Arch. Pharm. Chem. Life Sci. 2015, *348*, 408–420

ARCH PHARM
Archiv der Pharmazie

Full Paper

Design, Synthesis, and Anti-Thrombotic Evaluation of Some Novel Fluorinated Thrombin Inhibitor Derivatives

Haifeng Chen and Yujie Ren

College of Chemistry and Environmental Engineering, Shanghai Institute of Technology, Shanghai, China

Computer-aided simulation was used to design and synthesize nine novel fluorinated thrombin inhibitor derivatives. These compounds were confirmed by spectral analyses (^{1}H NMR, ^{13}C NMR, and FT-ICR-MS). Their inhibitory activities against thrombin enzyme were evaluated by chromogenic assay. All the derivatives demonstrated thrombin inhibitory activity *in vitro*. Five of these compounds exerted more potent effects against thrombin enzyme compared with the reference drug argatroban. Compound 3-(2-(((4-carbamimidoylphenyl)amino)methyl)-1-ethyl-*N*-(2-fluoro-phenyl)-1*H*-benzo[*d*]imidazole-5-carboxamido)propanoic acid ($IC_{50} = 3.52 \pm 0.32$ nmol/L) was a more potent inhibitor of thrombosis than argatroban ($IC_{50} = 9.46 \pm 0.92$ nmol/L).

Keywords: Argatroban / Chromogenic assay / Docking / Fluorine / Thrombin inhibitor

Received: December 16, 2014; Revised: February 21, 2015; Accepted: February 27, 2015

DOI 10.1002/ardp.201400460

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Introduction

Thrombosis occurs in the arterial or venous circulation; this condition has become one of the medical highlights. The pathophysiology of arterial thrombosis differs from that of venous thrombosis; this difference is reflected by the different ways by which these conditions are treated. Arterial thrombosis is generally treated with drugs that target platelets, whereas venous thrombosis is treated with drugs that target protein of the coagulation cascade [1].

A variety of thrombin inhibitory drugs have been listed on the stock market recently, including rivaroxaban (Bayer) [2–4], argatroban (Mitsubishi) [5], and dabigatran etexilate (Boehringer Ingelheim) [6–9] (Fig. 1). In particular, dabigatran etexilate was the first oral direct thrombin inhibitor, which broke the earlier 50-year monopoly of the anticoagulant drug market. However, the new series of anticoagulants have several disadvantages, including their low oral bioavailability and the probability of hemorrhage when used in large doses.

To date, fluorinated drugs account for a considerable proportion of the drugs used in clinical treatment [10–15]. With the introduction of fluorine atoms or fluoride groups, the physical and chemical properties of drug molecules could be changed, which, in turn, alters the pharmacokinetic properties of drug molecules. For instance, the distribution of a drug in the target tissue could be improved, which consequently increases its bioavailability. The changes in the structural conformation of compounds may enhance their capacity to bind with ligands and target proteins as well as their selectivity for target proteins. Fluorination could block the metabolic pathways of drugs in a particular site, change the rate of their metabolism, prolong their effects in the body, and improve their metabolic stability, among others. Furthermore, the introduction of fluorine in drug molecules may enhance the membrane permeability [16–19] and the specific loci formed with target proteins during hydrophobic interaction [20]. Since the introduction of 5-fluorouracil, the addition of fluorine atoms or fluoride groups into drug molecules has gradually become an important research strategy in drug design and structure transformation.

Therefore, to combine the benefits of anti-thrombin drugs and fluorine atoms during treatment, fluoride groups were introduced into thrombin inhibitory drugs to modify their

Correspondence: Prof. Yujie Ren, College of Chemistry and Environmental Engineering, Shanghai Institute of Technology, No. 100 Haiquan Road, Fengxian District, 201400 Shanghai, China.
E-mail: clab@sit.edu.cn
Fax: +021-6087-7210

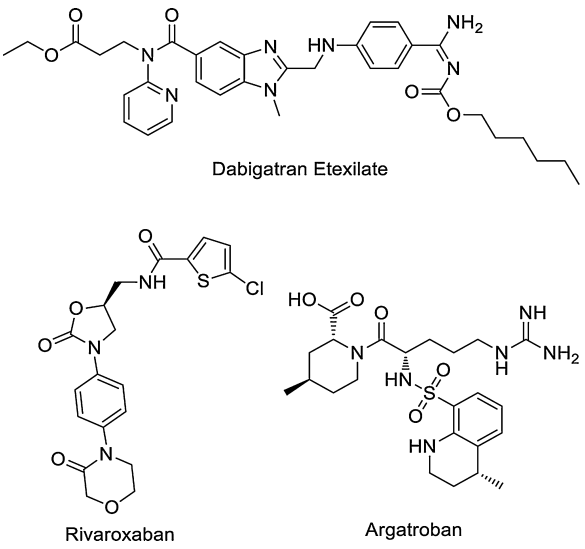


Figure 1. Structures of commercial thrombin inhibitory drugs.

structures. This modification was expected to generate a series of fluoride-modified anti-thrombin derivatives, which could maximize the selected characteristics of fluorine atoms and improve the shortcomings of thrombin inhibitory drugs, thereby providing more efficient and safer novel anti-clotting drug candidate compounds.

The research and development of novel compounds has become more efficient with the development of computer-aided simulation and design. In the present study, we designed and synthesized a series novel of fluoride anti-thrombin derivatives using dabigatran etexilate as the reference molecule, studied their anti-thrombin activities, and validated their activity using molecular modeling approaches (Fig. 2). Initially, docking experiments with designed structures at the active site (PDB entry code: 4JN2) [21] were performed *in silico* to assess their binding (docking scores) with respect to that of dabigatran. Dabigatran etexilate has a basic benzimidazole ring and a benzamidine group that is interlinked using amino linker. However, a 3-(pyridyl-2-amino)propionic acid ethyl ester is connected to the benzimidazole ring through carboxide. According to the literature [22], 3-(pyridyl-2-amino)propionic acid ethyl ester and its analogs account for its bioactivity. Therefore, we replaced the pyridine ring with a benzene ring and introduced various fluoride groups at different sites (Fig. 3). The effects of the introduction of fluorine atoms on the anti-coagulant activity were determined.

The designed compounds were synthesized and evaluated for their capacity to inhibit thrombin activity. Furthermore,

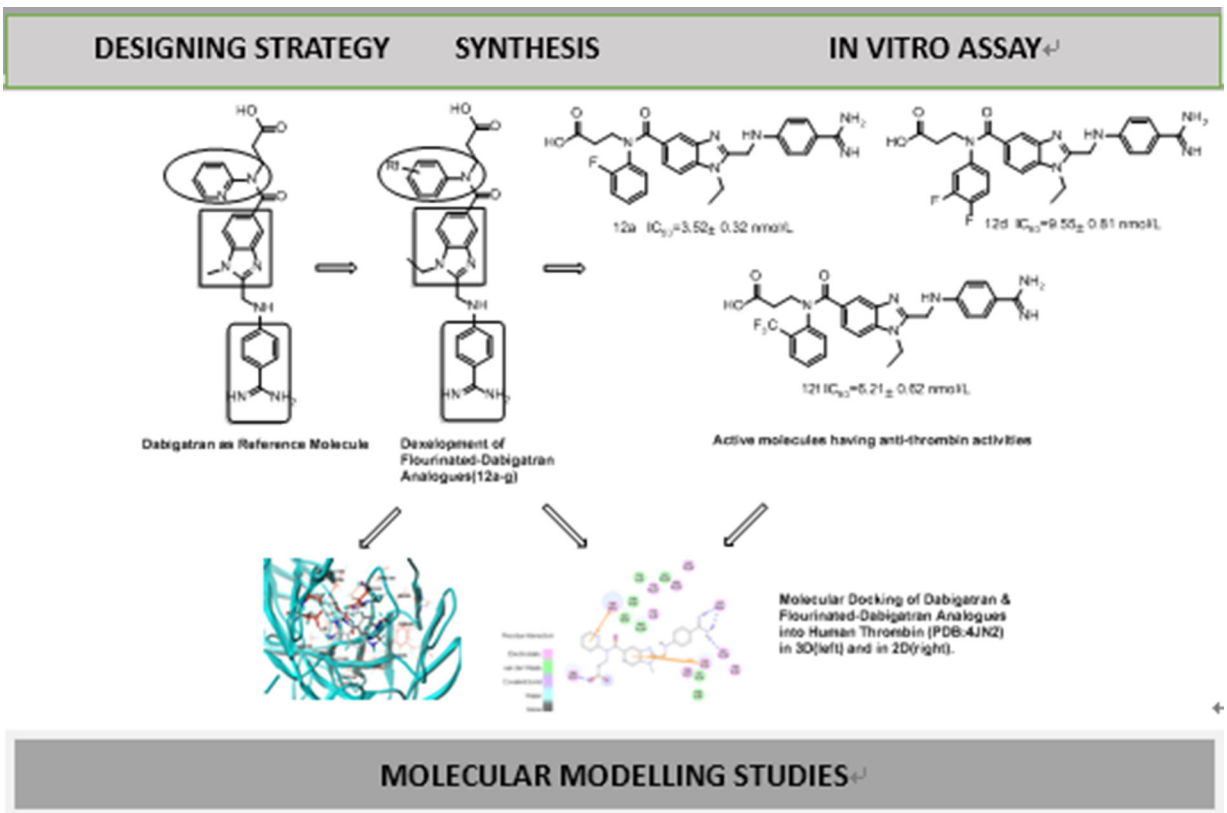


Figure 2. The outline of the various experimental and modeling techniques used in the study.

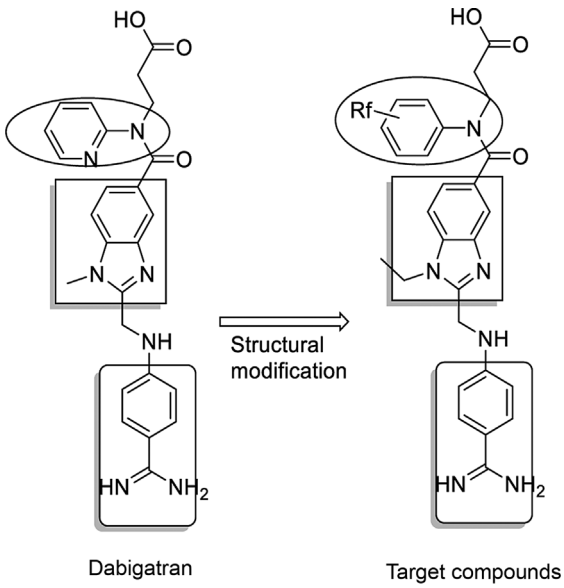


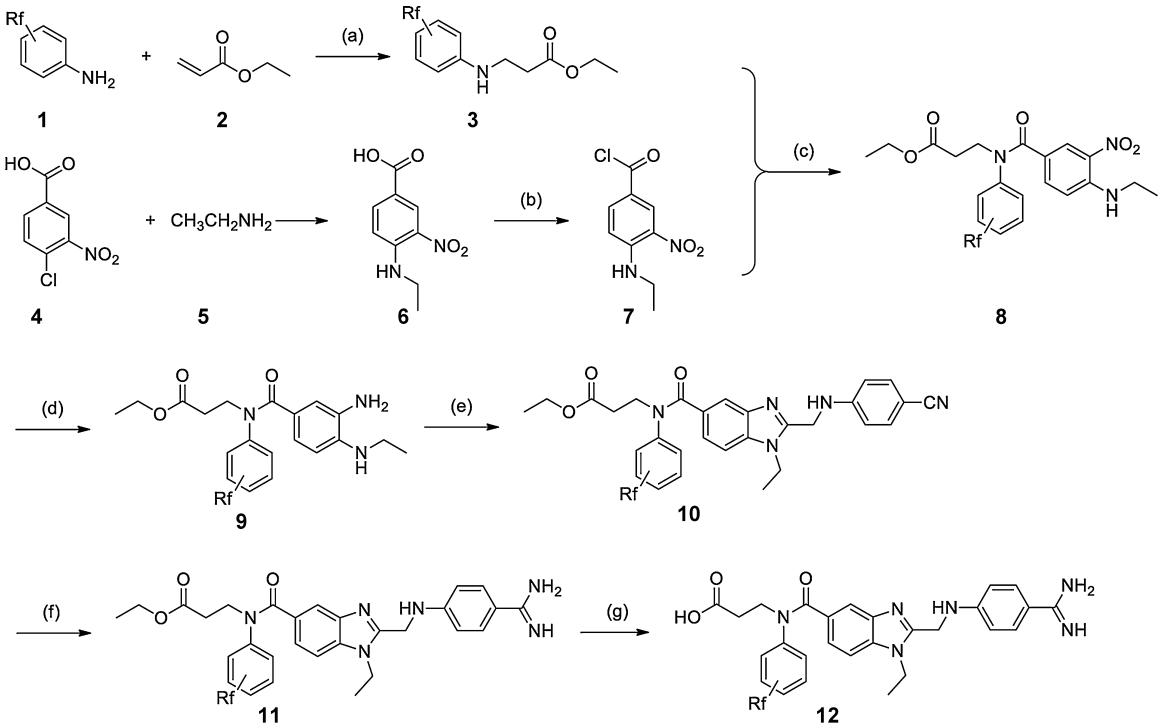
Figure 3. Design strategy of fluorinated thrombin inhibitor derivaties.

molecular modeling studies were conducted by molecular docking approaches to understand the inhibitory mechanism.

Results and discussion

Chemistry

Given the high pharmacological activity profile of thrombin inhibitory drugs and fluorinated compounds, we designed and synthesized fluorinated thrombin inhibitor derivatives through the synthetic process depicted in Scheme 1. The main intermediate ethyl 3-((fluorinated-phenyl)amino)propanoate (**3**) was synthesized from fluorinated anilines (**1**) in excess ethyl acrylate with the catalyst trifluoromethanesulfonic acid at 100°C. The synthesis of 4-(ethylamino)-3-nitrobenzoic acid (**6**) was achieved in good yield by the reaction between 4-chloro-3-nitrobenzoic acid and ethylamine in water (60%) without a solvent. The intermediate 4-(ethylamino)-3-nitrobenzoyl chloride (**7**) was synthesized from compound **6** and thionyl chloride in dichloromethane using *N*,*N*-dimethylformamide as a catalyst. The important intermediate ethyl 3-(*N*-(fluorinated-phenyl)-4-(ethylamino)-3-nitrobenzamido)propanoate (**8**) was synthesized from the intermediate



Scheme 1. Reagents, conditions, and range of yields: a) trifluoromethanesulfonic acid, yields: 38–98%; b) thionyl chloride, *N*,*N*-dimethylformamide, dichloromethane, yields: 100%; c) triethylamine, dichloromethane, yields: 24–79%; d) Zn powder, ammonium chloride, tetrahydrofuran/water, yields: 59–98%; e) (i) 2-((4-cyanophenyl)amino)acetic acid, EDCI, HoBt, tetrahydrofuran/*N*,*N*-dimethylformamide, (ii) acetic acid, ammonium hydroxide, yields: 46–89%; f) (i) hydroxylamine hydrochloride, triethylamine, ethanol, (ii) Pd/C, ammonium formate, acetic acid, nitrogen, yields: 54–88%; g) sodium hydroxide, ethanol/water, yields: 42–99%.

Table 1. Thrombin inhibitor activities of the synthesized compounds.

No.	Compound	Rf	IC_{50} (Mean ± SD)[a] (nmol/L)
1	**12a**	2-F	**3.52 ± 0.32**
2	**12b**	3-F	**7.49 ± 0.7**
3	**12c**	4-F	**4.26c ± 0.3**
4	**12d**	3,4-F_2	9.55 ± 0.81
5	**12e**	3,5-F_2	**7.9 ± 0.56**
6	**12f**	2-CF_3	**6.21 ± 0.62**
7	**12g**	3-CF_3	26.3 ± 0.33
8	**12h**	4-CF_3	199 ± 16.48
9	**12i**	4-OCF_3	117.7 ± 10.77
10	Argatroban[b]		9.46 ± 0.92

Bold face indicates significant anti-thrombin inhibitory activity.
[a] Values are expressed as the mean ± standard error of the mean of six experiments.
[b] Reference drug.

compounds **3** and **7** in dichloromethane using triethylamine as the base. The main intermediate ethyl 3-(3-amino-*N*-(fluorinated-phenyl)-4-(ethylamino)benzamido)propanoate (**9**) was obtained by the reductant of zinc powder from compound **8** in tetrahydrofuran with water as the solvent. The main intermediate compound **10** was obtained from compounds **9a–i** by condensation reactions. However, the most important intermediate compound **11** was achieved from intermediate **10** through the Pinner reaction. The target compound **12** was obtained from compounds **11a–i** by hydrolysis.

The structures of products **12a–i** were determined from their ^{1}H NMR and ^{13}C NMR spectra as well as by FT-ICR-MS.

Anti-thrombin activity *in vitro*

The potency of the anti-thrombin activity of the synthesized fluorinated thrombin inhibitor analogs was measured by chromogenic assay [6] to determine potential thrombin inhibitors of interest. Argatroban was used as the reference compound for the comparison of the results. The thrombin inhibitory activities of the synthesized compounds are shown in Table 1. Most of the designed compounds clearly exhibited moderate inhibitory activity toward thrombin. Compounds **12a–f** were found to be more potent than argatroban for thrombin inhibition. Their IC_{50} values differed because of the different attached substituents and different positions of the phenyl ring in **12a–i**. The substituent fluorinated thrombin inhibitor derivatives, including **12a–f**, showed significant activity with IC_{50} values of 3.52, 7.49, 4.26, 7.9, and 6.21 nmol/L, respectively. Among the newly synthesized derivatives, compound **12a** (3.52 nmol/L) showed the most potent thrombin inhibition; this compound had a (–F) group at the *ortho* position. When this (–F) group was attached at the *meta* and *para* positions, the thrombin inhibition activity was decreased, as observed in **12b** and **12c** with IC_{50} values of 7.49 and 4.26 nmol/L, respectively. However, when (–CF_3) was placed in the *ortho*, *meta*, and *para* positions, the thrombin inhibitory activity decreased to 6.21, 26.3, and 199 nmol/L, as in the case of **12f–h**. Compound **12d** showed moderate thrombin inhibitory potency when (–F_2) was attached at the 3,4-position; the inhibitory activity increased when this group was found in the 3,5-position (**12e**; 7.9 nmol/L). Compound **12i** has a strong electron donating (–OCF_3) group at the *para* position and a remarkably decreased IC_{50} value of 117.7 nmol/L.

Molecular modeling

The molecular modeling studies were performed using the molecular modeling package SYBYL-X2.0 (Tripos, Inc., USA). The 3D structures of all compounds were constructed with the Sketch Molecule module. Energy minimization was performed by the Powell gradient algorithm with the Tripos force field [23] and Gasteiger–Hückel charge [24]. The maximum number of iterations for the minimization was set to 10000. The minimization was terminated when the energy gradient convergence criterion of 0.005 kcal/mol · Å was reached [25].

Molecular docking

To study the binding mode of the inhibitors in the active site of thrombin protein, molecular docking was performed with the Surflex-Dock module of SYBYL-X 2.0. The crystal structure of the thrombin protein receptor complex was retrieved from the RCSB Protein Data Bank (PDB entry code: 4JN2). The ligands were docked to the binding site of the corresponding proteins by an empirical scoring function and a patented search engine in Surflex-Dock. Before the docking process, one natural ligand was extracted while the other natural ligands and water molecules were removed from the crystal structure. Subsequently, the protein was prepared with the Biopolymer module implemented in Sybyl. The polar hydrogen atoms were added and the Gasteiger–Hückel charges were assigned to the protein atoms. Automated docking was applied in the present work. Other parameters were established by default in the software. The Surflex-Dock total scores, which were expressed in $-\log_{10}(K_d)$ units to represent binding affinities, were applied to estimate the

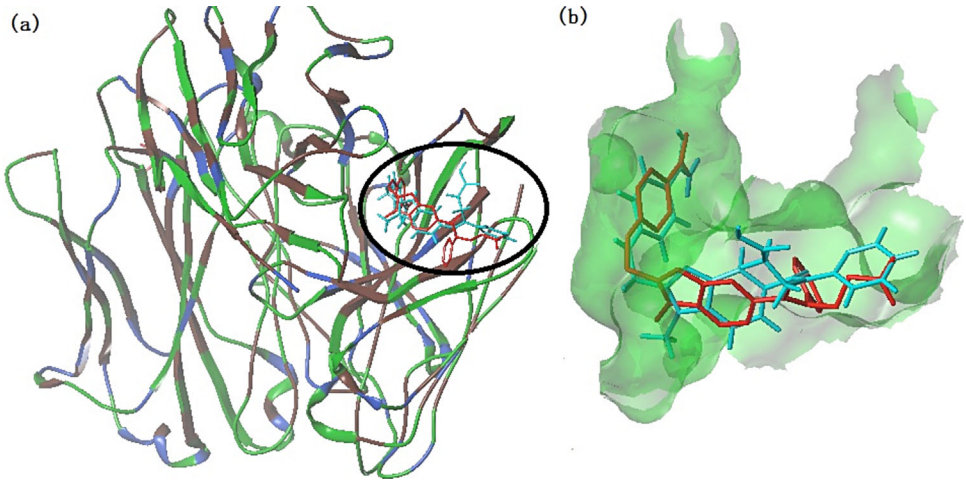


Figure 4. a) The comparison of dabigatran (red) in the crystal structure (PDB code 4JN2) and compound **12a** (cyan) in the docked complex by superimposing the coordinates of protein together. The ribbon is color-coded by lipophilic potential. b) A molecular surface of the active site of inhibitor–thrombin complexes depicted by transparent.

ligand–receptor interactions of the newly designed molecules.

Docking analysis

Dabigatran was re-docked to validate the docking reliability, as shown in Fig. 4. The binding mode of the re-docked and co-crystallized compound **12a** is almost similar in the active site of the thrombin receptor (PDB entry code: 4JN2) (Table 2). For example, the H atom of acetamidine interacts with the carbonyl group of His102 and the O atom of Trp53 by water-mediated hydrogen bonds. The O atom of the carbonyl group interacts with the hydroxyl group of Ser57. The benzimidazole rings of both compounds are bound to thrombin by a hydrophobic interaction in the same pocket. The pyridine ring is also positioned in the same pocket, except for the slight rotation of bonds. After validating the docking reliability, the compound with the highest activity was selected for exploring the probable binding conformation.

The interaction of compound **12a** with the binding site of the thrombin receptor is shown in Fig. 5. Compound **12a** was successfully docked in the binding cavity (Fig. 5a). This compound shares a similar binding mode with other compounds, as reported in the literature. The benzimidazole ring forms π–π stacking interactions with the conserved Tyr32, which is crucial for the binding of dabigatran derivatives with the thrombin protein. The central template is bound to thrombin by a hydrophobic interaction with the key residue Tyr40. The substituted benzene ring is positioned between Tyr104, Tyr107, and Asp108 in the same pocket. The carboxyl group and benzimidazole ring of compound **12a** exhibit van der Waals interaction with the key residues: Tyr40, His99, Thr98, and Phe100. Electrostatic interactions were observed between the amidino and carboxyl groups and the following important amino acids: Trp48, Phe110, Tyr107, and His102. Two hydrogen bonds were formed between the amidino group and the Asp36 and His102 residues, while two more hydrogen bonds were formed between the O and H atoms of the hydroxyl group and the Trp53 and Tyr101 residues, as shown in Fig. 5b. The observed hydrogen bond distances were 1.94 Å (Asp36–O…H–N–),

Table 2. Binding scores, amino acid interactions, and hydrogen bond length of the docked compounds on the active site of 4JN2.

Compound no.	Total score	Amino acid residue	H bond length A
12a	10.0853	Asp36, His102, Trp53, Tyr101	1.94, 2.18, 2.06, 2.02
12b	11.2988	Asp36, His102, Trp53, Tyr101	1.92, 2.17, 2.06, 2.06
12c	10.2847	Asp36, His102, Trp53, Tyr104	2.02, 2.09, 2.16, 1.91
12d	10.4239	Asp36, His102, Ser57	1.87, 1.97, 2.05
12e	10.0810	Asp36, His102, Trp53, Ser57	2.09, 2.22, 1.92, 2.60
12f	11.0723	Asp36, His102, Tyr32, Trp53,Tyr101	2.04, 2.11, 2.82, 2.04, 2.04
12g	9.0756	Asp36, His102	2.04, 2.26
12h	11.7752	Asp36, His102, Trp53	1.97, 2.29, 1.90
12i	10.1096	Asp36, His102, Phe100	2.01, 2.13, 2.16
Dabigatran	11.0146	Asp36, Tyr104	2.01, 1.77

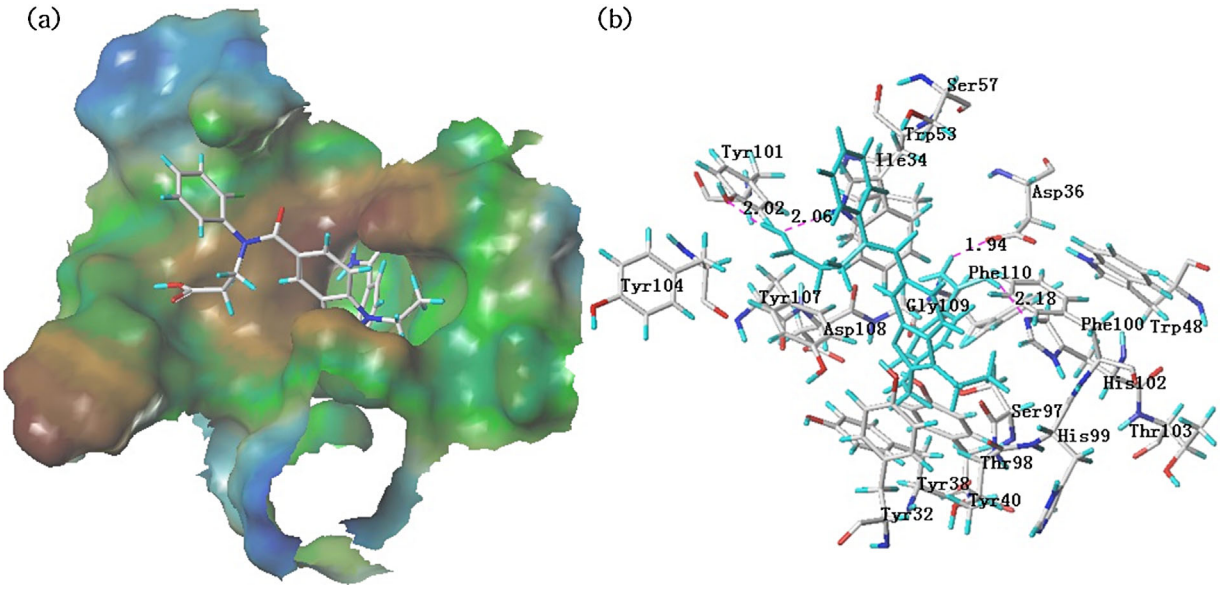


Figure 5. a) A surface of compound **12a** in complex with thrombin receptor. The lipophilic potential is mapped on the protein surface. b) Docking result of the compound **12a** into the binding site of thrombin protease. Hydrogen bonds are shown as pink dashed lines, with distance unit of Å. The inhibitor (cyan) and the important residues (atom type) are shown as stick model.

2.18 Å (His102–C–O…H–N–), 2.02 Å (Tyr101–O–H…H–N), and 2.06 Å (Trp53–N–H…O–H). These hydrogen bond interactions may have important roles in the interaction of dabigatran derivatives with the residues in the active site of thrombin.

Conclusion

A novel series of fluorinated thrombin inhibitor derivatives were designed and synthesized on the basis of a docking study at the 4JN2 site. The synthesized compounds were characterized and evaluated *in vitro* for thrombin inhibitory activity. The present investigation demonstrated the significant antithrombin activity in some of these novel compounds, which was comparable to argatroban. The results suggested that these novel compounds could represent alternative lead compounds for the synthesis of alternative thrombin inhibitors.

Experimental

General

All the chemicals and solvents were purchased from Darui and Titan Corporation and used without further purification. Melting points were measured on WRS-1B and were uncorrected. NMR spectra were carried out using a Bruker Avance 500 MHz NMR spectrometer, using TMS as internal reference. Chemical shifts are reported in δ scale (ppm). Mass spectra of the synthesized compounds were recorded by a Bruker FT-ICR-MS.

*General procedure for the synthesis of ethyl 3-((fluorinated-phenyl)amino)propanoates **3a–i***

To a solution of commercial fluorinated benzenamine (**1a–i**) (0.05 mol) and ethyl acrylate (0.09 mol) without solvent, trifluoromethanesulfonic acid (0.005 mol) was added. The resulting mixture was heated to 100°C for 24 h. The progress of the reaction was monitored by thin layer chromatography using petroleum ether/dichloromethane (2:1) as solvent system. The crude mixture was purified by silica gel column chromatography eluting 5:1 petroleum ether/dichloromethane to give the immediate compounds **3a–g**.

*Ethyl 3-((2-fluorophenyl)amino)propanoate (**3a**)*

Red liquid; yield: 86%; ^{1}H NMR (500 MHz, chloroform-*d*) δ 6.91 (t, J = 8.7 Hz, 2H), 6.61–6.55 (m, 2H), 4.18 (q, J = 7.2 Hz, 2H), 3.95 (s, 1H), 3.42 (t, J = 6.3 Hz, 2H), 2.62 (t, J = 6.3 Hz, 2H), 1.29 (t, J = 7.2 Hz, 3H). FT-MS *m/z* Calcd. for $C_{11}H_{14}FNO_2$ ($[M+H]^+$): 212.10813. Found: 212.10697.

*Ethyl 3-((3-fluorophenyl)amino)propanoate (**3b**)*

Brown-yellow liquid; yield: 92%; ^{1}H NMR (500 MHz, chloroform-*d*) δ 6.91 (t, J = 8.7 Hz, 2H), 6.61–6.55 (m, 2H), 4.18 (q, J = 7.2 Hz, 2H), 3.95 (s, 1H), 3.42 (t, J = 6.3 Hz, 2H), 2.62 (t, J = 6.3 Hz, 2H), 1.29 (t, J = 7.2 Hz, 3H). FT-MS *m/z* Calcd. for $C_{11}H_{14}FNO_2$ ($[M+H]^+$): 212.10813. Found: 212.10819.

*Ethyl 3-((4-fluorophenyl)amino)propanoate (**3c**)*
Red liquid; yield: 98%; ^{1}H NMR (500 MHz, chloroform-*d*) δ 6.91 (t, J = 8.7 Hz, 2H), 6.61–6.55 (m, 2H), 4.18 (q, J = 7.2 Hz, 2H), 3.95 (s, 1H), 3.42 (t, J = 6.3 Hz, 2H), 2.62 (t, J = 6.3 Hz, 2H), 1.29 (t, J = 7.2 Hz, 3H). FT-MS *m/z* Calcd. for $C_{11}H_{14}FNO_2$ ($[M+H]^+$): 212.10813. Found: 212.10811.

*Ethyl 3-((3,4-difluorophenyl)amino)propanoate (**3d**)*
Orange-yellow liquid; yield: 96%; ^{1}H NMR (500 MHz, chloroform-*d*) δ 6.95 (q, J = 9.1 Hz, 1H), 6.40 (ddd, J = 12.7, 6.6, 2.8 Hz, 1H), 6.28 (d, J = 8.9 Hz, 1H), 4.19–4.13 (m, 2H), 3.37 (t, J = 6.3 Hz, 2H), 2.59 (t, J = 6.3 Hz, 2H), 1.27 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{11}H_{13}F_2NO_2$ ($[M+H]^+$): 230.09871. Found: 230.09822.

*Ethyl 3-((3,5-difluorophenyl)amino)propanoate (**3e**)*
Yellow-green solid; yield: 93%; m.p.: 42.1–43.2°C; ^{1}H NMR (500 MHz, chloroform-*d*) δ 6.15 (tt, J = 9.2, 2.2 Hz, 1H), 6.11 (dd, J = 10.0, 2.1 Hz, 2H), 4.37 (s, 1H), 4.18 (q, J = 7.1 Hz, 2H), 3.47–3.37 (m, 2H), 2.62 (t, J = 6.2 Hz, 2H), 1.29 (t, J = 7.2 Hz, 3H). FT-MS *m/z* Calcd. for $C_{11}H_{13}F_2NO_2$ ($[M+H]^+$): 230.09871. Found: 230.09858.

*Ethyl 3-((2-(trifluoromethyl)phenyl)amino)propanoate (**3f**)*
Brown-yellow oil; yield: 62%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.42 (t, J = 7.5 Hz, 2H), 6.86 (d, J = 8.3 Hz, 1H), 6.75–6.68 (m, 1H), 5.38 (s, 1H), 4.07 (q, J = 6.8 Hz, 2H), 3.45 (q, J = 6.1 Hz, 2H), 2.60 (t, J = 6.4 Hz, 2H), 1.17 (t, J = 7.0 Hz, 3H). FT-MS *m/z* Calcd. for $C_{12}H_{14}F_3NO_2$ ($[M+H]^+$): 262.10494. Found: 262.10449.

*Ethyl 3-((3-(trifluoromethyl)phenyl)amino)propanoate (**3g**)*
Red liquid; yield: 85%; ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.30–7.27 (m, 1H), 6.96 (d, J = 7.6 Hz, 1H), 6.83 (s, 1H), 6.77 (dd, J = 8.2, 2.0 Hz, 1H), 4.31 (s, 1H), 4.19 (q, J = 7.1 Hz, 2H), 3.49 (t, J = 6.2 Hz, 2H), 2.64 (t, J = 6.3 Hz, 2H), 1.29 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{12}H_{14}F_3NO_2$ ($[M+H]^+$): 262.10494. Found: 262.10401.

*Ethyl 3-((4-(trifluoromethyl)phenyl)amino)propanoate (**3h**)*
Gray-green solid; yield: 38%; m.p.: 60.5–61.2°C; ^{1}H NMR (500 MHz, chloroform-*d*) δ 6.91 (t, J = 7.0 Hz, 2H), 6.62–6.54 (m, 2H), 4.18 (q, J = 5.7 Hz, 2H), 3.95 (s, 1H), 3.42 (t, J = 5.0 Hz, 2H), 2.62 (t, J = 5.0 Hz, 2H), 1.29 (t, J = 5.7 Hz, 3H). FT-MS *m/z* Calcd. for $C_{12}H_{14}F_3NO_2$ ($[M+H]^+$): 262.10494. Found: 262.10416.

*Ethyl 3-((4-(trifluoromethoxy)phenyl)amino)propanoate (**3i**)*
Red liquid; yield: 85%; ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.11 (q, J = 8.0 Hz, 1H), 7.06 (d, J = 8.7 Hz, 1H), 6.59 (d, J = 8.9 Hz, 1H), 6.41 (ddd, J = 13.8, 8.2, 2.0 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 3.45 (t, J = 6.2 Hz, 2H), 2.63 (t, J = 6.3 Hz, 2H), 1.29 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{12}H_{14}F_3NO_3$ ($[M+H]^+$): 278.09985, Found: 278.10087.

*Synthetic procedure for 4-(ethylamino)-3-nitrobenzoic acid (**6**)*
A 250 mL round-bottom flask was charged with 4-chloro-3-nitrobenzoic acid (20 g, 0.1 mol) and a solution of ethylamine in water (60%, 86 mL, 0.8 mol). The mixture was refluxed at 80°C for 5 h. The solution was adjusted to pH 4–5 with acetic acid. The precipitations of yellow solids were filtered to get products **5** (19.6 g, 100%).

*Synthetic procedure for 4-(ethylamino)-3-nitrobenzoyl chloride (**7**)*
A 250 mL round-bottom flask was charged with 4-(ethylamino)-3-nitrobenzoic acid (9.0 g, 0.046 mol). Thirty milliliters of dichloromethane and five drops of *N*,*N*-dimethylformamide were added in one portion. The mixture was stirred at room temperature (30°C). Thionyl chloride (20 mL, 0.23 mol) was added dropwise via addition funnel. The reaction solution was stirred at room temperature for 5–6 h. The progress of the reaction was monitored by thin layer chromatography using ethyl acetate/petroleum ether (1:1) as solvent system. The reaction solvent was evaporated *in vacuo* to dryness to give the product **6** without further operation.

*General procedure for the synthesis of ethyl 3-(N-(fluorinated-phenyl)-4-(ethylamino)-3-nitrobenzamido)-propanoates **8a–i***
To a vigorously stirred mixture of compound **3** (0.03 mol) and triethylamine (0.03 mol) in anhydrous dichloromethane (50 mL) at room temperature 4-(ethylamine)-3-nitrobenzoyl-chloride (0.03 mol) in anhydrous dichloromethane was added dropwise over 30 min. The reaction mixture was further stirred for 5 h at room temperature. The progress of the reaction was monitored by thin layer chromatography using ethyl acetate/petroleum ether (1:1) as solvent system. The reaction mixture was washed with aqueous sodium chloride solution three times and dried over anhydrous sodium sulfate. The crude product was purified by silica gel column chromatography eluting with 2:1 petroleum ether/ethyl acetate to give the product **8a–i**.

*Ethyl 3-(4-(ethylamino)-N-(2-fluorophenyl)-3-nitrobenzamido)propanoate (**8a**)*
Yellow solid; yield: 65%; m.p.: 91.2–92.3°C; ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.12 (d, J = 1.9 Hz, 1H), 8.03 (s, 1H), 7.48 (dd, J = 9.0, 1.7 Hz, 1H), 7.25–7.21 (m, 1H), 7.11 (t, J = 7.6 Hz, 1H), 7.02 (t, J = 9.1 Hz, 1H), 6.64 (d, J = 9.0 Hz, 1H), 4.16–4.09 (m, 2H), 4.05 (q, J = 7.1 Hz, 2H), 3.34–3.24 (m, 2H), 2.71 (d, J = 35.8 Hz, 2H), 1.31 (t, J = 7.2 Hz, 3H), 1.20 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{20}H_{22}FN_3O_5$ ($[M+H]^+$): 404.16163. Found: 404.16413.

*Ethyl 3-(4-(ethylamino)-N-(3-fluorophenyl)-3-nitrobenzamido)propanoate (**8b**)*
Red-orange solid; yield: 79%; m.p.: 95.8–96.3°C; ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.16 (d, J = 2.0 Hz, 1H), 7.42 (dd, J = 9.0, 1.9 Hz, 1H), 7.30–7.24 (m, 1H), 6.93 (td, J = 8.2, 2.0 Hz, 2H), 6.86 (dt, J = 9.5, 2.1 Hz, 1H), 6.63 (d, J = 9.1 Hz, 1H), 4.19 (t, J = 7.2 Hz, 2H), 4.08 (q, J = 7.1 Hz, 2H), 3.34–3.27 (m, 2H), 2.70 (t, J = 7.2 Hz, 2H), 1.33 (t, J = 7.2 Hz, 3H), 1.22 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{20}H_{22}FN_3O_5$ ($[M+H]^+$): 404.16163. Found: 404.16131.

*Ethyl 3-(4-(ethylamino)-N-(4-fluorophenyl)-3-nitrobenzamido)propanoate (**8c**)*
Yellow solid; yield: 65%; m.p.: 85.3–86.9°C; ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.18–7.99 (m, 2H), 7.33 (d, J = 8.8 Hz, 1H), 7.18–7.04 (m, 2H), 7.00–6.91 (m, 1H), 6.58 (d, J = 9.0 Hz, 1H), 4.21–4.10 (m, 2H), 4.02 (q, J = 6.9 Hz, 2H), 3.24 (dd, J = 13.1, 6.1 Hz, 2H), 2.64 (t, J = 7.0 Hz, 2H), 1.33–1.24 (m, 3H), 1.17 (q, J = 9.9, 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{20}H_{22}FN_3O_5$ ([M+H]$^+$): 404.16163. Found: 404.16423.

*Ethyl 3-(N-(3,4-difluorophenyl)-4-(ethylamino)-3-nitrobenzamido)propanoate (**8d**)*
Yellow solid; yield: 75%; m.p.: 110.3–110.7°C; ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.15 (d, J = 1.7 Hz, 1H), 7.41–7.36 (m, 1H), 7.09 (q, J = 8.9 Hz, 1H), 7.02 (td, J = 7.4, 3.5 Hz, 1H), 6.89 (d, J = 8.4 Hz, 1H), 6.65 (d, J = 9.0 Hz, 1H), 4.14 (t, J = 7.0 Hz, 2H), 4.08 (q, J = 7.1 Hz, 2H), 3.36–3.25 (m, 2H), 2.69 (t, J = 7.0 Hz, 2H), 1.33 (t, J = 7.2 Hz, 3H), 1.22 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{20}H_{21}F_2N_3O_5$ ([M+H]$^+$): 422.15220. Found: 422.15308.

*Ethyl 3-(N-(3,5-difluorophenyl)-4-(ethylamino)-3-nitrobenzamido)propanoate (**8e**)*
Yellow solid; yield: 59%; m.p.: 99.0–99.1°C; ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.19 (d, J = 1.6 Hz, 1H), 8.10 (s, 1H), 7.42 (d, J = 9.0 Hz, 1H), 6.71–6.65 (m, 3H), 4.15 (t, J = 7.1 Hz, 2H), 4.09 (q, J = 7.1 Hz, 2H), 3.32 (p, J = 7.1 Hz, 2H), 2.69 (t, J = 7.1 Hz, 2H), 1.33 (t, J = 7.2 Hz, 3H), 1.22 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{20}H_{21}F_2N_3O_5$ ([M+H]$^+$): 422.15220. Found: 422.15140.

*Ethyl 3-(4-(ethylamino)-3-nitro-N-(2-(trifluoromethyl)-phenyl)benzamido)propanoate (**8f**)*
Orange-yellow solid; yield: 49%; m.p.: 78.4–78.8°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.50 (d, J = 3.8 Hz, 2H), 7.47 (d, J = 6.5 Hz, 1H), 7.39–7.34 (m, 1H), 6.70 (d, J = 1.4 Hz, 1H), 6.28 (dd, J = 6.6, 1.4 Hz, 1H), 6.10 (d, J = 6.6 Hz, 1H), 4.07 (t, J = 5.5 Hz, 2H), 3.94 (q, J = 5.7 Hz, 2H), 2.98 (q, J = 5.4 Hz, 2H), 2.56 (t, J = 5.5 Hz, 2H), 1.12 (dt, J = 11.3, 5.7 Hz, 6H). FT-MS *m/z* Calcd. for $C_{21}H_{22}F_3N_3O_5$ ([M+H]$^+$): 454.15843. Found: 454.15842.

*Ethyl 3-(4-(ethylamino)-3-nitro-N-(3-(trifluoromethyl)-phenyl)benzamido)propanoate (**8g**)*
Orange-yellow solid; yield: 63%; m.p.: 81.2–82.1°C; ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.12 (d, J = 2.0 Hz, 1H), 8.07 (s, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.44 (t, J = 7.9 Hz, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.33–7.28 (m, 1H), 6.65 (d, J = 9.1 Hz, 1H), 4.23 (t, J = 7.1 Hz, 2H), 4.07 (q, J = 7.1 Hz, 2H), 3.36–3.25 (m, 2H), 2.73 (t, J = 7.1 Hz, 2H), 1.33 (t, J = 7.2 Hz, 3H), 1.21 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{21}H_{22}F_3N_3O_5$ ([M+H]$^+$): 454.15843. Found: 454.15764.

*Ethyl 3-(4-(ethylamino)-3-nitro-N-(4-(trifluoromethyl)-phenyl)benzamido)propanoate (**8h**)*
Orange-yellow oil; yield: 74%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.63 (d, J = 6.7 Hz, 2H), 7.30 (d, J = 6.6 Hz, 2H), 6.68 (d, J = 1.5 Hz, 1H), 6.34 (dd, J = 6.5, 1.4 Hz, 1H), 6.12 (d, J = 6.6 Hz, 1H), 4.06 (t, J = 5.6 Hz, 2H), 3.95 (q, J = 5.7 Hz, 2H), 2.98 (dt, J = 9.8, 4.9 Hz, 2H), 2.56 (t, J = 5.6 Hz, 2H), 1.13 (dt, J = 13.0, 5.7 Hz, 6H). FT-MS *m/z* Calcd. for $C_{21}H_{22}F_3N_3O_5$ ([M+H]$^+$): 454.15843. Found: 454.16151.

*Ethyl 3-(4-(ethylamino)-3-nitro-N-(4-(trifluoromethoxy)-phenyl)benzamido)propanoate (**8i**)*
Yellow oil; yield: 48%; ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.14 (d, J = 2.1 Hz, 1H), 7.38 (dd, J = 9.0, 1.9 Hz, 1H), 7.16 (s, 4H), 6.63 (d, J = 9.1 Hz, 1H), 4.18 (t, J = 7.1 Hz, 2H), 4.06 (q, J = 7.1 Hz, 2H), 3.34–3.27 (m, 2H), 2.70 (t, J = 7.1 Hz, 2H), 1.32 (t, J = 7.2 Hz, 3H), 1.20 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{21}H_{22}F_3N_3O_5$ ([M+H]$^+$): 470.15335. Found: 470.15766.

*General procedure for the synthesis of ethyl 3-(3-amino-N-(fluorinated-phenyl)-4-(methylamino)benzamido)-propanoates **9a–i***
A round bottom flask was charged with a solution of compound **8** (20 mmol) in tetrahydrofuran/water (40/80 mL). Zn powder (0.1 mol) and NH_4Cl (10 mmol) were added in turn. The reaction mixture was refluxed at 80°C for overnight under the atmosphere of nitrogen. The reaction process was monitored by thin layer chromatography using ethyl acetate/petroleum ether (1:1) as solvent system. The mixture was filtered through a pad to remove the Zn powder. The residue solution was extracted with dichloromethane three times. The combined organic layer was washed with an aqueous solution of sodium chloride solution three times and dried over anhydrous sodium sulfate. The crude product was purified by silica gel chromatography eluting with 1:1 ethyl acetate/petroleum ether to give product **9**.

*Ethyl 3-(3-amino-4-(ethylamino)-N-(2-fluorophenyl)-benzamido)propanoate (**9a**)*
Black solid; yield: 98%; m.p.: 118.8–120.1°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.32 (t, J = 7.9 Hz, 1H), 7.25 (q, J = 6.8, 6.1 Hz, 1H), 7.14 (t, J = 8.4 Hz, 2H), 6.71 (d, J = 1.7 Hz, 1H), 6.36 (d, J = 8.0 Hz, 1H), 6.08 (d, J = 8.3 Hz, 1H), 4.75 (s, 1H), 4.58 (s, 2H), 3.95 (q, J = 7.1 Hz, 4H), 2.97 (q, J = 7.0 Hz, 2H), 2.57 (t, J = 6.6 Hz, 2H), 1.13 (dt, J = 13.9, 7.1 Hz, 6H). FT-MS *m/z* Calcd. for $C_{20}H_{24}FN_3O_3$ ([M+H]$^+$): 374.18745. Found: 374.18959.

*Ethyl 3-(3-amino-4-(ethylamino)-N-(3-fluorophenyl)-benzamido)propanoate (**9b**)*
Brown solid; yield: 82%; m.p.: 114.6–115.4°C; ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.19 (q, J = 7.4 Hz, 1H), 6.85 (d, J = 10.1 Hz, 4H), 6.73 (d, J = 8.1 Hz, 1H), 6.34 (d, J = 8.3 Hz, 1H), 4.17 (t, J = 7.2 Hz, 2H), 4.08 (q, J = 7.1 Hz, 2H), 3.10 (q, J = 7.1 Hz, 2H), 2.71 (t, J = 7.2 Hz, 2H), 1.24 (dt, J = 17.8, 7.1 Hz, 6H). FT-MS *m/z* Calcd. for $C_{20}H_{24}FN_3O_3$ ([M+H]$^+$): 374.18745. Found: 374.18941.

*Ethyl 3-(3-amino-4-(ethylamino)-N-(4-fluorophenyl)-benzamido)propanoate (**9c**)*
Gray solid; yield: 84%; m.p.: 112.7–113.8°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.17–7.07 (m, 4H), 6.68 (d, J = 1.8 Hz,

1H), 6.33 (dd, $J = 8.2$, 1.7 Hz, 1H), 6.11 (d, $J = 8.3$ Hz, 1H), 4.73 (s, 1H), 4.55 (s, 2H), 3.98 (p, $J = 7.0$ Hz, 4H), 2.99 (q, $J = 6.9$ Hz, 2H), 2.54 (t, $J = 7.1$ Hz, 2H), 1.14 (q, $J = 7.2$ Hz, 6H). FT-MS *m/z* Calcd. for $C_{20}H_{24}FN_3O_3$ ($[M+H]^+$): 374.18745. Found: 374.18883.

*Ethyl 3-(3-amino-N-(3,4-difluorophenyl)-4-(ethylamino)-benzamido)propanoate (**9d**)*
Red-brown solid; yield: 66%; m.p.: 112.7–113.8°C; ^{1}H NMR (500 MHz,DMSO-d_6) δ 7.39–7.28 (m, 2H), 6.89 (d, $J = 8.8$ Hz, 1H), 6.64 (d, $J = 1.9$ Hz, 1H), 6.38 (dd, $J = 8.2$, 1.9 Hz, 1H), 6.15 (d, $J = 8.3$ Hz, 1H), 4.77 (s, 1H), 4.61 (s, 2H), 4.00 (d, $J = 7.1$ Hz, 2H), 3.99–3.96 (m, 2H), 3.00 (q, $J = 7.0$ Hz, 2H), 2.55 (t, $J = 7.1$ Hz, 2H), 1.17–1.11 (m, 6H). FT-MS *m/z* Calcd. for $C_{20}H_{23}F_2N_3O_3$ ($[M+H]^+$): 392.17802. Found: 392.17966.

*Ethyl 3-(3-amino-N-(3,5-difluorophenyl)-4-(ethylamino)-benzamido)propanoate (**9e**)*
Gray solid; yield: 95%; m.p.: 122.5–123.5°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.04 (d, $J = 9.2$ Hz, 1H), 6.92–6.86 (m, 2H), 6.68 (d, $J = 1.8$ Hz, 1H), 6.43 (dd, $J = 8.2$, 1.7 Hz, 1H), 6.19 (d, $J = 8.3$ Hz, 1H), 4.82 (s, 1H), 4.61 (s, 2H), 4.06–4.01 (m, 2H), 4.01–3.96 (m, 2H), 3.02 (q, $J = 6.5$ Hz, 2H), 2.57 (t, $J = 7.0$ Hz, 2H), 1.15 (dt, $J = 11.2$, 7.1 Hz, 6H). FT-MS *m/z* Calcd. for $C_{20}H_{23}F_2N_3O_3$ ($[M+H]^+$): 392.17802. Found: 392.17923.

*Ethyl 3-(3-amino-4-(ethylamino)-N-(2-(trifluoromethyl)-phenyl)benzamido)propanoate (**9f**)*
Dark green oil; yield: 59%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.52–7.46 (m, 3H), 7.36 (d, $J = 5.8$ Hz, 1H), 6.72–6.67 (m, 1H), 6.28 (dd, $J = 6.6$, 1.4 Hz, 1H), 6.10 (d, $J = 6.6$ Hz, 1H), 4.78 (s, 1H), 4.60 (s, 2H), 4.07 (t, $J = 5.5$ Hz, 2H), 3.94 (q, $J = 5.7$ Hz, 2H), 2.98 (q, $J = 5.4$ Hz, 2H), 2.56 (t, $J = 5.5$ Hz, 2H), 1.12 (dt, $J = 11.3$, 5.7 Hz, 6H). FT-MS *m/z* Calcd. for $C_{21}H_{24}F_3N_3O_3$ ($[M+H]^+$): 424.18425. Found: 424, 18649.

*Ethyl 3-(3-amino-4-(ethylamino)-N-(3-(trifluoromethyl)-phenyl)benzamido)propanoate (**9g**)*
Dark red oil; yield: 84%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.51–7.45 (m, 3H), 7.36 (d, $J = 7.3$ Hz, 1H), 6.70 (d, $J = 1.8$ Hz, 1H), 6.28 (dd, $J = 8.2$, 1.7 Hz, 1H), 6.10 (d, $J = 8.3$ Hz, 1H), 4.78 (s, 1H), 4.60 (s, 2H), 4.07 (t, $J = 6.9$ Hz, 2H), 3.94 (q, $J = 7.1$ Hz, 2H), 2.98 (q, $J = 6.8$ Hz, 2H), 2.56 (t, $J = 6.9$ Hz, 2H), 1.12 (dt, $J = 14.1$, 7.1 Hz, 6H). FT-MS *m/z* Calcd. for $C_{21}H_{24}F_3N_3O_3$ ($[M+H]^+$): 424.18425. Found: 424.18649.

*Ethyl 3-(3-amino-4-(ethylamino)-N-(4-(trifluoromethyl)-phenyl)benzamido)propanoate (**9h**)*
Dark gray oil; yield: 92%; ^{1}H NMR (500 MHz,DMSO-d_6) δ 7.63 (d, $J = 8.4$ Hz, 2H), 7.30 (d, $J = 8.3$ Hz, 2H), 6.68 (d, $J = 1.9$ Hz, 1H), 6.34 (dd, $J = 8.2$, 1.7 Hz, 1H), 6.12 (d, $J = 8.3$ Hz, 1H), 4.80 (t, $J = 4.9$ Hz, 1H), 4.60 (s, 2H), 4.06 (t, $J = 7.0$ Hz, 2H), 3.95 (q, $J = 7.1$ Hz, 2H), 2.98 (p, $J = 7.0$ Hz, 2H), 2.56 (t, $J = 7.0$ Hz, 2H), 1.13 (dt, $J = 16.3$, 7.1 Hz, 6H). FT-MS *m/z* Calcd. for $C_{21}H_{24}F_3N_3O_3$ ($[M+H]^+$): 424.18425. Found: 424.18480.

*Ethyl 3-(3-amino-4-(ethylamino)-N-(4-(trifluoromethoxy)-phenyl)benzamido)propanoate (**9i**)*
Dark green oil; yield: 79%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.26 (d, $J = 8.7$ Hz, 2H), 7.22 (d, $J = 8.9$ Hz, 2H), 6.69 (s, 1H), 6.32 (d, $J = 8.2$ Hz, 1H), 6.11 (d, $J = 8.3$ Hz, 1H), 4.76 (s, 1H), 4.57 (s, 2H), 4.02 (t, $J = 6.9$ Hz, 2H), 3.95 (q, $J = 7.1$ Hz, 2H), 2.99 (q, $J = 7.1$ Hz, 2H), 2.56 (t, $J = 6.9$ Hz, 2H), 1.13 (dt, $J = 14.4$, 7.1 Hz, 6H). FT-MS *m/z* Calcd. for $C_{21}H_{24}F_3N_3O_4$ ($[M+H]^+$): 440.17917. Found: 440.14178.

*General procedure for the synthesis of compounds **10a–i***
A round bottom flask was charged with a solution of 2-((4-cyanophenyl)amino)acetic acid (12 mmol), EDCI (12 mmol), and HoBt (12 mmol) in tetrahydrofuran/*N*,*N*-dimethylformamide (70/10 mL). The reaction mixture was stirred at ice bath for 35 min. Then a solution of compound **9** (10 mmol) in tetrahydrofuran (50 mL) was added dropwise with stirring at room temperature. The reaction solution was stirred for overnight. The reaction solution was evaporated *in vacuo* to dryness. The residual was dissolved in dichloromethane (100 mL), washed with water three times, and dried over anhydrous sodium sulfate.The organic layer was evaported *in vacuo* to dryness. Then the residual was dissolved in acetic acid (70 mL) and refluxed at 120°C for 2–3 h. The reaction solution was adjusted to pH 9–10 with ammonium hydroxide and stirred for 30 min. The reaction solution was extracted with dichloromethane three times, washed with water three times, and dried over anhydrous sodium sulfate. The reaction process was monitored by thin layer chromatography using dichloromethane/methyl alcohol (20:1) as solvent system. The crude product was purified by silica gel chromatography eluting with 2:1 ethyl acetate/petroleum ether to give product **10**.

*Ethyl 3-(2-(((4-cyanophenyl)amino)methyl)-1-ethyl-N-(2-fluorophenyl)-1H-benzo[d]imidazole-5-carboxamido)-propanoate (**10a**)*
Red-brown solid; yield: 89%; m.p.: 176.8–177.6°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.48 (t, $J = 7.6$ Hz, 3H), 7.43 (d, $J = 8.4$ Hz, 1H), 7.30 (t, $J = 5.2$ Hz, 1H), 7.24 (d, $J = 8.1$ Hz, 2H), 7.13 (dt, $J = 23.1$, 8.2 Hz, 2H), 6.82 (d, $J = 8.7$Hz, 2H), 4.59 (d, $J = 5.3$ Hz, 2H), 4.24 (q, $J = 6.8$ Hz, 2H), 4.04 (s, 2H), 3.96 (q, $J = 7.1$ Hz, 2H), 2.71–2.54 (m, 2H), 1.24 (t, $J = 7.0$ Hz, 3H), 1.12 (t, $J = 7.1$ Hz, 3H). FT-MS *m/z* Calcd. for $C_{29}H_{28}FN_5O_3$ ($[M+H]^+$): 514.22489. Found: 514.22582.

*Ethyl 3-(2-(((4-cyanophenyl)amino)methyl)-1-ethyl-N-(3-fluorophenyl)-1H-benzo[d]imidazole-5-carboxamido)-propanoate (**10b**)*
Red-brown oil; yield: 73%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.54 (s, 1H), 7.48 (d, $J = 8.7$ Hz, 2H), 7.44 (d, $J = 8.4$ Hz, 1H), 7.29–7.24 (m, 2H), 7.20 (d, $J = 10.2$ Hz, 1H), 6.99 (d, $J = 7.5$ Hz, 2H), 6.84 (d, $J = 8.7$ Hz, 2H), 4.61 (d, $J = 5.4$ Hz, 2H), 4.25 (q, $J = 7.0$ Hz, 2H), 4.13 (t, $J = 7.1$ Hz, 2H), 4.00 (q, $J = 7.1$ Hz, 2H), 2.64 (t, $J = 7.1$ Hz, 2H), 1.25 (t, $J = 7.2$ Hz, 3H), 1.14 (t, $J = 7.1$ Hz, 3H). FT-MS *m/z* Calcd. for $C_{29}H_{28}FN_5O_3$ ($[M+H]^+$): 514.22489. Found: 514.22602.

*Ethyl 3-(2-(((4-cyanophenyl)amino)methyl)-1-ethyl-N-(4-fluorophenyl)-1H-benzo[d]imidazole-5-carboxamido)-propanoate (**10c**)*
White solid; yield: 74%; m.p.: 161.7–162.4°C; ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.62 (s, 1H), 7.40 (d, J = 8.6 Hz, 2H), 7.29–7.24 (m, 1H), 7.11 (d, J = 8.4 Hz, 1H), 7.04 (dd, J = 8.6, 4.7 Hz, 2H), 6.88 (t, J = 8.4 Hz, 2H), 6.70 (d, J = 8.6 Hz, 2H), 5.70 (t, J = 4.7 Hz, 1H), 4.47 (d, J = 4.6 Hz, 2H), 4.20 (t, J = 7.2 Hz, 2H), 4.12 (q, J = 7.2 Hz, 2H), 4.07 (q, J = 7.1 Hz, 2H), 2.70 (t, J = 7.2 Hz, 2H), 1.36 (t, J = 7.2 Hz, 3H), 1.21 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{29}H_{28}FN_5O_3$ ([M+H]$^+$): 514.22489. Found: 514.22627.

*Ethyl 3-(2-(((4-cyanophenyl)amino)methyl)-N-(3,4-difluorophenyl)-1-ethyl-1H-benzo[d]imidazole-5-carboxamido)propanoate (**10d**)*
Red-brown oil; yield: 69%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.53 (s, 1H), 7.49–7.44 (m, 3H), 7.30 (dt, J = 15.2, 9.2 Hz, 2H), 7.21 (d, J = 8.5 Hz, 1H), 7.01 (d, J = 8.5 Hz, 1H), 6.83 (d, J = 8.7 Hz, 2H), 4.61 (d, J = 5.4 Hz, 2H), 4.25 (q, J = 6.7 Hz, 2H), 4.08 (t, J = 7.0 Hz, 2H), 4.00 (q, J = 7.1 Hz, 2H), 2.65–2.59 (m, 2H), 1.25 (t, J = 7.2 Hz, 3H), 1.14 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{29}H_{27}F_2N_5O_3$ ([M+H]$^+$): 532.21547. Found: 532.21860.

*Ethyl 3-(2-(((4-cyanophenyl)amino)methyl)-N-(3,5-difluorophenyl)-1-ethyl-1H-benzo[d]imidazole-5-carboxamido)propanoate (**10e**)*
Pink solid; yield: 93%; m.p.: 128.4–128.9°C; ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.67 (s, 1H), 7.43 (d, J = 8.7 Hz, 2H), 7.37–7.32 (m, 1H), 7.20 (d, J = 8.4 Hz, 1H), 6.72 (d, J = 8.7 Hz, 2H), 6.67–6.63 (m, 2H), 6.61–6.56 (m, 1H), 5.61 (s, 1H), 4.51 (d, J = 4.5 Hz, 2H), 4.21 (t, J = 7.1 Hz, 2H), 4.18–4.13 (m, 2H), 4.13–4.07 (m, 2H), 2.73 (t, J = 7.1 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H), 1.23 (t, J = 7.2 Hz, 3H). FT-MS *m/z* Calcd. for $C_{29}H_{27}F_2N_5O_3$ ([M+H]$^+$): 532.21547. Found: 532.21610.

*Ethyl 3-(2-(((4-cyanophenyl)amino)methyl)-1-ethyl-N-(2-(trifluoromethyl)phenyl)-1H-benzo[d]imidazole-5-carboxamido)propanoate (**10f**)*
Red solid; yield: 80%; m.p.: 152.3–152.9°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.72–7.67 (m, 1H), 7.61 (s, 2H), 7.47 (d, J = 8.1 Hz, 3H), 7.41 (s, 1H), 7.28 (s, 1H), 7.22 (d, J = 7.3 Hz, 1H), 6.82 (d, J = 7.5 Hz, 2H), 4.58 (s, 2H), 4.48 (s, 1H), 4.27–4.17 (m, 2H), 4.07–3.95 (m, 2H), 3.77–3.42 (m, 2H), 2.84–2.69 (m, 2H), 1.23 (s, 3H), 1.14 (s, 3H). FT-MS *m/z* Calcd. for $C_{30}H_{28}F_3N_5O_3$ ([M+H]$^+$): 564.22170. Found: 564.22165.

*Ethyl 3-(2-(((4-cyanophenyl)amino)methyl)-1-ethyl-N-(3-(trifluoromethyl)phenyl)-1H-benzo[d]imidazole-5-carboxamido)propanoate (**10g**)*
Brown-yellow oil; yield: 77%; ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.67 (s, 1H), 7.46 (d, J = 8.6 Hz, 2H), 7.38 (d, J = 11.2 Hz, 2H), 7.33 (t, J = 7.8 Hz, 1H), 7.31–7.25 (m, 2H), 7.17 (d, J = 8.4 Hz, 1H), 6.73(d, J = 8.6 Hz, 2H), 4.51 (d, J = 4.3 Hz, 2H), 4.28 (t, J = 7.0 Hz, 2H), 4.16 (q, J = 7.2 Hz, 2H), 4.08 (q, J = 7.1 Hz, 2H), 2.76 (t, J = 7.1 Hz, 2H), 1.39 (t, J = 7.2 Hz, 3H), 1.22 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{30}H_{28}F_3N_5O_3$ ([M+H]$^+$): 564.22170. Found: 564.21980.

*Ethyl 3-(2-(((4-cyanophenyl)amino)methyl)-1-ethyl-N-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazole-5-carboxamido)propanoate (**10h**)*
Yellow solid; yield: 68%; m.p.: 148.2–149.1°C; ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.69 (s, 1H), 7.46 (t, J = 8.2 Hz, 4H), 7.33–7.27 (m, 1H), 7.19 (dd, J = 15.9, 8.4 Hz, 3H), 6.72 (d, J = 8.6 Hz, 2H), 4.51 (d, J = 4.4 Hz, 2H), 4.28 (t, J = 7.1 Hz, 2H), 4.17 (q, J = 7.2 Hz, 2H), 4.08 (q, J = 7.1 Hz, 2H), 2.74 (t, J = 7.1 Hz, 2H), 1.41 (t, J = 7.3 Hz, 3H), 1.22 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{30}H_{28}F_3N_5O_3$ ([M+H]$^+$): 564.22170. Found: 564.22271.

*Ethyl 3-(2-(((4-cyanophenyl)amino)methyl)-1-ethyl-N-(4-(trifluoromethoxy)phenyl)-1H-benzo[d]imidazole-5-carboxamido)propanoate (**10i**)*
Pink solid; yield: 46%; m.p.: 162.3–163.1°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.51 (s, 1H), 7.44 (dd, J = 16.8, 8.6 Hz, 3H), 7.32 (t, J = 8.0 Hz, 2H), 7.26 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 8.5 Hz, 1H), 6.82 (d, J = 8.7 Hz, 2H), 4.61 (d, J = 5.3 Hz, 2H), 4.25 (q, J = 7.0 Hz, 2H), 4.12 (t, J = 6.9 Hz, 2H), 3.96 (q, J = 7.1 Hz, 2H), 2.62 (t, J = 7.0 Hz, 2H), 1.24 (t, J = 7.2 Hz, 3H), 1.11 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{30}H_{28}F_3N_5O_4$ ([M+H]$^+$): 580.21662. Found: 580.22290.

*General procedure for the synthesis of compounds **11a–i***
A round bottom flask was charged with a solution of compound **10** (5 mmol), hydroxylamine hydrochloride (10 mmol), and triethylamine (10 mmol) in ethanol (50 mL). The reaction mixture was refluxed at 80°C for 4–5 h. Then the reaction mixture was evaporated *in vacuo* to dryness to obtain the crude intermediate which was used without further purification in the next step. The crude intermediate was dissolved in acetic acid (50 mL). Then Pd/C (5%, 1.0 g) and ammonium formate (15 mmol) were added in turn. The reaction mixture was refluxed at 120°C for 5–6 h under the protection of nitrogen. The reaction process was monitored by thin layer chromatography using dichloromethane/methyl alcohol (10:1) as solvent system. The crude product was purified by silica gel chromatography eluting with 7:1 dichloromethane/methyl alcohol to give product **11**.

*Ethyl 3-(2-(((4-carbamimidoylphenyl)amino)methyl)-1-ethyl-N-(2-fluorophenyl)-1H-benzo[d]imidazole-5-carboxamido)propanoate (**11a**)*
White solid; yield: 51%; m.p.: 99.9–101.5°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.83 (s, 3H), 7.67 (d, J = 8.9 Hz, 2H), 7.50–7.46 (m, 2H), 7.43 (d, J = 8.3 Hz, 1H), 7.22 (d, J = 8.1 Hz, 2H), 7.13 (dt, J = 24.1, 8.1 Hz, 2H), 6.86 (d, J = 8.9 Hz, 2H), 4.64 (d, J = 5.3 Hz, 2H), 4.26 (q, J = 6.8 Hz, 2H), 4.07–3.99 (m, 2H), 3.96 (q, J = 7.1 Hz, 2H), 2.71–2.56 (m, 2H), 1.23 (t, J = 7.1 Hz, 3H), 1.12 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{29}H_{31}FN_6O_3$ ([M+H]$^+$): 531.25144. Found: 531.24776.

*Ethyl 3-(2-(((4-carbamimidoylphenyl)amino)methyl)-1-ethyl-N-(3-fluorophenyl)-1H-benzo[d]imidazole-5-carboxamido)propanoate (**11b**)*
White solid; yield: 42%; m.p.: 194.7–196.1°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.80 (s, 3H), 7.64 (d, J = 8.8 Hz, 2H), 7.51 (s, 1H), 7.44 (d, J = 8.1 Hz, 1H), 7.28–7.17 (m, 3H), 7.03–6.96 (m, 2H), 6.86 (d, J = 8.8 Hz, 2H), 4.64 (d, J = 5.3 Hz, 2H), 4.26 (q, J = 6.7 Hz, 2H), 4.10 (t, J = 7.0 Hz, 2H), 3.99 (q, J = 7.1 Hz, 2H), 2.62 (t, J = 7.0 Hz, 2H), 1.24 (t, J = 7.0 Hz, 3H), 1.14 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{29}H_{31}FN_6O_3$ ([M+H]$^+$): 531.25144. Found: 531.25389.

*Ethyl 3-(2-(((4-carbamimidoylphenyl)amino)methyl)-1-ethyl-N-(4-fluorophenyl)-1H-benzo[d]imidazole-5-carboxamido)propanoate (**11c**)*
White solid; yield: 51%; m.p.: 122.4–123.3°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.93 (s, 2H), 8.76 (s, 2H), 7.68 (d, J = 8.8 Hz, 2H), 7.51–7.48 (m, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.24 (dd, J = 8.7, 5.0 Hz, 2H), 7.18 (d, J = 8.3 Hz, 1H), 7.10 (t, J = 8.7 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 4.64 (d, J = 5.5 Hz, 2H), 4.27 (q, J = 6.9 Hz, 2H), 4.08 (t, J = 7.0 Hz, 2H), 3.99 (q, J = 7.1 Hz, 2H), 2.60 (t, J = 7.1 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H), 1.14 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{29}H_{31}FN_6O_3$ ([M+H]$^+$): 531.25144. Found: 531.25429.

*Ethyl 3-(2-(((4-carbamimidoylphenyl)amino)methyl)-N-(3,4-difluorophenyl)-1-ethyl-1H-benzo[d]imidazole-5-carboxamido)propanoate (**11d**)*
White solid; yield: 57%; m.p.: 105.6–106.3°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.61 (d, J = 8.8 Hz, 2H), 7.54 (s, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.31 (q, J = 9.2 Hz, 2H), 7.21 (d, J = 9.5 Hz, 1H), 7.01 (d, J = 8.8 Hz, 1H), 6.85 (d, J = 8.8 Hz, 2H), 4.64 (d, J = 5.4 Hz, 2H), 4.27 (q, J = 7.0 Hz, 2H), 4.08 (t, J = 7.1 Hz, 2H), 4.00 (q, J = 7.1 Hz, 2H), 2.62 (t, J = 7.1 Hz, 2H), 1.25 (t, J = 7.2 Hz, 3H), 1.14 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{29}H_{30}F_2N_6O_3$ ([M+H]$^+$): 549.24202. Found: 549.24301.

*Ethyl 3-(2-(((4-carbamimidoylphenyl)amino)methyl)-N-(3,5-difluorophenyl)-1-ethyl-1H-benzo[d]imidazole-5-carboxamido)propanoate (**11e**)*
White solid; yield: 55%; m.p.: 101.2–103.0°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.87 (s, 1H), 8.63 (s, 2H), 7.65 (d, J = 8.8 Hz, 2H), 7.56 (s, 1H), 7.46 (dd, J = 12.7, 7.0 Hz, 2H), 7.26–7.22 (m, 1H), 7.09–7.02 (m, 2H), 6.87 (d, J = 8.9 Hz, 2H), 4.66 (d, J = 5.5 Hz, 2H), 4.28 (q, J = 6.9 Hz, 2H), 4.10 (t, J = 7.0 Hz, 2H), 4.00 (q, J = 7.1 Hz, 2H), 2.64 (t, J = 7.0 Hz, 2H), 1.26 (t, J = 7.2 Hz, 3H), 1.17–1.14 (m, 3H). FT-MS *m/z* Calcd. for $C_{29}H_{30}F_2N_6O_3$ ([M+H]$^+$): 549.24202. Found: 549.24629.

*Ethyl 3-(2-(((4-carbamimidoylphenyl)amino)methyl)-1-ethyl-N-(2-(trifluoromethyl)phenyl)-1H-benzo[d]-imidazole-5-carboxamido)propanoate (**11f**)*
White solid; yield: 48%; m.p.: 105.3–106.4°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.82 (s, 4H), 7.67 (d, J = 7.1 Hz, 2H), 7.49 (q, J = 5.3, 4.7 Hz, 2H), 7.43 (d, J = 6.7 Hz, 1H), 7.22 (d, J = 6.5 Hz, 2H), 7.13 (dt, J = 19.2, 6.5 Hz, 2H), 6.86 (d, J = 7.1 Hz, 2H), 4.64 (d, J = 4.3 Hz, 2H), 4.26 (q, J = 5.4 Hz, 2H), 4.03 (s, 2H), 3.96 (q, J = 5.7 Hz, 2H), 2.70–2.55 (m, 2H), 1.23 (t, J = 5.7 Hz, 3H), 1.12 (t, J = 5.7 Hz, 3H). FT-MS *m/z* Calcd. for $C_{30}H_{31}F_3N_6O_3$ ([M+H]$^+$): 581.24825. Found: 581.25478.

*Ethyl 3-(2-(((4-carbamimidoylphenyl)amino)methyl)-1-ethyl-N-(3-(trifluoromethyl)phenyl)-1H-benzo[d]-imidazole-5-carboxamido)propanoate (**11g**)*
White solid; yield: 44%; m.p.: 126.1–126.9°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.77 (s, 4H), 7.76–7.71 (m, 1H), 7.70–7.66 (m, 1H), 7.64 (d, J = 8.6 Hz, 2H), 7.51 (s, 1H), 7.45 (d, J = 5.1 Hz, 2H), 7.44–7.40 (m, 1H), 7.19 (d, J = 8.5 Hz, 1H), 6.86 (d, J = 8.7 Hz, 2H), 4.65 (d, J = 5.4 Hz, 2H), 4.26 (q, J = 8.6, 7.7 Hz, 2H), 4.15 (t, J = 6.8 Hz, 2H), 3.96 (q, J = 7.1 Hz, 2H), 2.63 (t, J = 6.9 Hz, 2H), 1.24–1.19 (m, 3H), 1.12 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{30}H_{31}F_3N_6O_3$ ([M+H]$^+$): 581.24825. Found: 581.25271.

*Ethyl 3-(2-(((4-carbamimidoylphenyl)amino)methyl)-1-ethyl-N-(4-(trifluoromethyl)phenyl)-1H-benzo[d]-imidazole-5-carboxamido)propanoate (**11h**)*
White solid; yield: 48%; m.p.: 109.4–111.1°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.91 (s, 3H), 7.64 (t, J = 8.0 Hz, 4H), 7.53 (s, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.41 (d, J = 8.2 Hz, 2H), 7.22 (d, J = 8.5 Hz, 1H), 6.86 (d, J = 8.6 Hz, 2H), 4.65 (d, J = 5.2 Hz, 2H), 4.27 (q, J = 6.6 Hz, 2H), 4.15 (t, J = 6.9 Hz, 2H), 3.97 (q, J = 7.1 Hz, 2H), 2.63 (t, J = 6.9 Hz, 2H), 1.24 (t, J = 7.0 Hz, 3H), 1.12 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{30}H_{31}F_3N_6O_3$ ([M+H]$^+$): 581.24825. Found: 581.24708.

*Ethyl 3-(2-(((4-carbamimidoylphenyl)amino)methyl)-1-ethyl-N-(4-(trifluoromethoxy)phenyl)-1H-benzo[d]-imidazole-5-carboxamido)propanoate (**11i**)*
White solid; yield: 55%; m.p.: 142.6–145.3°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.91 (s, 1H), 8.47 (s, 2H), 7.60 (s, 2H), 7.52 (s, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.33 (d, J = 8.8 Hz, 2H), 7.26 (d, J = 8.5 Hz, 2H), 7.18 (d, J = 8.4 Hz, 1H), 6.85 (d, J = 8.8 Hz, 2H), 4.63 (d, J = 5.3 Hz, 2H), 4.26 (q, J = 6.8 Hz, 2H), 4.11 (t, J = 6.9 Hz, 2H), 3.96 (q, J = 7.1 Hz, 2H), 2.62 (t, J = 6.9 Hz, 2H), 1.71 (s, 2H), 1.24 (t, J = 7.1 Hz, 3H), 1.12 (t, J = 7.1 Hz, 3H). FT-MS *m/z* Calcd. for $C_{30}H_{31}F_3N_6O_4$ ([M+H]$^+$): 597.24317. Found: 597.24712.

*General procedure for the synthesis of compounds **12a–i***
A round bottom flask was charged with a solution of compound **11** (0.2 mmol), sodium hydroxide (0.6 mmol) in 6 mL of water and 3 mL of ethanol. The reaction mixture was stirred at ambient temperature for 2 h. The mixture was neutralized with acetic acid. The precipitate was isolated and washed with water and ether to afford the zwitterionic title compound **12**.

*3-(2-(((4-Carbamimidoylphenyl)amino)methyl)-1-ethyl-N-(2-fluorophenyl)-1H-benzo[d]imidazole-5-carboxamido)-propanoic acid (**12a**)*
White solid; yield: 51%; m.p.: 238–238.9°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.10 (s, 1H), 8.88 (s, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.74 (d, J = 8.7 Hz, 2H), 7.68 (s, 1H), 7.55 (t, J = 8.5 Hz, 1H), 7.48 (d, J = 7.1 Hz, 1H), 7.30–7.21 (m, 1H), 7.15 (dt, J = 24.6, 7.5 Hz, 2H), 6.92 (d, J = 8.8 Hz, 2H), 5.03 (s, 2H), 4.51–4.46 (m, 2H), 4.01 (s, 2H), 2.58 (d, J = 31.2 Hz, 2H), 1.36 (t, J = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.35, 170.17, 164.25, 164.17, 164.10,

155.71, 152.76, 152.65, 135.25, 130.25, 129.81, 129.67, 129.54, 129.46, 125.26, 122.95, 118.00, 116.37, 116.17, 113.44, 11277, 111.71, 110.02, 45.79, 32.08, 14.64. FT-MS *m/z* Calcd. for $C_{27}H_{27}FN_6O_3$ ($[M+H]^+$): 503.22014. Found: 503.22069.

*3-(2-(((4-Carbamimidoylphenyl)amino)methyl)-1-ethyl-N-(3-fluorophenyl)-1H-benzo[d]imidazole-5-carboxamido)-propanoic acid (**12b**)*
White solid; yield: 42%; m.p.: 226.9–229.4°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.11 (s, 1H), 8.84 (s, 1H), 7.89 (d, J = 8.6 Hz, 1H), 7.73 (d, J = 9.4 Hz, 3H), 7.48 (d, J = 8.4 Hz, 1H), 7.26 (d, J = 8.1 Hz, 2H), 7.06–6.99 (m, 2H), 6.91 (d, J = 8.6 Hz, 2H), 5.03 (s, 2H), 4.49 (d, J = 7.0 Hz, 2H), 4.04 (t, J = 7.0 Hz, 2H), 2.55 (t, J = 7.1 Hz, 2H), 1.35 (t, J = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.46, 168.28, 163.58, 163.06, 160.62, 152.70, 151.96, 133.96, 132.23, 130.87, 130.77, 129.78, 128.99, 125.89, 124.50, 115.27, 115.04, 114.35, 114.09, 112.47, 112.17, 46.12, 40.55, 31.96, 13.92. FT-MS *m/z* Calcd. for $C_{27}H_{27}FN_6O_3$ ($[M+H]^+$): 503.22014. Found: 503.21804.

*3-(2-(((4-Carbamimidoylphenyl)amino)methyl)-1-ethyl-N-(4-fluorophenyl)-1H-benzo[d]imidazole-5-carboxamido)-propanoic acid (**12c**)*
White solid; yield: 51%; m.p.: 249.8–251.9°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.02 (s, 1H), 8.79 (s, 1H), 7.69 (dd, J = 17.4, 8.4 Hz, 3H), 7.62 (s, 1H), 7.34 (d, J = 8.3 Hz, 1H), 7.30 (dd, J = 7.9, 5.2 Hz, 2H), 7.10 (t, J = 8.5 Hz, 2H), 6.90 (d, J = 8.6 Hz, 2H), 4.87 (s, 2H), 4.40 (q, J = 6.4 Hz, 2H), 4.04 (t, J = 7.2 Hz, 2H), 2.56 (t, J = 7.2 Hz, 2H), 1.31 (t, J = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.44, 168.28, 164.12, 161.62, 159.19, 152.63, 152.00, 134.13, 132.11, 130.34, 130.25, 129.78, 129.13, 125.83, 116.20, 115.98, 114.64, 114.40, 112.36, 112.16, 46.10, 40.47, 31.93, 13.95. FT-MS *m/z* Calcd. for $C_{27}H_{27}FN_6O_3$ ($[M+H]^+$): 503.22014. Found: 503.21761.

*3-(2-(((4-Carbamimidoylphenyl)amino)methyl)-N-(3,4-difluorophenyl)-1-ethyl-1H-benzo[d]imidazole-5-carboxamido)propanoic acid (**12d**)*
White solid; yield: 57%; m.p.: 228.5–229.0°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.12 (s, 1H), 8.89 (s, 1H), 7.91 (d, J = 8.5 Hz, 1H), 7.74 (d, J = 8.6 Hz, 3H), 7.59 (ddd, J = 10.0, 7.3, 2.2 Hz, 1H), 7.49 (d, J = 7.9 Hz, 1H), 7.33 (q, J = 9.3 Hz, 1H), 7.11 (d, J = 7.3 Hz, 1H), 6.93 (d, J = 8.8 Hz, 2H), 5.05 (s, 2H), 4.51 (d, J = 7.1 Hz, 2H), 4.04 (t, J = 6.4 Hz, 2H), 2.57 (t, J = 7.0 Hz, 2H), 1.36 (t, J = 7.2 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.44, 168.23, 164.14, 152.72, 152.03, 149.41, 149.28, 147.78, 147.64, 146.95, 146.83, 133.76, 132.31, 129.78, 129.34, 125.73, 117.77, 117.60, 114.59, 112.44, 112.16, 46.09, 40.45, 31.94, 13.97. FT-MS *m/z* Calcd. for $C_{27}H_{26}F_2N_6O_3$ ($[M+H]^+$): 521.21072. Found: 521.21102.

*3-(2-(((4-Carbamimidoylphenyl)amino)methyl)-N-(3,5-difluorophenyl)-1-ethyl-1H-benzo[d]imidazole-5-carboxamido)propanoic acid (**12e**)*
White solid; yield: 99%; m.p.: 254.5–257.0°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.09 (s, 1H), 8.86 (s, 1H), 7.91 (d, J = 8.6 Hz, 1H), 7.78–7.71 (m, 3H), 7.52 (d, J = 8.7 Hz, 1H), 7.19–7.08 (m, 3H), 6.93 (d, J = 8.7 Hz, 2H), 5.03 (s, 2H), 4.51 (q, J = 6.8 Hz, 2H), 4.06 (t, J = 7.2 Hz, 2H), 2.60 (t, J = 7.3 Hz, 2H), 1.37 (t, J = 7.2 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.43, 168.18, 164.13, 163.31, 163.16, 160.86, 160.71, 152.86, 152.05, 133.64, 132.44, 129.78, 129.26, 125.77, 114.54, 112.52, 112.17, 111.91, 46.07, 40.47, 31.99, 13.95. FT-MS *m/z* Calcd. for $C_{27}H_{26}F_2N_6O_3$ ($[M+H]^+$): 521.21072. Found: 521.20974.

*3-(2-(((4-Carbamimidoylphenyl)amino)methyl)-1-ethyl-N-(2-(trifluoromethyl)phenyl)-1H-benzo[d]imidazole-5-carboxamido)propanoic acid (**12f**)*
White solid; yield: 80%; m.p.: 258.3–259.0°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.10 (s, 1H), 8.86 (s, 1H), 7.89 (d, J = 8.6 Hz, 1H), 7.74 (d, J = 9.0 Hz, 2H), 7.70 (d, J = 7.9 Hz, 1H), 7.68–7.63 (m, 2H), 7.61 (s, 1H), 7.53–7.44 (m, 2H), 6.91 (d, J = 8.4 Hz, 2H), 5.01 (s, 2H), 4.63 (s, 2H), 3.49 (dt, J = 14.1, 7.4 Hz, 2H), 2.72 (qt, J = 15.8, 6.6 Hz, 2H), 1.34 (t, J = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.51, 168.65, 164.19, 152.85, 152.32, 139.81, 133.77, 133.28, 132.90, 132.04, 129.74, 129.16, 127.97, 124.84, 115.63, 114.26, 112.95, 112.02, 111.52, 46.66, 31.82, 14.16. FT-MS *m/z* Calcd. for $C_{28}H_{27}F_3N_6O_3$ ($[M+H]^+$): 553.21695. Found: 553.21460.

*3-(2-(((4-Carbamimidoylphenyl)amino)methyl)-1-ethyl-N-(3-(trifluoromethyl)phenyl)-1H-benzo[d]imidazole-5-carboxamido)propanoic acid (**12g**)*
White solid; yield: 44%; m.p.: 225.1–226.0°C; ^{1}H NMR (500 MHz, DMSO-*d6*) δ 9.11 (s, 1H), 8.85 (s, 1H), 7.89 (d, J = 7.5 Hz, 1H), 7.73 (s, 4H), 7.48 (d, J = 26.9 Hz, 4H), 6.90 (d, J = 8.5 Hz, 2H), 5.04 (s, 2H), 4.53–4.47 (m, 2H), 4.07 (s, 2H), 2.56 (s, 2H), 1.33 (d, J = 13.3 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.41, 168.47, 164.07 (t, J = 7.7 Hz), 152.63, 151.92, 133.94, 132.29, 132.13, 130.40, 129.98, 129.77, 129.66, 129.01, 125.75, 124.70, 123.76, 122.09, 114.65–114.47 (m), 114.29, 112.52, 112.15, 46.15, 40.55, 31.98, 13.91. FT-MS *m/z* Calcd. for $C_{28}H_{27}F_3N_6O_3$ ($[M+H]^+$): 553.21695. Found: 553.21798.

*3-(2-(((4-Carbamimidoylphenyl)amino)methyl)-1-ethyl-N-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazole-5-carboxamido)propanoic acid (**12h**)*
White solid; yield: 99%; m.p.: 213.8–215.1°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.09 (s, 1H), 8.86 (s, 1H), 7.88 (d, J = 8.7 Hz, 1H), 7.78–7.71 (m, 3H), 7.65 (d, J = 8.5 Hz, 2H), 7.48 (dd, J = 15.8, 8.5 Hz, 3H), 6.92 (d, J = 8.8 Hz, 2H), 5.03 (s, 2H), 4.50 (q, J = 7.0 Hz, 2H), 3.43 (q, J = 7.0 Hz, 2H), 2.58 (t, J = 7.3 Hz, 2H), 1.36 (t, J = 7.2 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.37, 168.50, 164.16 (t, J = 7.6 Hz), 152.84, 152.14, 146.33, 133.10, 132.91, 130.83, 129.74, 128.59, 127.05, 126.74, 126.46–126.21 (m), 125.54, 125.16, 115.42, 114.44 (d, J = 6.7 Hz), 112.09, 55.91, 46.28, 32.06, 18.40, 14.06. FT-MS *m/z* Calcd. for $C_{28}H_{27}F_3N_6O_3$ ($[M+H]^+$): 553.21695. Found: 553.21682.

*3-(2-(((4-Carbamimidoylphenyl)amino)methyl)-1-ethyl-N-(4-(trifluoromethoxy)phenyl)-1H-benzo[d]imidazole-5-carboxamido)propanoic acid (**12i**)*
White solid; yield: 55%; m.p.: 219.6–226.2°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.02 (s, 1H), 8.80 (s, 1H), 7.71 (d,

$J = 8.5$ Hz, 3H), 7.67 (s, 1H), 7.37 (dd, $J = 16.8$, 8.6 Hz, 3H), 7.27 (d, $J = 8.3$ Hz, 2H), 6.91 (d, $J = 8.6$ Hz, 2H), 4.90 (s, 2H), 4.42 (q, $J = 6.1$ Hz, 2H), 4.06 (t, $J = 7.2$ Hz, 2H), 2.57 (t, $J = 7.2$ Hz, 2H), 1.32 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.38, 168.26, 164.11 (q, $J = 7.6$ Hz), 152.72, 152.02, 146.54, 141.47, 133.80, 132.33, 130.00, 129.76, 129.54, 125.76, 121.75, 121.09, 118.54, 114.70, 112.27, 112.13, 55.91, 46.24, 31.98, 18.39, 13.95. FT-MS *m/z* Calcd. for $C_{28}H_{27}F_3N_6O_4$ ($[M+H]^+$): 569.21186. Found: 569.20958.

Thrombin assay

Different dilutions of the test compounds dissolved in DMSO were preincubated for 10 min at 37°C. The national standard, lyophilized human thrombin (5.4 μg/mL), was purified from human blood. After that, Ac-FVR-AMC (5 μM), a specific fluorogenic thrombin substrate, was added to above system. We detected the dynamic changes in relative fluorescence intensity using an Envision microplate reader (PerkinElmer) at room temperature within 10 min. The slope of the linear enzyme dynamics curve during the initial stage of the reaction was referred to as the initial velocity of enzyme reaction. The known thrombin inhibitor argatroban was used as a positive control. Instrument settings included the following: excitation wavelength, 355 nm and emission wavelength, 460 nm. Each well was measured 20 times every 20 s for about 10 min. The change in fluorescence within a predetermined time was measured under these conditions. The reaction kinetic curve slope (v_{max}) was used as an activity indicator.

The concentration that induced an IC_{50} was calculated. All measurements were performed in duplicate; the mean values of both determinations are presented.

This work is supported by the Science and Technology Commission of Shanghai Municipality (No. 13142201001, 13DZ1930402, 13DZ 1930403).

The authors have declared no conflict of interest.

References

[1] N. Mackman, *Nature* **2008**, *451*, 914–918.
[2] E. Perzborn, S. Roehring, A. Straub, D. Kubitza, F. Misselwitz, *Nat. Rev. Drug Discov.* **2011**, *10*, 61–75.
[3] A. Straub, S. Roehrig, A. Hillisch, *Curr. Top. Med. Chem.* **2010**, *10*, 257–269.
[4] F. Misselwitz, S. D. Berkowitz, E. Perzborn, *Ann. N. Y. Acad. Sci.* **2011**, *1222*, 64–75.
[5] M. W. Jeanine, *Pathophysiol. Haemost. Thromb.* **2002**, *32*, 9–14.
[6] N. H. Hauel, H. Nar, H. Priepke, U. Ries, J. M. Stassen, W. Wienen, *J. Med. Chem.* **2002**, *45*, 1757–1766.
[7] M. Ieko, *Curr. Opin. Invest. Drugs* **2007**, *8*, 758–768.
[8] J. V. Ryn, J. Stangier, S. Haertter, K. H. Liesenfeld, W. Wienen, M. Feuring, *Thromb. Haemost.* **2010**, *103*, 1116–1127.
[9] G. J. Hankey, J. W. Eikelboom, *Circulation* **2011**, *123*, 1436–1450.
[10] W. K. Hagmam, *J. Med. Chem.* **2008**, *51*, 4359–4369.
[11] S. Purser, P. R. Moore, S. Swallow, V. Gouverner, *Chem. Soc. Rev.* **2008**, *37*, 320–330.
[12] K. L. Kirk, *J. Fluorine. Chem.* **2006**, *127*, 1013–1029.
[13] C. Isanbor, D. O'Hagan, *J. Fluorine. Chem.* **2006**, *127*, 303–319.
[14] K. Müller, C. Faeh, F. Dicderich, *Science* **2007**, *317*, 1881–1886.
[15] K. L. Kirk, *Org. Process. Res. Dev.* **2008**, *12*, 305–321.
[16] H. Choo, Y. Chong, Y. Choi, J. Mathew, R. F. Schinazi, C. K. Chu, *J. Med. Chem.* **2003**, *46*, 389–398.
[17] M. D. Johnson, J. Chen, B. D. Anderson, *Drug Metabol. Dispos.* **2002**, *30*, 191–198.
[18] F. Tanaka, T. Fukuse, H. Wada, M. Fukushima, *Curr. Pharm. Biotechnol.* **2000**, *1*, 137–164.
[19] J. D. McCarter, M. J. Adam, N. G. Hartman, S. G. Withers, *Biochem. J.* **1994**, *301*(2), 343–348.
[20] J. Wang, H. Liu, *Chin. J. Org. Chem.* **2011**, *31*, 1785–1798.
[21] F. Schiele, J. V. Ryn., K. Canada, C. Newsome, E. Sepulveda, J. Park, H. Nar, T. Litzenburger, *Blood.* **2013**, *121*, 3554–3562.
[22] J. K. Moon, Y. S. Keun, E. C. Hwang, B. S. Park, H. R. Chang, Q. X. Li, J. K. Kim, *J. Agric. Food Chem.* **2007**, *55*, 5416–5422.
[23] M. Clark, R. D. Cramer III, N. V. Opdenbosch, *J. Comput. Chem.* **1989**, *10*, 982–1012.
[24] J. Gasteiger, M. Marsili, *Tetrahedron.* **1980**, *36*, 3219–3222.
[25] J. Sun, S. Cai, N. Yan, H. Mei, *Eur. J. Med. Chem.* **2010**, *45*, 1008–1014.