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Development of tryptase inhibitors derived from thalidomide

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ABSTRACT

A novel series of tryptase inhibitors with a *N*-phenylphthalimide skeleton structurally derived from thalidomide (**1**) has been developed. Structure–activity relationship studies led to a potent and selective tryptase inhibitor, 2-(4-cyanophenyl)isoindole-1,3-dione-5-yl 3-(2-aminopyridin-5-yl)propanoate (**7**), with the IC_{50} value of 78 nM.

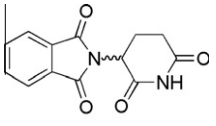
1. Introduction

Human tryptases are classified into serine proteases with a trypsin-like substrate specificity (cleaving adjacent to lysine or arginine residues).[1] There exist several different isozymes, among which β-tryptases represent the major type stored in mast cell secretory granules, where they account for 20–25% of the total protein; they are also the major isozymes in lung and skin tissues.[2–4] β-Tryptases are further divided into three subtypes (βI, βII, and βIII), but because the subtype composition of native β-tryptases isolated from tissues used in various studies is usually heterogeneous,[5] such preparations, except for recombinant β-tryptases, are generally referred to simply as tryptase in this paper.

Tryptase profoundly alters the behavior of certain cell types, provoking release of IL-8 from epithelial and endothelial cells,[6,7] activation of mast cells,[8,9] stimulation of proliferation of epithelial cells[6] and fibroblasts,[10] induction of cytokine production and release from human peripheral blood eosinophils,[11] and stimulation of TNF-α, IL-6, and IL-1β synthesis and release from peripheral blood mononuclear cells.[12] Furthermore, elevated tryptase levels have been observed in a number of disease states, such as asthma and inflammatory skin diseases, and tryptase may also function as a potent angiogenic factor.[3] Thus, tryptase has been considered as a causal factor in allergic and inflammatory diseases, including asthma. Therefore, potent and selective tryptase inhibitors are expected to be useful both for the treatment of such diseases, and as tools to study the role of tryptase in inflammation and other related bio-processes.

On the other hand, we have been utilizing thalidomide (**1**, Fig. 1), a sedative/hypnotic drug which was developed and marketed in the late 1950s, as a multi-template for the creation of various biologically active compounds.[13–17] The multi-template hypothesis is based on the idea that the number of protein fold structure types that comprise all the domains occurring in natural proteins is quite limited, in spite of the huge number of natural proteins.[18–20] Although thalidomide (**1**) subsequently had to be withdrawn from sale due to its teratogenicity,[21–26] it has since been discovered that the drug possesses various biological activities, including regulation of tumor necrosis factor-α (TNF-α) production, anti-inflammatory, antiangiogenic, and cyclooxygenase (COX)-inhibitory activities.[21–25,27,28] Our previous structural development studies of thalidomide (**1**), focusing on anti-inflammatory and enzyme-inhibitory properties, including TNF-α production regulation,[23–25,29–31] COX inhibition,[32–34] and nitric oxide synthase (NOS) inhibition,[35,36] led us to hypothesize that thalidomide (**1**) has potential as a lead structure for the development of novel tryptase inhibitors.

In this paper, we describe the synthesis and tryptase-inhibitory activity of a series of N-substituted phthalimides structurally derived from thalidomide (**1**), as well as their structure–activity



Thalidomide (**1**)

Figure 1. Structure of thalidomide (**1**).

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relationships, selectivity for tryptase versus other serine proteases, and inhibitory mechanism.

2. Results and discussion

2.1. *N*-Methylphthalimide derivatives possessing aniline moiety as a basic fragment

Previous tryptase inhibition studies have suggested that formation of a salt bridge between the basic fragment of an inhibitor molecule and the Asp189 residue deep in the slender pocket of the enzyme active site of tryptase is required for binding of the inhibitor in the active site and for inactivation of the enzyme. On the basis of this information, we designed slender molecules possessing an aniline moiety as a basic fragment, bound to the *N*-methylphthalimide skeleton [derived from thalidomide (**1**) as a pharmacophore scaffold] at the 5-position via an ester linkage (Fig. 2).

Our designed compounds were prepared as shown in Scheme 1. Briefly, 4-hydroxyphthalic acid was dehydrated at a high temperature, then coupled with methylamine to give 5-hydroxy-*N*-methylphthalimide (**8**). Next, **8** was coupled with an appropriate carboxylic acid possessing a terminal nitrophenyl group, except for compound **2a** which was prepared by the coupling of **8** and anthranilic acid under Mitsunobu reaction conditions. The nitro group of the resultant ester-linked compounds (**9**) was reduced by hydrogenation to give the desired compounds (**2**). In the case of cinnamate compounds (**9f** and **9g**), the double bond was also reduced at the same time to give **2f** and **2g**.

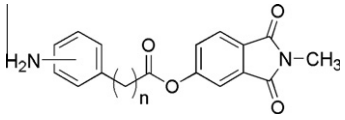
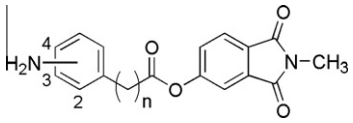
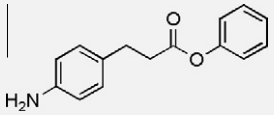


Figure 2. Design of tryptase inhibitor candidates.

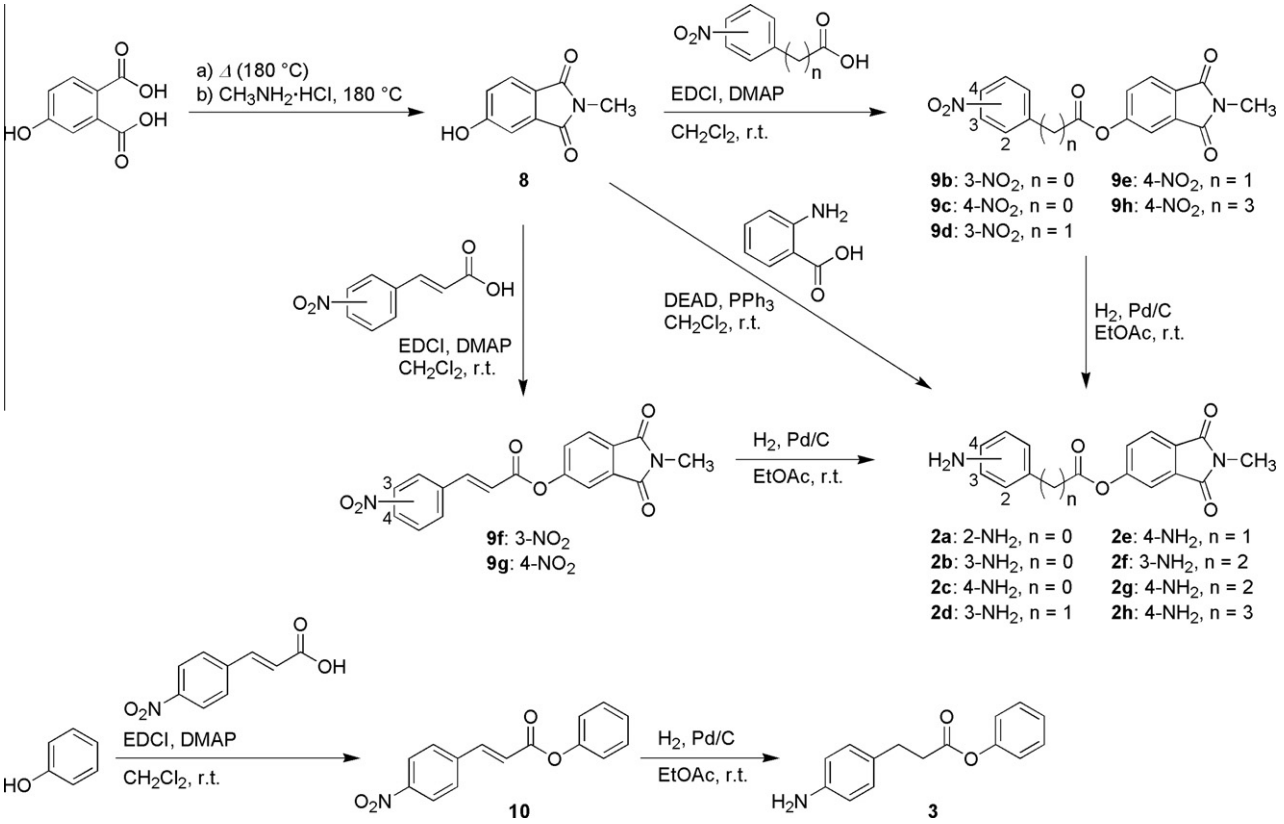
First, we measured the enzyme inhibition rate of the synthesized compounds (**2a**–**h**) at a fixed concentration of 30 μM. As shown Table 1, only **2d** and **2g** showed moderate and potent tryptase-inhibitory activity, respectively, suggesting that the length of

Table 1
Tryptase-inhibitory activity of *N*-phthalimide derivatives **2a**–**h** and **3**



	n	Position	% Inhibitor[a]
2a	0	2	N.A.[b]
2b	0	3	N.A.[b]
2c	0	4	N.A.[b]
2d	1	3	46%
2e	1	4	N.A.[b]
2f	2	3	N.A.[b]
2g	2	4	98% (1.5 μM)[c]
2h	3	4	N.A.[b]
3			N.A.[b]

[a] At 30 μM.
[b] No activity at 30 μM.
[c] IC_{50} value of compound **2g** is shown in parentheses.



Scheme 1. Synthesis of **2a**–**h**, and **3**.

the ester chain and the position of the amino group are critical for the activity. Benzoate derivatives (**2a**–**c**) are inactive regardless of the position of the amino group. Concerning phenylacetate derivatives (**2d** and **2e**), the *meta*-amino derivative (**2d**) showed moderate activity, while the corresponding *para*-amino regioisomer (**2e**) was inactive. In the case of phenylpropionate derivatives (**2f** and **2g**), the *para*-amino derivative (**2g**) inhibited the enzyme activity almost completely, while the corresponding *meta*-amino regioisomer (**2f**) was inactive. Elongation of the ester linkage moiety of the potent inhibitor **2g** by one carbon unit, that is **2h**, resulted in loss of the activity. Based on these results, as well as the fact that phenyl 4-aminocinnamate (**3**) is inactive (suggesting that the *N*-methylphthalimide moiety is essential for the activity), we chose **2g**, which almost completely inhibited tryptase activity at 30 μM under our experimental conditions, as a lead compound for further structural development. The IC_{50} value of **2g** was estimated to be 1.5 μM.

2.2. Optimization of the basic fragment

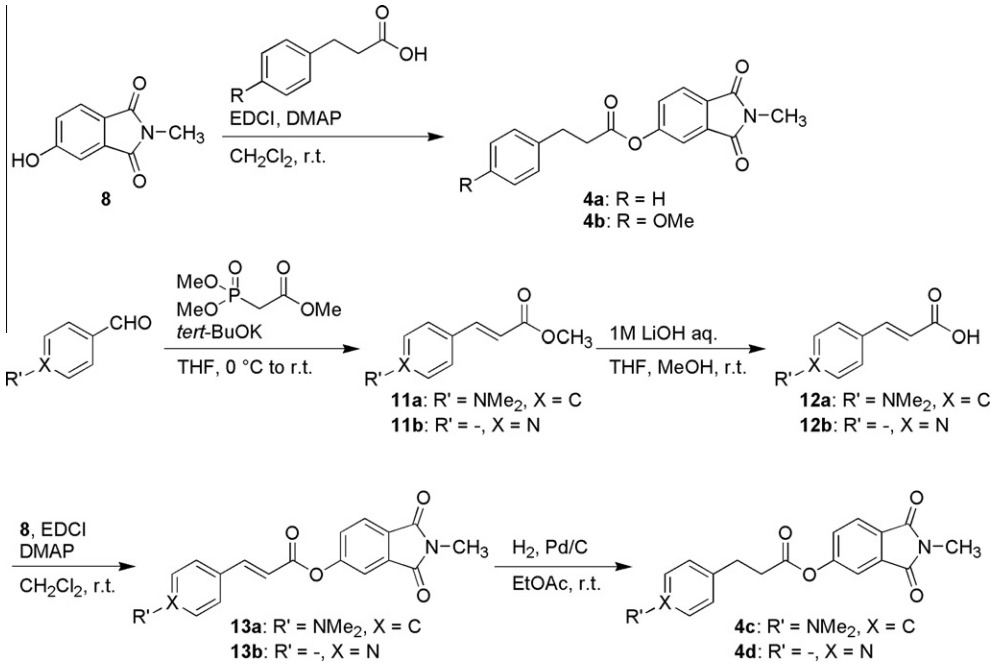
Based on the discovery of tryptase-inhibitory activity of compound **2g**, the effects of the basic fragments were analyzed. Several hydrocinnamate compounds (**4a**–**f**) and their aza-analogs (**4g** and **4h**) were prepared as shown in Schemes 2 and 3, and their enzyme-inhibitory activity was measured (Table 2).

As shown in Table 2, compounds **4a** and **4b**, which do not possess an amino group at the end of the ester chain, did not exhibit tryptase-inhibitory activity, suggesting that a basic amino group is essential for the activity, and an electron-donating group with weak basicity, such as a methoxy group (**4b**), cannot substitute for the amino group. *N*,*N*-Dimethylanilino and pyridyl derivatives (**4c** and **4d**, respectively) were also inactive or very weakly active, suggesting that a free amino group is essential for the activity. Compounds **4e** and **4f** were designed so as to enhance the basicity of the amino group of **2g** by the introduction of a methoxy group at the *ortho*- and *meta*-position (in relation to the amino group-introduced position), respectively. Neither of these modifications improved the activity, that is, introduction of a methoxy group at the *ortho*-position (**4e**) resulted in a marked decrease of the activity, while introduction at the *meta*-position (**4f**) slightly decreased the activity. These results suggest that the effect of steric hindrance around the basic nitrogen atom is larger than that of the basicity of the nitrogen atom. Therefore, compounds without steric hindrance around the amino group, that is, aza-analogs of **2g** (**4g** and **4h**) were designed. The pK_a values of the basic fragment of these compounds[37] decreased in the order of **4g** (6.67) > **2g** (4.61) > **4f** (4.17) > **4h** (3.87). The tryptase-inhibitory activity of these compounds decreased in the exactly the same order, that is, **4g** > **2g** ⩾ **4f** > **4h**. Thus, the 2-aminopyridyl analog **4g** showed the most potent tryptase-inhibitory activity, with an IC_{50} value of 0.40 μM.

2.3. Optimization of the substituent introduced at the nitrogen atom of the phthalimide moiety

Next, the effect of N-substituents of phthalimide was examined. Fifteen compounds bearing an alkyl chain (**5a**–**d**) or a substituted phenyl group (**6a**–**k**) on the nitrogen atom of the phthalimide moiety instead of the methyl group of **2g** were synthesized, as illustrated in Scheme 4.

As shown in Table 3, all of the N-alkylated compounds **5a**–**d** showed similar tryptase-inhibitory activity with IC_{50} values of 1.2–1.5 μM. Substitution at the same position with a phenyl group (**6a**) also resulted in similar activity to that of **2g**. On the other hand, there seemed to be a substituent effect of the phenyl ring of **6a** on the activity. As shown in Table 4, introduction of a methoxy group (**6b**–**d**) enhanced the activity position-dependently. Introduction of a methoxy substituent at the *ortho*-position (**6b**) resulted in slight enhancement of the activity compared to non-substituted **6a**. Introduction of the same substituent at *meta*- and *para*-positions greatly increased the activity, with the *para*-isomer (**6d**) being more potent that the corresponding *meta*-isomer (**6c**), suggesting that the *para*-position is the best position at which to introduce a substituent. For *para*-substituted compounds (**6d**–**k**), an apparent structure–activity relationship was observed. Analogs with a *tert*-butyl (**6g**) or trifluoromethyl group (**6h**) had little or no



Scheme 2. Synthesis of **4a**-**d**.

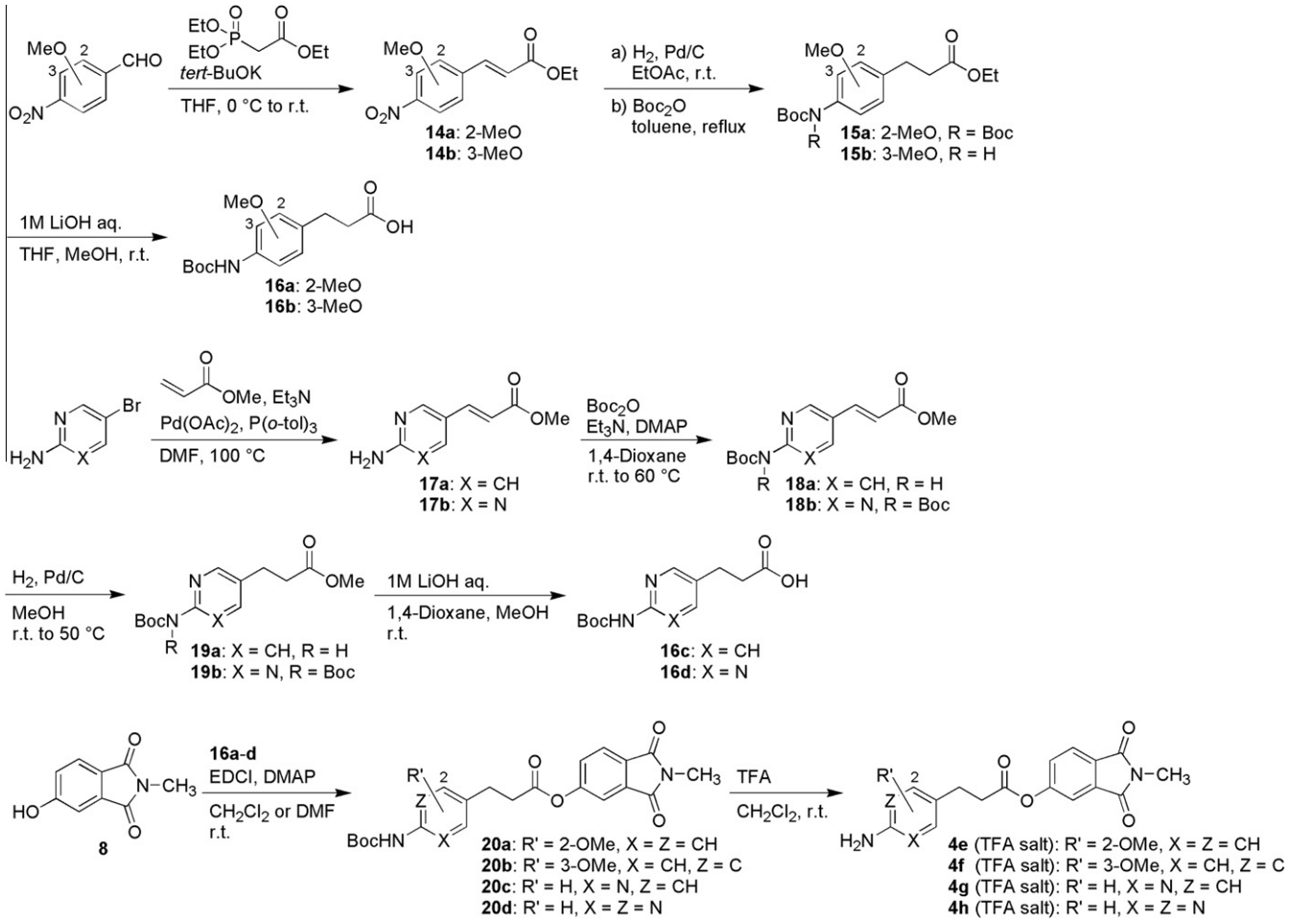
inhibitory activity, whereas analogs with a methyl (**6e**), isopropyl (**6f**), or fluoro (**6i**) group showed moderate activity. These results suggest that introduction of a bulky and hydrophobic group at the *para*-position is unfavorable for the activity. Compounds with a heteroatomic group, including methoxy (**6d**), cyano (**6j**), and amino (**6k**) groups, exhibited potent tryptase-inhibitory activity with IC_{50} values of 0.39, 0.34, and 0.82 μM, respectively. These results suggest that the ability of a substituent to serve as a hydrogen-bond-donating/accepting functionality seems to be important for enhancing the enzyme-inhibitory activity. A *para*-cyanophenyl group (**6j**) seemed to be the best substituent among those we investigated.

2.4. Inhibition mode analysis of phthalimide analogs

To examine whether or not the mode of tryptase inhibition of our compounds is competitive, we carried out Lineweaver–Burk plot analysis for compounds **4g** and **6j** (Fig. 3). Compounds **4g** and **6j** both showed competitive inhibition (the inhibition curves cross the vertical axis). These results indicate that compounds **4g** and **6j** inhibit tryptase by binding competitively to the substrate-binding pocket.

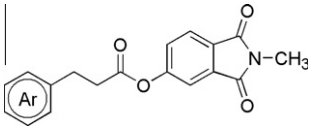
2.5. Tryptase-inhibitory activity of hybrid compound

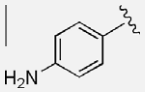
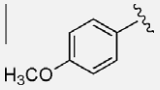
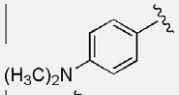
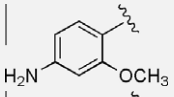
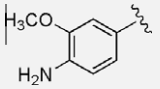
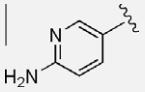
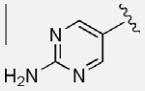
Compounds **4g** and **6j** were developed independently, focusing on the basic fragment moiety (2-aminopyridyl group) and N-substituent moiety (*para*-cyanophenyl group), respectively. From our inhibition mode analysis mentioned above, the binding site of compounds **4g** and **6j** is thought to be identical. Simply considered, the 2-aminopyridyl group of **4g** and the *para*-cyanophenyl group of **6j** seem to interact at different loci in the same substrate-binding pocket, and the former and the latter groups seem to contribute to the enhancement of the tryptase-inhibitory activity of **2g** by factors of 3.75-fold [IC_{50} value of **2g** (1.5 μM)/ that of **4g** (0.40 μM)] and 4.41-fold [IC_{50} value of **2g** (1.5 μM)/ that of **6j** (0.34 μM)], respectively. Therefore, it is expected that a molecule which possesses a 2-aminopyridyl group at the basic fragment moiety and a *para*-cyanophenyl group at the phthalimide moiety would be a much more potent tryptase inhibitor, possibly by a factor of 16.5-fold (3.75×4.41) compared to **2g**. According to this fragment-based drug design-like idea, we synthesized compound **7**, which is a hybrid molecule of **4g** with a 2-aminopyridyl moiety and **6j** with a *para*-cyanophenyl moiety. Compound **7** was prepared by condensation of **16c** and **21n**, and subsequent deprotection of the Boc group with TFA, as summarized in Scheme 5. As expected, the hybrid compound **7** exhibited potent, dose-dependent tryptase-inhibitory activity, with the IC_{50} value of 78 nM (Fig. 4). This IC_{50} value (78 nM) is in good coincidence with the product of the substituent effects elicited by the 2-aminopyridyl group of **4g** and the *para*-cyanophenyl group of **6j**, that is, 1.5 μM/16.5 = 90 nM. Inhibition mode analysis confirmed that compound **7** showed competitive inhibition, like compounds **4g** and **6j** (Fig. 4).



Scheme 3. Synthesis of **4e–h**.

Table 2
Effects of base fragments on tryptase-inhibitory activity



	Ar	IC_{50} (μM)
2g		1.5
4a		N.A.[a]
4b		N.A.[a]
4c		N.A.[a]
4d		>30[b]
4e		1.8
4f		>30[b]
4g		0.40
4h		7.8

[a] No activity at 30 μM.
[b] Failed to reach 50% inhibition at 30 μM.

2.6. Enzymatic selectivity

In order to investigate the enzymatic selectivity of our compounds, we examined the binding affinity of **2g**, **4g**, **6j**, and **7** for tryptase, thrombin and trypsin, which are representative serine proteases. As shown in Table 5, the K_i values of these compounds for tryptase were in good accordance with the IC_{50} values, and decreased in the order of **7** > **6j** > **4g** > **2g**. Concerning the affinity for thrombin, these compounds are rather similar, with K_i values of 7.0–16 μM. Thus, the enzymatic selectivity of the compounds for tryptase over thrombin increases in proportion to the affinity of the compounds for tryptase. In particular, compound **7** possesses 364-fold higher affinity for tryptase than thrombin. In contrast, the affinity of these compounds for trypsin showed the same tendency as the affinity for tryptase, that is, their K_i values for trypsin decreased in the order of **7** > **4g** ⩾ **6j** > **2g**. As for enzymatic selectivity for tryptase over trypsin, the 4-cyanophenyl analogs **6j** and **7** tended to be more selective for tryptase in comparison with the methyl analogs **2g** and **4g**. These results suggest that introduction of a 4-cyanophenyl group at the nitrogen atom of the phthalimide moiety is favorable for increasing selectivity for tryptase over thrombin and trypsin.

To interpret the enzymatic selectivity of **7**, crystal structures of trypsin[38] and thrombin[39] were compared with the structure of tryptase[40] docked with **7** using AutoDock 4.2 docking program[41] (Fig. 5). The docking model of the complex (compound **7** was docked into the active site of tryptase), including the centrally located Ser195, shows the 2-aminopyridyl group occupying the S1 pocket of tryptase. The *para*-cyanophenyl group of **7** is shown to be fitted neatly into a small pocket, surrounded by Lys60D, Asp60E, Ala63, Val35, and Phe41 (Fig. 5A). In trypsin, the corresponding small pocket does not exist, because of the difference of amino acid sequence, that is, Met39 in tryptase is replaced by Tyr39. This single amino acid difference results in hydrogen-bonding interaction between Lys60 and Tyr39 in trypsin, and the resulting conformational change around Lys60 of trypsin prevents the formation of a cavity corresponding to the small pocket in tryptase (Fig. 5B). As for thrombin, the protein structure around the active site is quite different from that of tryptase/trypsin, that is, a bridge constructed between Tyr60A-Trp60D and Glu192 caps the site corresponding to the small pocket of tryptase (Fig. 5C). Therefore, the tryptase selectivity of **7** can be attributed to the presence in tryptase of a small pocket, which accommodates the *para*-cyanophenyl group of **7**, and the absence of a corresponding pocket in trypsin/thrombin.

3. Conclusion

We have developed a series of potent tryptase inhibitors with high selectivity for tryptase over thrombin and trypsin, which are representative serine proteases. We found that among the compounds we prepared, compound **7** showed the most potent tryptase-inhibitory activity, having an IC_{50} value of 78 nM, with high selectivity.

4. Experimental

4.1. General comments

Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL JNM-GX500 (500 MHz) spectrometer. Chemical shifts are expressed in δ (ppm) values with tetramethylsilane (TMS) as an internal reference. Mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded on a JEOL JMS-DX303 spectrometer.

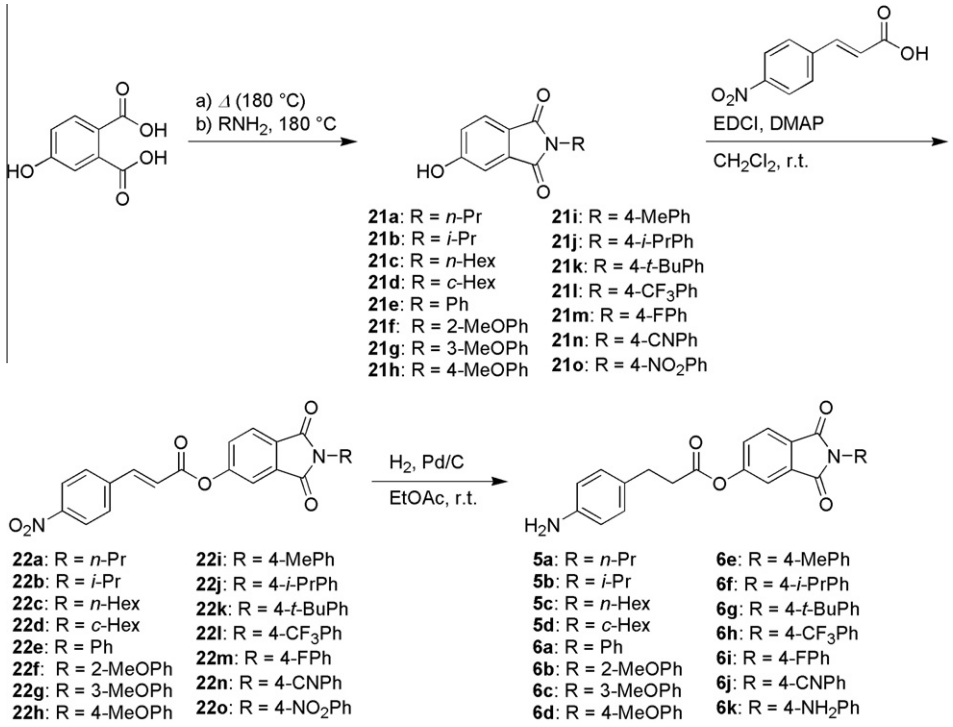
4.2. Materials

Human β-tryptase (5.2 U/mg protein) was purchased from Wako Pure Chemicals, Inc. (Osaka, Japan). Bovine thrombin (74.0 U/mg protein) was purchased from Calbiochem Co. (San Diego, CA, USA). Bovine type-1 trypsin (9590 U/mg protein) was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). *tert*-Butoxycarbonyl-L-phenylalanyl-L-seryl-L-arginine-4-methylcoumaryl-7-amide (*t*-Boc-Phe-Ser-Arg-MCA) was purchased from Peptide Institute, Inc. (Osaka, Japan).

4.3. Chemistry

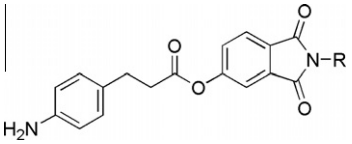
4.3.1. 5-Hydroxy-2-methylisoindoline-1,3-dione (8)

4-Hydroxyphthalic acid (182 mg, 1.00 mmol) was heated at 180 °C for 4.5 h, then cooled to room temperature. To this solution was added methylamine hydrochloride (81.3 mg, 1.20 mmol), and the mixture was heated at 180 °C for 1.5 h. After cooling to room temperature, the mixture was diluted with CH_2Cl_2, and then washed with 1 N HCl, water, and brine. The organic layer was dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/AcOEt = 2:1)



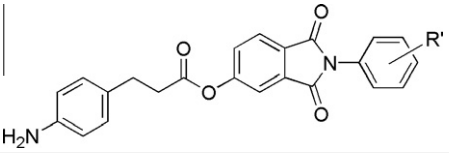
Scheme 4. Synthesis of **5a–d** and **6a–k**.

Table 3
Effects of N-substituents of phthalimide on tryptase-inhibitory activity



	R	IC_{50} (μM)
2a	Me	1.5
5a	*n*-Pr	1.2
5b	*i*-Pr	1.2
5c	*n*-Hex	1.5
5d	*c*-Hex	1.2
6a	Ph	1.3

Table 4
Effects of substituents of *N*-phenylphthalimide on tryptase-inhibitory activity



	R′	IC_{50} (μM)
6a	H	1.3
6b	2-OMe	1.1
6c	3-OMe	0.65
6d	4-OMe	0.39
6e	4-Me	1.2
6f	5-*i*-Pr	1.8
6g	4-*t*-Bu	>30[a]
6h	4-CF_3	N.A.[b]
6i	4-F	1.7
6j	4-CN	0.34
6k	4-NH_2	0.82

[a] Failed to reach 50% inhibition at 30 μM.
[b] No activity at 30 μM.

to give **8** (160 mg, 0.903 mmol, 90%) as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.88 (s, 1H), 7.67 (d, J = 7.9 Hz, 1H), 7.11 (d, J = 2.4 Hz, 1H), 7.08 (dd, J = 7.9, 2.4 Hz, 1H), 2.97 (s, 3H); mp 247–250 °C; MS (FAB) m/z 178 $(M+H)^+$.

4.3.2. 2-Methylisoindole-1,3-dione-5-yl 2-aminobenzoate (2a)

To a solution of anthranilic acid (45.3 mg, 0.330 mmol), **8** (48.2 mg, 0.272 mmol), and PPh_3 (88.9 mg, 0.339 mmol) in THF (3.0 mL) was added DEAD (140 μL, 2.2 M in toluene, 0.308 mmol) at room temperature, and the mixture was stirred at the same temperature. After 3 h, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (*n*-hexane/AcOEt = 6:1 to 4:1) followed by recrystallization from *n*-hexane/AcOEt to give **2a** (35.1 mg, 0.118 mmol, 44%) as a yellow powder. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.94–7.92 (m, 2H), 7.79 (d, J = 2.4 Hz, 1H), 7.67 (dd, J = 7.9, 2.4 Hz, 1H), 7.35 (dd, J = 7.9, 7.9 Hz, 1H), 6.84 (d, J = 7.9 Hz, 1H), 6.76 (br s, 2H), 6.61 (dd, J = 7.9, 7.9 Hz, 2H), 3.04 (s, 3H); mp 198–200 °C; HRMS (FAB) calcd for $C_{16}H_{13}N_2O_4$ 297.0875; found: 297.0874 $(M+H)^+$.

4.3.3. 2-Methylisoindole-1,3-dione-5-yl 3-nitrobenzoate (9b): General procedure for the synthesis of 9c–h

To a solution of 3-nitrobenzoic acid (1.2 equiv) in CH_2Cl_2 was added **8** (1 equiv), EDCI (1.5 equiv) and DMAP (0.2 equiv), and the mixture was stirred for 4 h at room temperature, then washed with saturated aqueous $NaHCO_3$, water, and brine. The organic layer was dried over $MgSO_4$, and concentrated under reduced pressure. The residue was recrystallized from *n*-hexane/CH_2Cl_2 to give **9b** (96%) as a white powder; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.05 (s, 1H), 8.56–8.53 (m, 2H), 7.95 (d, J = 7.9 Hz, 1H), 7.80–7.77 (m, 2H), 7.58 (dd, J = 7.9, 1.8 Hz, 1H), 2.81 (s, 3H); MS (FAB) m/z 327 $(M+H)^+$.

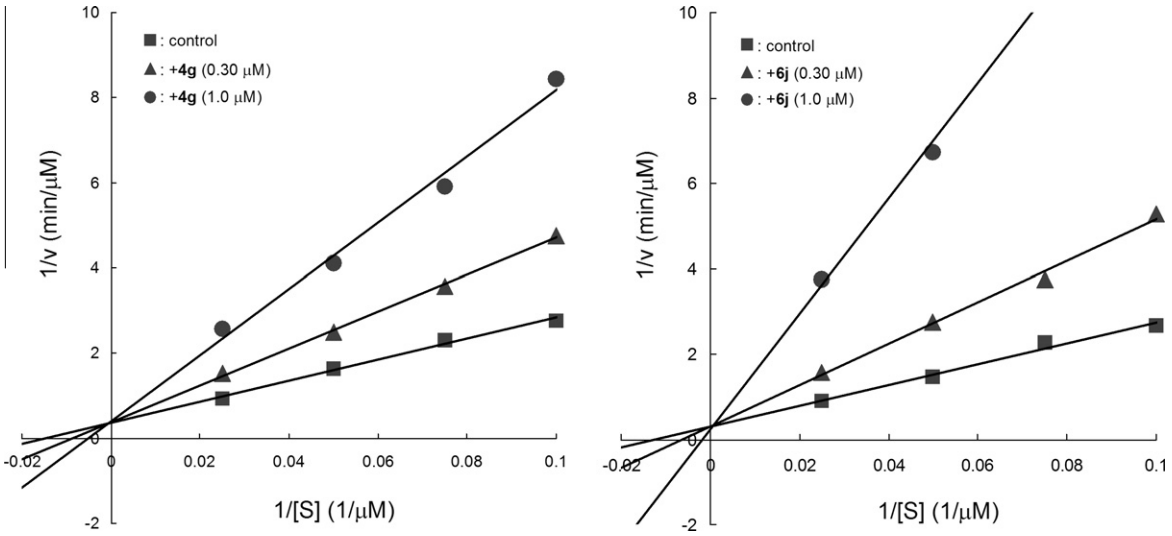
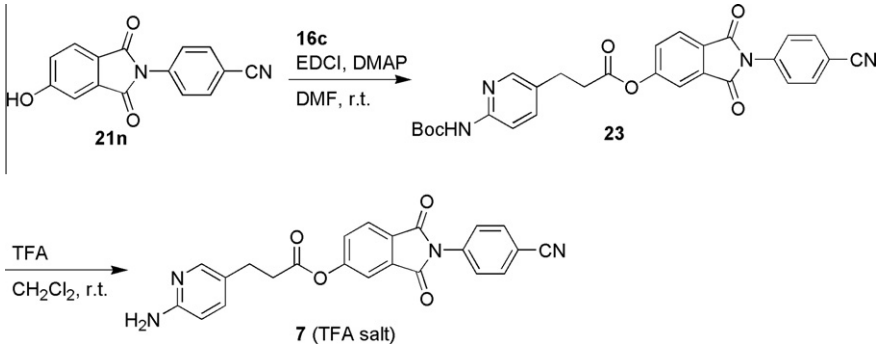


Figure 3. Lineweaver–Burk plot analysis of **4g** and **6j**.



Scheme 5. Synthesis of hybrid compound **7**.

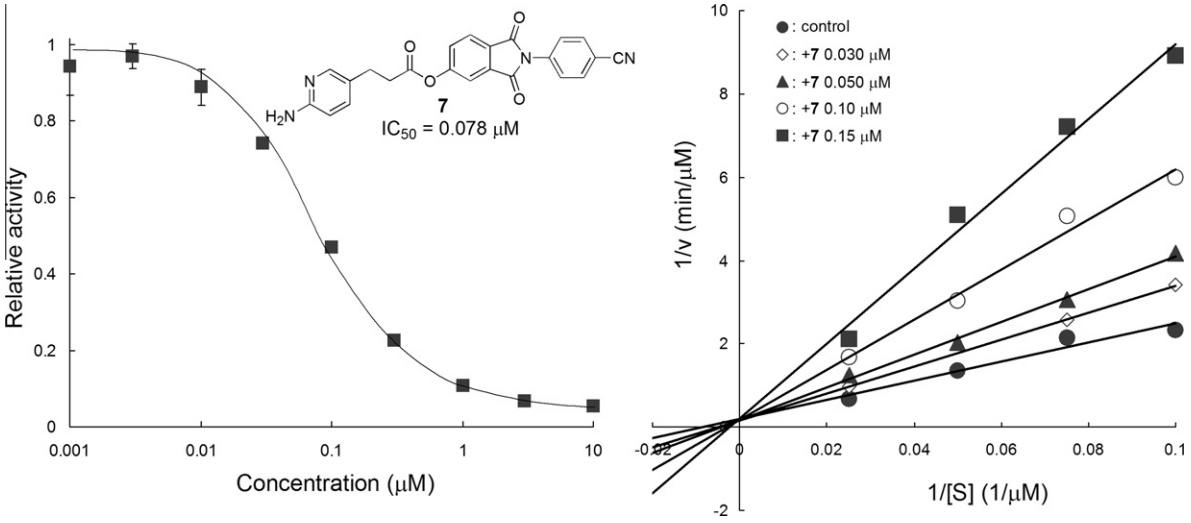


Figure 4. Tryptase-inhibitory activity and Lineweaver–Burk plot analysis for **7**.

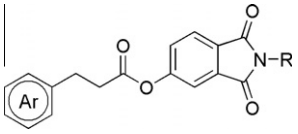
4.3.4. 2-Methylisoindole-1,3-dione-5-yl 4-nitrobenzoate (9c)

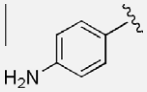
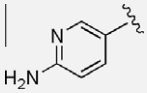
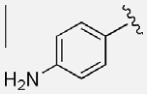
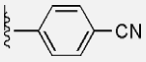
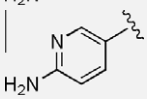
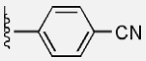
White powder (88%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.40 (m, 4H), 7.95 (d, J = 7.9 Hz, 1H), 7.76 (d, J = 1.8 Hz, 1H), 7.57 (dd, J = 7.9, 1.8 Hz, 1H), 3.21 (s, 3H); MS (FAB) m/z 327 $(M+H)^+$.

4.3.5. 2-Methylisoindole-1,3-dione-5-yl 2-(3-nitrophenyl) acetate (9d)

White powder (98%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.27 (s, 1H), 8.23 (d, J = 7.9 Hz, 1H), 7.86 (d, J = 7.9 Hz, 1H), 7.73 (d, J = 7.9 Hz,

Table 5
Selectivity of compounds **2g**, **4g**, **6j**, and **7** for serine proteases



	Ar	R	IC_{50} (μM)	K_i (μM)		
			Tryptase	Tryptase	Thrombin	Trypsin
2g		Me	1.5	1.7	15 (8.82)	13 (7.65)
4g		Me	0.40	0.34	7.0 (20.6)	2.9 (8.53)
6j			0.34	0.19	8.6 (45.2)	4.3 (22.6)
7			0.078	0.044	16 (364)	0.88 (20.0)

Selectivity index relative to tryptase is shown in parentheses.

1H), 7.61–7.58 (m, 2H), 7.41 (dd, *J* = 7.9, 1.8 Hz, 1H), 4.04 (s, 2H), 3.21 (s, 3H); MS (FAB) *m/z* 341 $(M+H)^+$.

4.3.6. 2-Methylisoindole-1,3-dione-5-yl 2-(4-nitrophenyl) acetate (9e)

White powder (74%); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.27 (d, *J* = 7.9 Hz, 2H), 7.86 (d, *J* = 7.9 Hz, 1H), 7.58–7.56 (m, 2H), 7.39 (dd, *J* = 7.9, 1.8 Hz, 1H), 4.04 (s, 2H), 3.18 (s, 3H); MS (FAB) *m/z* 341 $(M+H)^+$.

4.3.7. 2-Methylisoindole-1,3-dione-5-yl (*E*)-3-(3-nitrophenyl) acrylate (9f)

White powder (98%); ^1H NMR (DMSO-d_6, 500 MHz) δ 8.68 (dd, *J* = 1.8, 1.8 Hz, 1H), 8.33 (dd, *J* = 7.9, 1.8 Hz, 1H), 8.30 (dd, *J* = 7.9, 1.8 Hz, 1H), 8.08 (d, *J* = 15.9 Hz, 1H), 7.95 (d, *J* = 7.9 Hz, 1H), 7.79 (d, *J* = 1.8 Hz, 1H), 7.76 (dd, *J* = 7.9, 7.9 Hz, 1H), 7.66 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.17 (d, *J* = 15.9 Hz, 1H), 3.04 (s, 3H); MS (FAB) *m/z* 353 $(M+H)^+$.

4.3.8. 2-Methylisoindole-1,3-dione-5-yl (*E*)-3-(4-nitrophenyl) acrylate (9g)

White powder (80%); ^1H NMR (DMSO-d_6, 500 MHz) δ 8.29 (d, *J* = 9.1 Hz, 2H), 8.12 (d, *J* = 9.1 Hz, 2H), 8.04 (d, *J* = 15.9 Hz, 1H), 7.95 (d, *J* = 7.9 Hz, 1H), 7.80 (d, *J* = 1.8 Hz, 1H), 7.67 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.16 (d, *J* = 15.9 Hz, 1H), 3.04 (s, 3H); mp 194–196 °C; MS (FAB) *m/z* 353 $(M+H)^+$.

4.3.9. 2-Methylisoindole-1,3-dione-5-yl 4-(4-nitrophenyl) butanoate (9h)

White powder (94%); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.18 (d, *J* = 8.5 Hz, 2H), 7.86 (d, *J* = 7.9 Hz, 1H), 7.57 (d, *J* = 1.8 Hz, 1H), 7.39–7.37 (m, 3H), 3.18 (s, 3H), 2.87 (t, *J* = 7.3 Hz, 2H), 2.66 (t, *J* = 7.3 Hz, 2H), 2.13 (tt, *J* = 7.3, 7.3 Hz, 2H); MS (FAB) *m/z* 369 $(M+H)^+$.

4.3.10. 2-Methylisoindole-1,3-dione-5-yl 3-aminobenzoate (2b): General procedure for the synthesis of 2c–h

To a solution of **9b** (1 equiv) in ethyl acetate (5.0 mL) was added 10% Pd/C (10 wt%), and the mixture was stirred for 6 h at room temperature under an H_2 atmosphere. The reaction mixture was filtered through a pad of Celite®, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography followed by recrystallization from *n*-hexane/CH_2Cl_2 to give **2b** (69%) as a white powder; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.91 (d, *J* = 7.9 Hz, 1H), 7.72 (d, *J* = 2.4 Hz, 1H), 7.59 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.53 (dd, *J* = 7.9, 2.4 Hz, 1H), 7.48 (dd, *J* = 1.8, 1.8 Hz, 1H), 7.31 (dd, *J* = 7.9, 7.9 Hz, 1H), 6.97 (dd, *J* = 7.9, 1.8 Hz, 1H), 3.87 (br s, 2H), 3.20 (s, 3H); mp 165–166 °C; HRMS (FAB) calcd for $C_{16}H_{13}N_2O_4$ 297.0875; found: 297.0899 $(M+H)^+$.

4.3.11. 2-Methylisoindole-1,3-dione-5-yl 4-aminobenzoate (2c)

Yellow powder (78%); ^1H NMR (DMSO-d_6, 500 MHz) δ 7.91 (d, *J* = 7.9 Hz, 1H), 7.81 (d, *J* = 7.9 Hz, 2H), 7.73 (d, *J* = 1.8 Hz, 1H), 7.64 (d, *J* = 7.3 Hz, 1H), 6.64 (d, *J* = 7.3 Hz, 2H), 6.26 (br s, 2H), 3.03 (s, 3H); mp 216–218 °C; HRMS (FAB) calcd for $C_{16}H_{13}N_2O_4$ 297.0875; found: 297.0853 $(M+H)^+$.

4.3.12. 2-Methylisoindole-1,3-dione-5-yl 2-(3-nitrophenyl) acetate (2d)

White powder (80%); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.83 (d, *J* = 7.9 Hz, 1H), 7.56 (d, *J* = 2.4 Hz, 1H), 7.38 (dd, *J* = 7.9, 2.4 Hz, 1H), 7.16 (dd, *J* = 7.3, 7.3 Hz, 1H), 6.76 (d, *J* = 7.3 Hz, 1H), 6.70 (s, 1H), 6.65 (d, *J* = 7.3 Hz, 1H), 3.79 (s, 1H), 3.73 (br s, 2H), 3.17 (s, 3H); mp 92–94 °C; HRMS (FAB) calcd for $C_{17}H_{15}N_2O_4$ 311.1032; found: 311.1041 $(M+H)^+$.

4.3.13. 2-Methylisoindole-1,3-dione-5-yl 2-(4-aminophenyl) acetate (2e)

Yellow powder (69%); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.83 (d, *J* = 8.5 Hz, 1H), 7.55 (d, *J* = 1.8 Hz, 1H), 7.36 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.15 (d, *J* = 7.9 Hz, 2H), 6.69 (d, *J* = 7.9 Hz, 2H), 3.77 (s, 2H), 3.17 (s, 3H); mp 127–129 °C; HRMS (FAB) calcd for $C_{17}H_{14}N_2O_4$ 310.0954; found: 310.0955 $(M)^+$.

4.3.14. 2-Methylisoindole-1,3-dione-5-yl 3-(3-aminophenyl) propionate (2f)

White powder (59%); ^1H NMR (DMSO-d_6, 500 MHz) δ 7.89 (d, *J* = 7.9 Hz, 1H), 7.60 (d, *J* = 1.8 Hz, 1H), 7.47 (dd, *J* = 7.9, 1.8 Hz,

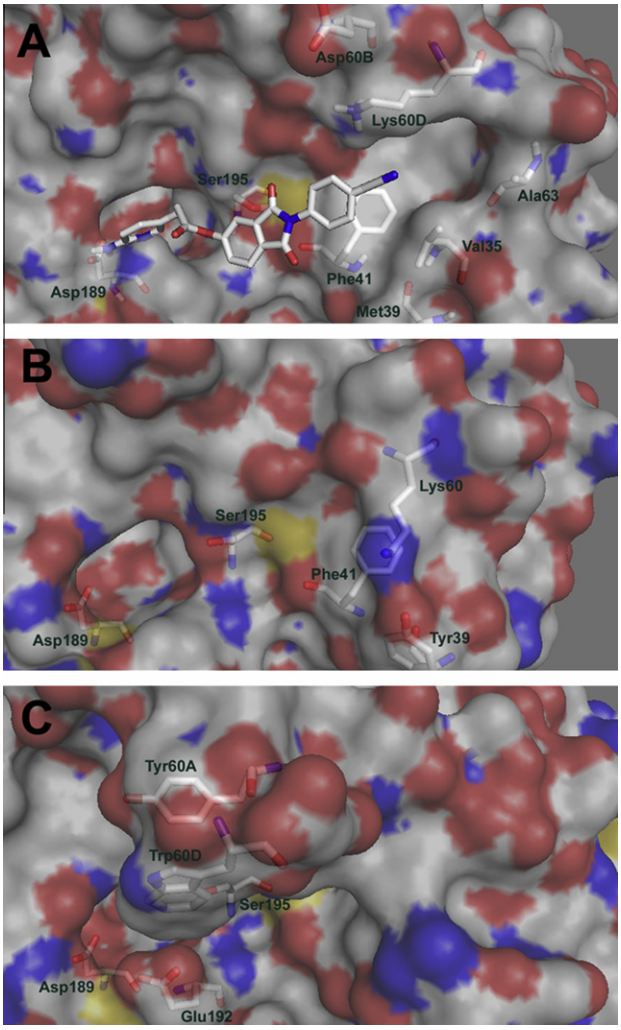


Figure 5. Catalytic core domains of tryptase, trypsin, and thrombin. (A) Compound **7** docked into the X-ray crystal structure of tryptase (PDB ID: 2FXR[40]) using AutoDock 4.2 docking program.[41] (B) Surface map of the X-ray crystal structure of trypsin (PDB ID: 1TPP[38]). (C) Surface map of the X-ray crystal structure of thrombin (PDB ID: 1UVU[39]). The images were generated by PyMOL.[42]

1H), 6.94 (dd, J = 7.9, 7.3 Hz, 1H), 6.46 (s, 1H), 6.41–6.40 (m, 2H), 4.99 (br s, 2H), 3.02 (s, 3H), 2.88 (t, J = 6.7 Hz, 2H), 2.82 (t, J = 6.7 Hz, 2H); mp 100–102 °C; HRMS (FAB) calcd for $C_{18}H_{17}N_2O_4$ 325.1188; found: 325.1210 $(M+H)^+$.

4.3.15. 2-Methylisoindole-1,3-dione-5-yl 3-(4-aminophenyl) propionate (2g)

Yellow powder (94%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.83 (d, J = 7.9 Hz, 1H), 7.48 (d, J = 1.8 Hz, 1H), 7.31 (dd, J = 7.9, 1.8 Hz, 1H), 7.05 (d, J = 8.5 Hz, 2H), 6.67 (d, J = 8.5 Hz, 2H), 3.63 (br s, 2H), 3.18 (s, 3H), 2.98 (t, J = 7.3 Hz, 2H), 2.87 (t, J = 7.3 Hz, 2H); mp 127–129 °C; HRMS (FAB) calcd for $C_{18}H_{17}N_2O_4$ 325.1188; found: 325.1173 $(M+H)^+$.

4.3.16. 2-Methylisoindole-1,3-dione-5-yl 4-(4-aminophenyl) butanoate (2h)

White powder (62%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.84 (d, J = 7.9 Hz, 1H), 7.53 (d, J = 1.8 Hz, 1H), 7.35 (dd, J = 7.9, 1.8 Hz, 1H), 7.01 (d, J = 8.5 Hz, 2H), 6.67 (d, J = 8.5 Hz, 2H), 3.18 (s, 3H), 2.65 (t, J = 7.3 Hz, 2H), 2.60 (t, J = 7.3 Hz, 2H), 2.05 (tt, J = 7.3, 7.3 Hz, 2H); mp 80–82 °C; HRMS (FAB) calcd for $C_{19}H_{19}N_2O_4$ 339.1345; found: 339.1317 $(M+H)^+$.

4.3.17. Phenyl 3-(4-aminophenyl)propionate (3)

This compound was prepared by a similar method to that used for the synthesis of **2b** via preparation of phenyl 4-nitrocinnamate (**10**), starting from phenol and 4-nitrocinnamic acid. White solid (87%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36 (dd, J = 8.5, 7.3 Hz, 2H), 7.21 (t, J = 7.3 Hz, 1H), 7.06 (d, J = 7.3 Hz, 2H), 7.02 (d, J = 7.3 Hz, 2H), 6.66 (d, J = 8.5 Hz, 2H), 3.60 (br s, 2H), 2.97 (t, J = 7.3 Hz, 2H) 2.82 (t, J = 7.3 Hz, 2H); mp 95–97 °C; HRMS (FAB) calcd for $C_{15}H_{15}NO_2$ 241.1103; found: 241.1095 $(M)^+$.

4.3.18. Methyl (*E*)-3-[4-(dimethylamino)phenyl]acrylate (11a)

To a stirred solution of trimethyl phosphonoacetate (400 μL, 2.48 mmol) in THF (10 mL) was added *t*-BuOK (270 mg, 2.41 mmol) at 0 °C, and the mixture was stirred at the same temperature. After 30 min, 4-(dimethylamino)benzaldehyde (308 mg, 2.06 mmol) was further added, and the mixture was stirred for 4.5 h at room temperature. The reaction was quenched by the addition of saturated aqueous NH_4Cl and the whole was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by Chromatorex® column chromatography (*n*-hexane/AcOEt = 10:1) to give **11a** (340 mg, 1.66 mmol, 81%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.63 (d, J = 15.9 Hz, 1H), 7.42 (d, J = 9.1 Hz, 2H), 6.67 (d, J = 9.1 Hz, 2H), 6.22 (d, J = 15.9 Hz, 1H), 3.78 (s, 3H), 3.02 (s, 6H); MS (FAB) m/z 205 $(M)^+$.

4.3.19. (*E*)-Methyl 3-(pyridin-4-yl)acrylate (11b)

This compound was prepared by a similar method to that used for the synthesis of **11a**, starting from 4-pyridinecarboxaldehyde. White solid (quant.); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.65 (d, J = 6.1 Hz, 2H), 7.60 (d, J = 15.9 Hz, 1H), 7.36 (d, J = 6.1 Hz, 2H), 6.59 (d, J = 15.9 Hz, 1H), 3.83 (s, 3H); MS (FAB) m/z 164 $(M)^+$.

4.3.20. (*E*)-3-[4-(Dimethylamino)phenyl]acrylic acid (12a)

To a solution of **11a** (330 mg, 1.61 mmol) in a mixture of THF (5.0 mL) and MeOH (5.0 mL) was added 1 M LiOH aq (5.0 mL), and the mixture was stirred for 24 h at room temperature. The reaction was quenched by acidification with 1 N HCl and then extracted with ethyl acetate. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give **12a** (290 mg, 1.52 mmol, 94%) as a yellow solid. This material was used in the next step without further purification. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.47 (d, J = 9.1 Hz, 2H), 7.45 (d, J = 15.9 Hz, 1H), 6.69 (d, J = 9.1 Hz, 2H), 6.19 (d, J = 15.9 Hz, 1H), 2.95 (s, 6H); mp 134–135 °C; MS (FAB) m/z 191 $(M)^+$.

4.3.21. (*E*)-3-(Pyridin-4-yl)acrylic acid (12b)

This compound was prepared by a similar method to that used for the synthesis of **12a**, starting from **11b**. White solid (78%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.71 (s, 1H), 8.60 (d, J = 6.7 Hz, 2H), 7.65 (d, J = 6.7 Hz, 2H), 7.55 (d, J = 15.9 Hz, 1H), 6.77 (d, J = 15.9 Hz, 1H); mp 277–280 °C; MS (FAB) m/z 150 $(M+H)^+$.

4.3.22. 2-Methylisoindole-1,3-dione-5-yl 3-phenylpropionate (4a)

This compound was prepared by a similar method to that used for the synthesis of **9b**, starting from hydrocinnamic acid. White powder (86%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.83 (d, J = 7.9 Hz, 1H), 7.50 (d, J = 1.8 Hz, 1H), 7.36–7.33 (m, 2H), 7.30 (dd, J = 7.9, 1.8 Hz, 1H), 7.27–7.24 (m, 3H), 3.18 (s, 3H), 3.09 (t, J = 7.3 Hz, 2H), 2.94 (t, J = 7.3 Hz, 2H); mp 83–85 °C; HRMS (FAB) calcd for $C_{18}H_{16}NO_4$ 310.1079; found: 310.1063 $(M+H)^+$.

4.3.23. 2-Methylisoindole-1,3-dione-5-yl 3-(4-methoxyphenyl) propionate (4b)

This compound was prepared by a similar method to that used for the synthesis of **9b**, starting from 4-methoxyhydrocinnamic acid as a starting material. White powder (80%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.83 (d, J = 7.9 Hz, 1H), 7.49 (d, J = 1.8 Hz, 1H), 7.31 (dd, J = 7.9, 1.8 Hz, 1H), 7.18 (d, J = 8.5 Hz, 2H), 6.87 (d, J = 8.5 Hz, 2H), 3.81 (s, 3H), 3.18 (s, 3H), 3.03 (t, J = 7.3 Hz, 2H), 2.90 (t, J = 7.3 Hz, 2H); mp 105–107 °C; HRMS (FAB) calcd for $C_{19}H_{18}NO_5$ 340.1185; found: 340.1184 $(M+H)^+$.

4.3.24. 2-Methylisoindole-1,3-dione-5-yl (*E*)-3-[4-(dimethylamino)phenyl]acrylate (13a)

This compound was prepared by a similar method to that used for the synthesis of **9b**, starting from **12a**. Yellow solid (72%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.87 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 15.9 Hz, 1H), 7.67 (d, J = 1.8 Hz, 1H), 7.50–7.47 (m, 3H), 6.70 (d, J = 8.5 Hz, 2H), 6.38 (d, J = 15.9 Hz, 1H), 3.19 (s, 3H), 3.06 (s, 6H); mp 182–184 °C; MS (FAB) m/z 350 $(M)^+$, 351 $(M+H)^+$.

4.3.25. 2-Methylisoindole-1,3-dione-5-yl (*E*)-3-(pyridin-4-yl) acrylate (13b)

This compound was prepared by similar method to that used for the synthesis of **9b**, starting from **12b**. White solid (74%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.73 (d, J = 6.1 Hz, 2H), 7.91 (d, J = 7.9 Hz, 1H), 7.83 (d, J = 15.9 Hz, 1H), 7.69 (d, J = 1.8 Hz, 1H), 7.50 (dd, J = 7.9, 1.8 Hz, 1H), 7.45 (d, J = 6.1 Hz, 2H), 6.80 (d, J = 15.9 Hz, 1H), 3.20 (s, 3H); MS (FAB) m/z 309 $(M+H)^+$.

4.3.26. 2-Methylisoindole-1,3-dione-5-yl 3-[4-(dimethylamino) phenyl]propionate (4c)

This compound was prepared by a similar method to that used for the synthesis of **2b**, starting from **13a**. White needles (54%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.81 (d, J = 8.5 Hz, 1H), 7.48 (d, J = 1.8 Hz, 1H), 7.29 (dd, J = 8.5, 1.8 Hz, 1H), 7.11 (d, J = 8.5 Hz, 2H), 6.70 (d, J = 8.5 Hz, 2H), 3.15 (s, 3H), 2.98 (d, J = 7.3 Hz, 2H), 2.92 (s, 6H), 2.86 (d, J = 7.3 Hz, 2H); mp 130–131 °C; HRMS (FAB) calcd for $C_{20}H_{20}N_2O_4$ 352.1423; found: 352.1404 $(M)^+$.

4.3.27. 2-Methylisoindole-1,3-dione-5-yl 3-(pyridin-4-yl) propionate (4d)

This compound was prepared by a similar method to that used for the synthesis of **2b**, starting from **13b**. White powder (68%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.57 (d, J = 6.1 Hz, 2H), 7.85 (d, J = 7.9 Hz, 1H), 7.55 (d, J = 1.8 Hz, 1H), 7.33 (dd, J = 7.9, 1.8 Hz, 1H), 7.20 (d, J = 6.1 Hz, 2H), 3.18 (s, 3H), 3.08 (t, J = 7.3 Hz, 2H), 2.98 (t, J = 7.3 Hz, 2H); mp 136–138 °C; HRMS (FAB) calcd for $C_{17}H_{15}N_2O_4$ 311.1032; found: 311.1024 $(M+H)^+$.

4.3.28. Ethyl (*E*)-3-(2-methoxy-4-nitrophenyl)acrylate (14a)

This compound was prepared by a similar method to that used for the synthesis of **11a**, starting from 2-methoxy-4-nitrobenzaldehyde and triethyl phosphonoacetate. Yellow solid (95%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ; 7.94 (d, J = 16.5 Hz, 1H), 7.84 (dd, J = 7.9, 1.8 Hz, 1H), 7.76 (d, J = 1.8 Hz, 1H), 7.63 (d, J = 7.9 Hz, 1H), 6.62 (d, J = 16.5 Hz, 1H), 4.29 (q, J = 7.3 Hz, 2H), 4.00 (s, 3H), 1.35 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 252 $(M+H)^+$.

4.3.29. Ethyl (*E*)-3-(3-methoxy-4-nitrophenyl)acrylate (14b)

This compound was prepared by a similar method to that used for the synthesis of **11a**, starting from 3-methoxy-4-nitrobenzaldehyde and triethyl phosphonoacetate. Yellow solid (96%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ; 7.87 (d, J = 7.9 Hz, 1H), 7.64 (d, J = 15.9 Hz, 1H), 7.19–7.17 (m, 2H), 6.51 (d, J = 15.9 Hz, 1H), 4.29 (q, J = 7.3 Hz, 2H), 4.00 (s, 3H), 1.35 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 252 $(M+H)^+$.

4.3.30. Ethyl 3-[4-(*tert*-butoxycarbonyl)amino-3-methoxyphenyl]propionate (15b)

To a solution of **14b** (252 mg, 1.00 mmol) in ethyl acetate was added 10% Pd/C (21.3 mg), and the mixture was stirred for 4 h at room temperature under an H_2 atmosphere. The reaction was filtered through a pad of Celite, which was washed with ethyl acetate, and the combined filtrate was concentrated under reduced pressure. The residue was dissolved in toluene, and Boc_2O (300 μL, 1.31 mmol) was added. The reaction was refluxed for 4 h at 120 °C. The reaction mixture cooled to room temperature, then concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 8:1) to give **15b** (298 mg, 0.922 mmol, 92% [two steps]) as a yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ; 7.94 (m, 1H), 6.99 (s, 1H), 6.77 (dd, J = 7.9, 1.8 Hz, 1H), 6.69 (d, J = 1.8 Hz, 1H), 4.12 (q, J = 7.3 Hz, 2H), 3.85 (s, 3H), 2.89 (t, J = 7.9 Hz, 2H), 2.59 (t, J = 7.9 Hz, 2H), 1.51 (s, 9H), 1.24 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 323 $(M)^+$.

4.3.31. Ethyl 3-[4-(di-*tert*-butoxycarbonyl)amino-2-methoxyphenyl]propionate (15a)

This compound was prepared by a similar method to that used for the synthesis of **15b**, starting from **14a**. White solid (quant., two steps); ^{1}H NMR ($CDCl_3$, 500 MHz) δ; 7.11 (d, J = 7.9 Hz, 1H), 6.67 (dd, J = 7.9, 1.8 Hz, 1H), 6.62 (d, J = 1.8 Hz, 1H), 4.12 (q, J = 7.3 Hz, 2H), 3.80 (s, 3H), 2.92 (t, J = 7.3 Hz, 2H), 2.58 (t, J = 7.3 Hz, 2H), 1.43 (s, 18H), 1.24 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 423 $(M)^+$.

4.3.32. 3-[4-(*tert*-Butoxycarbonyl)amino-2-methoxyphenyl]propionic acid (16a)

This compound was prepared by a similar method to that used for the synthesis of **12a**, starting from **15a**. White solid (96%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ; 7.18 (m, 1H), 7.03 (d, J = 7.9 Hz, 1H), 6.64 (dd, J = 7.9, 1.8 Hz, 1H), 6.51 (br s, 1H), 3.82 (s, 3H), 2.88 (t, J = 7.6 Hz, 2H), 2.62 (t, J = 7.6 Hz, 2H), 1.52 (s, 9H); MS (FAB) m/z 295 $(M)^+$.

4.3.33. 3-[4-(*tert*-Butoxycarbonyl)amino-3-methoxyphenyl]propionic acid (16b)

This compound was prepared by a similar method to that used for the synthesis of **12a**, starting from **15b**. Yellow oil (quant.); ^{1}H NMR ($CDCl_3$, 500 MHz) δ; 7.95 (m, 1H), 7.01 (s, 1H), 6.78 (dd, J = 7.9, 1.8 Hz, 1H) 6.70 (d, J = 1.8 Hz, 1H), 3.84 (s, 3H), 2.91 (t, J = 7.9 Hz, 2H), 2.65 (d, J = 7.9 Hz, 2H), 1.51 (s, 9H); MS (FAB) m/z 295 $(M)^+$.

4.3.34. Methyl (*E*)-3-(6-aminopyridin-3-yl)acrylate (17a)

To a solution of 2-amino-5-bromopyridine (346 mg, 2.00 mmol), $Pd(OAc)_2$ (20.3 mg, 90.4 μmol), P(*o*-tol)$_3$ (60.1 mg, 0.197 mmol), TEA (340 μL, 2.43 mmol) in DMF (10 mL) was added methyl acrylate (200 μL, 2.21 mmol), and the mixture was stirred at 100 °C for 6 h. The reaction was quenched by the addition of water and the whole was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/ethyl acetate/MeOH = 4:1:0 to 2:1:0 to 2:1:0.1) to give **17a** (237 mg, 1.33 mmol, 66%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.15 (d, J = 2.4 Hz, 1H), 7.78 (dd, J = 8.5, 2.4 Hz, 1H), 7.49 (d, J = 15.9 Hz, 1H), 6.57 (br s, 2H), 6.44 (d, J = 8.5 Hz, 1H), 6.31 (d, J = 15.9 Hz, 1H), 3.67 (s, 3H); mp 173–174 °C; MS (FAB) m/z 179 $(M+H)^+$.

4.3.35. Methyl (*E*)-3-(2-aminopyrimidin-5-yl)acrylate (17b)

This compound was prepared by a similar method to that used for the synthesis of **17a**, starting from 2-amino-5-bromopyrimidine. Yellow solid (70%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.60 (s,

2H), 7.47 (d, J = 15.9 Hz, 1H), 7.19 (br s, 2H), 6.51 (d, J = 15.9 Hz, 1H), 3.68 (s, 3H); MS (FAB) m/z 180 $(M+H)^+$.

4.3.36. Methyl 3-(6-*tert*-butoxycarbonylaminopyridin-3-yl)acrylate (18a)

To a solution of **17a** (333 mg, 1.87 mmol), TEA (280 μL, 2.00 mmol), DMAP (8.1 mg, 66.3 μmol) in dioxane (8 mL) was added Boc_2O (460 μL, 2.00 mmol), and the mixture stirred at room temperature. After 4 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/ethyl acetate = 4:1) to give **18a** (305 mg, 1.10 mmol, 59%) as a white solid. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.36 (d, J = 2.4 Hz, 1H), 7.99 (d, J = 8.5 Hz, 1H), 7.84 (dd, J = 8.5, 2.4 Hz, 1H), 7.64 (br s, 1H), 7.62 (d, J = 15.9 Hz, 1H), 6.40 (d, J = 15.9 Hz, 1H), 3.81 (s, 3H), 1.54 (s, 9H); MS (FAB) m/z 279 $(M+H)^+$.

4.3.37. Methyl 3-(2-di-*tert*-butoxycarbonylaminopyrimidin-5-yl)acrylate (18b)

This compound was prepared by a similar method to that used for the synthesis of **18a**, starting from **17b**, at 60 °C. White solid (59%); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.85 (s, 2H), 7.62 (d, J = 15.9 Hz, 1H), 6.56 (d, J = 15.9 Hz, 1H), 3.84 (s, 3H), 1.48 (s, 18H); MS (FAB) m/z 380 $(M+H)^+$.

4.3.38. Methyl 3-(6-*tert*-butoxycarbonylaminopyridin-3-yl) propionate (19a)

To a solution of **18a** (305 mg, 1.10 mmol) in MeOH (10 mL) was added 10% Pd/C (28.3 mg), and the mixture was stirred for 2 h at 50 °C under a H_2 atmosphere, then cooled to room temperature, and filtered through a pad of Celite®. The filtrate was concentrated under reduced pressure to give **19a** (302 mg, 1.08 mmol, 98%) as a white solid. This material was used in the next step without further purification. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.08 (d, J = 2.4 Hz, 1H), 7.85 (d, J = 8.5 Hz, 1H), 7.50 (dd, J = 8.5, 2.4 Hz, 1H), 7.30 (br s, 1H), 3.66 (s, 3H), 2.89 (t, J = 7.3 Hz, 2H), 2.60 (t, J = 7.3 Hz, 2H), 1.52 (s, 9H); MS (FAB) m/z 281 $(M+H)^+$.

4.3.39. Methyl 3-(2-di-*tert*-butoxycarbonylaminopyrimidin-5-yl)propionate (19b)

This compound was prepared by a similar method ti that used for the synthesis of **19a**, starting from **18b**, at room temperature. Colorless oil (quant.); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.62 (s, 2H), 3.67 (s, 3H), 2.97 (t, J = 7.3 Hz, 2H), 2.67 (t, J = 7.3 Hz, 2H), 1.45 (s, 18H); MS (FAB) m/z 382 $(M+H)^+$.

4.3.40. 3-(6-*tert*-Butoxycarbonylaminopyridin-3-yl)propionic acid (16c)

To a solution of **19a** (302 mg, 1.08 mmol) in dioxane (3 mL) and MeOH (5 mL) was added 1 M LiOH aq (3 mL), and the mixture was stirred for 12 h at room temperature. The reaction was quenched by the addition of water, and the whole was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was recrystallized from ethyl acetate and *n*-hexane to give **16c** (139 mg, 0.522 mmol, 48%) as a white solid. ^1H NMR (DMSO-d_6, 500 MHz) δ 12.14 (br s, 1H), 9.62 (br s, 1H), 8.08 (d, J = 2.4 Hz, 1H), 7.67 (d, J = 8.5 Hz, 1H), 7.58 (dd, J = 8.5, 2.4 Hz, 1H), 2.77 (t, J = 7.3 Hz, 2H), 2.61 (t, J = 7.3 Hz, 2H), 1.44 (s, 9H); mp >230 °C (decompose); MS (FAB) m/z 267 $(M+H)^+$.

4.3.41. 3-(2-*tert*-Butoxycarbonylaminopyrimidin-5-yl) propionic acid (16d)

This compound was prepared by a similar method to that used for the synthesis of **16c**, starting from **19b**. White solid (45%); ^1H NMR (DMSO-d_6, 500 MHz) δ 12.21 (br s, 1H), 9.92 (br s, 1H), 8.46 (s, 2H), 2.73 (t, J = 7.3 Hz, 2H), 2.56 (t, J = 7.3 Hz, 2H), 1.44 (s, 9H); MS (FAB) m/z 268 $(M+H)^+$.

4.3.42. 2-Methylisoindole-1,3-dione-5-yl 3-[4-(*tert*-Butoxycarbonyl)amino-2-methoxyphenyl]propionate (20a)

This compound was prepared by a similar method to that used for the synthesis of **4a**, starting from **8** and **16a**. White solid (89%); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.83 (d, J = 7.9 Hz, 1H), 7.51 (d, J = 2.4 Hz, 1H), 7.32 (dd, J = 7.9, 2.4 Hz, 1H), 7.25 (d, J = 1.8 Hz, 1H), 7.07 (d, J = 7.9 Hz, 1H), 6.66 (dd, J = 7.9, 1.8 Hz, 1H), 6.48 (br s, 1H), 3.86 (s, 3H), 3.17 (s, 3H), 3.00 (t, J = 7.3 Hz, 2H), 2.87 (t, J = 7.3 Hz, 2H), 1.52 (s, 9H); MS (FAB) m/z 454 $(M)^+$, 455 $(M+H)^+$.

4.3.43. 2-Methylisoindole-1,3-dione-5-yl 3-[4-(*tert*-butoxycarbonyl)amino-3-methoxyphenyl]propionate (20b)

This compound was prepared by a similar method to that used for the synthesis of **4a**, starting from **8** and **16b**. White solid (86%); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.01 (m, 1H), 7.84 (d, J = 7.9 Hz, 1H), 7.53 (dd, J = 1.8 Hz, 1H), 7.32 (dd, J = 7.9, 1.8 Hz, 1H), 7.03 (br s, 1H), 6.83 (dd, J = 7.9, 1.8 Hz, 1H), 6.74 (d, J = 1.8 Hz, 1H), 3.86 (s, 1H), 3.18 (s, 3H), 3.03 (t, J = 7.3 Hz, 2H), 2.91 (t, J = 7.3 Hz, 2H), 1.52 (s, 9H); MS (FAB) m/z 454 $(M)^+$, 455 $(M+H)^+$.

4.3.44. 2-Methylisoindole-1,3-dione-5-yl 3-(6-*tert*-butoxycarbonylaminopyridin-3-yl)propionate (20c)

This compound was prepared by a similar method to that used for the synthesis of **4a**, starting from **8** and **16c**. White solid (87%); ^1H NMR (DMSO-d_6, 500 MHz) δ 9.66 (br s, 1H), 8.17 (d, J = 2.4 Hz, 1H), 7.90 (d, J = 7.9 Hz, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.68 (dd, J = 8.5, 2.4 Hz, 1H), 7.62 (d, J = 1.8 Hz, 1H), 7.48 (dd, J = 7.9, 1.8 Hz, 1H), 3.02 (s, 3H), 2.98–2.91 (m, 4H), 1.46 (s, 9H); mp 178–180 °C; MS (FAB) m/z 426 $(M+H)^+$.

4.3.45. 2-Methylisoindole-1,3-dione-5-yl 3-(2-*tert*-butoxycarbonylaminopyrimidin-5-yl)propionate (20d)

This compound was prepared by a similar method to that used for the synthesis of **4a**, starting from **8** and **16d**. White solid (88%); ^1H NMR (DMSO-d_6, 500 MHz) δ 9.95 (br s, 1H), 8.55 (s, 2H), 7.90 (d, J = 9.1 Hz, 1H), 7.65 (s, 1H), 7.50 (d, J = 9.1 Hz, 1H), 3.02–3.00 (m, 5H), 2.91 (t, J = 7.3 Hz, 2H), 1.44 (s, 9H); mp 158–160 °C; MS (FAB) m/z 427 $(M+H)^+$.

4.3.46. 2-Methylisoindole-1,3-dione-5-yl 3-(4-amino-3-methoxyphenyl)propionate TFA salt (4f)

To a solution of **20b** (74.3 mg, 0.163 mmol) in CH_2Cl_2 (2.0 mL) was added TFA (300 μL), and the mixture was stirred at room temperature. After 1 h, the reaction mixture was concentrated under reduced pressure. The residue was recrystallized from MeOH and CH_2Cl_2 to give **4f** (60.0 mg, 0.133 mmol, 82%) as a white powder. ^1H NMR (CD_3OD, 500 MHz) δ 7.87 (d, J = 7.9 Hz, 1H), 7.51 (d, J = 1.2 Hz, 1H), 7.43 (dd, J = 7.9, 1.2 Hz, 1H), 7.26 (d, J = 7.9 Hz, 1H), 7.16 (d, J = 1.8 Hz, 1H), 7.00 (dd, J = 7.9, 1.8 Hz, 1H), 3.97 (s, 3H), 3.13 (s, 3H), 3.11 (t, J = 7.3 Hz, 2H), 3.02 (t, J = 7.3 Hz, 2H); mp 159–162 °C; HRMS (FAB) calcd for $C_{19}H_{19}N_2O_5$ 355.1294; found: 355.1306 $(M+H)^+$.

4.3.47. 2-Methylisoindole-1,3-dione-5-yl 3-(4-amino-2-methoxyphenyl)propionate TFA salt (4e)

This compound was prepared by a similar method to that used for the synthesis of **4f**, starting from **20a**. White powder (84%); ^1H NMR (CD_3OD, 500 MHz) δ 7.86 (d, J = 7.9 Hz, 1H), 7.50 (d, J = 1.8 Hz, 1H), 7.42 (dd, J = 7.9, 1.8 Hz, 1H), 7.33 (d, J = 7.9 Hz, 1H), 6.89–6.84 (m, 2H), 3.91 (s, 3H), 3.13 (s, 3H), 3.06 (t, J = 7.3 Hz, 2H), 2.93 (t, J = 7.3 Hz, 2H); mp 154–155 °C; HRMS (FAB) calcd for $C_{19}H_{19}N_2O_5$ 355.1294; found: 355.1287 $(M+H)^+$.

4.3.48. 2-Methylisoindole-1,3-dione-5-yl 3-(6-aminopyridin-3-yl)propionate TFA salt (4g)

This compound was prepared by a similar method to that used for the synthesis of **4f**, starting from **20c**. White powder (59%); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.97 (dd, J = 9.1, 1.8 Hz, 1H), 7.88 (d, J = 7.9 Hz, 1H), 7.78 (d, J = 1.8 Hz, 1H), 7.60 (d, J = 1.8 Hz, 1H), 7.48 (dd, J = 7.9, 1.8 Hz, 1H), 7.01 (d, J = 9.1 Hz, 1H), 3.13 (s, 3H), 3.04–2.97 (m, 4H); mp 157–159 °C; HRMS (FAB) calcd for $C_{17}H_{16}N_3O_4$ 326.1141; found: 326.1115 $(M+H)^+$.

4.3.49. 2-Methylisoindole-1,3-dione-5-yl 3-(2-aminopyrimidin-5-yl)propionate TFA salt (4h)

This compound was prepared by a similar method to that used for the synthesis of **4f**, starting from **20d**. White powder (54%); ^{1}H NMR (CD_3OD, 500 MHz) δ 8.44 (s, 2H), 7.88 (d, J = 7.9 Hz, 1H), 7.61 (d, J = 1.8 Hz, 1H), 7.48 (dd, J = 7.9, 1.8 Hz, 1H), 3.13 (s, 3H), 3.02 (t, J = 6.7 Hz, 2H), 2.96 (t, J = 6.7 Hz, 2H); mp 154–165 °C; HRMS (FAB) calcd for $C_{16}H_{15}N_4O_4$ 327.1093; found: 327.1128 $(M+H)^+$.

4.3.50. 5-Hydroxy-2-(1-propyl)isoindole-1,3-dione (21a)

This compound was prepared by a similar method to that used for the synthesis of **8**, starting from *n*-propylamine hydrochloride. White solid (90%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.72 (d, J = 7.9 Hz, 1H), 7.30 (d, J = 1.8 Hz, 1H), 7.11 (dd, J = 7.9, 1.8 Hz, 1H), 6.53 (br s, 1H), 3.62 (t, J = 7.3 Hz, 2H), 1.69 (qt, J = 7.3, 7.3 Hz, 2H), 0.94 (d, J = 7.3 Hz, 3H); MS (FAB) m/z 206 $(M+H)^+$.

4.3.51. 5-Hydroxy-2-(2-propyl)isoindole-1,3-dione (21b)

This compound was prepared by a similar method to that used for the synthesis of **8**, starting from isopropylamine hydrochloride. White solid (87%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.69 (d, J = 7.9 Hz, 1H), 7.26 (d, J = 1.8 Hz, 1H), 7.09 (dd, J = 7.9, 1.8 Hz, 1H), 6.34 (br s, 1H), 4.50 (sept, J = 6.7 Hz, 1H), 1.48 (d, J = 6.7 Hz, 6H); mp 181–182 °C; MS (FAB) m/z 206 $(M+H)^+$.

4.3.52. 2-(1-Hexyl)-5-hydroxyisoindole-1,3-dione (21c)

This compound was prepared by a similar method to that used for the synthesis of **8**, starting from *n*-hexylamine hydrochloride. White solid (81%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.72 (d, J = 7.9 Hz, 1H), 7.28 (d, J = 2.4 Hz, 1H), 7.10 (dd, J = 7.9, 2.4 Hz, 1H), 6.07 (br s, 1H), 3.64 (t, J = 7.3 Hz, 2H), 1.68–1.63 (m, 2H), 1.34–1.27 (m, 6H), 0.87 (t, J = 6.7 Hz, 3H); mp 106–107 °C; MS (FAB) m/z 270 $(M+H)^+$.

4.3.53. 2-Cyclohexyl-5-hydroxyisoindole-1,3-dione (21d)

This compound was prepared by a similar method to that used for the synthesis of **8**, starting from cyclohexylamine hydrochloride. White solid (83%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.69 (d, J = 7.9 Hz, 1H), 7.23 (d, J = 1.8 Hz, 1H), 7.07 (dd, J = 7.9, 1.8 Hz, 1H), 5.80 (br s, 1H), 4.10–4.03 (m, 1H), 2.23–2.14 (m, 2H), 1.88–1.84 (m, 2H), 1.73–1.67 (m, 2H), 1.38–1.25 (m, 4H); mp >272 °C (sublime); MS (FAB) m/z 246 $(M+H)^+$.

4.3.54. 5-Hydroxy-2-methylisoindole-1,3-dione (21e)

This compound was prepared by a similar method to that used for the synthesis of **8**, starting from aniline. Yellow solid (84%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.03 (s, 1H), 7.79 (d, J = 7.9 Hz, 1H), 7.50 (t, J = 7.9 Hz, 2H), 7.42–7.39 (m, 3H), 7.21 (d, J = 1.8 Hz, 1H), 7.18 (dd, J = 7.9, 1.8 Hz, 1H); mp 261–262 °C; MS (FAB) m/z 240 $(M+H)^+$.

4.3.55. 5-Hydroxy-2-(2-methoxyphenyl)isoindole-1,3-dione (21f)

This compound was prepared by a similar method to that used for the synthesis of **8**, starting from 2-methoxyaniline. Pale yellow solid (72%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.81 (d, J = 7.9 Hz, 1H), 7.42 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 7.35 (d, J = 1.8 Hz, 1H), 7.25 (dd, J = 7.9, 1.8 Hz, 1H), 7.14 (dd, J = 7.9, 1.8 Hz, 1H), 7.08–7.03 (m, 2H), 6.43 (br s, 1H), 3.80 (s, 3H); mp 237–238 °C; MS (FAB) m/z 270 $(M+H)^+$.

4.3.56. 5-Hydroxy-2-(3-methoxyphenyl)isoindole-1,3-dione (21g)

This compound was prepared by a similar method to that used for the synthesis of **8**, starting from 3-methoxyaniline. Pale yellow solid (93%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.84 (d, J = 7.9 Hz, 1H), 7.40 (dd, J = 7.9, 7.9 Hz, 1H), 7.36 (d, J = 2.4 Hz, 1H), 7.17 (dd, J = 7.9, 2.4 Hz, 1H), 7.01 (dd, J = 7.9, 2.4 Hz, 1H), 6.97 (dd, J = 2.4, 2.4 Hz, 1H), 6.94 (dd, J = 7.9, 2.4 Hz, 1H), 6.17 (br s, 1H), 3.84 (s, 3H); MS (FAB) m/z 270 $(M+H)^+$.

4.3.57. 5-Hydroxy-2-(4-methoxyphenyl)isoindole-1,3-dione (21h)

This compound was prepared by a similar method to that used for the synthesis of **8**, starting from 4-methoxyaniline. White solid (93%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.83 (d, J = 8.5 Hz, 1H), 7.35 (d, J = 2.4 Hz, 1H), 7.32 (d, J = 9.1 Hz, 2H), 7.16 (dd, J = 8.5, 2.4 Hz, 1H), 7.01 (d, J = 9.1 Hz, 2H), 5.79 (br s, 1H), 3.85 (s, 3H); MS (FAB) m/z 270 $(M+H)^+$.

4.3.58. 5-Hydroxy-2-(4-tolyl)isoindole-1,3-dione (21i)

This compound was prepared by a similar method to that used for the synthesis of **8**, starting from *p*-toluidine. Pale yellow solid (95%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.01 (s, 1H), 7.77 (d, J = 8.5 Hz, 1H), 7.30–7.25 (m, 4H), 7.20 (d, J = 1.8 Hz, 1H), 7.17 (dd, J = 8.5, 1.8 Hz, 1H), 2.35 (s, 3H); MS (FAB) m/z 254 $(M+H)^+$.

4.3.59. 5-Hydroxy-2-(4-isopropylphenyl)isoindole-1,3-dione (21j)

This compound was prepared by a similar method to that used for the synthesis of **8**, starting from 4-isopropylaniline. Pale yellow solid (82%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.02 (s, 1H), 7.78 (d, J = 7.9 Hz, 1H), 7.36 (d, J = 8.5 Hz, 2H), 7.30 (d, J = 8.5 Hz, 2H), 7.20 (d, J = 1.8 Hz, 1H), 7.17 (dd, J = 7.9, 1.8 Hz, 1H), 2.94 (sept, J = 6.7 Hz, 1H), 1.23 (d, J = 6.7 HZ, 6H); mp 236–237 °C; MS (FAB) m/z 282 $(M+H)^+$.

4.3.60. 5-Hydroxy-2-(4-*tert*-butylphenyl)isoindole-1,3-dione (21k)

This compound was prepared by a similar method to that used for the synthesis of **8**, starting from 4-*tert*-butylaniline. White solid (85%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.02 (s, 1H), 7.78 (d, J = 8.5 Hz, 1H), 7.51 (d, J = 7.9 Hz, 2H), 7.31 (d, J = 7.9 Hz, 2H), 7.21 (s, 1H), 7.18 (d, J = 8.5 Hz, 1H), 1.31 (s, 9H); MS (FAB) m/z 296 $(M+H)^+$.

4.3.61. 5-Hydroxy-2-[4-(trifluoromethyl)phenyl]isoindole-1,3-dione (21l)

This compound was prepared by a similar method to that used for the synthesis of **8**, starting from 4-(trifluoromethyl)aniline. Pale yellow solid (54%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.09 (s, 1H), 7.90 (d, J = 8.5 Hz, 2H), 7.82 (d, J = 7.9 Hz, 1H), 7.69 (d, J = 8.5 Hz, 2H), 7.24 (d, J = 1.8 Hz, 1H), 7.20 (dd, J = 7.9, 1.8 Hz, 1H); mp 250–253 °C; MS (FAB) m/z 308 $(M+H)^+$.

4.3.62. 2-(4-Fluorophenyl)-5-hydroxyisoindole-1,3-dione (21m)

This compound was prepared by a similar method to that used for the synthesis of **8**, starting from 4-fluoroaniline. Gray solid (86%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.03 (s, 1H), 7.79 (d, J = 8.5 Hz, 1H), 7.47–7.44 (m, 2H), 7.36–7.33 (m, 2H), 7.21 (d, J = 2.4 Hz, 1H), 7.18 (dd, J = 8.5, 2.4 Hz, 1H); MS (FAB) m/z 258 $(M+H)^+$.

4.3.63. 2-(4-Cyanophenyl)-5-hydroxyisoindole-1,3-dione (21n)

This compound was prepared by a similar method to that used for the synthesis of **8**, starting from 4-aminobenzonitrile. Pale yellow solid (92%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.10 (s, 1H), 8.00 (d, J = 9.1 Hz, 2H), 7.82 (d, J = 7.9 Hz, 1H), 7.67 (d, J = 9.1 Hz, 2H), 7.24 (d, J = 2.4 Hz, 1H), 7.20 (dd, J = 7.9, 2.4 Hz, 1H); MS (FAB) m/z 265 (M+H)$^+$.

4.3.64. 5-Hydroxy-2-(4-nitrophenyl)isoindole-1,3-dione (21o)

This compound was prepared by a similar method to that used for the synthesis of **8**, starting from 4-nitroaniline. Yellow solid (74%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.02 (s, 1H), 8.38 (d J = 8.5 Hz, 2H), 7.84 (d, J = 7.9 Hz, 1H), 7.76 (d J = 8.5 Hz, 2H), 7.25 (d, J = 1.8 Hz, 1H), 7.21 (dd, J = 7.9, 1.8 Hz, 1H).

4.3.65. 2-(1-Propyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-nitrophenyl)acrylate (22a)

This compound was prepared by a similar method to that used for the synthesis of **9g**, starting from **21a**. White solid (79%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.29 (d, J = 9.1 Hz, 2H), 8.13 (d, J = 9.1 Hz, 2H), 8.04 (d, J = 15.9 Hz, 1H), 7.95 (d, J = 7.9 Hz, 1H), 7.80 (d, J = 1.8 Hz, 1H), 7.68 (dd, J = 7.9, 1.8 Hz, 1H), 7.15 (d, J = 15.9 Hz, 1H), 3.53 (t, J = 7.3 Hz, 2H), 1.60 (qt, J = 7.3, 7.3 Hz, 2H), 0.87 (t, J = 7.3 Hz, 3H); mp 149–151 °C; MS (FAB) m/z 381 (M+H)$^+$.

4.3.66. 2-Isopropylisoindole-1,3-dione-5-yl (*E*)-3-(4-nitrophenyl)acrylate (22b)

This compound was prepared by a similar method to that used for the synthesis of **9g**, starting from **21b**. White solid (95%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.31 (d, J = 9.1 Hz, 2H), 7.94 (d, J = 15.9 Hz, 1H), 7.87 (d, J = 7.9 Hz, 1H), 7.77 (d, J = 9.1 Hz, 2H), 7.65 (d, J = 1.8 Hz, 1H), 7.48 (dd, J = 7.9, 1.8 Hz, 1H), 6.77 (d, J = 15.9 Hz, 1H), 4.54 (sept, J = 6.7 Hz, 2H), 1.50 (d, J = 6.7 Hz, 6H); mp 150–152 °C; MS (FAB) m/z 381 (M+H)$^+$.

4.3.67. 2-(1-Hexyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-nitrophenyl)acrylate (22c)

This compound was prepared by a similar method to that used for the synthesis of **9g**, starting from **21c**. White solid (65%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.31 (d, J = 8.5 Hz, 2H), 7.95 (d, J = 15.9 Hz, 1H), 7.90 (d, J = 7.9 Hz, 1H), 7.77 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 1.8 Hz, 1H), 7.50 (dd, J = 7.9, 1.8 Hz, 1H), 6.77 (d, J = 15.9 Hz, 1H), 3.69 (t, J = 7.3 Hz, 2H), 1.69–1.64 (m, 2H), 1.36–1.29 (m, 6H), 0.88 (t, J = 7.0 Hz, 3H); mp 127–132 °C; MS (FAB) m/z 423 (M+H)$^+$.

4.3.68. 2-Cyclohexylisoindole-1,3-dione-5-yl (*E*)-3-(4-nitrophenyl)acrylate (22d)

This compound was prepared by a similar method to that used for the synthesis of **9g**, starting from **21d**. White solid (92%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.31 (d, J = 9.1 Hz, 2H), 7.94 (d, J = 15.9 Hz, 1H), 7.87 (d, J = 7.9 Hz, 1H), 7.77 (d, J = 9.1 Hz, 2H), 7.65 (d, J = 1.8 Hz, 1H), 7.48 (dd, J = 7.9, 1.8 Hz, 1H), 6.77 (d, J = 15.9 Hz, 1H), 4.14–4.09 (m, 1H), 2.25–2.17 (m, 2H), 1.90–1.86 (m, 2H), 1.76–1.69 (m, 2H), 1.41–1.26 (m, 4H); mp 177.5–179 °C; MS (FAB) m/z 421 (M+H)$^+$.

4.3.69. 2-Phenylisoindole-1,3-dione-5-yl (*E*)-3-(4-nitrophenyl)acrylate (22e)

This compound was prepared by a similar method to that used for the synthesis of **9g**, starting from **21e**. White solid (60%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.30 (d, J = 9.1 Hz, 2H), 8.13 (d, J = 9.1 Hz, 2H), 8.06 (d, J = 8.5 Hz, 1H), 8.05 (d, J = 16.5 Hz, 1H), 7.92 (d, J = 1.8 Hz, 1H), 7.76 (dd, J = 8.5, 1.8 Hz, 1H), 7.55–7.52 (m, 2H), 7.46–7.43 (m, 3H), 7.18 (d, J = 16.5 Hz, 1H); mp 207.5–209 °C; MS (FAB) m/z 415 (M+H)$^+$.

4.3.70. 2-(2-Methoxyphenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-nitrophenyl)acrylate (22f)

This compound was prepared by a similar method to that used for the synthesis of **9g**, starting from **21f**. White solid (78%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.30 (d, J = 9.1 Hz, 2H), 8.14 (d, J = 9.1 Hz, 2H), 8.06 (d, J = 15.9 Hz, 1H), 8.06 (d, J = 7.9 Hz, 1H), 7.92 (d, J = 1.8 Hz, 1H), 7.77 (dd, J = 7.9, 1.8 Hz, 1H), 7.49 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 7.38 (dd, J = 7.9, 1.8 Hz, 1H), 7.22 (dd, J = 7.9, 1.8 Hz, 1H), 7.18 (d, J = 15.9 Hz, 1H), 7.09 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 3.75 (s, 3H); mp 204–204.5 °C; MS (FAB) m/z 445 (M+H)$^+$.

4.3.71. 2-(3-Methoxyphenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-nitrophenyl)acrylate (22g)

This compound was prepared by a similar method to that used for the synthesis of **9g**, starting from **21g**. White solid (74%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.30 (d, J = 9.1 Hz, 2H), 8.13 (d, J = 9.1 Hz, 2H), 8.06 (d, J = 15.9 Hz, 1H), 8.05 (d, J = 7.9 Hz, 1H), 7.91 (d, J = 1.8 Hz, 1H), 7.75 (dd, J = 7.9, 1.8 Hz, 1H), 7.43 (dd, J = 7.9, 7.9 Hz, 1H), 7.18 (d, J = 15.9 Hz, 1H), 7.07–7.02 (m, 3H), 3.78 (s, 3H); MS (FAB) m/z 445 (M+H)$^+$.

4.3.72. 2-(4-Methoxyphenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-nitrophenyl)acrylate (22h)

This compound was prepared by a similar method to that used for the synthesis of **9g**, starting from **21h**. White solid (74%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.32 (d, J = 8.5 Hz, 2H), 8.01 (d, J = 7.9 Hz, 1H), 7.96 (d, J = 15.9 Hz, 1H), 7.79 (d, J = 1.8 Hz, 1H), 7.78 (d, J = 8.5 Hz, 2H), 7.58 (dd, J = 7.9, 1.8 Hz, 1H), 7.34 (d, J = 8.5 Hz, 2H), 7.03 (d, J = 8.5 Hz, 2H), 6.79 (d, J = 15.9 Hz, 1H), 3.86 (s, 3H); mp 206–208 °C; MS (FAB) m/z 445 (M+H)$^+$.

4.3.73. 2-(4-Methylphenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-nitrophenyl)acrylate (22i)

This compound was prepared by a similar method to that used for the synthesis of **9g**, starting from **21i**. White solid (74%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.32 (d, J = 8.5 Hz, 2H), 8.01 (d, J = 7.9 Hz, 1H), 7.96 (d, J = 15.9 Hz, 1H), 7.79 (d, J = 1.8 Hz, 1H), 7.78 (d, J = 8.5 Hz, 2H), 7.58 (dd, J = 7.9, 1.8 Hz, 1H), 7.34 (d, J = 8.5 Hz, 2H), 7.03 (d, J = 8.5 Hz, 2H), 6.79 (d, J = 15.9 Hz, 1H), 3.86 (s, 3H); mp 207–209 °C; MS (FAB) m/z 445 (M+H)$^+$.

4.3.74. 2-(4-Isopropylphenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-nitrophenyl)acrylate (22j)

This compound was prepared by a similar method to that used for the synthesis of **9g**, starting from **21j**. Pale yellow crystals (76%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.30 (d, J = 9.1 Hz, 2H), 8.14 (d, J = 9.1 Hz, 2H), 8.06 (d, J = 15.9 Hz, 1H), 8.05 (d, J = 7.9 Hz, 1H), 7.90 (d, J = 1.8 Hz, 1H), 7.75 (dd, J = 7.9, 1.8 Hz, 1H), 7.40 (d, J = 8.5 Hz, 2H), 7.35 (d, J = 8.5 Hz, 2H), 7.18 (d, J = 15.9 Hz, 1H), 2.96 (sept, J = 6.7 Hz, 1H), 1.32 (d, J = 6.7 Hz, 6H); mp 187–188 °C; MS (FAB) m/z 457 (M+H)$^+$.

4.3.75. 2-(4-*tert*-Butylphenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-nitrophenyl)acrylate (22k)

This compound was prepared by a similar method to that used for the synthesis of **9g**, starting from **21k**. Pale yellow crystals (80%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.30 (d, J = 8.5 Hz, 2H), 8.14 (d, J = 8.5 Hz, 2H), 8.06 (d, J = 16.4 Hz, 1H), 8.05 (d, J = 7.9 Hz, 1H), 7.90 (d, J = 1.8 Hz, 1H), 7.75 (dd, J = 7.9, 1.8 Hz, 1H), 7.54 (d, J = 8.5 Hz, 2H), 7.37 (d, J = 8.5 Hz, 2H), 7.18 (d, J = 16.4 Hz, 1H), 1.32 (s, 9H); mp 209–210 °C; MS (FAB) m/z 471 (M+H)$^+$.

4.3.76. 2-[4-(Trifluoromethyl)phenyl]isoindole-1,3-dione-5-yl (*E*)-3-(4-nitrophenyl)acrylate (22l)

This compound was prepared by a similar method to that used for the synthesis of **9g**, starting from **21l**. Yellow solid (85%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.30 (d, J = 8.5 Hz, 2H), 8.14 (d, J = 8.5 Hz, 2H), 8.09 (d, J = 7.9 Hz, 1H), 8.06 (d, J = 15.9 Hz, 1H), 7.95 (d, J = 1.8 Hz, 1H), 7.94 (d, J = 8.5 Hz, 2H), 7.78 (dd, J = 7.9, 1.8 Hz, 1H), 7.73 (d, J = 8.5 Hz, 2H), 7.19 (d, J = 15.9 Hz, 1H); mp 222–224 °C; MS (FAB) m/z 483 (M+H)$^+$.

4.3.77. 2-(4-Fluorophenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-nitrophenyl)acrylate (22m)

This compound was prepared by a similar method to that used for the synthesis of **9g**, starting from **21m**. White solid (74%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.30 (d, J = 9.1 Hz, 2H), 8.14 (d, J = 9.1 Hz, 2H), 8.06 (d, J = 7.9 Hz, 1H), 8.05 (d, J = 15.9 Hz, 1H), 7.92 (d, J = 1.8 Hz, 1H), 7.76 (dd, J = 7.9, 1.8 Hz, 1H), 7.53–7.50 (m, 2H), 7.40–7.36 (m, 2H), 7.18 (d, J = 15.9 Hz, 1H); mp 198–200 °C; MS (FAB) m/z 433 (M+H)$^+$.

4.3.78. 2-(4-Cyanophenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-nitrophenyl)acrylate (22n)

This compound was prepared by a similar method to that used for the synthesis of **9g**, starting from **21n**. White solid (87%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.10 (s, 1H), 8.30 (d, J = 8.5 Hz, 2H), 8.13 (d, J = 8.5 Hz, 2H), 8.10–8.07 (m, 2H), 8.03 (d, J = 8.5 Hz, 2H), 7.95 (d, J = 1.8 Hz, 1H), 7.78 (dd, J = 7.9, 1.8 Hz, 1H), 7.71 (d, J = 8.5 Hz, 2H), 7.18 (d, J = 16.5 Hz, 1H); mp 290–292 °C; MS (FAB) m/z 440 (M+H)$^+$.

4.3.79. 2-(4-Nitrophenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-nitrophenyl)acrylate (22o)

This compound was prepared by a similar method to that used for the synthesis of **9g**, starting from **21o**. White solid (93%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.41 (d, J = 8.5 Hz, 2H), 8.30 (d, J = 8.5 Hz, 2H), 8.14 (d, J = 8.5 Hz, 2H), 8.11 (d, J = 7.9 Hz, 1H), 8.06 (d, J = 15.9 Hz, 1H), 7.97 (d, J = 1.8 Hz, 1H), 7.80–7.78 (m, 3H), 7.18 (d, J = 15.9 Hz, 1H); MS (FAB) m/z 460 (M+H)$^+$.

4.3.80. 2-(1-Propyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-aminophenyl)propionate (5a)

This compound was prepared by a similar method to that used for the synthesis of **2g**, starting from **22a**. White solid (95%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.82 (d, J = 7.9 Hz, 1H), 7.47 (d, J = 1.8 Hz, 1H), 7.30 (dd, J = 7.9, 1.8 Hz, 1H), 7.05 (d, J = 7.9 Hz, 2H), 6.67 (d, J = 7.9 Hz, 2H), 3.64 (t, J = 7.3 Hz, 2H), 2.98 (t, J = 7.3 Hz, 2H), 2.87 (t, J = 7.3 Hz, 2H), 1.70 (qt, J = 7.3, 7.3 Hz, 2H), 0.94 (t, J = 7.3 Hz, 3H); mp 86–87 °C; HRMS (FAB) calcd for $C_{20}H_{21}N_2O_4$ 353.1501; found: 353.1483 (M+H)$^+$.

4.3.81. 2-Isopropylisoindole-1,3-dione-5-yl (*E*)-3-(4-aminophenyl)propionate (5b)

This compound was prepared by a similar method to that used for the synthesis of **2g**, starting from **22b**. White solid (65%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.79 (d, J = 7.9 Hz, 1H), 7.43 (d, J = 1.8 Hz, 1H), 7.29 (dd, J = 7.9, 1.8 Hz, 1H), 7.05 (d, J = 8.5 Hz, 2H), 6.67 (d, J = 8.5 Hz, 2H), 4.51 (sept, J = 6.7 Hz, 1H), 3.63 (br s, 2H), 2.97 (t, J = 7.3 Hz, 2H), 2.87 (t, J = 7.3 Hz, 2H), 1.48 (d, J = 6.7 Hz, 6H); mp 93–94 °C; HRMS (FAB) calcd for $C_{20}H_{21}N_2O_4$ 353.1501; found: 353.1483 (M+H)$^+$.

4.3.82. 2-(1-Hexyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-aminophenyl)propionate (5c)

This compound was prepared by a similar method to that used for the synthesis of **2g**, starting from **22c**. Yellow oil (67%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.82 (d, J = 7.9 Hz, 1H), 7.46 (d, J = 2.4 Hz, 1H), 7.30 (dd, J = 7.9, 2.4 Hz, 1H), 7.05 (d, J = 8.5 Hz, 2H), 6.67 (d, J = 8.5 Hz, 2H), 3.66 (t, J = 7.3 Hz, 2H), 2.98 (t, J = 7.3 Hz, 2H), 2.87 (t, J = 7.3 Hz, 2H), 1.68–1.62 (m, 2H), 1.34–1.29 (m, 6H), 0.88 (t, J = 7.3 Hz, 3H); HRMS (FAB) calcd for $C_{23}H_{27}N_2O_4$ 395.1971; found: 395.2011 (M+H)$^+$.

4.3.83. 2-Cyclohexylisoindole-1,3-dione-5-yl (*E*)-3-(4-aminophenyl)propionate (5d)

This compound was prepared by a similar method to that used for the synthesis of **2g**, starting from **22d**. Yellow oil (66%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.79 (d, J = 7.9 Hz, 1H), 7.43 (d, J = 1.8 Hz, 1H), 7.28 (dd, J = 7.9, 1.8 Hz, 1H), 7.05 (d, J = 7.9 Hz, 2H), 6.67 (d, J = 7.9 Hz, 2H), 4.12–4.06 (m, 1H), 3.66 (br s, 2H), 2.97 (t, J = 7.3 Hz, 2H), 2.87 (t, J = 7.3 Hz, 2H), 2.22–2.14 (m, 2H), 1.90–1.85 (m, 2H), 1.73–1.68 (m, 2H), 1.38–1.25 (m, 4H); HRMS (FAB) calcd for $C_{23}H_{25}N_2O_4$ 393.1814; found: 393.1807 (M+H)$^+$.

4.3.84. 2-Phenylisoindole-1,3-dione-5-yl (*E*)-3-(4-aminophenyl) propionate (6a)

This compound was prepared by a similar method to that used for the synthesis of **2g**, starting from **22e**. Yellow powder (57%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.95 (d, J = 7.9 Hz, 1H), 7.58 (d, J = 1.8 Hz, 1H), 7.53–7.50 (m, 2H), 7.43–7.41 (m, 3H), 7.39 (dd, J = 7.9, 1.8 Hz, 1H), 7.06 (d, J = 7.9 Hz, 2H), 6.68 (d, J = 7.9 Hz, 2H), 3.63 (br s, 2H), 2.99 (t, J = 7.3 Hz, 2H), 2.89 (t, J = 7.9 Hz, 2H); mp 162–163.5 °C; HRMS (FAB) calcd for $C_{23}H_{19}N_2O_4$ 387.1345; found: 387.1310 (M+H)$^+$.

4.3.85. 2-(2-Methoxyphenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-aminophenyl)propionate (6b)

This compound was prepared by a similar method to that used for the synthesis of **2g**, starting from **22f**. Yellow powder (58%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.93 (d, J = 7.9 Hz, 1H), 7.56 (d, J = 1.8 Hz, 1H), 7.44 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 7.37 (dd, J = 7.9, 1.8 Hz, 1H), 7.09–7.04 (m, 4H), 6.68 (d, J = 7.9 Hz, 2H), 3.80 (s, 3H), 3.00 (t, J = 7.3 Hz, 2H), 2.89 (t, J = 7.3 Hz, 2H); mp 106–107 °C; HRMS (FAB) calcd for $C_{24}H_{21}N_2O_5$ 417.1450; found: 417.1468 (M+H)$^+$.

4.3.86. 2-(3-Methoxyphenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-aminophenyl)propionate (6c)

This compound was prepared by a similar method to that used for the synthesis of **2g**, starting from **22g**. Yellow powder (45%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.94 (d, J = 8.5 Hz, 1H), 7.57 (d, J = 1.8 Hz, 1H), 7.42–7.38 (m, 2H), 7.06 (d, J = 7.9 Hz, 2H), 7.01 (d, J = 8.5 Hz, 1H), 6.96–6.95 (m, 2H), 6.68 (d, J = 7.9 Hz, 2H), 3.84 (s, 3H), 2.99 (t, J = 7.3 Hz, 2H), 2.89 (t, J = 7.3 Hz, 2H); mp 125–127 °C; HRMS (FAB) calcd for $C_{24}H_{21}N_2O_5$ 417.1450; found: 417.1474 (M+H)$^+$.

4.3.87. 2-(4-Methoxyphenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-aminophenyl)propionate (6d)

This compound was prepared by a similar method to that used for the synthesis of **2g**, starting from **22h**. Pale yellow powder (72%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.93 (d, J = 7.9 Hz, 1H), 7.56 (d, J = 2.4 Hz, 1H), 7.38 (dd, J = 7.9, 2.4 Hz, 1H), 7.32 (d, J = 8.5 Hz, 2H), 7.06 (d, J = 8.5 Hz, 2H), 7.02 (d, J = 8.5 Hz, 2H), 6.67 (d, J = 8.5 Hz, 2H), 3.85 (s, 3H), 2.99 (t, J = 7.3 Hz, 2H), 2.89 (t, J = 7.3 Hz, 2H); mp 129–131 °C; HRMS (FAB) calcd for $C_{24}H_{21}N_2O_5$ 417.1450; found: 417.1409 (M+H)$^+$.

4.3.88. 2-(4-Methylphenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-aminophenyl)propionate (6e)

This compound was prepared by a similar method to that used for the synthesis of **2g**, starting from **22i**. Yellow powder (73%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.93 (d, J = 7.9 Hz, 1H), 7.56 (d, J = 1.8 Hz, 1H), 7.38 (dd, J = 7.9, 1.8 Hz, 1H), 7.32–7.27 (m, 4H), 7.06 (d, J = 8.5 Hz, 2H), 6.67 (d, J = 8.5 Hz, 2H), 3.64 (br s, 2H), 2.99 (t,

J = 7.3 Hz, 2H), 2.89 (t, J = 7.3 Hz, 2H), 2.41 (s, 3H); mp 137–139 °C; HRMS (FAB) calcd for $C_{24}H_{21}N_2O_4$ 401.1501; found: 401.1532 $(M+H)^+$.

4.3.89. 2-(4-Isopropylphenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-aminophenyl)propionate (6f)

This compound was prepared by a similar method to that used for the synthesis of **2g**, starting from **22j**. Pale yellow powder (79%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.94 (d, J = 7.9 Hz, 1H), 7.57 (d, J = 1.8 Hz, 1H), 7.39–7.31 (m, 5H), 7.06 (d, J = 8.5 Hz, 2H), 6.67 (d, J = 8.5 Hz, 2H), 3.65 (br s, 2H), 2.99 (t, J = 7.3 Hz, 2H), 2.97 (sept, J = 6.7 Hz, 1H), 2.89 (t, J = 7.3 Hz, 2H), 1.28 (d, J = 6.7 Hz, 6H); mp 112–113 °C; HRMS (FAB) calcd for $C_{26}H_{25}N_2O_4$ 429.1814; found: 429.1805 $(M+H)^+$.

4.3.90. 2-(4-*tert*-Butylphenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-aminophenyl)propionate (6g)

This compound was prepared by a similar method to that used for the synthesis of **2g**, starting from **22k**. Pale yellow powder (77%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.94 (d, J = 7.9 Hz, 1H), 7.57 (d, J = 1.8 Hz, 1H), 7.52 (d, J = 8.5 Hz, 2H), 7.38 (dd, J = 7.9, 1.8 Hz, 1H), 7.33 (d, J = 8.5 Hz, 2H), 7.06 (d, J = 8.5 Hz, 2H), 6.68 (d, J = 8.5 Hz, 2H), 3.49 (br s, 2H), 2.99 (t, J = 7.3 Hz, 2H), 2.89 (t, J = 7.3 Hz, 2H), 1.35 (s, 9H); mp 126–128 °C; HRMS (FAB) calcd for $C_{27}H_{27}N_2O_4$ 443.1971; found: 443.2001 $(M+H)^+$.

4.3.91. 2-[4-(Trifluoromethyl)phenyl]isoindole-1,3-dione-5-yl (*E*)-3-(4-aminophenyl)propionate (6h)

This compound was prepared by a similar method to that used for the synthesis of **2g**, starting from **22l**. Yellow powder (35%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.97 (d, J = 7.9 Hz, 1H), 7.78 (d, J = 8.5 Hz, 2H), 7.63 (d, J = 8.5 Hz, 2H), 7.61 (d, J = 1.8 Hz, 1H), 7.42 (dd, J = 7.9, 1.8 Hz, 1H), 7.06 (d, J = 8.5 Hz, 2H), 6.68 (d, J = 8.5 Hz, 2H), 3.64 (br s, 2H), 3.00 (t, J = 7.3 Hz, 2H), 2.90 (t, J = 7.3 Hz, 2H); mp 189–191 °C; HRMS (FAB) calcd for $C_{24}H_{18}F_3N_2O_4$ 455.1219; found: 455.1192 $(M+H)^+$.

4.3.92. 2-(4-Fluorophenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-aminophenyl)propionate (6i)

This compound was prepared by a similar method to that used for the synthesis of **2g**, starting from **22m**. White powder (65%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.94 (d, J = 8.5 Hz, 1H), 7.58 (d, J = 2.4 Hz, 1H), 7.42–7.39 (m, 3H), 7.21–7.18 (m, 2H), 7.06 (d, J = 7.9 Hz, 2H), 6.67 (d, J = 7.9 Hz, 2H), 3.65 (br s, 2H), 2.99 (t, J = 7.3 Hz, 2H), 2.90 (t, J = 7.3 Hz, 2H); mp 157–158 °C; HRMS (FAB) calcd for $C_{23}H_{18}FN_2O_4$ 405.1251; found: 405.1291 $(M+H)^+$.

4.3.93. 2-(4-Cyanophenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-aminophenyl)propionate (6j)

This compound was prepared by a similar method to that used for the synthesis of **2g**, starting from **22n**. Pale yellow powder (72%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.97 (d, J = 8.5 Hz, 1H), 7.80 (d, J = 8.5 Hz, 2H), 7.67 (d, J = 8.5 Hz, 2H), 7.61 (d, J = 1.8 Hz, 1H), 7.43 (dd, J = 8.5, 1.8 Hz, 1H), 7.06 (d, J = 8.5 Hz, 2H), 6.67 (d, J = 8.5 Hz, 2H), 3.66 (br s, 2H), 2.99 (t, J = 7.0 Hz, 2H), 2.90 (t, J = 7.0 Hz, 2H); mp 158–160 °C; HRMS (FAB) calcd for $C_{24}H_{18}N_3O_4$ 412.1297; found: 412.1263 $(M+H)^+$.

4.3.94. 2-(4-Aminophenyl)isoindole-1,3-dione-5-yl (*E*)-3-(4-aminophenyl)propionate (6k)

This compound was prepared by a similar method to that used for the synthesis of **2g**, starting from **22o**. Yellow powder (56%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.91 (d, J = 7.9 Hz, 1H), 7.54 (d, J = 1.8 Hz, 1H), 7.36 (dd, J = 7.9, 1.8 Hz, 1H), 7.15 (d, J = 8.5 Hz, 2H), 7.06 (d, J = 7.9 Hz, 2H), 6.77 (d, J = 8.5 Hz, 2H), 6.67 (d, J = 7.9 Hz, 2H), 3.81 (br s, 2H), 3.65 (br s, 2H), 2.99 (t, J = 7.3 Hz, 2H), 2.89 (t, J = 7.3 Hz, 2H); mp >300 °C; HRMS (FAB) calcd for $C_{23}H_{20}N_2O_4$ 402.1454; found: 402.1433 $(M+H)^+$.

4.3.95. 2-(4-Cyanophenyl)isoindole-1,3-dione-5-yl 3-(6-*tert*-butoxycarbonylaminopyridin-3-yl)propionate (23)

This compound was prepared by a similar method to that used for the synthesis of **20c**, starting from **21n**. White powder (93%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.16 (d, J = 1.8 Hz, 1H), 7.99 (d, J = 7.9 Hz, 1H), 7.92 (d, J = 8.5 Hz, 1H), 7.81 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 1.8 Hz, 1H), 7.67 (d, J = 8.5 Hz, 2H), 7.59 (dd, J = 8.5, 1.8 Hz, 1H), 7.45 (dd, J = 7.9, 1.8 Hz, 1H), 7.37 (br s, 1H), 3.04 (t, J = 7.9 Hz, 2H), 2.95 (t, J = 7.9 Hz, 2H), 1.53 (s, 9H); mp 175–177 °C; MS (FAB) m/z 513 $(M+H)^+$.

4.3.96. 2-(4-Cyanophenyl)isoindole-1,3-dione-5-yl 3-(6-aminopyridin-3-yl)propionate TFA salt (7)

This compound was prepared by a similar method to that used for the synthesis of **4g**, starting from **23**. White powder (68%); ^{1}H NMR (CD_3OD, 500 MHz) δ 8.02 (d, J = 7.9 Hz, 1H), 7.96 (dd, J = 9.1, 1.8 Hz, 1H), 7.89 (d, J = 8.5 Hz, 2H), 7.79 (d, J = 1.8 Hz, 1H), 7.75–7.73 (m, 3H), 7.59 (dd, J = 7.9, 1.8 Hz, 1H), 7.00 (d, J = 9.1 Hz, 1H), 3.04 (t, J = 6.7 Hz, 2H), 2.99 (t, J = 6.7 Hz, 2H); mp 178–180 °C; HRMS (FAB) calcd for $C_{23}H_{17}N_4O_4$ 413.1250; found: 413.1232 $(M+H)^+$.

4.4. Bioassay

4.4.1. Tryptase inhibition assay

Tryptase inhibition assay was performed by a slight modification of the literature method.[43] The effect of test compounds on tryptase activity was determined by incubating tryptase (1 nM final concentration) with its peptide substrate *t*-Boc-Phe-Ser-Arg-MCA (37.5 μM final concentration) in 100 mM Tris–HCl buffer (pH 7.8) in the absence or presence of test compound. To minimize the loss of tryptase activity, 20 mM heparin was included in the reaction buffer. The reaction was carried at 37 °C for 5 min, then terminated by the addition of 50% aqueous AcOH solution. The degradation product MCA was measured fluorimetrically at the excitation wavelength of 355 nm and the emission wavelength of 460 nm. The inhibition constant (K_i) values of test compounds were obtained from reciprocal plots of the reaction velocity versus inhibitor concentration at various *t*-Boc-Phe-Ser-Arg-MCA concentrations (10–40 μM final concentration) for 3 min at 37 °C.

4.4.2. Thrombin inhibition assay

Thrombin inhibition assay was performed by a slight modification of the literature method.[44] The effect of test compounds on thrombin activity was determined by incubating thrombin (150 U/mL final concentration) with its peptide substrate *t*-Boc-Val-Pro-Arg-MCA (37.5 μM final concentration) in 100 mM Tris–HCl buffer (pH 7.8) in the absence or presence of test compound. The reaction was carried at 37 °C for 5 min, then terminated by the addition of 50% aqueous AcOH solution. The degradation product MCA was measured fluorimetrically at the excitation wavelength of 355 nm and the emission wavelength of 460 nm. The inhibition constant (K_i) values of test compounds were obtained from reciprocal plots of the reaction velocity versus inhibitor concentration at various *t*-Boc-Val-Pro-Arg-MCA concentrations (10–40 μM final concentration) for 3 min at 37 °C.

4.4.3. Trypsin inhibition assay

Trypsin inhibition assay was performed by a slight modification of the literature method.[45] The effect of test compounds on tryptase activity was determined by incubating trypsin (1 nM final concentration) with its peptide substrate *t*-Boc-Phe-Ser-Arg-MCA (37.5 μM final concentration) and $CaCl_2$ (10 mM final concentra-

tion) in 50 mM Tris–HCl buffer (pH 8.0) in the absence or presence of test compound. The reaction was carried at 37 °C for 5 min, then terminated by the addition of 50% aqueous AcOH solution. The degradation product MCA was measured fluorimetrically at the excitation wavelength of 355 nm and the emission wavelength of 460 nm. The inhibition constant (K_i) values of test compounds were obtained from reciprocal plots of the reaction velocity versus inhibitor concentration at various *t*-Boc-Phe-Ser-Arg-MCA concentrations (10–40 μM final concentration) for 3 min at 37 °C.

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