 132.34, 131.56, 130.29, 128.93, 128.66, 128.58, 127.60, 126.47, 118.95, 118.10, 118.03, 117.55, 117.24, 116.02, 115.99, 111.07, 110.88, 103.08, 54.41, 43.85, 36.67, 28.52, 24.50, 13.19.

*4.1.98. Synthesis of (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-{4-[(isohexylcarbonyl)amino]phenyl}propanamido}-1H-indole-2-carboxylic acid (**44j**)*

Compound **44j** was prepared from **43j** in the same manner as described for **28a-28k**. A white solid, yield: 18.96%. Mp 171.5–172.9 °C. ESI-HRMS calcd for $C_{36}H_{34}ClFN_6O_6$ [M + Na]$^+$ 723.2105 found: 723.2103; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.49 (s, 1H), 9.91 (s, 1H), 9.69 (s, 1H), 8.32 (s, 1H), 7.92 (s, 1H), 7.50 (m, 3H), 7.27 (m, 5H), 6.93 (s, 1H), 4.82 (dd, J = 14.3, 7.2 Hz, 1H), 3.06 (dd, J = 13.4, 6.0 Hz, 1H), 2.91 (dd, J = 12.5, 7.1 Hz, 1H), 2.39–2.17 (m, 4H), 1.83–1.67 (m, 4H), 1.50–1.05 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.54, 170.21, 163.77, 161.09, 155.22, 153.54, 138.30, 134.32, 132.61, 132.03, 130.02, 129.81, 129.65, 129.40, 127.26, 124.97, 120.56, 120.44, 120.22, 119.27, 118.65, 112.94, 112.67, 112.28, 106.81, 54.86, 45.28, 38.52, 29.59, 25.87, 25.70, 14.41.

*4.1.99. Synthesis of (S)-5-{3-{4-[(phenylcarbonyl)amino]phenyl}-2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]propanamido}-1H-indole-2-carboxylic acid (**44k**)*

Compound **44k** was prepared from **43k** in the same manner as described for **28a-28k**. An off yellow solid, yield: 26.28%. Mp 163.4–165.1 °C. ESI-HRMS calcd for $C_{36}H_{28}ClFN_6O_6$ [M − H]$^-$ 693.1670 found: 693.1710; ^{1}H NMR (600 MHz, DMSO-d_6) δ13.08 (s, 1H), 11.70 (s, 1H), 11.21 (s, 1H), 10.15 (d, J = 13.8 Hz, 2H), 8.40 (s, 1H), 7.99 (s, 1H), 7.92 (d, J = 6.7 Hz, 2H), 7.75 (s, 1H), 7.68 (d, J = 7.3 Hz, 2H), 7.55 (m, 4H), 7.45–7.32 (m, 3H), 7.28 (d, J = 7.4 Hz, 2H), 7.06 (s, 1H), 4.95 (d, J = 6.0 Hz, 1H), 3.22–3.10 (m, 1H), 3.01 (s, 1H), 2.51 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.61, 165.88, 163.19, 159.41, 154.97, 153.31, 138.09, 135.48, 134.68, 132.99, 132.30, 132.07, 131.94, 129.84, 129.46, 128.82, 128.05, 127.14, 126.02, 121.20, 120.83, 120.72, 120.66, 119.17, 115.13, 112.94, 112.50, 107.78, 54.71, 38.59, 11.96.

*4.1.100. Synthesis of (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-{4-{[(pyridin-2-yl)carbonyl]amino}phenyl}propanamido}-1H-indole-2-carboxylic acid (**44l**)*

Compound **44l** was prepared from **43l** in the same manner as described for **28a-28k**. An off yellow solid, yield: 17.32%. Mp 177.2–178.8 °C. ESI-HRMS calcd for $C_{35}H_{27}ClFN_6O_6$ [M + Na]$^+$ 718.1588 found: 718.1587; ^{1}H NMR (400 MHz, DMSO-d_6) δ11.82 (d, J = 5.2 Hz, 1H), 10.66 (d, J = 3.9 Hz, 1H), 10.08 (d, J = 4.6 Hz, 1H), 9.02 (dd, J = 7.2, 4.9 Hz, 3H), 7.89–7.82 (m, 1H), 7.81–7.70 (m, 4H), 7.66 (d, J = 8.4 Hz, 1H), 7.47–7.28 (m, 6H), 7.24 (d, J = 8.4 Hz, 1H), 4.70 (dd, J = 13.5, 7.8 Hz, 1H), 3.12 (dd, J = 12.1, 8.9 Hz, 1H), 3.03 (d, J = 5.9 Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ169.66, 163.17, 162.77, 160.16, 159.47, 157.67, 150.37, 148.84, 138.55, 137.27, 134.71, 133.36, 132.53, 132.45, 132.08, 130.64, 129.94, 129.45, 127.30, 127.16, 125.02, 122.76, 121.04, 120.53, 119.23, 116.55, 116.34, 112.94, 112.57, 107.80, 54.73, 38.59, 12.07.

*4.1.101. Synthesis of (S)-5-{2-[2-(3-chloro-2-fluorophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxamido]-3-{4-{[(pyrimidin-2-yl)carbonyl]amino}phenyl}propanamido}-1H-indole-2-carboxylic acid (**44m**)*

Compound **44m** was prepared from **43m** in the same manner as described for **28a-28k**. An off yellow solid, yield: 18.14%. Mp 181.1–182.6°C. ESI-HRMS calcd for $C_{34}H_{26}ClFN_6O_6$ [M + H]$^+$ 697.1721 found: 697.1958; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.85 (d, J = 1.3 Hz, 1H), 10.64 (s, 1H), 10.26 (s, 1H), 9.02 (d, J = 4.9 Hz, 2H), 8.32 (d, J = 7.8 Hz, 1H), 8.06 (s, 1H), 7.80 (d, J = 8.5 Hz, 2H), 7.73 (t, J = 4.9 Hz, 1H), 7.72–7.64 (m, 1H), 7.36–7.32 (m, 1H), 7.27–7.18 (m, 2H), 7.08 (t, J = 7.7 Hz, 2H), 6.86 (d, J = 7.1 Hz, 2H), 4.98 (td, J = 8.2, 5.3 Hz, 1H), 3.25 (dd, J = 13.9, 4.9 Hz, 1H), 3.12 (dd, J = 13.8, 9.3 Hz, 1H), 2.29 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ169.90, 161.69, 158.97, 158.29, 155.76, 154.08, 138.75, 137.38, 137.16, 134.84, 133.71, 132.71, 132.26, 130.37, 130.20, 130.05, 129.69, 128.72, 128.63, 128.40, 127.07, 125.67, 123.60, 119.52, 113.15, 112.55, 108.14, 55.53, 37.63, 14.78.

*4.1.102. Synthesis of trans-N-{(S)-1-[4-(3-amino-1H-indazol-6-yl)-5-chloro-1H-imidazole-2-yl]-2-phenylethyl}-4-(aminomethyl) cyclohexanecarboxamide, bis-hydrochloric acid salt (**BMS-724296**)*

Compound **BMS-724296** was synthesized by the reported literature [2],^{1}H NMR (400 MHz, DMSO-d_6) δ 12.70 (s, 1H), 10.57 (s, 1H), 8.39 (d, J = 7.6 Hz, 1H), 8.18–8.04 (m, 2H), 7.70–7.59 (m, 1H), 7.31 (td, J = 6.5, 3.3 Hz, 1H), 7.27–7.09 (m, 4H), 5.28–5.03 (m, 2H), 4.73 (dd, J = 12.7, 9.6 Hz, 1H), 1.88–1.72 (m, 2H), 1.69–1.55 (m, 2H), 1.49–1.30 (m, 1H), 1.24 (s, 2H), 1.06–0.72 (m, 8H). ESI-MS (m/z) = 492.31[M + H] $^+$

4.2. Enzyme affinity assays

FXa, FXIa and substrate of FXIa (*p*-Glu-Pro-Arg-pNa, HCl) were purchased from Haematologic Technologies [43]. The substrates (CH_3SO_2-D-CHA-But-Arg-pNA, AcOH and CH_3OCO-D-CHA-Gly-Arg-pNA, AcOH) of FVIIa and FXa were purchased from Pentapharm (Basel, Switzerland). The substrates (H-D-Phe-Pip-Arg-pNa, 2HCl and D-Pro-Phe-Arg-pNa, 2HCl) of thrombin and plasma kallikrein as well as thrombin were purchased from Hyphen BioMed. FVIIa and plasma kallikrein were purchased from Enzyme Research Laboratories.

According to manufacturer's manual, FXIa was suspended in 0.9% NaCl for a concentration of 31.5 pM (stored at −20°C). Thrombin, FVIIa, FXa, plasma kallikrein and their corresponding substrates were diluted with water to final concentrations of 4NIH/mL, 0.025 mg/mL, 10 nM, 33.3 nM, 4 mM, 4 mM, 4 mM and 0.475 mM, respectively. The substrate of FXIa was dissolved and diluted with 0.9% NaCl to provide the substrate solution (425 μM). The buffers used by these enzymes: thrombin, FVIIa, FXa, FXIa and plasma kallikrein, included a (50 mM Tris and 0.3 M NaCl, pH 8.4); b (0.1 M Tris, 0.1 M NaCl, 5 mM $CaCl_2$ and 0.1%BSA, pH 8.4); c (0.1 M Tris, 0.2 M NaCl and 0.1%BSA, pH 7.4); d (50 mM Tris and 0.3 M NaCl, pH 7.4); e (50 mM Tris-HCl, 0.25 M NaCl and 1.5 μM BSA, pH 7.5). All target compounds and positive control **BMS-724296** were dissolved in DMSO (initial concentration: 10 mM), which were then further diluted with the corresponding buffers to obtain the required concentrations. The test protocols for serine proteases were different, as follows: 1. the buffer (100 μL), FXIa (15 μL) and sample (15 μL) or blank solution (the buffer) were added to 96-well plates (COSTAR 3599) and incubated at 37°C for 5 min. Then, the mixture was added with substrate (30 μL) to the plates, which was

further measured at 405 nm [20,43]; 2. the buffer (70 μL), FVIIa (10 μL) and sample (10 μL) or blank solution (the buffer) were added to 96-well plates (COSTAR 3599) and incubated at 37°C for 10 min. Then, the mixture was added with substrate (10 μL) to the plates, which was further measured at 405 nm [44]; 3. the buffer (70 μL), plasma kallikrein (10 μL) and sample (10 μL) or blank solution (the buffer) were added to 96-well plates (COSTAR 3599) and incubated at 37°C for 15 min. Then, the mixture was added with substrate (10 μL) to the plates, which was further measured at 405 nm [45]; 4. the buffer (35 μL), FXa (5 μL) and sample (5 μL) or blank solution (the buffer) were added to 96-well plates (COSTAR 3599) and incubated at 25°C for 10 min. Then, the mixture was added with substrate (5 μL) to the plates, which was further measured at 405 nm [46]; 5. the buffer (70 μL), thrombin (10 μL) and sample (10 μL) or blank solution (the buffer) were added to 96-well plates (COSTAR 3599) and incubated at 25°C for 10 min. Then, the mixture was added with substrate (10 μL) to the plates, which was further measured at 405 nm [44]. The test compounds that presented binding effects over 50% at a concentration of 30 μM were further tested at a wide range of concentrations to calculate their IC_{50} values using SPSS 20.0 software. Then, The Michaelis–Menten constant, K_m, for substrate hydrolysis was determined by the following relationship: Michaelis–Menten equation,

$$v = \frac{V_{max} S}{K_m + S}$$

where v represents velocity of the reaction, V_{max} is the maximal velocity, S is the final concentration of substrate and K_m is the Michaelis–Menten constant for the substrate [20].

Competitive inhibition was assumed for all proteases. The Ki values were further derived from IC_{50} values by the following relationship [20]:

$$Ki = \frac{IC_{50}}{1 + \frac{[S]}{K_m}}$$

4.3. Molecular modeling

Molecular modeling studies were progressed using GLIDE (2016, Schrödinger Suite) [39]. The crystal structures (PDB code: 4TY7 and 5E2O) of FXIa were retrieved from the RCSB Protein Data Bank, which was further prepared using the Protein Preparation Wizard tool (2016, Schrödinger Suite) [39] by adding all hydrogen atoms as well as the missed side chains of residues and deleting all bound water. The ligands were built within Maestro BUILD (2016, Schrödinger Suite) [39] and prepared by the LIGPREP module (2016, Schrödinger Suite) [39]. The tautomeric forms of ligands, which included the keto and enol forms of ligands, were generated at a physiological pH (7.0 ± 2.0). The crystal structure 4TY7 was selected for molecular model, since its cocrystallized ligand **BMS-724296** was used for comparison in biological evaluation. In addition, the crystal structure 5E2O with cocrystallized ligand FXIa inhibitor **7** was used to display the active pockets of FXIa. The Glide Grid was built using an inner box of dimensions 24 × 24 × 24 $Å^3$ around the centroid of the ligand, assuming that the ligands to be docked were of a size similar to the cocrystallized ligand. This docking methodology has been validated by extracting the crystallographic bound **BMS-724296** and redocking it with the Glide module using extra precision (XP) [39]. Different docking poses of ligands were generated and analyzed by Accelrys Discovery Studio Visualizer 2017 [47] for interpretation of the final results. Accelrys Discovery Studio Visualizer 2017 and Pymol [48] were used for graphic display.

4.4. Steady-state kinetic analysis

An enzyme kinetics study of the most potent compound **44g** was carried out to further evaluate and identify the type of inhibition, and enzyme kinetic assays were performed in the same way as the *in vitro* FXIa inhibitory activity but with varying concentrations of the substrate at 212.5, 425, 850 and 1700 μM (final concentrations of the substrate were 42.5, 85, 170 and 340 μM, respectively). The Lineweaver-Burk plot was established from which we could calculate the K_m and V_{max} of the inhibitor [17,41].

4.5. In vivo efficacy in the rabbit arteriovenous shunt model

The AV shunt model was initiated according to the previously described method with minor modifications [2,21]. Male New Zealand White rabbits (2–2.5 kg) were obtained from the Animal Center of Shenyang Pharmaceutical University (Shenyang, China). Animal maintenance and treatment met the protocols approved by the Ethics Review Committee for Animal Experimentation of Shenyang Pharmaceutical University. The rabbits had free access to food and water and were maintained on a 12-h light/dark cycle in a temperature- and humidity-controlled room for one week.

Briefly, male New Zealand White rabbits were anesthetized urethane (1.25 g/kg, i. v.) and then placed on a heating pad to maintain body temperature. Then, their femoral artery, jugular vein and femoral vein were catheterized to an AV-shunt device through a PE60 cannula, and significant thrombosis formation was induced through contact with a 4.8 cm long 2-0 silk thread. After 40 min, the AV-shunt was disconnected from the arterial and venous catheters, and a mixture of silk thread and thrombosis were removed and weighed. The wet weight of thrombosis was obtained by subtraction, which was used as the control [2,21]. Subsequently, after the compound or saline vehicle was given as a bolus intravenously followed by a continuous IV infusion via the jugular vein, the thrombosis formation was induced by a new AV-shunt for 40 min immediately, and the measurement method was as described above. Data were reported as % inhibition of thrombosis formation in the treatment period versus in control period. Compound **44g** was tested at three doses: 0.5 mg/kg + 0.8 mg kg^{-1} h^{-1}, 2.5 mg/kg + 4.0 mg kg^{-1} h^{-1}, 5.0 mg/kg + 8.0 mg $kg^{-1}\cdot$ h^{-1}using a dosing vehicle of saline, and **BMS-724296** was used as a positive control with a dose of 0.5 mg/kg + 0.8 mg $kg^{-1}\cdot$ h^{-1}. The saline vehicle did not significantly inhibit the AV-shunt-induced thrombosis formation. A total of three rabbits were used for each group. The ED_{50} represented the dose that produced 50% inhibition of thrombus formation and was estimated by linear regression.

Declaration of competing interest

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

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Appendix ASupplementary data

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