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Pyrrolopyrimidine-inhibitors with hydantoin moiety as spacer can explore P4/S4 interaction on plasmin

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ABSTRACT

In the development of plasmin inhibitors, a novel chemotype, pyrrolopyrimidine scaffold possessing two motifs, a hydantoin-containing P4 moiety and a warhead-containing P1 moiety, is uncovered. A unique feature of the new line of the plasmin inhibitors is that the interaction between the plasmin inhibitors and key subsites in plasmin can be controlled by a spacer like hydantoin. The application of the novel chemotype is demonstrated by **1n** and provides further evidence on the importance of hydantoin as the spacer.

1. Introduction

Plasminogen is the main component of the fibrinolytic system and can be converted to the active enzyme, plasmin, by distinct plasminogen activators, which leads to fibrinolysis. Plasmin directly degrades extracellular matrix components including the glycoproteins fibronectin, laminin, and elastin, as well as proteoglycans.[1] Failure of the extracellular matrix is crucial for cancer invasion and metastasis. It is accomplished by the concerted action of several proteases, including the serine protease plasmin and a number of matrix metalloproteases (MMPs). The MMPs and plasminogen activator-plasmin systems have also been implicated in experimental vascular tumor formation.[2] The activity of each of these proteases is regulated by an array of activators, inhibitors and cellular receptors,[3] resulting in serious diseases that arise from an imbalance in physiological substances. Thus, antiproteolysis, which is performed by plasmin inhibitors, has become a key target in therapeutic strategies aimed at inhibiting tumor growth.[4] Additionally, plasmin inhibitors, which have a different mode-of-action from prior anti-cancer drugs, have become regarded as a potential therapy for the treatment of cancer.[4] However, the necessary proof-of-concept validation of the therapeutic usefulness of plasmin inhibitors has not been provided. This would be due to the lack of bioavailable and low-molecular weight plasmin inhibitors.

The number of reports on plasmin inhibitors has recently increased. The peptidic aldehyde has been recently utilized as an irreversible warhead of plasmin inhibitor consisting of a tetrapeptide.[5] The S3 pocket of plasmin is electropositive due to the guanidino group, Arg719, which donates a hydrogen bond to a P3 methionine residue of the peptidic aldehyde inhibitors. These observations for the P3/S3 interaction were in agreement with a previous report published by Backes et al.[6] As distinct types of plasmin inhibitors were developed, it has been noted that a macrolide structure of peptidomimetic plasmin inhibitors interacts with the S2 and S3 binding pockets of plasmin,[7] however, they were not sufficiently selective to avoid activity toward plasma kallikrein (PK) which shares a high degree of sequence homology with plasmin. Our efforts in this area have recently focused on the optimization of peptidic and non-peptidic plasmin inhibitors having the nitrile warhead in Lys (P1 moiety) since P1/S1 interaction primarily

Abbreviations: MMP, matrix metalloproteinase; PK, plasma kallikreine; UK, urokinase; DABCO, 1,4-diazabicyclo[2.2.2]octane; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DKP, diketopiperazine; <Glu, pyroglutamic acid.

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contributes to its affinity for plasmin.[8,9] New non-peptidic P2 and P3 moieties enhanced the inhibitory potency toward plasmin with the selectivity over PK and urokinase (UK).[8] Through these studies, we are able to acquire more information on P2/S2 and P3/S3 interactions from the investigation on inhibitors using a substrate library, while the plasmin inhibitors containing P4 moiety have not been studied in this context other than recent aldehyde-containing inhibitors.[5] Since a few information on P4/S4 interaction between plasmin and molecule and on how the interaction affects the potency of plasmin inhibition and the selectivity against PK and UK are available,[5,6] we sought to exploit structure–activity relationships (SAR) developed from studies using P4-containing inhibitors.

With regard to the novel type of cysteine proteases (cathepsins K and S) inhibitors, heterocyclic core structures like purine and pyrimidine were utilized as the scaffold in which the cathepsin K and S inhibitors possessed a nitrile warhead for the P2 and P3 moieties.[10–13] The structural features of the core structure gave us a clue to the discovery of the novel chemotype for plasmin inhibitors unlike previously published plasmin inhibitors. Since the heterocyclic structure of pyrrolopyrimidine[10–13] has three potential attachment sites, we considered it as the prime scaffold for plasmin inhibitors in order to exploit the SAR toward P4/S4 interaction (Fig. 1). Therefore, our strategy toward the SAR studies on P4/S4 interaction for plasmin inhibitors included the assessment of pyrrolopyrimidine as the scaffold that would allow the attachment of P1 and P4 substituents.

2. Chemistry

The synthesis of plasmin inhibitors **1a**–**1t** containing pyrrolopyrimidine as the scaffold is illustrated in Scheme 1. The synthetic approach allowed us to access compounds with structural diversity by using an almost unlimited choice of commercially available, **2a** or synthetic hydantoin derivatives, **2b**,[14] **2c**,[14] **2d**–**2f**, **2h**, **2i**, **2k**, **2l** and **2n**–**2t**. Condensation of 1-methylhydantoin (**2a**) with **3** was performed by using sodium hydride to yield **4a**. Other hydantoin analogues, **2b**–**2f**, **2h**, **2i**, **2k**, **2l** and **2n**–**2t**, were coupled with the common intermediate **3** in DMF in the presence of K_2CO_3 to afford **4b**–**4f**, **4h**, **4i**, **4k**, **4l** and **4n**–**4t**. Regardless of the structure of R (in the case of **4f**, **4h**, **4i**, **4k** and **4q**–**4t**) in Scheme 1, the hydrolysis of methylester and hydantoin were detected simultaneously by hydrolysis with $LiOH{\cdot}H_2O$. However, the product immediately rearranged to form the hydantoin moiety by using 1,1′-carbodiimidazole (CDI). In contrast, the methylesters of **4a**–**4e**, **4l** and **4n**–**4p** were readily hydrolyzed and their hydantoins were intact in the same way for **4f**, **4h**, **4i**, **4k** and **4q**–**4t** shown above. The hydrolyzed **4a**–**4f**, **4h**, **4i**, **4k**, **4l**, **4n**–**4t** were coupled with **5** to give the corresponding **6a**–**6f**, **6h**, **6i**, **6k**, **6l** and **6n**–**6t**, respectively. Treatment of **6f**, **6i** and **6l** with trifluoroacetic anhydride and triethylamine[9] yielded the corresponding **6g**, **6j** and **6m**. Removal of Fmoc group of **6a**–**6t** with 20% piperidine in DMF provided the desired products **1a**–**t**, respectively.

The derivatives having imidazolidinone (**1u** and **1v**) or diketopiperazine (DKP) (**1w**) as a spacer were prepared in the same way as for **1a** as shown in Scheme 2.

Commercially available or synthetic aniline derivatives were alkylated by ethyl bromoacetae in the presence of K_2CO_3 or diisopropyl ethylamine and subsequently the N-alkylated aniline derivatives were treated with sodium cyanate in acetic acid[15] to yield hydantoin derivatives **2d**–**2f**, **2h**, **2i**, **2k**, **2l** and **2n**–**2t**. Commercially available aniline derivatives were alkylated by 2-chloroethyl isocyanate[16] and formation of the imidazolidinone ring was carried out by using sodium hydride to give **2u** and **2v**. The building block with DKP (**2w**) was prepared based on the reference.[17] Experimental procedures for the synthesis and characterization (^{1}H NMR) of **2d**–**2f**, **2h**, **2i**, **2k**, **2l** and **2n**–**2w** are available in Supplementary data.

We improved the method[10,12,15] for preparing the building block, methyl 6-(bromomethyl)-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (**3**), that suits our goals depicted as in Scheme 3. Regioselective substitution of the chloride at the 4-position of 5-bromo-2,4-dichloropyrimidine gave the intermediate **3a**. The second chloride was exchanged with cyanide to give the pyrimidine **3b**. The nitrile group of **3b** was converted to methyl ester (**3c**) using 5–10% HCl/MeOH. Bromide **3c** was coupled with 2-prop-2-ynyloxy-tetrahydro-pyran[18] by using $Pd(PPh_3)_2Cl_2$ and CuI as catalysts to yield **3d**. Formation of the pyrrolopyrimidine ring (**3e**) was carried out by using DBU, followed by removal of tetrahydropyran using 6 M HCl and MeOH to give **3f**. Alcohol **3f** was converted to the bromide **3**.

Another building block with nitrile moiety as the warhead were prepared from the starting material, H-Lys(Fmoc)-NH_2 as shown in Scheme 4. The N-terminal group was protected by trityl group (**5a**) and subsequent treatment with trifluoroacetic anhydride and triethylamine[9] yielded **5b**. The acid labile group of **5b** was removed to give the building block **5** as the P1 moiety.

3. Results and discussion

The strategy that we pursued, schematically outlined in Figure 1, was to find a heterocyclic scaffold which can combine the Lys-nitrile as the P1-warhead and additionally extend the P2, P3 or P4 group. We first focused on analyzing synthetically feasible heteroaromatic compounds combined with Lys-nitrile by using computer-assisted molecular modeling to see how they are tolerated in an active site of plasmin. Molecular modeling of pyrropyrimidine-Lys-nitrile (**7**) into the active site of plasmin (Fig. 2) suggested that pyrropyrimidine at the α amino group of Lys would be directed toward non-prime sites other than the S1 pocket. Additionally, the 6-position of pyrrolopyrimidine could be used to introduce the

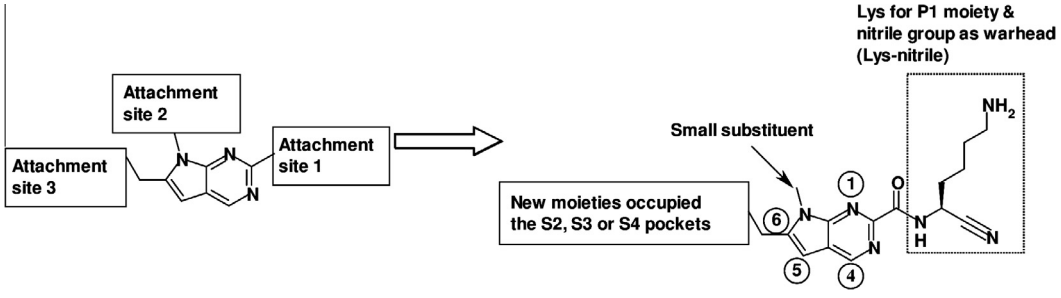
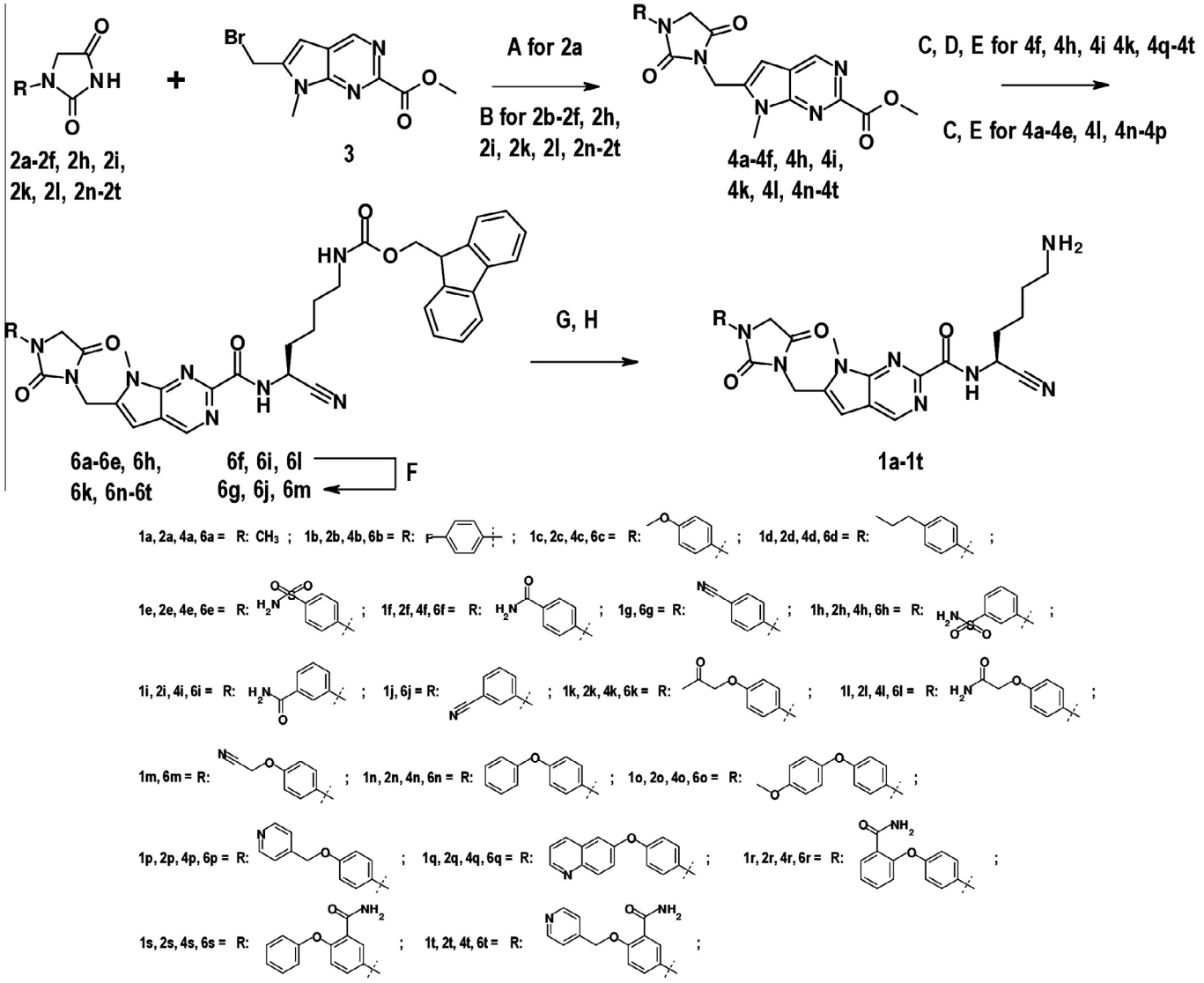


Figure 1. Schematic outline for developing new chemotype of plasmin inhibitors.

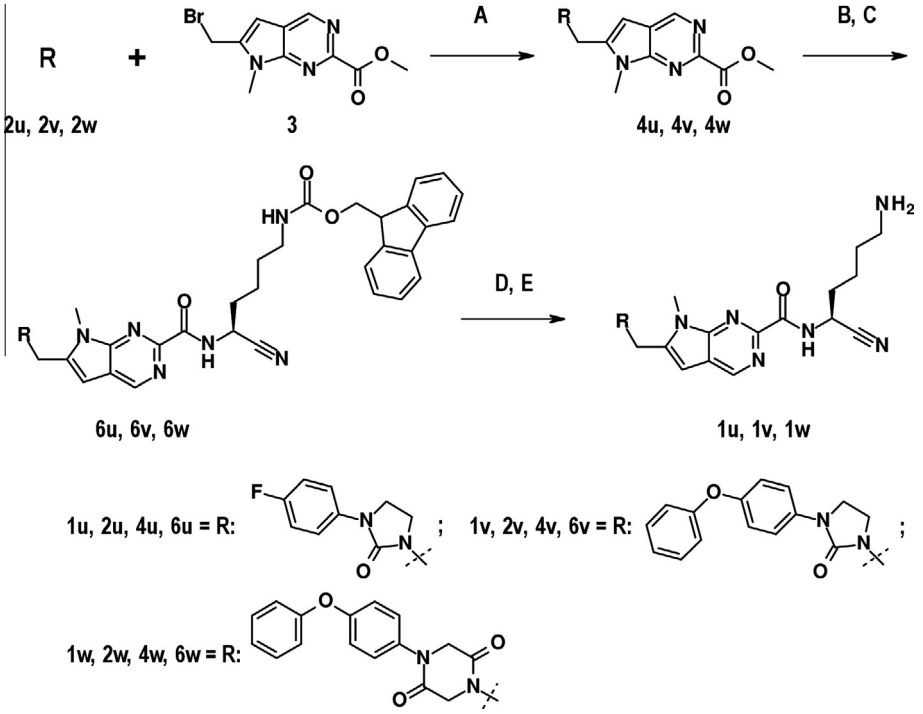


Scheme 1. Reagents and conditions: (A) 60% NaH, DMF, rt, 15 h; (B) K_2CO_3, DMF, rt, 5 h; (C) LiOH, H_2O, THF/MeOH/H_2O, rt, 3–15 h; (D) CDI, DMF, rt, 15 h; (E) H-Lys(Fmoc)-CN(5), HOAt, WSCI·HCl, DMF, rt, 15 h; (F) $(CF_3CO)_2O$, Et_3N, THF/DMF, 0 °C, 5–10 min; (G) 20% Piperidine/DMF, rt, 40 min; (H) Preparative HPLC.

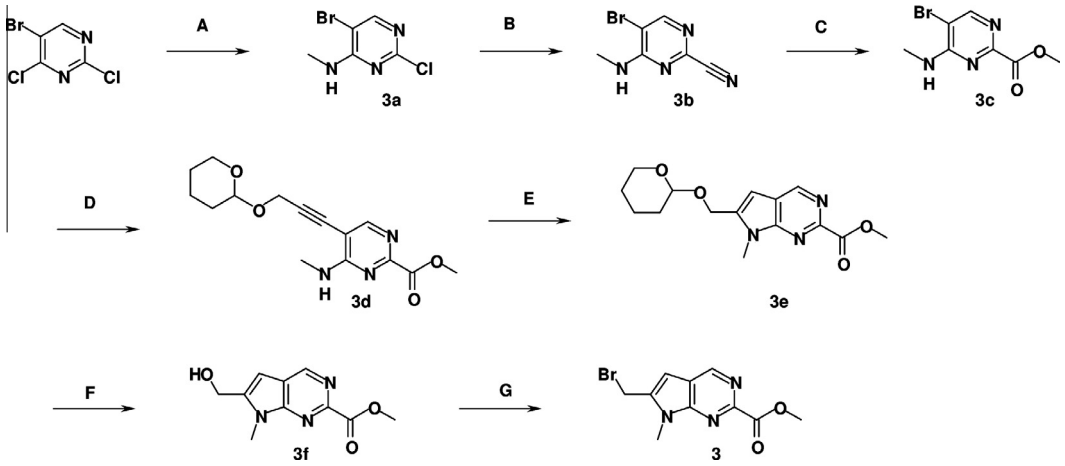
substituents, which should extend into the non-prime subsites, suggesting an application of pyrrolopyrimidine as the scaffold.

As a simple entry onto the pyrrolopyrimidine scaffold, we designed **1a** with hydantoin, which would enable to the introduction of a wide range of substituents at 1-position of hydantoin. Pyrrolopyrimidine-based inhibitor **1a** exhibited no inhibitory activity against plasmin (Table 1) and molecular modeling of **1a** (pink) suggested that 1-methylhydantoin moiety on pyrrolopyrimidine directed toward the S2 and S3 pockets (Fig. 3). Replacement of methyl with 4-fluorobenzene (**1b**) showed an increased potency against plasmin. The binding mode of **1b** (green) complexed with plasmin was shown to be different from **1a** (pink) as depicted in Figure 3. 4-Fluorobenzene linearly extended from hydantoin pointed toward the S4 pocket unlike the 1-methylhydantoin moiety in **1a** but did not reach so deep into the S4 subsite. Indeed, replacement of 4-fluorobenzene with 4-fluorobenzyl that enhanced free rotation fully lost the inhibitory activity against plasmin (data not shown). Compound **1c** inhibited plasmin at a similar level to that of **1b** while having the selectivity against PK (**1b** vs **1c**). A similar effect of the introduction of the aromatic moiety on the potency and selectivity was also found in another derivative, **1d**. As indicated in Figure 3, two Arg residues (Arg719 and Arg767) are located close to the S4 subsite and their positive charge could make a favorable electrostatic interaction with the functional group in the P4 interacting region of **1e**–**1j** (Table 2). Compound **1i** with the amide substituents at the *meta*-position of the aromatic ring marginally increased their potency for plasmin compared to **1b**–**1d** yet retained the selectivity against PK and UK, but other compounds **1e**–**1h** and **1j** caused no substantial changes in the potency against a targeted enzyme. Extension by one methylene and oxygen groups onto the P4 moiety as in **1k**–**1l** lost their inhibitory activity against plasmin (Table 2). Conversion of amide (**1l**) to nitrile (**1m**) resulted in the retention of potency to a similar level to that of **1b**.

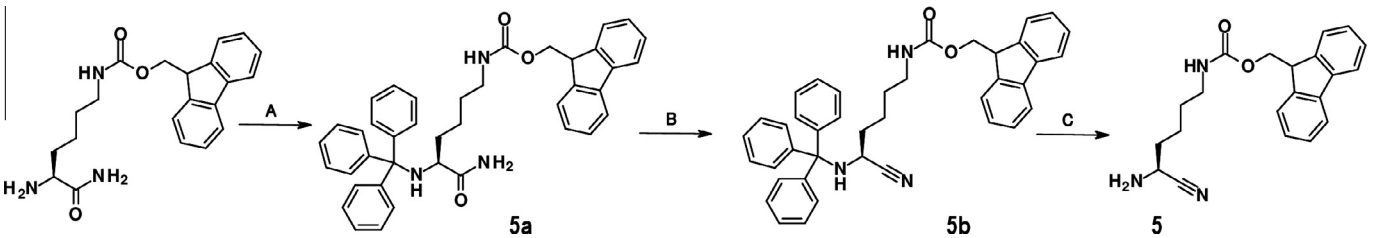
In order to pursue the size of the S4 subsite, we focused on substituents at the 4-position of benzene extended from hydantoin. In this context, **1n**–**1q** were designed to elucidate whether the appropriate residue at the 4-position of the benzene was well tolerated in the S4 subsite of plasmin as listed in Table 3. Inhibitors **1n** and **1o** displayed potent plasmin inhibition while having a significant selectivity for plasmin over PK and UK. Molecular modeling of **1n** (yellow) suggested that an aromatic ring occupied the S4 pocket in plasmin in Figure 4a. Replacement of the aromatic ring with a picolyl group (**1p**) marginally decreased the potency for plasmin



Scheme 2. Reagents and conditions: (A) 60% NaH, DMF, rt, 15 h; (B) LiOH·H_2O, THF/MeOH/H_2O, rt, 3–15 h; (C) H-Lys(Fmoc)-CN(5), HOAt, WSCI·HCl, DMF, rt, 15 h; (D) 20% Piperidine/DMF, rt, 40 min; (E) Preparative HPLC.



Scheme 3. Reagents and conditions: (A) Methylamine, MeOH, 0 °C, 20 min → rt, 3 h; (B) NaCN, DABCO, DMSO, 60 °C, 2 h; (C) 5–10% HCl/MeOH; (D) 2-Prop-2-ynyloxy-tetrahydro-pyran, Et_3N, CuI, $(Ph_3P)_2PdCl_2$, DMF, 80 °C, 4 h; (E) DBU, DMF, 100 °C, 2 h; (F) 6 M HCl, MeOH, rt, 3 h; (G) CBr_4, Ph_3P, CH_2Cl_2, 0 °C, 0.5 h → rt, 15 h.



Scheme 4. Reagents and conditions: (A) Tr-Cl, Et_3N, CH_2Cl_2, rt, 4 h; (B) $(CF_3CO)_2O$, Et_3N, THF, 0 °C, 5 min; (C) CH_3COOH, Trifluoroethanol, CH_2Cl_2, rt, 4 h.

compared to **1n** but caused no substantial change in the selectivity against untargeted enzymes. The introduction of quinoline (**1q**) resulted in the loss of the inhibitory activity against plasmin. The amide moiety was introduced as H-acceptor on the P4 moiety of **1n** and **1p** (**1r**–**1t** in Table 3). Inhibitors **1r** having the amide group on the external aromatic ring inhibited plasmin nearly equipotent

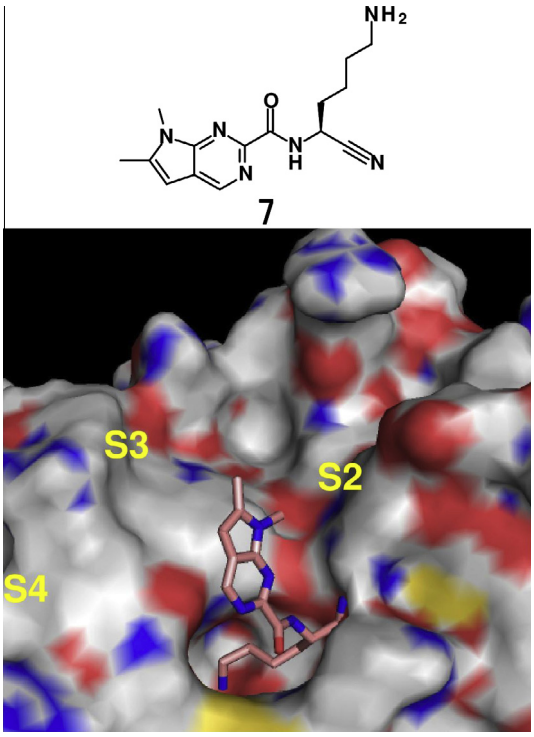
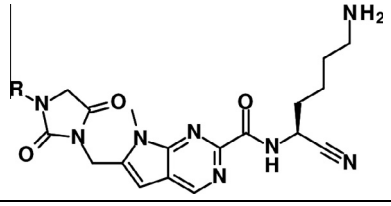
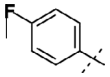
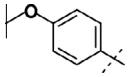
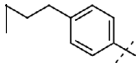


Figure 2. Pyrrolopyrimidine core structure **7** (pink) docked into the plasmin active site.

Table 1
Inhibition of plasmin, plasma kallikrein and urokinase by **1a**–**1d**



Compounds	R	IC_{50} (μM)		
		Plasmin	Plasma kallikrein	Urokinase
1a	CH_3	>1000	>1000	>1000
1b		130	740	>1000
1c		180	>1000	>1000
1d		330	>1000	>1000

to **1p**, but **1s** and **1t** showed a decreased trend for potency against plasmin. Molecular modeling of **1n** and **1s** implied that the P4 moieties of both compounds were directed toward the S4 pocket of plasmin (see Supplementary data). However, a detailed breakdown showed that the external aromatic rings of both compounds were pointed in the different direction and the internal aromatic ring of **1s** was slightly displaced from the surface of the S4 subsite compared to that of **1n**. The results on the P4 moieties suggest that the S4 pocket in plasmin possesses a deep binding site but a fused aromatic ring is not acceptable to the pocket (**1n**, **1o**, **1p** vs **1q**).

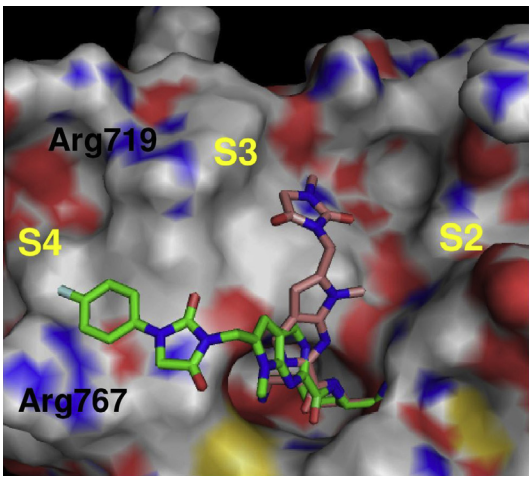
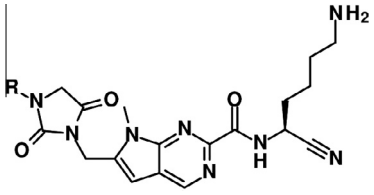


Figure 3. Compounds **1a** (pink) and **1b** (green) docked into the plasmin active site.

Table 2
Inhibition of plasmin, plasma kallikrein and urokinase by **1b** and **1e**–**1m**



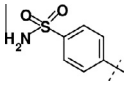
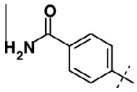
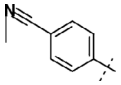
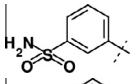
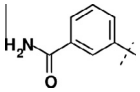
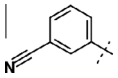
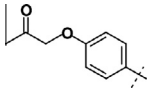
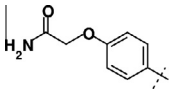
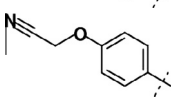
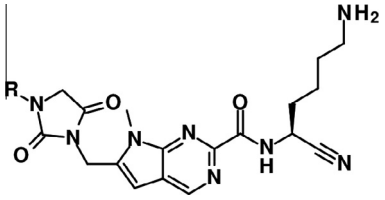
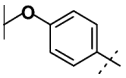
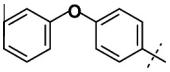
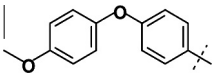
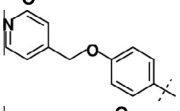
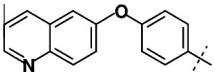
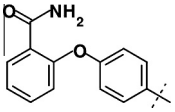
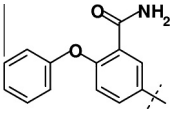
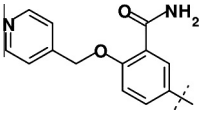
Compounds	R	IC_{50} (μM)		
		Plasmin	Plasma kallikrein	Urokinase
1b		130	740	>1000
1e		260	>1000	>400
1f		160	>1000	>1000
1g		210	>1000	>1000
1h		220	>1000	>1000
1i		100	>1000	>1000
1j		220	>1000	>1000
1k		250	>1000	>1000
1l		400	>1000	>1000
1m		130	>1000	>1000

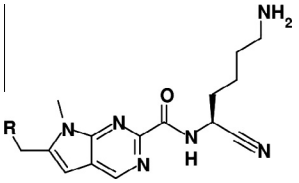
Table 3
Inhibition of plasmin, plasma kallikrein and urokinase by **1c** and **1n–1t**

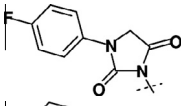
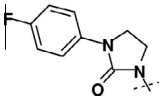
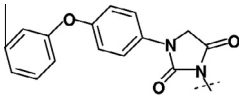
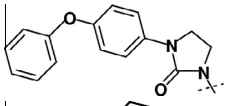
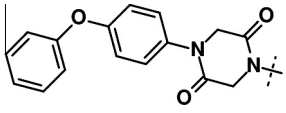


Compounds	R	IC_{50} (μM)		
		Plasmin	Plasma kallikrein	Urokinase
1c		180	>1000	>1000
1n		51	>1000	>400
1o		64	>1000	>400
1p		87	>1000	>1000
1q		>250	>1000	>400
1r		99	>1000	>1000
1s		510	>1000	>1000
1t		170	>1000	>1000

Given the results described above, it seems that the preferences in the S4 subsite have no distinctive feature. However, Swedberg et al. has disclosed that the S4 pocket tends to prefer a Lys residue in the peptidic aldehyde inhibitors.[5] On the basis of their report, alternative P4 moieties combined with pyrrolopyrimidine scaffold will be covered in future publications.

Table 4
Inhibition of plasmin, plasma kallikrein and urokinase by **1b**, **1n** and **1u–1w**



Compounds	R	IC_{50} (μM)		
		Plasmin	Plasma kallikrein	Urokinase
1b		130	740	>1000
1u		510	>1000	>1000
1n		51	>1000	>400
1v		>250	>1000	>200
1w		960	>1000	>1000

Derivatives with imidazolidinone (**1u** and **1v**) or DKP (**1w**) as the spacer were prepared to determine how the binding mode and the inhibitory activity against plasmin change by the type of spacer employed. Compounds **1u** and **1v**, which differ from **1b** and **1n** only by the replacement of carbonyl with methylene, had decreased potency against plasmin, respectively, as listed in Table 4. Molecular modeling supported that 4-fluorobenzene in **1u** (pink) occupied the S2 and S3 pockets (Fig. 4b) unlike that in **1b** (sky blue). Likewise, as depicted in Figure 4a, phenoxybenzene combined with hydantoin in **1n** (yellow, IC_{50} = 51 μM for plasmin) pointed toward the S4 subsite whereas those in **1v** (sky blue, IC_{50} >250 μM for plasmin) and **1w** (blue, IC_{50} = 960 μM for plasmin) directed toward other pockets, S2 and S3, suggesting that **1v** and **1w**

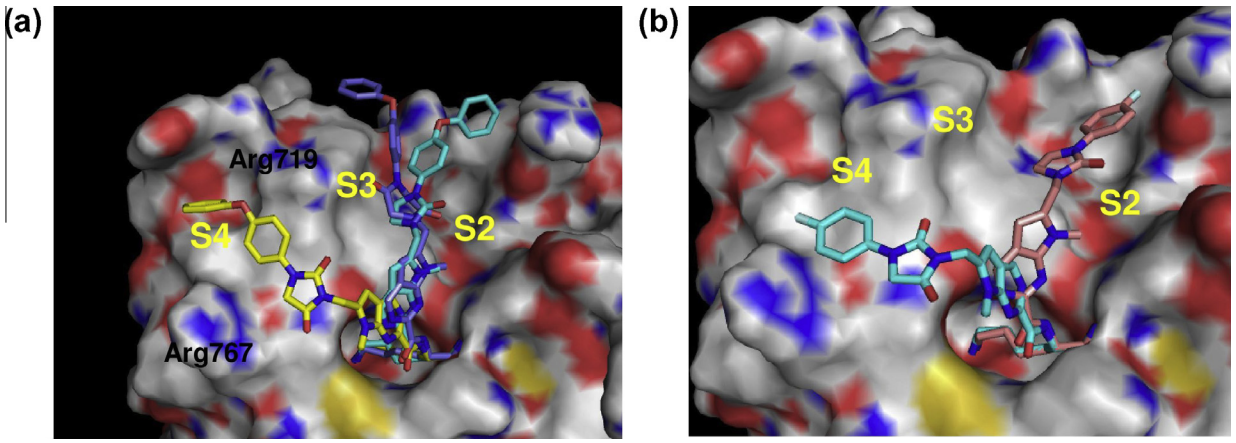


Figure 4. (a) Compounds **1n** (yellow), **1v** (sky blue) and **1w** (blue) docked into the plasmin active site. (b) Compounds **1b** (sky blue) and **1u** (pink) docked into the plasmin active site.

did not much interact with them. The results reveal that phenoxybenzene in **1n** is accepted by the S4 subsite in plasmin because hydantoin directs phenoxybenzene to interact with the S4 pocket; however, imidazolidinone and DKP in **1v** and **1w** do not allow for the interaction between phenoxybenzene and the S4 subsite.

Appropriate moieties extending from the pyrrolopyrimidine scaffold engaged with S4 and S1 pockets in the active site of plasmin when having hydantoin as the spacer. The application of pyrrolopyrimidine scaffold as the novel plasmin inhibitors has been demonstrated by the derivatives represented by **1n**.

4. Conclusion

In the course of our exploration of the SAR studies on the P4/S4 interaction, we designed, synthesized and evaluated a new series of plasmin inhibitors that have two motifs, the P1 and P4 moieties, arranged in the prrrolopyrimidine scaffold. The representative inhibitor **1n** of this series revealed the application of pyrrolopyrimidine scaffold as the novel plasmin inhibitors. With respect to the selectivity, it turned out that the structure of this series was not totally tolerated for PK and UK. In addition, the interaction between plasmin inhibitors and the key pockets of plasmin could be controlled by the type of modified heterocyclic moiety extending away from the pyrrolopyrimidine (Fig. 4a and b). In this way, a novel structural class of plasmin inhibitors may provide a starting point for the design of the potent inhibitors having S4/P4 interaction. We anticipate that the SAR developed from our studies of the new chemotypes described in our report will lead to further discoveries of novel inhibitors for plasmin as well as other serine proteases.

5. Experimental

5.1. Materials and methods

All chemicals were purchased from Tokyo Chemical Industry Co., Ltd or Wako Pure Chemical Industries, Ltd and used without further purification. All protected amino acids and the coupling reagents were purchased from Watanabe Chemical Industries, Ltd. ^{1}H NMR experiments were recorded on a JMTC-600 (JEOL Ltd) 600 NMR spectrometer with $CDCl_3$, DMSO-d_6 or D_2O as solvents. Chemical shifts are expressed in parts per million (ppm, δ) and referred to the solvent signal. HRMS spectra were recorded on The AccuTOF (JMS-T100LC) equipped with an electrospray ion source (JEOL Ltd). The preparative HPLC system consisted of a SHINADZU CBM-20A System Controller and a SHINADZU Pump Unit LC-20AT, a SHIMADZU In-Line Degasser DGU-20A$_{3R}$, a SHIMADZU SPD-20A Absorbance Detector, a SHIMADZU FCV-11AL Valve Unit and a SHIMADZU FRC-10A Fraction Collector (SHIMADZU Corporation). The absorbance detector was operated at 254 nm. The mobile phase for preparation and analysis was a combinations of water (A) and acetonitrile (B), both containing 0.1% TFA, and the flow rate was 8 and 1 ml/min, respectively. TSK gel ODS columns (21.5 × 300 mm for preparation and 4.6 × 150 mm for analysis, TOSOH Corporation) were used.

5.1.1. 5-Bromo-2-chloro-*N*-methyl-pyrimidin-4-amine (3a)

Methylamine (9.8 M MeOH solution) (8.9 ml, 87.7 mmol) was added dropwise at 0 °C over 20 min to a solution of 5-bromo-2,4-dichloropyrimidine (10 g, 43.9 mmol) in MeOH (150 ml). After stirring for 20 min at 0 °C, the mixture was warmed to room temperature, stirred for 3 h, and evaporated down. The residue was suspended in EtOAc, washed with satd $NaHCO_3$ and brine, dried with $MgSO_4$, and evaporated down. The residue was chromatographed on silica gel column to give 10 g of **3a** in quantitative yield. R_f = 0.15 (*n*-hexane/EtOAc = 5:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.12 (s, 1H), 5.58 (br s, 1H), 3.10 (d, J = 4.8 Hz, 3H).

5.1.2. 5-Bromo-4-methylamino-pyrimidine-2-carbonitrile (3b)

At room temperature, to a solution of NaCN (3.3 g, 67.3 mmol) in H_2O (10 ml) was added successively DMSO (30 ml), DABCO (1.2 g, 11.2 mmol), and a solution of **3a** (10 g, 44.9 mmol) in DMSO (50 ml). The mixture was stirred for 2 h at 60 °C, poured into an ice water, and extracted with EtOAc. The combined extracts were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give 7.0 g of **3b** in 74% yield. R_f = 0.18 (*n*-hexane/EtOAc = 3:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.31 (s, 1H), 5.69 (br s, 1H), 3.11 (d, J = 4.8 Hz, 3H).

5.1.3. Methyl 5-bromo-4-methylamino-pyrimidine-2-carboxylate (3c)

To **3b** (7 g, 32.8 mmol) was added hydrochloric acid (5–10% methanol solution, 70 ml) and the mixture was stirred for 15 h at room temperature. The solvent was evaporated down and the residue was extracted with EtOAc. The combined extracts were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give 6.4 g of **3c** in 80% yield. R_f = 0.15 (*n*-hexane/EtOAc = 1:2). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.40 (s, 1H), 5.61 (br s, 1H), 4.00 (s, 3H), 3.18 (d, J = 4.8 Hz, 3H).

5.1.4. Methyl 4-methylamino-5-(3-tetrahydropyran-2-yloxyprop-1-ynyl)pyrimidine -2-carboxylate (3d)

At room temperature, a solution of **3c** (6.4 g, 26.2 mmol) and 2-prop-2-ynyloxy-tetrahydro-pyran[17] (5.5 g, 39.3 mmol) in DMF (65 ml) was treated with triethylamine (11 ml, 78.5 mmol), CuI (0.5 g, 2.6 mmol) and $(Ph_3P)_2PdCl_2$ (0.9 g, 1.3 mmol). The mixture was stirred for 4 h at 80 °C, poured into an ice water, and extracted with EtOAc. The combined extracts were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give 6.7 g of **3d** in 84% yield. R_f = 0.48 (*n*-hexane/EtOAc = 1:3). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.34 (s, 1H), 5.94 (br s, 1H), 4.87–4.86 (m, 1H), 4.55 (dd, J = 16.2 Hz, 7.2 Hz, 2H), 4.00 (s, 3H), 3.92–3.88 (m, 1H), 3.59–3.57 (m, 1H), 3.16 (d, J = 4.8 Hz, 3H), 1.86–1.77 (m, 3H), 1.69–1.58 (m, 3H).

5.1.5. Methyl 7-methyl-6-(tetrahydropyran-2-yloxymethyl)pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (3e)

At room temperature, a solution of **3d** (6.74 g, 22 mmol) in DMF (45 ml) was treated with DBU (1.1 ml, 7.5 mmol). The mixture was stirred for 2 h at 100 °C, poured into an ice water, and extracted with EtOAc. The combined extracts were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give 5.1 g of **3e** in 76% yield. R_f = 0.12 (*n*-hexane/EtOAc = 3:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.05 (s, 1H), 6.65 (s, 1H), 4.96 (d, J = 13.2 Hz, 1H), 4.74–4.72 (m, 2H), 4.09 (s, 3H), 3.99 (s, 3H), 3.91–3.87 (m, 1H), 3.60–3.57 (m, 1H), 1.84–1.83 (m, 1H), 1.76–1.72 (m, 1H), 1.64–1.57 (m, 4H).

5.1.6. Methyl 6-(hydroxymethyl)-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (3f)

At room temperature, a solution of **3e** (4.0 g, 13.1 mmol) in MeOH (120 ml) was treated with 6 M HCl (4.4 ml, 26.2 mmol), stirred for 3 h at room temperature, treated with $NaHCO_3$ (2.2 g, 26.2 mmol). The solvents were concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give 1.6 g of desired **3f** in 55% yield. R_f = 0.60 (CH_2Cl_2/MeOH = 9:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.03 (s, 1H), 6.60 (s,

1H), 4.96 (d, J = 5.4 Hz, 2H), 4.09 (s, 3H), 4.01 (s, 3H), 1.97–1.96 (m, 1H).

5.1.7. Methyl 6-(bromomethyl)-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (3)

A solution of CBr_4 (4.8 g, 14.5 mmol) in CH_2Cl_2 (10 ml) was added dropwise over 15 min to a solution of **3f** (1.6 g, 7.23 mmol) and Ph_3P (1.9 g, 7.23 mmol) in CH_2Cl_2 (50 ml) at 0 °C. After stirring for 30 min at 0 °C, the mixture was warmed to room temperature, stirred for 3 h. The mixture was diluted with CH_2Cl_2, washed with satd $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give 1.7 g of **3** in 90% yield. R_f = 0.55 (EtOAc). ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.07 (s, 1H), 6.71 (s, 1H), 4.68 (s, 2H), 4.09 (s, 3H), 4.02 (s, 3H).

5.1.8. Methyl 7-methyl-6-[(3-methyl-2,5-dioxo-imidazolidin-1-yl)methyl]pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4a)

To a solution of 1-methylhydantoin (80 mg, 0.70 mmol) in DMF (3 ml), 60% NaH (30 mg, 0.84 mmol) was added at 0 °C and the mixture was stirred at room temperature for 30 min. Compound **3** (100 mg, 0.35 mmol) in DMF (2 ml) was added at 0 °C and the reaction mixture was stirred for 15 h at ambient temperature. The reaction mixture was quenched with satd NH_4Cl and extracted with EtOAc. The combined extracts were washed with brine, dried over $MgSO_4$ and evaporated down. The residue was purified by silica gel column chromatography to give 67 mg of **4a** in 30% yield. R_f = 0.25 (EtOAc/MeOH = 95:5). ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.03 (s, 1H), 6.74 (s, 1H), 4.91 (s, 2H), 4.08 (s, 3H), 4.05 (s, 3H), 3.94 (s, 2H), 3.03 (s, 3H).

5.1.9. Methyl 6-[[3-(4-fluorophenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4b)

To a solution of **2b**[14] (68 mg, 0.35 mmol) in DMF (10 ml), K_2CO_3 (150 mg, 1.1 mmol) and **3** (100 mg, 0.35 mmol) were added at ambient temperature. The reaction mixture was stirred for 15 h at ambient temperature. The reaction solvent was evaporated and the residue was extracted with EtOAc. The combined extracts were washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give 90 mg of **4b** in 65% yield. R_f = 0.48 (EtOAc), ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.08 (s, 1H), 7.70–7.68 (m, 2H), 7.29 (t, J = 9.0 Hz, 2H), 6.79 (s, 1H), 5.01 (s, 2H), 4.61 (s, 2H), 3.94 (s, 3H), 3.92 (s, 3H).

5.1.10. Methyl 6-[[3-(4-methoxyphenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4c)

Compound **4c** was prepared from **2c**[14] and **3** in a manner similar to that described for **4b** in 74% yield. R_f = 0.15 (EtOAc). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.08 (s, 1H), 7.65–7.62 (m, 2H), 6.99 (d, J = 9.0 Hz, 2H), 6.78 (s, 1H), 5.00 (s, 2H), 4.58 (s, 2H), 3.94 (s, 3H), 3.92 (s, 3H), 3.76 (s, 3H).

5.1.11. Methyl 6-[[2,5-dioxo-3-(4-propylphenyl)imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4d)

Compound **4d** was prepared in a manner similar to that described for **4b** in 81% yield (containing impurity). R_f = 0.72 (EtOAc).

5.1.12. Methyl 6-[[2,5-dioxo-3-(4-sulfamoylphenyl)imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4e)

Compound **4e** was prepared in a manner similar to that described for **4b** in 29% yield. R_f = 0.55 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.09 (s, 1H), 7.85 (s, 4H), 7.35 (s, 2H), 6.82 (s, 1H), 5.03 (s, 2H), 4.65 (s, 2H), 3.94 (s, 3H), 3.92 (s, 3H).

5.1.13. Methyl 6-[[3-(4-carbamoylphenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4f)

Compound **4f** was prepared in a manner similar to that described for **4b** in 81% yield. R_f = 0.74 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.08 (s, 1H), 7.97 (br s, 1H), 7.94 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 8.4 Hz, 2H), 7.35 (br s, 1H), 6.82 (s, 1H), 5.03 (s, 2H), 4.64 (s, 2H), 3.94 (s, 3H), 3.93 (s, 3H).

5.1.14. Methyl 6-[[2,5-dioxo-3-(3-sulfamoylphenyl)imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4h)

Compound **4h** was prepared in a manner similar to that described for **4b** in 56% yield. R_f = 0.62 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.09 (s, 1H), 8.32 (s, 1H), 7.73–7.71 (m, 1H), 7.64–7.59 (m, 2H), 7.47 (s, 2H), 6.82 (s, 1H), 5.03 (s, 2H), 4.64 (s, 2H), 3.94 (s, 3H), 3.92 (s, 3H).

5.1.15. Methyl 6-[[3-(3-carbamoylphenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4i)

Compound **4i** was prepared in a manner similar to that described for **4b** in 77% yield. R_f = 0.35 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.05 (br s, 2H), 7.91 (d, J = 7.8 Hz, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.49–7.51 (m, 2H), 6.18 (s, 1H), 5.03 (s, 2H), 4.65 (s, 2H), 3.95 (s, 3H), 3.92(s, 3H).

5.1.16. Methyl 6-[[3-(4-acetonyloxyphenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4k)

Compound **4k** was prepared in a manner similar to that described for **4b** in 69% yield. R_f = 0.75 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.08 (s, 1H), 7.54 (d, J = 8.4 Hz, 2H), 6.96 (d, J = 8.4 Hz, 2H), 6.78 (s, 1H), 5.00 (s, 2H), 4.83 (s, 2H), 4.58 (s, 2H), 3.93 (s, 3H), 3.92 (s, 3H), 2.16 (s, 3H).

5.1.17. Methyl 6-[[3-[4-(2-amino-2-oxo-ethoxy)phenyl]-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4l)

Compound **4l** was prepared in a manner similar to that described for **4b** in 77% yield. R_f = 0.14 (EtOAc). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.08 (s, 1H), 7.57 (d, J = 9.0 Hz, 2H), 7.55 (br s, 1H), 7.43 (br s, 1H), 7.01 (d, J = 9.0 Hz, 2H), 6.78 (s, 1H), 5.00 (s, 2H), 4.58 (s, 2H), 4.43 (s, 2H), 3.94 (s, 3H), 3.92 (s, 3H).

5.1.18. Methyl 6-[[2,5-dioxo-3-(4-phenoxyphenyl)imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4n)

Compound **4n** was prepared in a manner similar to that described for **4b** in 60% yield. R_f = 0.48 (EtOAc). ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.05 (s, 1H), 7.49 (d, J = 8.4 Hz, 2H), 7.34 (t, J = 7.8 Hz, 2H), 7.12 (t, J = 7.2 Hz, 1H), 7.05 (t, J = 8.4 Hz, 2H), 6.99 (t, J = 7.8 Hz, 2H), 6.81 (s, 1H), 5.02 (s, 2H), 4.37 (s, 2H), 4.09 (s, 3H), 4.08 (s, 3H).

5.1.19. Methyl 6-[[3-[4-(4-methoxyphenoxy)phenyl]-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4o)

Compound **4o** was prepared in a manner similar to that described for **4b** in 65% yield. R_f = 0.48 (EtOAc). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.05 (s, 1H), 7.60 (d, J = 9.0 Hz, 2H), 6.97 (d, J = 8.4 Hz, 2H), 6.95–6.93 (m, 4H), 6.76 (s, 1H), 4.98 (s, 2H), 4.57 (s, 2H), 3.90 (s, 3H), 3.89 (s, 3H), 3.73 (s, 3H).

5.1.20. Methyl 6-[[2,5-dioxo-3-[4-(4-pyridylmethoxy)phenyl]imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4p)

Compound **4p** was prepared in a manner similar to that described for **4b** in 42% yield. R_f = 0.35 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.58 (d, J = 5.4 Hz, 2H), 7.58 (d, J = 9.0 Hz, 2H), 7.44 (d, J = 5.4 Hz, 2H), 7.08 (d, J = 9.0 Hz, 2H), 6.78 (s, 1H), 5.21 (s, 2H), 5.00 (s, 2H), 4.58 (s, 2H), 3.93 (s, 3H), 3.92 (s, 3H).

5.1.21. Methyl 6-[[2,5-dioxo-3-[4-(6-quinolyloxy)phenyl] imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4q)

Compound **4q** was prepared in a manner similar to that described for **4b** in 82% yield. R_f=0.58 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.09 (s, 1H), 8.83 (d, J = 3.0 Hz, 1H), 8.27 (d, J = 8.4 Hz, 1H), 8.06 (d, J = 9.0 Hz, 1H), 7.74 (d, J = 9.0 Hz, 2H), 7.55 (dd, J = 3.0, 6.0 Hz, 1H), 7.51 (dd, J = 4.2 Hz, 1H), 7.35 (d, J = 3.0 Hz, 1H), 7.25 (d, J = 9.6 Hz, 2H), 6.80 (s, 1H), 5.03 (s, 2H), 4.65 (s, 2H), 3.95 (s, 3H), 3.93 (s, 3H).

5.1.22. Methyl 6-[[3-[4-(2-carbamoylphenoxy)phenyl]-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4r)

Compound **4r** was prepared in a manner similar to that described for **4b** in 48% yield. R_f=0.65 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.08 (s, 1H), 7.72 (d, J = 6.0 Hz, 1H), 7.68 (d, J = 9.0 Hz, 2H), 7.67 (br s, 1H), 7.57 (br s, 1H), 7.45 (t, J = 6.6 Hz, 1H), 7.21 (t, J = 7.8 Hz, 1H), 7.12 (d, J = 9.0 Hz, 2H), 6.84 (d, J = 8.4 Hz, 1H), 6.79 (s, 1H), 5.02 (s, 2H), 4.62 (s, 2H), 3.94 (s, 3H), 3.92 (s, 3H).

5.1.23. Methyl 6-[[3-(3-carbamoyl-4-phenoxy-phenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4s)

Compound **4s** was prepared in a manner similar to that described for **4b** in 74% yield. R_f=0.80 (EtOAc/MeOH = 8:2). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.09 (s, 1H), 8.03 (d, J = 2.4 Hz, 1H), 7.71 (dd, J = 9.0, 3.0 Hz, 1H), 7.69 (br s, 1H), 7.66 (br s, 1H), 7.40 (t, J = 7.8 Hz, 2H), 7.15 (t, J = 7.8 Hz, 1H), 7.01 (t, J = 7.8 Hz, 3H), 6.80 (s, 1H), 5.02 (s, 2H), 4.64 (s, 2H), 3.94 (s, 3H), 3.93 (s, 3H).

5.1.24. Methyl 6-[[3-[3-carbamoyl-4-(4-pyridylmethoxy)phenyl] -2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4t)

Compound **4t** was prepared in a manner similar to that described for **4b** in 70% yield. R_f=0.52 (CH_2Cl_2/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.60 (d, J = 6.0 Hz, 2H), 8.04 (d, J = 3.0 Hz, 1H), 7.76 (br s, 1H), 7.69 (br s, 1H), 7.64–7.62 (m, 1H), 7.48 (d, J = 5.4 Hz, 2H), 7.19 (d, J = 9.0 Hz, 1H), 6.78 (s, 1H), 5.35 (s, 2H), 5.00 (s, 2H), 4.60 (s, 2H), 3.94 (s, 3H), 3.92 (s, 3H).

5.1.25. Methyl 6-[[3-(4-fluorophenyl)-2-oxo-imidazolidin-1-yl] methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4u)

Compound **4u** was prepared in a manner similar to that described for **4a** in 25% yield. R_f = 0.30 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.07 (s, 1H), 7.61 (q, J = 4.8 Hz, 2H), 7.18 (d, J = 9.0 Hz, 2H), 6.75 (s, 1H), 4.72 (s, 2H), 3.89 (s, 3H), 3.84–3.82 (m, 5H), 3.42 (t, J = 8.4 Hz, 2H).

5.1.26. Methyl 7-methyl-6-[[2-oxo-3-(4-phenoxyphenyl)imidazolidin-1-yl]methyl]pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4v)

Compound **4v** was prepared in a manner similar to that described for **4a** in 31% yield (containing impurity). R_f = 0.15 (EtOAc).

5.1.27. Methyl 6-[[2,5-dioxo-4-(4-phenoxyphenyl)piperazin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (4w)

Compound **4w** was prepared in a manner similar to that described for **4a** in 67% yield. R_f = 0.52 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.13 (s, 1H), 7.43 (t, J = 7.8 Hz, 2H), 7.39 (d, J = 9.0 Hz, 2H), 7.20–7.19 (m, 1H), 7.07–7.05 (m, 4H), 6.87 (s, 1H), 4.97 (s, 2H), 4.44 (s, 2H), 4.14 (s, 2H), 3.93 (s, 3H), 3.85 (s, 3H).

5.1.28. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-6-amino-6-oxo-5-(tritylamino)hexyl]carbamate (5a)

H-Lys(Fmoc)-NH_2·HCl (2.41 g, 5.97 mmol) was suspended in CH_2Cl_2 (60 ml), and triethylamine (1.75 ml, 12.5 mmol) and trityl chloride (1.83 g, 6.57 mmol) were added under ice cold conditions. The mixture was stirred at room temperature for 6 h. The reaction mixture was quenched with saturated ammonium chloride and extracted with ethyl acetate. The combined extracts were washed with water and brine, dried over magnesium sulfate and concentrated under reduced pressure to give 3.9 g of crude **5a** (containing impurity) in 98% yield. R_f = 0.21 (*n*-hexane/EtOAc = 1:2).

5.1.29. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-(tritylamino)pentyl]carbamate (5b)

Compound **5a** (3.9 g, 6.41 mmol) was dissolved in THF (80 ml), and triethylamine (3.6 ml, 25.6 mmol) and trifluoroacetic anhydride (1.3 ml, 9.6 mmol) were added under ice cold conditions. The mixture was stirred under the same conditions for 5 min. The reaction mixture was quenched with saturated ammonium chloride and extracted with ethyl acetate. The combined extracts were washed with water and brine, dried over magnesium sulfate and concentrated under reduced pressure to give 3.7 g of desired **5b** in 98% yield. R_f = 0.85 (*n*-hexane/EtOAc = 1:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.91–7.89 (m, 2H), 7.69–7.68 (m, 2H), 7.47–7.41 (m, 8H), 7.38–7.21 (m, 13H), 4.31 (d, J = 4.2 Hz, 2H), 4.22 (t, J = 6.6 Hz, 1H), 3.29–3.25 (m, 1H), 2.93 (q, J = 6.0 Hz, 2H), 1.69–1.27 (m, 6H).

5.1.30. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-amino-5-cyano-pentyl]carbamate (5)

Compound **5b** (3.7 g, 6.29 mmol) was dissolved in CH_2Cl_2 (60 ml), and trifluoroehanol (15 ml) and acetic acid (15 ml) were added at room temperature. The mixture was stirred under the same conditions for 4 h. The mixture was evaporated and the residue was purified by silica gel column chromatography to give 1.9 g of desired **5** in 87% yield. R_f = 0.61 (CH_2Cl_2/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.91 (d, J = 7.2 Hz, 2H), 7.70 (d, J = 7.2 Hz, 2H), 7.43 (t, J = 6.6 Hz, 2H), 7.36–7.28 (m, 4H), 7.23–7.20 (m, 1H), 4.31 (d, J = 6.6 Hz, 2H), 4.23 (t, J = 7.2 Hz, 1H), 3.66 (t, J = 7.2 Hz, 1H), 3.00–2.98 (m, 2H), 1.62–1.58 (m, 2H), 1.42–1.39 (m, 4H).

5.1.31. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-[[7-methyl-6-[(3-methyl-2,5-dioxo-imidazolidin-1-yl)methyl]pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]pentyl]carbamate (6a)

To a solution of **4a** (67 mg, 0.21 mmol) in MeOH/THF/H_2O (2 ml/2 ml/2 ml), LiOH·H_2O (13 mg, 0.32 mmol) was added at room temperature and the mixture was stirred at room temperature for 3 h. The reaction mixture was neutralized with 1 M HCl and evaporated down. The residue in DMF (2 ml) was added to a solution of **3** (100 mg, 0.25 mmol) and triethylamine (35 ml, 0.25 mmol) in DMF (3 ml) at 0 °C. Subsequently, HOAt (55 mg, 0.42 mmol) and WSCI·HCl (80 mg, 0.42 mmol) were added to the former solution at 0 °C. The reaction mixture was evaporated down and H_2O was added to the residue to afford powder. The residue was purified by silica gel column chromatography to give 61 mg of desired **6a**

in 45% yield. R_f = 0.18 (EtOAc/MeOH = 95:5). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.58 (d, J = 7.8 Hz, 1H), 9.04 (s, 1H), 7.88 (d, J = 7.2 Hz, 2H), 7.67 (d, J = 7.2 Hz, 2H), 7.41 (t, J = 7.8 Hz, 2H), 7.33–7.31 (m, 3H), 6.64 (s, 1H), 4.97 (q, J = 7.8 Hz, 1H), 4.90 (s, 2H), 4.30–4.28 (m, 2H), 4.21–4.18 (m, 1H), 4.09 (s, 2H), 3.93 (s, 3H), 3.00–2.99 (m, 2H), 2.91 (s, 3H), 2.01–1.97 (m, 2H), 1.46–1.39 (m, 4H).

5.1.32. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-[[6-[[3-(4-fluorophenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]pentyl]carbamate (6b)

Compound **6b** was prepared in a manner similar to that described for **6a** in 87% yield. R_f = 0.48 (EtOAc). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.58 (d, J = 8.4 Hz, 1H), 9.05 (s, 1H), 7.88 (d, J = 7.8 Hz, 2H), 7.71–7.68 (m, 4H), 7.41 (t, J = 7.2 Hz, 2H), 7.33–7.27 (m, 5H), 6.77 (s, 1H), 5.01 (s, 2H), 4.97 (q, J = 7.2 Hz, 1H), 4.62 (s, 2H), 4.30–4.28 (m, 2H), 4.20–4.19 (m, 1H), 3.96 (s, 3H), 3.00–2.99 (m, 2H), 2.00–1.98 (m, 2H), 1.46–1.39 (m, 4H).

5.1.33. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-[[6-[[3-(4-methoxyphenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]pentyl]carbamate (6c)

Compound **6c** was prepared in a manner similar to that described for **6a** in 73% yield. R_f = 0.81 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.58 (d, J = 7.8 Hz, 1H), 9.05 (s, 1H), 7.97 (s, 1H), 7.87 (d, J = 7.8 Hz, 2H), 7.66 (d, J = 7.8 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.41 (t, J = 7.2 Hz, 2H), 7.34–7.31 (m, 2H), 7.01 (d, J = 9.0 Hz, 2H), 6.76 (s, 1H), 5.00 (s, 2H), 4.99–4.97 (m, 1H), 4.59 (s, 2H), 4.31–4.27 (m, 2H), 4.23–4.18 (m, 1H), 3.96 (s, 3H), 3.76 (s, 3H), 3.02–2.97 (m, 2H), 2.02–2.00 (m, 2H), 1.49–1.40 (m, 4H).

5.1.34. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-[[6-[[2,5-dioxo-3-(4-propylphenyl)imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]pentyl]carbamate (6d)

Compound **6d** was prepared in a manner similar to that described for **6a** in 73% yield. R_f = 0.65 (EtOAc). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.58 (d, J = 8.4 Hz, 1H), 9.04 (s, 1H), 7.88 (d, J = 7.2 Hz, 2H), 7.67 (d, J = 7.8 Hz, 2H), 7.56 (d, J = 7.2 Hz, 2H), 7.41 (t, J = 6.6 Hz, 2H), 7.33–7.32 (m, 3H), 7.23 (d, J = 7.8 Hz, 2H), 6.76 (s, 1H), 5.00 (s, 2H), 4.99–4.97 (m, 1H), 4.60 (s, 2H), 4.31–4.27 (m, 2H), 4.23–4.19 (m, 1H), 3.96 (s, 3H), 3.02–2.97 (m, 2H), 2.62–2.59 (m, 2H), 2.00–1.98 (m, 2H), 1.58 (q, J = 7.2 Hz, 2H), 1.46–1.39 (m, 4H), 0.88 (t, J = 7.2 Hz, 3H).

5.1.35. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-[[6-[[2,5-dioxo-3-(4-sulfamoylphenyl)imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]pentyl]carbamate (6e)

Compound **6e** was prepared in a manner similar to that described for **6a** in 31% yield. R_f = 0.75 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.58 (d, J = 9.0 Hz, 1H), 9.05 (s, 1H), 7.88 (d, J = 7.2 Hz, 2H), 7.85 (s, 4H), 7.67 (d, J = 7.2 Hz, 2H), 7.41 (t, J = 7.8 Hz, 2H), 7.35–7.32 (m, 4H), 6.80 (s, 1H), 5.03 (s, 2H), 4.99–4.97 (m, 1H), 4.66 (s, 2H), 4.30–4.27 (m, 2H), 4.21–4.19 (m, 1H), 3.96 (s, 3H), 3.06–2.97 (m, 2H), 2.01–1.98 (m, 2H), 1.46–1.38 (m, 4H).

5.1.36. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-[[6-[[3-(4-carbamoylphenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]-5-cyano-pentyl]carbamate (6f)

To a solution of **4f** (72 mg, 0.17 mmol) in MeOH/THF/H_2O (4 ml/5 ml/4 ml), LiOH·H_2O (8.6 mg, 0.26 mmol) was added at room temperature and the mixture was stirred at room temperature for 3 h. The reaction mixture was neutralized with 1 M HCl and evaporated down. The reaction mixture was evaporated down and the residue was dissolved in DMF (20 ml). To a solution, CDI (55 mg, 0.34 mmol) was added at room temperature and stirred at ambient temperature for 15 h. The reaction mixture was evaporated down. The residue in DMF (2 ml) was added to a solution of **3** (100 mg, 0.24 mmol) and triethylamine (34 μl, 0.24 mmol) in DMF (3 ml) at 0 °C. Subsequently, HOAt (65 mg, 0.48 mmol) and WSCI·HCl (92 mg, 0.48 mmol) were added to the former solution at 0 °C. The mixture was stirred at room temperature for 15 h. The reaction mixture was evaporated down and H_2O was added to the residue to afford powder. The residue was purified by silica gel column chromatography to give 53 mg of desired **6f** in 41% yield. R_f = 0.18 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.60 (d, J = 8.4 Hz, 1H), 9.05 (s, 1H), 7.97–7.94 (m, 3H), 7.88 (d, J = 7.8 Hz, 2H), 7.74 (d, J = 8.4 Hz, 2H), 7.67 (d, J = 7.2 Hz, 2H), 7.41 (t, J = 7.8 Hz, 2H), 7.35–7.32 (m, 4H), 6.79 (s, 1H), 5.02 (s, 2H), 4.99–4.97 (m, 1H), 4.65 (s, 2H), 4.30–4.27 (m, 2H), 4.21–4.19 (m, 1H), 3.96 (s, 3H), 3.06–2.97 (m, 2H), 2.01–1.98 (m, 2H), 1.46–1.39 (m, 4H).

5.1.37. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-[[6-[[3-(4-cyanophenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]pentyl]carbamate (6g)

Compound **6f** (18 mg, 0.024 mmol) was dissolved in THF/DMF (4 ml/0.5 ml), and triethylamine (14 μl, 0.097 mmol) and trifluoroacetic anhydride (5 μl, 0.037 mmol) were added under ice cold conditions. The mixture was stirred under the same conditions for 5 min. The reaction mixture was quenched with satd NH_4Cl and extracted with EtOAc. The combined extracts were washed with water and brine, dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give 14 mg of desired **6g** in 82% yield. R_f = 0.23 (EtOAc), ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.58 (d, J = 8.4 Hz, 1H), 9.05 (s, 1H), 7.92–7.86 (m, 7H), 7.66 (d, J = 7.8 Hz, 2H), 7.41 (t, J = 7.2 Hz, 2H), 7.33–7.30 (m, 2H), 6.80 (s, 1H), 5.03 (s, 2H), 4.99–4.97 (m, 1H), 4.66 (s, 2H), 4.30–4.27 (m, 2H), 4.21–4.19 (m, 1H), 3.96 (s, 3H), 3.06–2.97 (m, 2H), 2.01–1.98 (m, 2H), 1.46–1.39 (m, 4H).

5.1.38. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-[[6-[[2,5-dioxo-3-(3-sulfamoylphenyl)imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]pentyl]carbamate (6h)

Compound **6h** was prepared in a manner similar to that described for **6f** in 59% yield. R_f = 0.67 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.58 (d, J = 8.4 Hz, 1H), 9.05 (s, 1H), 8.32 (s, 1H), 7.88 (d, J = 7.8 Hz, 2H), 7.72–7.61 (m, 5H), 7.47 (s, 2H), 7.42 (m, 6H), 6.80 (s, 1H), 5.03 (s, 2H), 4.98–4.95 (m, 1H), 4.65 (s, 2H), 4.30–4.27 (m, 2H), 4.21–4.19 (m, 1H), 3.97 (s, 3H), 3.03–2.99 (m, 2H), 2.01–1.98 (m, 2H), 1.46–1.41 (m, 4H).

5.1.39. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-[[6-[[3-(3-carbamoylphenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]-5-cyano-pentyl]carbamate (6i)

Compound **6i** was prepared in a manner similar to that described for **6f** in 60% yield. R_f = 0.40 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.59 (d, J = 8.4 Hz, 1H), 9.05 (s, 1H), 8.05 (br s, 2H), 7.88–7.92 (m, 3H), 7.66–7.68 (m, 3H), 7.49–7.52 (m, 2H), 7.39–7.42 (m, 2H), 7.32 (br s, 3H), 6.79 (s, 1H), 5.02 (s, 2H), 4.98 (br s, 1H), 4.66 (s, 2H), 4.29 (br s, 2H), 4.19 (br s, 1H), 3.97 (s, 3H), 3.00 (d, J = 6.0 Hz, 2H), 1.98 (m, 2H), 1.40–1.46 (m, 4H).

5.1.40. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-[[6-[[3-(3-cyanophenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]pentyl]carbamate (6j)

Conversion of **6i**–**6j** was performed in a manner similar to that described for **6g** in 79% yield. R_f = 0.30 (EtOAc). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.59 (d, J = 8.4 Hz, 1H), 9.05 (s, 1H), 8.08 (br s, 2H), 7.88 (d, J = 7.8 Hz, 2H), 7.64–7.68 (m, 4H), 7.41 (t, J = 7.8 Hz, 2H), 7.32 (m, 3H), 6.80 (s, 1H), 5.02 (s, 2H), 4.98 (br s, 1H), 4.65 (s, 2H), 4.29 (br s, 2H), 4.19 (br s, 1H), 3.96 (s, 3H), 3.00 (m, 2H), 2.00 (m, 2H), 1.40–1.46 (m, 4H).

5.1.41. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-[[6-[[3-(4-acetonyloxyphenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]-5-cyano-pentyl]carbamate (6k)

Compound **6h** was prepared in a manner similar to that described for **6f** in 17% yield (containing impurity). R_f = 0.70 (EtOAc/MeOH = 9:1).

5.1.42. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-[[6-[[3-[4-(2-amino-2-oxo-ethoxy)phenyl]-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]-5-cyano-pentyl]carbamate (6l)

Compound **6l** was prepared in a manner similar to that described for **6a** in 52% yield. R_f = 0.48 (CH_2Cl_2/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.58 (d, J = 7.8 Hz, 1H), 9.04 (s, 1H), 7.88 (d, J = 7.8 Hz, 2H), 7.66 (d, J = 7.8 Hz, 2H), 7.58–7.55 (m, 3H), 7.42–7.39 (m, 3H), 7.33–7.31 (m, 3H), 7.01 (d, J = 9.6 Hz, 2H), 6.76 (s, 1H), 5.00 (s, 2H), 4.98–4.95 (m, 1H), 4.59 (s, 2H), 4.43 (s, 2H), 4.30–4.28 (m, 2H), 4.20–4.19 (m, 1H), 3.96 (s, 3H), 3.00–2.99 (m, 2H), 2.01–1.96 (m, 2H), 1.46–1.40 (m, 4H).

5.1.43. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-[[6-[[3-[4-(cyanomethoxy)phenyl]-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]pentyl]carbamate (6m)

Conversion of **6l**–**6m** was performed in a manner similar to that described for **6g** in 83% yield. R_f = 0.50 (EtOAc). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.58 (d, J = 8.4 Hz, 1H), 9.05 (s, 1H), 7.97 (s, 1H), 7.88 (d, J = 7.8 Hz, 2H), 7.68–7.65 (m, 3H), 7.41 (t, J = 7.2 Hz, 2H), 7.33–7.31 (m, 3H), 7.14 (d, J = 9.6 Hz, 2H), 6.76 (s, 1H), 5.19 (s, 2H), 5.00 (s, 2H), 4.98–4.95 (m, 1H), 4.61 (s, 2H), 4.30–4.28 (m, 2H), 4.20–4.18 (m, 1H), 3.96 (s, 3H), 3.00–2.99 (m, 2H), 2.01–1.96 (m, 2H), 1.46–1.41 (m, 4H).

5.1.44. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-[[6-[[2,5-dioxo-3-(4-phenoxyphenyl)imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]pentyl]carbamate (6n)

Compound **6n** was prepared in a manner similar to that described for **6a** in 85% yield. R_f = 0.83 (EtOAc). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.59 (d, J = 8.4 Hz, 1H), 9.05 (s, 1H), 7.88 (d, J = 7.2 Hz, 2H), 7.70–7.66 (m, 4H), 7.40–7.38 (m, 4H), 7.33–7.30 (m, 3H), 7.15–7.12 (m, 3H), 7.00 (d, J = 7.8 Hz, 2H), 6.77 (s, 1H), 5.01 (s, 2H), 4.98–4.96 (m, 1H), 4.63 (s, 2H), 4.29–4.27 (m, 2H), 4.20–4.18 (m, 1H), 3.96 (s, 3H), 3.00–2.99 (m, 2H), 2.01–1.96 (m, 2H), 1.46–1.40 (m, 4H).

5.1.45. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-[[6-[[3-[4-(4-methoxyphenoxy)phenyl]-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]pentyl]carbamate (6o)

Compound **6o** was prepared in a manner similar to that described for **6a** in 83% yield (containing impurity). R_f = 0.52 (EtOAc).

5.1.46. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-[[6-[[2,5-dioxo-3-[4-(4-pyridylmethoxy)phenyl]imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]pentyl]carbamate (6p)

Compound **6p** was prepared in a manner similar to that described for **6a** in 41% yield. R_f = 0.60 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.59 (d, J = 8.4 Hz, 1H), 9.04 (s, 1H), 8.58 (d, J = 4.8 Hz, 2H), 7.88 (d, J = 7.8 Hz, 2H), 7.68 (d, J = 7.8 Hz, 2H), 7.58 (d, J = 8.4 Hz, 2H), 7.44 (d, J = 4.8 Hz, 2H), 7.41 (t, J = 7.8 Hz, 2H), 7.34–7.30 (m, 3H), 7.09 (d, J = 4.8 Hz, 2H), 6.75 (s, 1H), 5.21 (s, 2H), 5.00 (s, 2H), 4.98–4.96 (m, 1H), 4.59 (s, 2H), 4.29–4.27 (m, 2H), 4.20–4.18 (m, 1H), 3.96 (s, 3H), 3.00–2.99 (m, 2H), 2.01–1.98 (m, 2H), 1.46–1.40 (m, 4H).

5.1.47. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-[[6-[[2,5-dioxo-3-[4-(6-quinolyloxy)phenyl]imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]pentyl]carbamate (6q)

Compound **6q** was prepared in a manner similar to that described for **6f** in 41% yield. R_f = 0.75 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.58 (d, J = 7.8 Hz, 1H), 9.06 (s, 1H), 8.84–8.83 (m, 1H), 8.27 (d, J = 7.8 Hz, 1H), 8.06 (d, J = 9.0 Hz, 1H), 7.88 (d, J = 9.0 Hz, 2H), 7.75 (d, J = 9.0 Hz, 2H), 7.66 (d, J = 7.8 Hz, 2H), 7.55 (dd, J = 3.0 Hz, 6.0 Hz, 1H), 7.52–7.50 (m, 1H), 7.41 (t, J = 6.6 Hz, 2H), 7.36–7.31 (m, 4H), 7.25 (d, J = 9.0 Hz, 2H), 6.73 (s, 1H), 5.03 (s, 2H), 4.98–4.96 (m, 1H), 4.66 (s, 2H), 4.30–4.28 (m, 2H), 4.21–4.19 (m, 1H), 3.97 (s, 3H), 3.00–2.99 (m, 2H), 2.01–1.98 (m, 2H), 1.46–1.40 (m, 4H).

5.1.48. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-[[6-[[3-[4-(2-carbamoylphenoxy)phenyl]-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]-5-cyano-pentyl]carbamate (6r)

Compound **6r** was prepared in a manner similar to that described for **6f** in 88% yield. R_f = 0.75 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.58 (d, J = 8.4 Hz, 1H), 9.05 (s, 1H), 7.88 (d, J = 7.8 Hz, 2H), 7.73–7.66 (m, 6H), 7.46–7.39 (m, 3H), 7.33–7.30 (m, 4H), 7.21 (t, J = 7.8 Hz, 1H), 7.13 (d, J = 9.0 Hz, 2H), 6.84 (d, J = 7.8 Hz, 1H), 6.77 (s, 1H), 5.01 (s, 2H), 4.98–4.96 (m, 1H), 4.63 (s, 2H), 4.30–4.28 (m, 2H), 4.21–4.19 (m, 1H), 3.96 (s, 3H), 3.00–2.99 (m, 2H), 2.01–1.98 (m, 2H), 1.46–1.40 (m, 4H).

5.1.49. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-[[6-[[3-(3-carbamoyl-4-phenoxy-phenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]-5-cyano-pentyl]carbamate (6s)

Compound **6s** was prepared in a manner similar to that described for **6f** in 53% yield. R_f = 0.80 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.59 (d, J = 7.8 Hz, 1H), 9.50 (s, 1H), 8.04 (br s, 1H), 7.88 (d, J = 7.8 Hz, 2H), 7.66–7.72 (m, 5H), 7.39–7.41 (m, 4H), 7.31–7.33 (m, 3H), 7.15 (t, J = 7.8 Hz, 1H), 7.00–7.03 (m, 3H), 6.78 (s, 1H), 5.02 (s, 2H), 4.98 (br s, 1H), 4.65 (s, 2H), 4.29 (br s, 2H), 4.19 (br s, 1H), 3.96 (s, 3H), 3.00 (m, 2H), 1.98 (m, 2H), 1.41–1.46 (m, 4H).

5.1.50. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-[[6-[[3-[3-carbamoyl-4-(4-pyridylmethoxy)phenyl]-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]-5-cyano-pentyl]carbamate (6t)

Compound **6t** was prepared in a manner similar to that described for **6f** in 89% yield. R_f = 0.48 (CH_2Cl_2/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.59 (d, J = 9.0 Hz, 1H), 9.05 (s, 1H), 8.59 (d, J = 5.4 Hz, 2H), 8.05 (d, J = 2.4 Hz, 1H), 7.88 (d, J = 7.8 Hz, 2H), 7.77 (br s, 1H), 7.70 (br s, 1H), 7.66 (d, J = 7.8 Hz, 2H), 7.64–7.63 (m, 1H), 7.48 (d, J = 6.6 Hz, 2H), 7.41 (t, J = 7.2 Hz, 2H), 7.33–7.31 (m, 3H), 7.19 (d, J = 9.6 Hz, 1H), 6.76 (s, 1H), 5.35 (s, 2H),

5.00 (s, 2H), 4.98–4.96 (m, 1H), 4.60 (s, 2H), 4.30–4.28 (m, 2H), 4.21–4.19 (m, 1H), 3.96 (s, 3H), 3.00–2.99 (m, 2H), 2.01–1.98 (m, 2H), 1.46–1.40 (m, 4H).

5.1.51. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-[[6-[[3-(4-fluorophenyl)-2-oxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]pentyl]carbamate (6u)

Compound **6u** was prepared in a manner similar to that described for **6a** in 96% yield (including impurity). R_f = 0.17 (EtOAc).

5.1.52. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-[[7-methyl-6-[[2-oxo-3-(4-phenoxyphenyl)imidazolidin-1-yl]methyl] pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]pentyl]carbamate (6v)

Compound **6v** was prepared in a manner similar to that described for **6a** in 75% yield (including impurity). R_f = 0.43 (EtOAc/MeOH = 9:1).

5.1.53. 9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-cyano-5-[[6-[[2,5-dioxo-4-(4-phenoxyphenyl)piperazin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carbonyl]amino]pentyl]carbamate (6w)

Compound **6a** was prepared in a manner similar to that described for **6w** in 96% yield. R_f = 0.51 (EtOAc/MeOH = 9:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.60 (d, J = 8.4 Hz, 1H), 9.10 (s, 1H), 7.89 (d, J = 7.2 Hz, 2H), 7.67 (d, J = 7.2 Hz, 2H), 7.45–7.40 (m, 6H), 7.33–7.31 (m, 3H), 7.19 (t, J = 7.2 Hz, 1H), 7.07–7.05 (m, 4H), 6.86 (s, 1H), 4.99–4.96 (m, 3H), 4.44 (s, 2H), 4.30–4.28 (m, 2H), 4.21–4.19 (m, 1H), 4.14 (s, 2H), 3.88 (s, 3H), 3.00–2.99 (m, 2H), 2.01–1.98 (m, 2H), 1.46–1.40 (m, 4H).

5.1.54. *N*-[(1*S*)-5-Amino-1-cyano-pentyl]-7-methyl-6-[(3-methyl-2,5-dioxo-imidazolidin-1-yl)methyl]pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1a)

Compound **6a** (60 mg, 0.095 mmol) was dissolved in 20% piperidine in DMF (940 μl) at room temperature. The reaction mixture was stirred under the same conditions for 40 min. The mixture was evaporated and pale yellow products were precipitated by ether. The products were purified with HPLC system (column; TSK gel ODS-120T, Φ 21.5 × 300 mm, eluent CH_3CN containing 0.1% TFA/H_2O containing 0.1% TFA) to give **1a** in 32% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.91 (s, 1H), 6.69 (s, 1H), 4.97 (t, J = 7.2 Hz, 1H), 4.85 (s, 2H), 4.02 (s, 2H), 3.81 (s, 3H), 2.86–2.65 (m, 2H), 2.84 (s, 3H), 2.00–1.94 (m, 2H), 1.61–1.58 (m, 2H), 1.47–1.44 (m, 2H), HR-ESI-MS $[M+H]^+$ 413.20471 for $C_{19}H_{25}N_8O_3$ (calcd 413.20496).

5.1.55. *N*-[(1*S*)-5-Amino-1-cyano-pentyl]-6-[[3-(4-fluorophenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1b)

Compound **1b** was prepared in a manner similar to that described for **1a** in 41% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.90 (s, 1H), 7.35–7.33 (m, 2H), 7.02 (t, J = 9.0 Hz, 2H), 6.74 (s, 1H), 4.96 (t, J = 7.2 Hz, 1H), 4.93 (s, 2H), 4.45 (s, 2H), 3.83 (s, 3H), 2.86 (t, J = 7.2 Hz, 2H), 2.01–1.92 (m, 2H), 1.61–1.58 (m, 2H), 1.47–1.44 (m, 2H), HR-ESI-MS $[M+H]^+$ 493.21387 for $C_{24}H_{26}FN_8O_3$ (calcd 493.21119).

5.1.56. *N*-[(1*S*)-5-Amino-1-cyano-pentyl]-6-[[3-(4-methoxyphenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1c)

Compound **1c** was prepared in a manner similar to that described for **1a** in 52% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.90 (s, 1H), 7.24 (d, J = 9.0 Hz, 2H), 6.84 (d, J = 9.6 Hz, 2H), 6.76 (s, 1H), 4.95 (t, J = 7.8 Hz, 1H), 4.91 (s, 2H), 4.40 (s, 2H), 3.83 (s, 3H), 3.66 (s, 3H), 2.86 (t, J = 7.8 Hz, 2H), 2.03–1.90 (m, 2H), 1.64–1.56 (m, 2H), 1.49–1.41 (m, 2H), HR-ESI-MS $[M+Na]^+$ 527.21589 for $C_{25}H_{28}N_8NaO_4$ (calcd 527.21312).

5.1.57. *N*-[(1*S*)-5-Amino-1-cyano-pentyl]-6-[[2,5-dioxo-3-(4-propylphenyl)imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1d)

Compound **1d** was prepared in a manner similar to that described for **1a** in 46% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.62 (d, J = 7.8 Hz, 1H), 9.08 (s, 1H), 7.66 (br s, 2H), 7.57 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 8.4 Hz, 2H), 6.78 (s, 1H), 5.03–5.02 (m, 1H), 5.01 (s, 2H), 4.61 (s, 2H), 3.98 (s, 3H), 2.82–2.80 (m, 2H), 2.55 (t, J = 7.8 Hz, 2H), 2.02–1.99 (m, 2H), 1.61–1.56 (m, 4H), 1.47–1.45 (m, 2H), 0.89 (t, J = 7.8 Hz, 3H), HR-ESI-MS $[M+H]^+$ 517.26512 for $C_{27}H_{33}N_8O_3$ (calcd 517.26756).

5.1.58. *N*-[(1*S*)-5-Amino-1-cyano-pentyl]-6-[[2,5-dioxo-3-(4-sulfamoylphenyl)imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1e)

Compound **1e** was prepared in a manner similar to that described for **1a** in 55% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.79 (s, 1H), 7.72 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 6.61 (s, 1H), 4.93–4.91 (m, 3H), 4.49 (s, 2H), 3.77 (s, 3H), 2.84 (t, J = 7.8 Hz, 2H), 1.96–1.91 (m, 2H), 1.58–1.49 (m, 2H), 1.45–1.42 (m, 2H), HR-ESI-MS $[M+H]^+$ 554.19632 for $C_{24}H_{28}N_9O_5S$ (calcd 554.19341).

5.1.59. *N*-[(1*S*)-5-Amino-1-cyano-pentyl]-6-[[3-(4-carbamoylphenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1f)

Compound **1e** was prepared in a manner similar to that described for **1a** in 51% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.85 (s, 1H), 7.67–7.65 (m, 2H), 7.48–7.47 (m, 2H), 6.70 (s, 1H), 4.94–4.91 (m, 3H), 4.48 (s, 2H), 3.80 (s, 3H), 2.84 (t, J = 7.2 Hz, 2H), 1.97–1.92 (m, 2H), 1.59–1.47 (m, 2H), 1.44–1.42 (m, 2H), HR-ESI-MS $[M+H]^+$ 518.22796 for $C_{25}H_{28}N_9O_4$ (calcd 518.22642).

5.1.60. *N*-[(1*S*)-5-Amino-1-cyano-pentyl]-6-[[3-(4-cyanophenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1g)

Compound **1g** was prepared in a manner similar to that described for **1a** in 61% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.86 (s, 1H), 7.62–7.60 (m, 2H), 7.52–7.49 (m, 2H), 6.70 (s, 1H), 4.93–4.90 (m, 3H), 4.47 (s, 2H), 3.81 (s, 3H), 2.84 (t, J = 7.8 Hz, 2H), 1.98–1.91 (m, 2H), 1.60–1.57 (m, 2H), 1.46–1.42 (m, 2H), HR-ESI-MS $[M+H]^+$ 500.21735 for $C_{25}H_{26}N_9O_3$ (calcd 500.21586).

5.1.61. *N*-[(1*S*)-5-Amino-1-cyano-pentyl]-6-[[2,5-dioxo-3-(3-sulfamoylphenyl)imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1h)

Compound **1h** was prepared in a manner similar to that described for **1a** in 47% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.86 (s, 1H), 7.97 (s, 1H), 7.56–7.53 (m, 2H), 7.47–7.44 (m, 1H), 6.73 (s, 1H), 4.94–4.92 (m, 3H), 4.50 (s, 2H), 3.82 (s, 3H), 2.84 (t, J = 7.8 Hz, 2H), 1.98–1.91 (m, 2H), 1.60–1.56 (m, 2H), 1.46–1.42 (m, 2H), HR-ESI-MS $[M+H]^+$ 554.19408 for $C_{24}H_{28}N_9O_5S$ (calcd 554.19341).

5.1.62. *N*-[(1*S*)-5-Amino-1-cyano-pentyl]-6-[[3-(3-carbamoylphenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1i)

Compound **1i** was prepared in a manner similar to that described for **1a** in 43% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.81 (br s, 1H), 7.79 (s, 1H), 7.58 (br s, 1H), 7.47 (br s, 1H), 7.40 (br s, 1H), 6.63 (s, 1H), 4.93 (s, 3H), 4.50 (s, 2H), 3.79 (s, 3H), 2.84 (br s, 2H), 1.93 (br s, 2H), 1.59 (br s, 2H), 1.43 (br s, 2H). HR-ESI-MS $[M+Na]^+$ 540.20996 for $C_{25}H_{27}N_9NaO_4$ (calcd 540.20837).

5.1.63. *N*-[(1*S*)-5-Amino-1-cyano-pentyl]-6-[[3-(3-cyanophenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1j)

Compound **1j** was prepared in a manner similar to that described for **1a** in 38% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.83 (s, 1H), 7.83 (s, 1H), 7.66 (br s, 1H), 7.43 (br s, 2H), 6.64 (s, 1H), 4.94 (s, 3H), 4.48 (s, 2H), 3.80 (s, 3H), 2.84 (br s, 2H), 1.96 (br s, 2H), 1.59 (br s, 2H), 1.44 (br s, 2H). HR-ESI-MS $[M+Na]^+$ 522.20008 for $C_{25}H_{25}N_9NaO_3$ (calcd 522.19780).

5.1.64. 6-[[3-(4-Acetonyloxyphenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-*N*-[(1S)-5-amino-1-cyano-pentyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1k)

Compound **1k** was prepared in a manner similar to that described for **1a** in 44% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.85 (s, 1H), 7.24 (d, J = 9.0 Hz, 2H), 6.80 (d, J = 9.0 Hz, 2H), 6.69 (s, 1H), 4.93 (t, J = 7.2 Hz, 1H), 4.89 (s, 2H), 4.75 (s, 2H), 4.39 (s, 2H), 3.80 (s, 3H), 2.84 (t, J = 7.8 Hz, 2H), 2.10 (s, 3H), 1.98–1.91 (m, 2H), 1.60–1.56 (m, 2H), 1.46–1.42 (m, 2H), HR-ESI-MS $[M+H]^+$ 547.24336 for $C_{27}H_{31}N_8O_5$ (calcd 547.24174).

5.1.65. *N*-[(1S)-5-Amino-1-cyano-pentyl]-6-[[3-[4-(2-amino-2-oxo-ethoxy)phenyl]-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1l)

Compound **1l** was prepared in a manner similar to that described for **1a** in 44% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.86 (s, 1H), 7.29 (d, J = 8.4 Hz, 2H), 6.87 (d, J = 8.4 Hz, 2H), 6.68 (s, 1H), 4.94 (t, J = 7.8 Hz, 1H), 4.91 (s, 2H), 4.46 (s, 2H), 4.42 (s, 2H), 3.80 (s, 3H), 2.84 (t, J = 7.2 Hz, 2H), 1.97–1.91 (m, 2H), 1.60–1.56 (m, 2H), 1.45–1.42 (m, 2H), HR-ESI-MS $[M+H]^+$ 548.23666 for $C_{26}H_{30}N_9O_5$ (calcd 548.23699).

5.1.66. *N*-[(1S)-5-Amino-1-cyano-pentyl]-6-[[3-[4-(cyanomethoxy)phenyl]-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1m)

Compound **1m** was prepared in a manner similar to that described for **1a** in 60% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.87 (s, 1H), 7.32 (d, J = 9.0 Hz, 2H), 6.95 (d, J = 9.0 Hz, 2H), 6.71 (s, 1H), 4.94 (t, J = 7.8 Hz, 1H), 4.91 (s, 2H), 4.85 (s, 2H), 4.42 (s, 2H), 3.81 (s, 3H), 2.85 (t, J = 7.2 Hz, 2H), 1.98–1.92 (m, 2H), 1.60–1.56 (m, 2H), 1.45–1.42 (m, 2H), HR-ESI-MS $[M+Na]^+$ 552.20948 for $C_{26}H_{27}N_9NaO_4$ (calcd 552.20837).

5.1.67. *N*-[(1S)-5-Amino-1-cyano-pentyl]-6-[[2,5-dioxo-3-(4-phenoxyphenyl)imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1n)

Compound **1n** was prepared in a manner similar to that described for **1a** in 54% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.63 (d, J = 8.4 Hz, 1H), 9.09 (s, 1H), 7.70–7.68 (m, 4H), 7.40 (t, J = 7.2 Hz, 2H), 7.16–7.11 (m, 3H), 7.00 (d, J = 7.2 Hz, 2H), 6.79 (s, 1H), 5.04–5.00 (m, 3H), 4.63 (s, 2H), 3.99 (s, 3H), 2.82–2.80 (m, 2H), 2.02–2.00 (m, 2H), 1.61–1.58 (m, 2H), 1.47–1.45 (m, 2H), HR-ESI-MS $[M+Na]^+$ 589.22935 for $C_{30}H_{30}N_8NaO_4$ (calcd 589.22877).

5.1.68. *N*-[(1S)-5-Amino-1-cyano-pentyl]-6-[[3-[4-(4-methoxyphenoxy)phenyl]-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1o)

Compound **1o** was prepared in a manner similar to that described for **1a** in 62% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.63 (d, J = 8.4 Hz, 1H), 9.09 (s, 1H), 7.66–7.63 (m, 4H), 7.02–6.96 (m, 6H), 6.78 (s, 1H), 5.03–5.00 (m, 3H), 4.61 (s, 2H), 3.98 (s, 3H), 3.76 (s, 3H), 2.83–2.80 (m, 2H), 2.02–2.00 (m, 2H), 1.61–1.58 (m, 2H), 1.47–1.44 (m, 2H), HR-ESI-MS $[M+H]^+$ 597.25750 for $C_{31}H_{33}N_8O_5$ (calcd 597.25739).

5.1.69. *N*-[(1S)-5-Amino-1-cyano-pentyl]-6-[[2,5-dioxo-3-[4-(4-pyridylmethoxy)phenyl]imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1p)

Compound **1p** was prepared in a manner similar to that described for **1a** in 42% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.83 (s, 1H), 8.57 (d, J = 7.2 Hz, 2H), 7.95 (d, J = 6.0 Hz, 2H), 7.28 (d, J = 8.4 Hz, 2H), 6.94 (d, J = 9.0 Hz, 2H), 6.64 (s, 1H), 5.32 (s, 2H), 4.93 (t, J = 7.8 Hz, 1H), 4.90 (s, 2H), 4.41 (s, 2H), 3.79 (s, 3H), 2.84 (t, J = 7.8 Hz, 2H), 1.98–1.92 (m, 2H), 1.60–1.56 (m, 2H), 1.45–1.42 (m, 2H), HR-ESI-MS $[M+H]^+$ 587.25798 for $C_{30}H_{32}N_9O_4$ (calcd 582.25772).

5.1.70. *N*-[(1S)-5-Amino-1-cyano-pentyl]-6-[[2,5-dioxo-3-[4-(7-quinolyloxy)phenyl]imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1q)

Compound **1q** was prepared in a manner similar to that described for **1a** in 40% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.87 (s, 1H), 8.79 (d, J = 5.4 Hz, 1H), 8.66–8.65 (m, 1H), 8.00 (d, J = 9.6 Hz, 1H), 7.80 (t, J = 6.6 Hz, 1H), 7.67 (br s, 1H), 7.43–7.42 (m, 2H), 7.31 (s, 1H), 7.05–7.04 (m, 2H), 6.80 (s, 1H), 4.96–4.93 (m, 3H), 4.48 (s, 2H), 3.85 (s, 3H), 2.84 (t, J = 7.8 Hz, 2H), 2.00–1.912 (m, 2H), 1.60–1.56 (m, 2H), 1.46–1.42 (m, 2H), HR-ESI-MS $[M+H]^+$ 618.25698 for $C_{33}H_{32}N_9O_4$ (calcd 618.25772).

5.1.71. *N*-[(1S)-5-Amino-1-cyano-pentyl]-6-[[3-[4-(2-carbamoylphenoxy)phenyl]-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1r)

Compound **1r** was prepared in a manner similar to that described for **1a** in 62% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.62 (d, J = 8.4 Hz, 1H), 9.09 (s, 1H), 7.73–7.65 (m, 7H), 7.58 (br s, 1H), 7.45 (t, J = 6.6 Hz, 1H), 7.22 (d, J = 7.2 Hz, 1H), 7.12 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.4 Hz, 1H), 6.79 (s, 1H), 5.02–5.00 (m, 3H), 4.63 (s, 2H), 3.97 (s, 3H), 2.82–2.80 (m, 2H), 2.02–2.00 (m, 2H), 1.60–1.58 (m, 2H), 1.47–1.44 (m, 2H), HR-ESI-MS $[M+Na]^+$ 632.23626 for $C_{31}H_{31}N_9NaO_5$ (calcd 632.23458).

5.1.72. *N*-[(1S)-5-Amino-1-cyano-pentyl]-6-[[3-(3-carbamoyl-4-phenoxy-phenyl)-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1s)

Compound **1s** was prepared in a manner similar to that described for **1a** in 47% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.63 (d, J = 7.8 Hz, 1H), 9.09 (s, 1H), 8.05 (d, J = 2.4 Hz, 1H), 7.66–7.72 (m, 5H), 7.40 (t, J = 7.8 Hz, 2H), 7.16 (t, J = 7.8 Hz, 1H), 7.02 (t, J = 9.0 Hz, 3H), 6.80 (s, 1H), 5.03 (s, 2H), 5.02 (br s, 1H), 4.65 (s, 2H), 3.99 (s, 3H), 2.82 (m, 2H), 2.01 (m, 2H), 1.59 (m, 2H), 1.46 (m, 2H). HR-ESI-MS $[M+Na]^+$ 632.23530 for $C_{31}H_{31}N_9NaO_5$ (calcd 632.23458).

5.1.73. *N*-[(1S)-5-Amino-1-cyano-pentyl]-6-[[3-[3-carbamoyl-4-(4-pyridylmethoxy)phenyl]-2,5-dioxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1t)

Compound **1t** was prepared in a manner similar to that described for **1a** in 52% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.85 (s, 1H), 8.60 (d, J = 4.8 Hz, 2H), 7.94 (d, J = 4.8 Hz, 2H), 7.69 (s, 1H), 7.46–7.44 (m, 1H), 6.98–6.97 (m, 1H), 6.68 (s, 1H), 5.43 (s, 2H), 4.94–4.90 (m, 3H), 4.43 (s, 2H), 3.81 (s, 3H), 2.84 (t, J = 7.2 Hz, 2H), 1.96–1.92 (m, 2H), 1.59–1.57 (m, 2H), 1.44–1.42 (m, 2H), HR-ESI-MS $[M+H]^+$ 625.26459 for $C_{31}H_{33}N_{10}O_5$ (calcd 625.26354).

5.1.74. *N*-[(1S)-5-Amino-1-cyano-pentyl]-6-[[3-(4-fluorophenyl)-2-oxo-imidazolidin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1u)

Compound **1u** was prepared in a manner similar to that described for **1a** in 45% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.88 (s,

1H), 7.25 (q, J = 4.8 Hz, 2H), 6.98 (t, J = 8.2 Hz, 2H), 6.73 (s, 1H), 4.95 (t, J = 7.2 Hz, 1H), 4.59 (s, 2H), 3.76–3.73 (m, 5H), 3.38 (t, J = 8.4 Hz, 2H), 2.84 (t, J = 7.8 Hz, 2H), 1.98–1.92 (m, 2H), 1.58–1.56 (m, 2H), 1.46–1.42 (m, 2H), HR-ESI-MS $[M+H]^+$ 479.23274 for $C_{24}H_{28}FN_8O_2$ (calcd 479.23192).

5.1.75. *N*-[(1*S*)-5-Amino-1-cyano-pentyl]-7-methyl-6-[[2-oxo-3-(4-phenoxyphenyl)imidazolidin-1-yl]methyl]pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1v)

Compound **1v** was prepared in a manner similar to that described for **1a** in 38% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.63 (d, J = 9.0 Hz, 1H), 9.11 (s, 1H), 7.66–7.64 (m, 4H), 7.39 (t, J = 7.8 Hz, 2H), 7.12 (t, J = 7.2 Hz, 1H), 7.07 (d, J = 8.4 Hz, 2H), 6.97 (d, J = 8.4 Hz, 2H), 6.79 (s, 1H), 5.02–5.00 (m, 1H), 4.77 (s, 2H), 3.90–3.86 (m, 5H), 2.82–2.80 (m, 2H), 2.02–1.99 (m, 2H), 1.61–1.58 (m, 2H), 1.47–1.44 (m, 2H), HR-ESI-MS $[M+H]^+$ 553.26677 for $C_{30}H_{33}N_8O_3$ (calcd 553.26756).

5.1.76. *N*-[(1*S*)-5-Amino-1-cyano-pentyl]-6-[[2,5-dioxo-4-(4-phenoxyphenyl)piperazin-1-yl]methyl]-7-methyl-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (1w)

Compound **1w** was prepared in a manner similar to that described for **1a** in 32% yield. ^{1}H NMR (600 MHz, D_2O): δ 8.99 (s, 1H), 7.24 (t, J = 7.8 Hz, 2H), 7.14 (d, J = 9.0 Hz, 2H), 7.04 (t, J = 6.6 Hz, 1H), 6.94–6.89 (m, 5H), 4.97 (t, J = 7.8 Hz, 1H), 4.88 (s, 2H), 4.38 (s, 2H), 4.15 (s, 2H), 3.80 (s, 3H), 2.84 (t, J = 7.2 Hz, 2H), 2.00–1.92 (m, 2H), 1.61–1.57 (m, 2H), 1.47–1.43 (m, 2H), HR-ESI-MS $[M+H]^+$ 581.26157 for $C_{31}H_{33}N_8O_4$ (calcd 581.26193).

5.2. Docking studies

Plasmin coordinates used in this study were prepared from micro-plasmin complex with streptokinase (PDB ID: 1BML) structure. The preparation procedure was described in detail in Ref. 19. The docked conformation of compound **3** was deduced by Monte Carlo multiple minimum method[20] with the BatchMin molecular mechanics engine[21] with standard parameters. The MMFF94s parameter was used as the force field.[22] The MMFF and AMBER94[23] atomic charges were loaded for inhibitor and enzyme atoms, respectively. Manipulation of molecular structures was done using the MAESTRO 9.0 molecular modeling package (Schrodinger Inc., Portland, USA) on a single Pentium IVcentral processing unit (3.0 GHz) computer with Linux/CentOS4.0.

5.3. Inhibition of plasmin, plasma kallikrein, and urokinase

Human plasmin was purchased from Chromogenix (Sweden) and human urokinase from Green Cross (Japan). Human plasma kallikrein was purified from human plasma.[24] H-D-Val-Leu-Lys-*p*NA (S-2251), H-D-Phe-Pro-Arg-*p*NA (S-2302), and <Glu-Gly-Arg-*p*NA (S-2444) were purchased from Chromogenix (Sweden). Inhibitory activities were determined by measuring the effect of the inhibitor on the hydrolytic activities of enzyme on synthetic peptide substrate. Plasmin activity was measured with 0.3 mM S-2251, plasma kallikrein with 0.2 mM S-2302, and urokinase with 0.1 mM S-2444. The IC_{50} value was taken as the concentration of inhibitor that reduced the absorbance at 405 nm by 50% compared with the absorbance measured under the same conditions without inhibitor.

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Supplementary data

Supplementary data (experimental procedures for the synthesis and characterization (^{1}H NMR) of **2d**–**2f**, **2h**, **2i**, **2k**, **2l**, **2n**–**2w**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.02.002.

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