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# Synthesis and Biological Evaluation of Some New 2,5-Substituted 1-Ethyl-1*H*-benzoimidazole Fluorinated Derivatives as Direct Thrombin Inhibitors

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A new series of fluorinated 2,5-substituted 1-ethyl-1*H*-benzimidazole derivatives were synthesized from starting compounds **3a–i**, which were prepared from acrylic acid ethyl ester and the appropriate amines using trifluoromethanesulfonic acid as a catalyst. A total of 9 novel derivatives were synthesized through 9 steps. All of them were evaluated for thrombin inhibition activity *in vitro* for the first time. We have altered their structures using different substituents on the amines to assess their structure–activity relationships as direct thrombin inhibitors. All the compounds were effective thrombin inhibitors, with $IC_{50}$ values ranging from 3.39 to 23.30 nM. Among the compounds synthesized, compounds **14a**, **14b**, **14d**, **14e**, and **14h** exhibited greater anticoagulant activity than argatroban ($IC_{50} = 9.36$ nM). Furthermore, compound **14h** synthesized starting with 2-amino-pyridine was the most potent thrombin inhibitor with an $IC_{50}$ value of 3.39 nM. Molecular modeling studies were performed to determine the probable interactions of the most potent compounds **14a**, **14e**, and **14h** with their protein receptor (PDB ID: 1KTS). Docking data show that the active compounds inhibit thrombin in a similar mode to that of the potent anticoagulant dabigatran.

**Keywords:** 1-Ethyl-1*H*-benzoimidazole fluorinated derivatives / Direct thrombin inhibitors / Molecular docking / Synthesis

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## Introduction

Thrombin, a multifunctional serine protease, is an important effector protease in the blood coagulation cascade that exhibits procoagulant and anticoagulant properties. It activates platelets and catalyses the conversion of fibrinogen to fibrin, which promotes the stability of blood clots and causes the blood to coagulate [1–3]. Thrombin plays an important role in blood clotting and thrombus formation and exerts a major effect on cardiovascular and cerebrovascular disease processes [4, 5]. When the body is in a hypercoagulable state or anticoagulant and fibrinolytic weakened, thromboembolic disease occurs [6]. Indirect thrombin inhibitors, such as heparin, have potential side effects [7, 8]. Direct thrombin inhibitors are an emerging class of anticoagulants [9, 10] that can not only inhibit thrombin but can also inhibit the activity of thrombin-mediated factors V, VIII, and XII, fibrinogen and platelets; they do not cause heparin-induced thrombocytopenia. In addition, direct thrombin inhibitors can inhibit platelet aggregation and exert anti-inflammatory effects [11], suggesting broad application in the clinical treatment of thrombotic-related diseases. Therefore, the development of direct thrombin inhibitors has become a topic of great interest [12]. Despite the promise of the potential therapeutic drugs argatroban [13], melagatran [14], efegatran and dabigatran [15, 16] (Fig. 1) as direct thrombin inhibitors [17], they are accompanied by complications during the treatment process, namely hemorrhage, particularly severe and fatal hemorrhage [18]. Although laboratory and clinical studies support the assertion that the hemorrhage risk of dabigatran is greatly reduced compared with those of the traditional anticoagulants [19–21], the RE-LY trial [22] showed that high-dose dabigatran increases the risk of hemorrhage by 0.4% compared with low-dose dabigatran [18]. The study found

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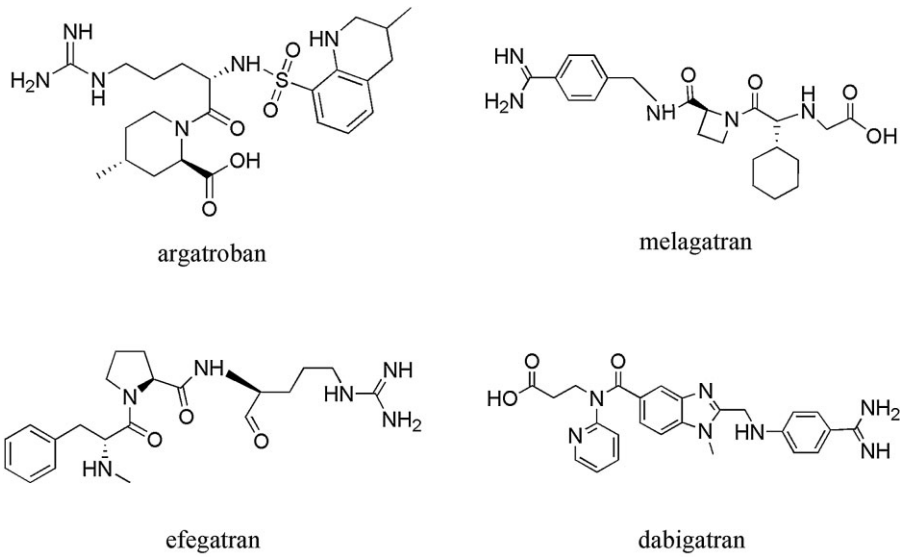



**Figure 1.** Chemical structures of some known direct thrombin inhibitors.

that the amount of hemorrhage is related to dosage [23] and that it can be controlled by reducing blood concentrations of thrombin inhibitors [24].

Drugs containing fluorine constitute a considerable proportion of the drugs used in clinical practice [25–30]. In 2009, the US FDA approved 19 new molecules; four fluorinated drugs were among them [31]. The introduction of a fluorine atom or a fluorine-containing group to a small-molecule drug is an important strategy for transforming the chemical structure of drugs. Fluorine introduction can adjust the physical and chemical properties of a small molecule drug, changing its pharmacokinetic properties to improve the bioavailability of the drug and its stability toward drug metabolism. For example, the hydrogen atom of uracil is substituted with fluorine to obtain fluorouracil, which attributes to the increasing lipophilicity and biological permeability and makes the efficacy enhanced. Kirk [27, 30] summarized the clinical use of fluorine atoms in drugs for the treatment of cardiovascular diseases. Therefore, it is necessary and significant to synthesize a number of new and efficient direct thrombin inhibitors with fluorine-containing structural modifications which would overcome the side effect of hemorrhage by reducing the dose.

During the past 2 years, in order to identify the structural features required for anticoagulant efficacy, we have studied the structures of existing drugs by docking their molecular structures with the protein receptor [32] (Fig. 2). The structural features include: (1) there is an open-chain carboxylic acid attached to amine group; (2) the sites for hydrophobic interactions, electrostatic interactions and hydrogen bonding interactions; (3) the presence of a carbonyl group and a terminal amidine which can form hydrogen

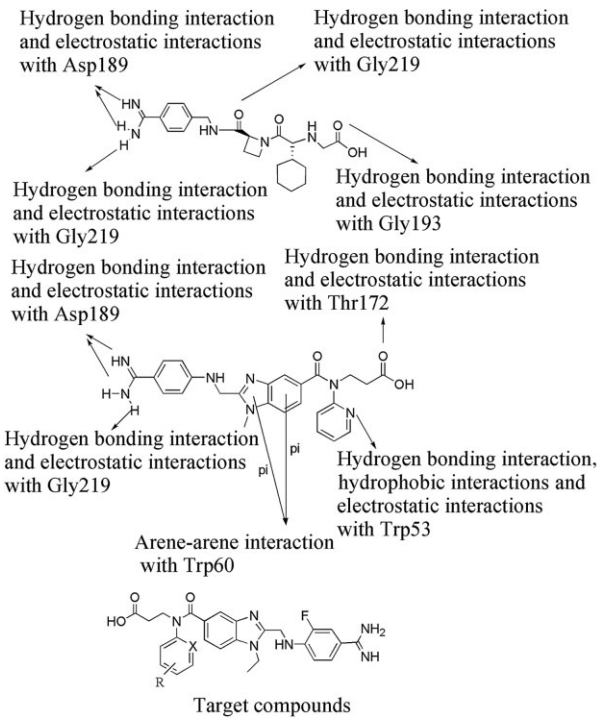



**Figure 2.** Structural features and interactions for direct thrombin inhibitors.

bonding interactions and electrostatic interactions with amino acid residues.

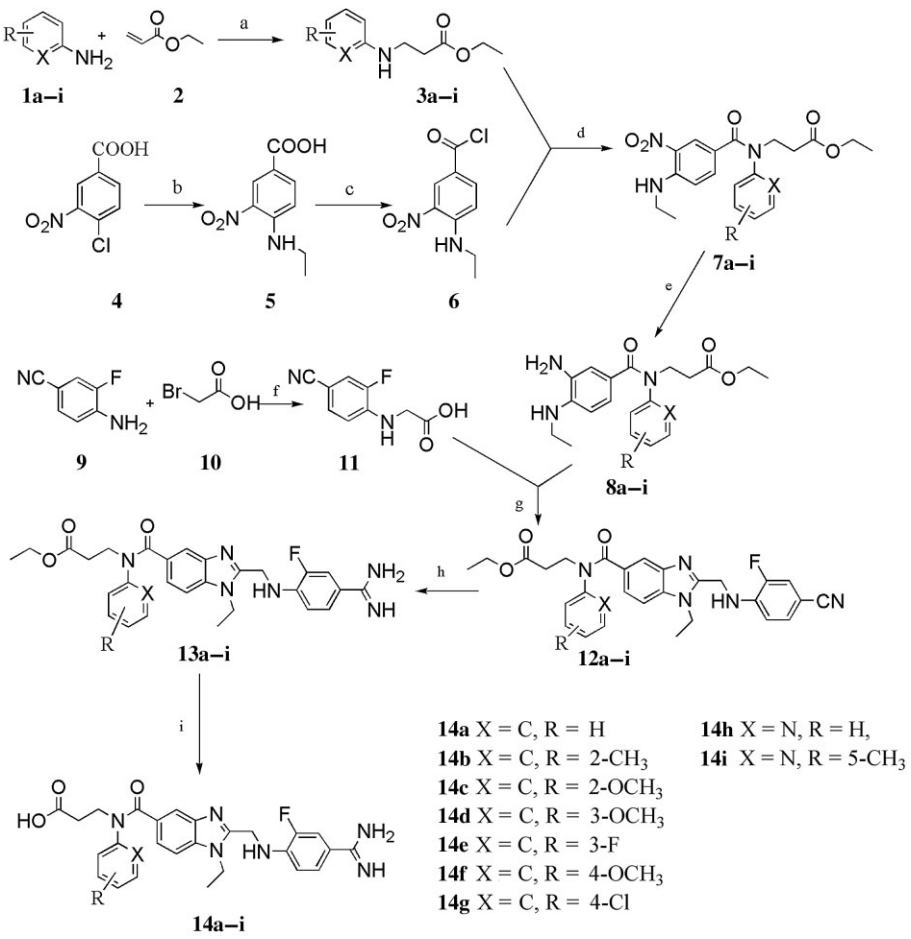
Hauel et al. [32] found that benzimidazole is indispensable for anticoagulant activity by investigating the crystal structure of the inhibitor with human α-thrombin. In the same way, they found the last phenyl ring of the inhibitor was bound to the D-pocket through a hydrophobic interaction and the N-substituted group was quite suitable for the P-pocket. Thus, we hope to optimize these structures by retaining the benzimidazole template and modifying the terminal phenyl ring with fluorine atom, which has become a widespread method in molecular design.

Here a series of fluorinated 2,5-substituted 1-ethyl-1*H*-benzoimidazole derivatives have been designed and synthetized. Their probable biological activity as direct thrombin inhibitors is predicted in this paper. Further, molecular modeling studies were performed by molecular docking approaches to understand the inhibitory mechanism of active compounds.

## Results and discussion

### Chemistry

A series of new fluorinated 2,5-substituted 1-ethyl-1*H*-benzimidazole derivatives were synthesized. After several experiments [33–35], we finally adopted a high yield reaction route. As a part of our research on the synthesis of these target compounds, a series of compounds with different substituents (**3a–i**) were synthetized using ethyl acrylate (**2**) and the corresponding amines (**1a–i**) with trifluoromethanesulfonic acid (TfOH) (10 mol%) as a catalyst. The reaction mixtures were heated at reflux overnight. Compounds **3a–i** were purified by column chromatography on silica gel. To reduce the cost and




**Scheme 1.** Synthesis of compounds **14a–i**. Reagents and conditions: (a) TfOH (10 mol%), 12 h, 100°C; (b) $CH_3CH_2NH_2$, 80°C, 5 h; AcOH, pH 4–5; (c) DCM, $SOCl_2$, rt, 2 h; (d) DCM, $NEt_3$, rt, 3 h; (e) Zn, $NH_4Cl$, $THF/H_2O$, 80°C, $N_2$; (f) $H_2O$, 100°C; (g) EDCI, HOBt, DMF/THF; (h) $NH_2OH{\cdot}HCl$, $NEt_3$, anhydrous ethanol; Pd/C, $HCOONH_4$, AcOH, $N_2$; (i) NaOH, $H_2O$/EtOH, rt, 2 h.

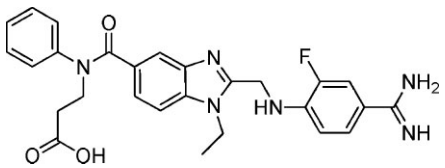
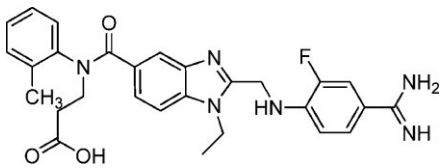
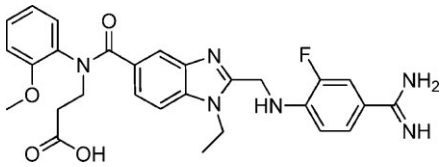
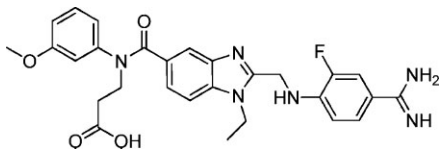
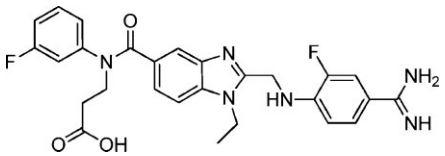
realize the reduction reactions of **7a–i** to **8a–i**, we used zinc dust to replace Pd/C. In addition, we chose hydroxylamine hydrochloride and triethylamine instead of HCl and ammonia used in the literature [34], which improved the safety of experiments. The reaction route is shown in Scheme 1.

The structures of the synthesized compounds were fully analyzed and characterized by $^{1}H$, $^{13}C$ nuclear magnetic resonance (NMR) and high resolution mass spectrometry (HRMS) before they were subjected to biological evaluation. All the spectral data were provided in detail in the experimental section, they were consistent with the presumed structures.

## Anticoagulant activity *in vitro*

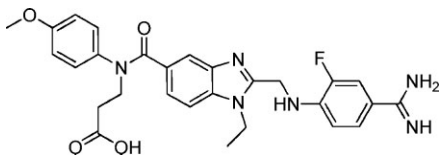
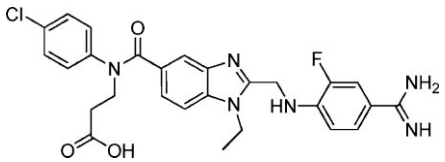
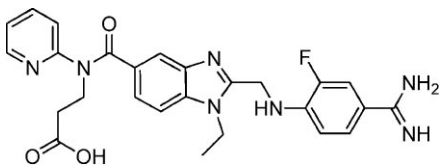
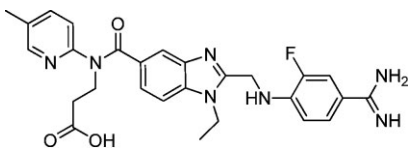
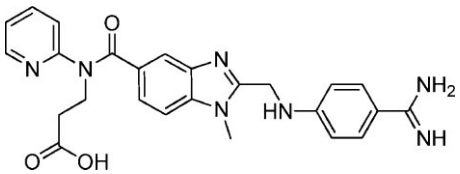
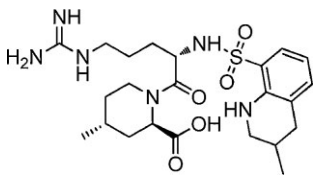
The synthesized compounds **14a–i** were evaluated for thrombin inhibitory activity *in vitro* by chromogenic assays. Argatroban and dabigatran were used as reference compounds. The $IC_{50}$ values of compounds **14a–i** were determined and the corresponding data are listed in Table 1. Most of them exhibited moderate to good thrombin inhibitory activity compared to argatroban. Compounds **14a**, **14b**, **14d**, and **14e** showed better inhibitory activity than the reference compound argatroban, with $IC_{50}$ values of 5.63, 7.26, 8.40, and 4.92 nM, respectively. Furthermore, compound **14h** with a pyridinyl ring was found to be the most potent thrombin inhibitor among all these new compounds with an $IC_{50}$ value

**Table 1. Thrombin inhibitory activity ($IC_{50}$) of new 2,5-substituted 1-ethyl-1*H*-benzoimidazole fluorinated derivatives.**

| Compound | Structure | Mean $IC_{50} \pm SD$ (nM)[a)] |
|---|---|---|
| **14a** |  | $5.63 \pm 0.10$ |
| **14b** |  | $7.26 \pm 0.91$ |
| **14c** |  | $21.55 \pm 0.22$ |
| **14d** |  | $8.40 \pm 0.09$ |
| **14e** |  | $4.92 \pm 0.29$ |

(*Continued*)

**Table 1.** (***Continued***)

| Compound | Structure | Mean $IC_{50} \pm SD$ (nM)[a)] |
|---|---|---|
| **14f** |  | 23.30 ± 0.02 |
| **14g** |  | 13.61 ± 0.67 |
| **14h** |  | 3.39 ± 0.10 |
| **14i** |  | 19.16 ± 1.04 |
| Dabigatran |  | 2.61 ± 0.84 |
| Argatroban |  | 9.46 ± 0.92 |

[a)]The values represent the means ± SD (nM) of at least two data.

of 3.39 nM, which is comparable to dabigatran. The introduction of a methoxy, a chlorine or a methyl group at the *para* position of the phenyl ring led to a reduction of efficacy against thrombin. As observed in **14f**, **14g**, and **14i**, the $IC_{50}$ values of them are 23.30, 13.61, and 19.16 nM, respectively.

## Structure–activity relationship

Based on the activity data in Table 1, some preliminary structure–activity relationship of the new fluorinated 2,5-substituted 1-ethyl-1*H*-benzoimidazole derivatives could be summarized: The R-substituent group on the ring of the

compounds played an important role for antithrombin activity. (a) The introduction of substituent group at the *para* position of the amino group of a benzene ring or a heterocyclic ring had a serious impact on activity and it led to a reduction of anticoagulant activity with respect to unsubstituted analogs (**14f** and **14g** compared to **14a**, **14i** compared to **14h**); (b) the electron-withdrawing substituent at benzene ring was advantageous to the activity. However, electron-donating substituent would reduce its antithrombin activity (**14d** and **14e** compared to **14a**). This conclusion also applied to **14b** and **14c** and was equally suitable for 14g and **14f**; (c) the positions of the substituent groups on the ring also affected the activity. When the substituent group was the same, the structure–activity relationship was: *meta-* > *ortho-* > *para-*, such as in compounds **14c**, **14d**, and **14f**; (d) pyridine ring is better than benzene ring for activity (**14h** compared to **14a**), and pyridine ring without substituents made the antithrombin activity of **14h** be comparable to that of dabigatran.

## Molecular modeling studies

The structures of compounds **14a–i**, which had potential thrombin inhibitory activity when combined with the apparent common pharmacophore of the direct thrombin inhibitor dabigatran, prompted us to guess that their anticoagulant activity was due to their binding affinity with amino acid residues in the active site of thrombin protein. In order to identify the recognition processes of the three promising compounds (**14a**, **14e**, and **14h**), we selected the thrombin crystal structure (PDB ID: 1KTS) as a docking protein [32]. Flexible docking experiments were conducted using SYBYL-2.0X software (Fig. 3). Compounds **14a**, **14e**, and **14h**, as well as dabigatran, were docked into the active site of thrombin protein (1KTS). The interactions with the amino acid residues of these compounds are summarized in Table 2. In the binding conformation for all of them, the π-electrons of the benzimidazole ring get engaged in a face-to-face stacking interaction with the π-electrons of Trp60. The central template is bound to thrombin through a hydrophobic interaction with the P-pocket [32]. This provides stability to the benzimidazole ring in the binding cavity. The H atoms of terminal amidine group interact with Asp189 and Gly219 through hydrogen bondings and electrostatic interactions. The O atom of carboxyl of compound **14a**, **14e**, or **14h** also forms hydrogen bond with the hydroxyl group of Thr172. Moreover, compound **14h** and dabigatran which have a pyridine ring exhibit additional electrostatic interaction and hydrophobic interaction with Trp53 and the N atom of the pyridine ring forms a additional hydrogen bond with Trp53 than other two compounds. From the docking results, we suspect that the most potent activity of compound **14h** is attributed to its pyridine ring, which makes it better than other new compounds and be comparable to dabigatran. The binding mode of these active compounds in the active site of the protein (1KTS) is similar to that of dabigatran. They all exert efficacy through the direct inhibition of thrombin.

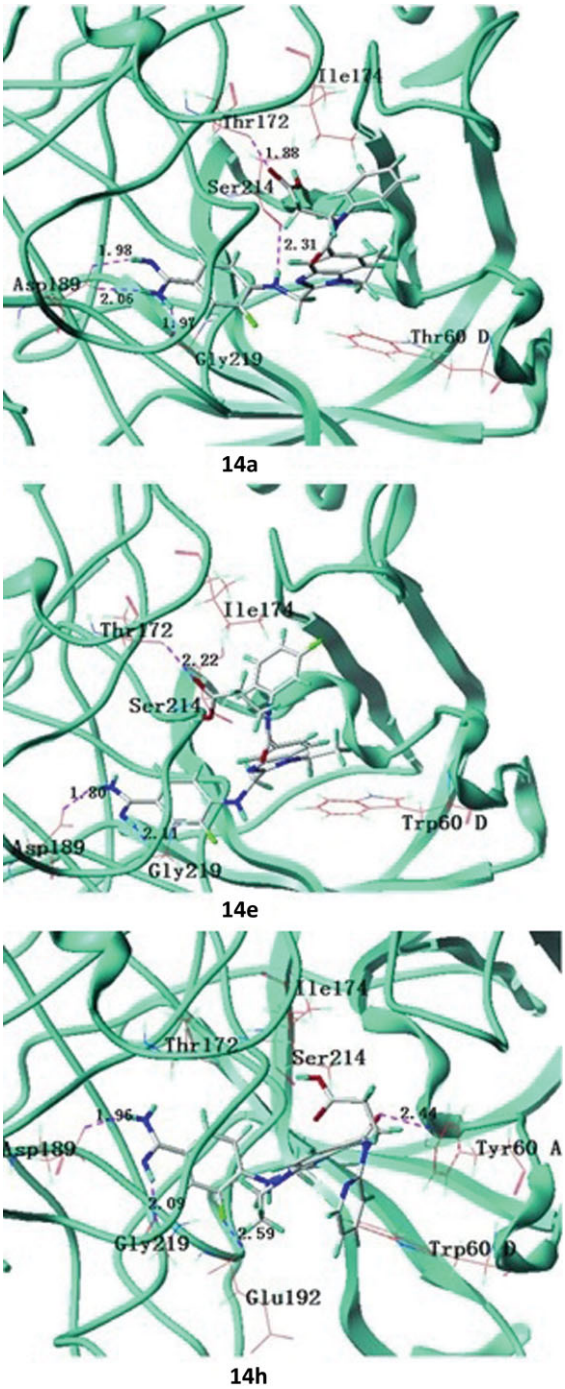



**Figure 3.** Binding modes of **14a**, **14e**, and **14h** at the binding site of thrombin (PDB code: 1KTS).

## Conclusion

A series of novel fluorinated 2,5-substituted 1-ethyl-1*H*-benzimidazole derivatives (**14a–i**) were synthesized and

**Table 2. Prediction of interactions of compounds in the active site of thrombin protein.**

| Compound | Protein–ligand interaction | Type of interactions |
|---|---|---|
| **14a** | Asp189, Thr172, Gly219, Trp60 | Hydrogen bonding & $\pi$–$\pi$ interactions |
| **14e** | Asp189, Thr172, Gly219, Trp60 | Hydrogen bonding & $\pi$–$\pi$ interactions |
| **14h** | Asp189, Thr172, Gly219, Trp60, Trp53 | Hydrogen bonding & $\pi$–$\pi$ interactions |
| Dabigatran | Asp189, Thr172, Gly219, Trp60, Trp53 | Hydrogen bonding & $\pi$–$\pi$ interactions |

evaluated for their *in vitro* thrombin inhibition activity. Structure–activity relationship revealed that the substituent groups on the benzene ring or heterocyclic ring of the compounds seriously affected thrombin inhibitory activity. Electron-donating substituents on the ring were detrimental to the inhibitory activity; however, electron-withdrawing substituents enhanced the anticoagulant activity. The positions of the substituent groups also had some impact on activity. The pyridine ring is better than benzene ring for activity. Molecular docking studies provided an idea about the binding of promising compounds, which in turn helped us to understand the role of the pharmacophore. Docking results showed that the active compounds exerted efficacy in a similar mode to that of the potent direct thrombin inhibitor dabigatran. Thus, it was confirmed that fluorinated 2,5-substituted 1-ethyl-1*H*-benzimidazole derivatives are a new series of potential direct thrombin inhibitors. The results provide guidance and reference for us to design newer molecular entities towards developing potent thrombin inhibitors.

## Experimental

### Chemistry

The melting points were recorded on a WPS-2A digital melting point apparatus and were uncorrected. The NMR spectra were recorded on a Bruker Avance 500 magnetic resonance spectrometer at 500 MHz for $^{1}H$ and 125 MHz for $^{13}C$, respectively, using TMS as internal standard. The HR-MS spectra were recorded on a SolariX-70FT-MS Bruker spectrometer. All reagents were of analytical grade and purchased from Shanghai Chemical Reagent Company in China. The reaction was monitored by thin layer chromatography (TLC). Spots on the TLC plates were visualized using UV light (254 nm). Column chromatography was performed with silica gel (200–300 mesh).

*General procedure for the synthesis of compounds **3a–i***

Amino compounds (**1a–i**) (45 mmol), ethyl acrylate (**2**) (80 mmol), and trifluoromethanesulfonic acid as catalyst (10 mol% with respect to amine) were mixed and the reaction mixture was heated under reflux temperature overnight. The reaction was detected by TLC. The solvent was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with PE/EtOAc = (6:1–1:1) to give compounds **3a–i** (75–85%) as red oil or white solid.

*General procedure for the synthesis of compound **6***

4-Chloro-3-nitrobenzoic acid (**4**) (23 mmol) was dissolved in an aqueous solution of ethylamine (391 mmol). The reaction mixture was stirred and heated to reflux at 80°C for 5–8 h. After the completion of reaction, the mixture was cooled to room temperature and was adjusted to pH 4–5 with acetic acid. The precipitation of yellow solid was filtered to get compound **5**. Yield: 70%. Compound **5** (14.3 mmol) obtained from previous step was dissolved in dichloromethane and 3 drops of *N*,*N*-dimethylformamide were added in this system. The mixture was stirred at room temperature for 5 min, then thionyl chloride (67 mmol) was added dropwise into the reaction mixture. The solution was stirred at room temperature for 3–8 h.The solvent was evaporated to afford yellow solid compound **6**. Yield: 70–80%.

*General procedure for the synthesis of compounds **8a–i***

To a vigorously stirred mixture of compounds **3a–i** (24 mmol) and trimethylamine (5 mL) dissolved in dichloromethane, a solution of compound **6** (36 mmol) dissolved in dichloromethane was added dropwise. Stirring was continued at room temperature for 3 h until completion of the reaction. Upon completion, the reaction mixture was diluted with water (15 mL) and extracted with dichloromethane (3 × 25 mL). The combined organic extracts were washed with brine, dried over $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography using PE/EtOAc = (3:1–1:1) as eluent to afford pure orange oil (**7a–i**) in 60–70% yield. To a solution of compounds **7a–i** (10 mmol) in THF/$H_2O$ (75 mL, 1:2 v/v), zinc powder (20 mmol) and ammonia chloride (20 mmol) were added with vigorous stirring. The mixture was refluxed at 80°C for 5 h under $N_2$. The progress of the reaction was monitored by TLC. The reaction mixture was filtered and the filtrate was concentrated and extracted with acetic ether (3 × 25 mL). The combined organic extracts were washed with brine, dried over $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography using PE/EtOAc = (2:1–1:1) as eluent to afford brown solid (**8a–i**) in 80–85% yield.

*3-[(3-Amino-4-ethylamino-benzoyl)-phenyl-amino]-propionic acid ethyl ester (**8a**)*

Yield: 82%; m.p. 112–113°C; $^{1}H$ NMR ($CDCl_3$, 500 MHz) $\delta$: 1.20 (t, 3H, $-CH_3$), 1.27 (t, 3H, $-CH_3$), 2.73 (t, $J$ = 7.4 Hz, 2H, $-CH_2$), 3.11 (q, $J$ = 7.1 Hz, 2H, $-CH_2$), 4.07 (q, $J$ = 7.1 Hz, 2H, $-CH_2$), 4.35 (t, $J$ = 7.4 Hz, 2H, $-CH_2$), 6.37 (d, $J$ = 8.3 Hz, 1H), 6.68 (d,

$J = 8.1$ Hz, 1H), 7.02 (dd, $J = 8.2$, 1.9 Hz, 1H), 7.46 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.48 (d, $J = 1.9$ Hz, 1H), 7.57 (dd, $J = 8.1$, 2.0 Hz, 1H), 7.64 (d, $J = 1.7$ Hz, 1H). HRMS (ESI) *m/z*: 356.1976 $[M+H]^+$; calcd. for $C_{20}H_{26}N_3O_3$ (M+H) 356.1929.

*3-[(3-Amino-4-ethylamino-benzoyl)-o-tolyl-amino]-propionic acid ethyl ester (**8b**)*
Yield: 80%; m.p. 115–117°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.21 (t, 3H, $-CH_3$), 1.26 (t, 3H, $-CH_3$), 2.34 (s, 3H, $-CH_3$), 2.74 (t, $J = 7.4$ Hz, 2H, $-CH_2$), 3.10 (q, $J = 7.1$ Hz, 2H, $-CH_2$), 4.07 (q, $J = 7.1$ Hz, 2H, $-CH_2$), 4.32 (t, $J = 7.4$ Hz, 2H, $-CH_2$), 6.37 (d, $J = 8.3$ Hz, 1H), 6.67 (d, $J = 8.1$ Hz, 1H), 7.02 (dd, $J = 8.2$, 1.9 Hz, 1H), 7.46 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.55 (dd, $J = 8.1$, 2.0 Hz, 1H), 7.64 (d, $J = 1.7$ Hz, 1H). HRMS (ESI) *m/z*: 370.2072 $[M+H]^+$; calcd. for $C_{21}H_{28}N_3O_3$ (M+H) 370.2086.

*3-[(3-Amino-4-ethylamino-benzoyl)-(2-methoxy-phenyl)-amino]-propionic acid ethyl ester (**8c**)*
Yield: 83%; m.p. 112–115°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.20 (t, 3H, $-CH_3$), 1.25 (t, 3H, $-CH_3$), 2.74 (t, $J = 7.4$ Hz, 2H, $-CH_2$), 3.11 (q, $J = 7.1$ Hz, 2H, $-CH_2$), 3.77 (s, 3H, $-CH_3$), 4.06 (q, $J = 7.1$ Hz, 2H, $-CH_2$), 4.34 (t, $J = 7.4$ Hz, 2H, $-CH_2$), 6.35 (d, $J = 8.3$ Hz, 1H), 6.67 (d, $J = 8.1$ Hz, 1H), 7.02 (dd, $J = 8.2$, 1.9 Hz, 1H), 7.46 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.56 (dd, $J = 8.1$, 2.0 Hz, 1H), 7.64 (d, $J = 1.7$ Hz, 1H). HRMS (ESI) *m/z*: 386.2056 $[M+H]^+$; calcd. for $C_{21}H_{28}N_3O_4$ (M+H) 386.2035.

*3-[(3-Amino-4-ethylamino-benzoyl)-(3-methoxy-phenyl)-amino]-propionic acid ethyl ester (**8d**)*
Yield: 79%; m.p. 118–120°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.21 (t, 3H, $-CH_3$), 1.27 (t, 3H, $-CH_3$), 2.74 (t, $J = 7.4$ Hz, 2H, $-CH_2$), 3.10 (q, $J = 7.1$ Hz, 2H, $-CH_2$), 3.77 (s, 3H, $-CH_3$), 4.07 (q, $J = 7.1$ Hz, 2H, $-CH_2$), 4.34 (t, $J = 7.4$ Hz, 2H, $-CH_2$), 6.35 (d, $J = 8.3$ Hz, 1H), 6.68 (d, $J = 8.1$ Hz, 1H), 7.02 (dd, $J = 8.2$, 1.9 Hz, 1H), 7.44 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.57 (dd, $J = 8.1$, 2.0 Hz, 1H), 7.65 (d, $J = 1.7$ Hz, 1H). HRMS (ESI) *m/z*: 386.2079 $[M+H]^+$; calcd. for $C_{21}H_{28}N_3O_4$ (M+H) 386.2035.

*3-[(3-Amino-4-ethylamino-benzoyl)-(3-fluoro-phenyl)-amino]-propionic acid ethyl ester (**8e**)*
Yield: 83%; m.p. 121–123°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.21 (t, 3H, $-CH_3$), 1.27 (t, 3H, $-CH_3$), 2.76 (t, $J = 7.4$ Hz, 2H, $-CH_2$), 3.10 (q, $J = 7.1$ Hz, 2H, $-CH_2$), 4.06 (q, $J = 7.1$ Hz, 2H, $-CH_2$), 4.32 (t, $J = 7.4$ Hz, 2H, $-CH_2$), 6.35 (d, $J = 8.3$ Hz, 1H), 6.68 (d, $J = 8.1$ Hz, 1H), 7.02 (dd, $J = 8.2$, 1.9 Hz, 1H), 7.48 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.58 (d, $J = 8.5$ Hz, 2H, Ar-H), HRMS (ESI) *m/z*: 374.1867 $[M+H]^+$; calcd. for $C_{20}H_{25}FN_3O_3$ (M+H) 374.1835.

*3-[(3-Amino-4-ethylamino-benzoyl)-(4-methoxy-phenyl)-amino]-propionic acid ethyl ester (**8f**)*
Yield: 85%; m.p. 116–118°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.21 (t, 3H, $-CH_3$), 1.27 (t, 3H, $-CH_3$), 2.74 (t, $J = 7.4$ Hz, 2H, $-CH_2$), 3.10 (q, $J = 7.1$ Hz, 2H, $-CH_2$), 3.77 (s, 3H, $-CH_3$), 4.06 (q, $J = 7.1$ Hz, 2H, $-CH_2$), 4.34 (t, $J = 7.4$ Hz, 2H, $-CH_2$), 6.35 (d, $J = 8.3$ Hz, 1H), 6.67 (d, $J = 8.1$ Hz, 1H), 7.02 (dd, $J = 8.2$, 1.9 Hz, 1H), 7.46 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.55 (dd, $J = 8.1$, 2.0 Hz, 1H), 7.64 (d, $J = 1.7$ Hz, 1H). HRMS (ESI) *m/z*: 386.2069 $[M+H]^+$; calcd. for $C_{21}H_{28}N_3O_4$ (M+H) 386.2035.

*3-[(3-Amino-4-ethylamino-benzoyl)-(4-chloro-phenyl)-amino]-propionic acid ethyl ester (**8g**)*
Yield: 81%; m.p. 117–119°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.20 (t, 3H, $-CH_3$), 1.26 (t, 3H, $-CH_3$), 2.74 (t, $J = 7.4$ Hz, 2H, $-CH_2$), 3.10 (q, $J = 7.1$ Hz, 2H, $-CH_2$), 4.06 (q, $J = 7.1$ Hz, 2H, $-CH_2$), 4.34 (t, $J = 7.4$ Hz, 2H, $-CH_2$), 6.34 (d, $J = 8.3$ Hz, 1H), 6.63 (d, $J = 8.1$ Hz, 1H), 6.85 (dd, $J = 8.2$, 1.9 Hz, 1H), 6.94 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.28 (dd, $J = 8.1$, 2.0 Hz, 1H), 7.46 (d, $J = 1.7$ Hz, 1H). HRMS (ESI) *m/z*: 391.2001 $[M+H]^+$; calcd. for $C_{20}H_{25}ClN_3O_3$ (M+H) 391.1477.

*3-[(3-Amino-4-ethylamino-benzoyl)-pyridin-2-yl-amino]-propionic acid ethyl ester (**8h**)*
Yield: 88%; m.p. 122–123°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.21 (t, 3H, $-CH_3$), 1.26 (t, 3H, $-CH_3$), 2.74 (t, $J = 7.4$ Hz, 2H, $-CH_2$), 3.10 (q, $J = 7.1$ Hz, 2H, $-CH_2$), 4.04 (q, $J = 7.1$ Hz, 2H, $-CH_2$), 4.35 (t, $J = 7.4$ Hz, 2H, $-CH_2$), 6.33 (d, $J = 8.3$ Hz, 1H), 6.62 (d, $J = 8.1$ Hz, 1H), 6.71 (dd, $J = 8.2$, 1.9 Hz, 1H), 6.94 (d, $J = 1.7$ Hz, 1H), 7.23 (dd, $J = 8.1$,2.0 Hz, 1H), 7.46 (d, $J = 1.7$ Hz, 1H), 8.24 (d, $J = 1.7$ Hz, 1H). HRMS (ESI) *m/z*: 357.1952 $[M+H]^+$; calcd. for $C_{19}H_{25}N_4O_3$ (M+H) 357.1882.

*3-[(3-Amino-4-ethylamino-benzoyl)-(5-methyl-pyridin-2-yl)-amino]-propionic acid ethyl ester (**8i**)*
Yield: 85%; m.p. 120–121°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.21 (t, 3H, $-CH_3$), 1.26 (t, 3H, $-CH_3$), 2.27 (s, 3H, $-CH_3$), 2.74 (t, $J = 7.4$ Hz, 2H, $-CH_2$), 3.10 (q, $J = 7.1$ Hz, 2H, $-CH_2$), 4.06 (q, $J = 7.1$ Hz, 2H, $-CH_2$), 4.34 (t, $J = 7.4$ Hz, 2H, $-CH_2$), 6.34 (d, $J = 8.3$ Hz, 1H), 6.63 (d, $J = 8.1$ Hz, 1H), 6.72 (dd, $J = 8.2$, 1.9 Hz, 1H), 6.85 (d, $J = 1.7$ Hz, 1H), 7.22 (dd, $J = 8.1$, 2.0 Hz, 1H), 8.26 (d, $J = 1.7$ Hz, 1H). HRMS (ESI) *m/z*: 371.2013 $[M+H]^+$; calcd. for $C_{20}H_{27}N_4O_3$ (M+H) 371.2039.

*General procedure for the synthesis of compound **11***
Compound **9** (50 mmol) and **10** (75 mmol) were mixed in $H_2O$ (150 mL) and allowed to stir at 100°C until completion of the reaction (about 12 h). Then the reaction mixture was cooled to room temperature and filtered. The filter cake was washed with $H_2O$ ($2 \times 20$ mL), and dried in vacuum to give light brown compound **11**. Yield: 70–80%.

*General procedure for the synthesis of compounds **12a–i***
A solution of compound **11** (16 mmol), EDCI (20 mmol), HOBt (20 mmol) in DMF/THF (1:7 v/v) was stirred at 0°C for 30 min. Then a freshly prepared solution of compounds **8a–i** (12 mmol) in THF was added dropwise with stirring for 12 h at room temperature. The solvent was concentrated and extracted with dichloromethane ($3 \times 25$ mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated under reduced pressure. To the residue, acetic acid (50 mL) was added, and the reaction mixture was heated at 120°C for 2–3 h until completion of the reaction.

The solvent was removed under reduced pressure and the residue was neutralized to pH 7–8 with ammonia. Stirring was continued for 30 min, and the residue was extracted with dichlorometh (3 × 25 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography using DCM/MeOH = (30:1–20:1) as eluent to afford pure **12a–i**.

*3-({2-[(4-Cyano-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-phenyl-amino)-propionic acid ethyl ester (**12a**)*
Yield: 52%; m.p. 181–182°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.13 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 1.28 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 2.65 (t, $J$ = 7.2 Hz, 2H, –$CH_2$–), 3.99 (t, 2H, –$NCH_2$), 4.05 (q, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.17 (q, $J$ = 7.2 Hz, 2H, –$OCH_2$–), 4.44 (d, 2H, –$NCH_2$–), 5.48 (brs, H, –NH–), 6.74 (m, 1H, Ar-H), 7.00 (m, 1H, Ar-H), 7.03 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.12 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.16 (m, 1H, Ar-H), 7.18 (m, 1H, Ar-H), 7.23 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.59 (m, 1H, Ar-H). HRMS (ESI) *m/z*: 514.2260 $[M+H]^+$; calcd. for $C_{29}H_{28}F_2N_5O_3$ (M+H) 514.2249.

*3-({2-[(4-Cyano-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-o-tolyl-amino)-propionic acid ethyl ester (**12b**)*
Yield: 55%; m.p. 160–162°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.23 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 1.37 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 2.36 (s, 3H, –$CH_3$), 2.73 (t, $J$ = 7.2 Hz, 2H, –$CH_2$–), 4.09 (t, 2H, –$NCH_2$), 4.17 (q, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.23 (q, $J$ = 7.2 Hz, 2H, –$OCH_2$–), 4.56 (d, 2H, –$NCH_2$–), 5.61 (brs, H, –NH–), 6.83 (m, 1H, Ar-H), 6.96 (m, 1H, Ar-H), 7.01 (m, 1H, Ar-H), 7.09 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.16 (m, 1H, Ar-H), 7.26 (m, 1H, Ar-H), 7.33 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.69 (m, 1H, Ar-H). HRMS (ESI) *m/z*: 528.2460 $[M+H]^+$; calcd. for $C_{30}H_{30}FN_5O_3$ (M+H) 528.2405.

*3-[{2-[(4-Cyano-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(2-methoxy-phenyl)-amino]-propionic acid ethyl ester (**12c**)*
Yield: 58%; m.p. 170–172°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.23 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 1.39 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 2.72 (t, $J$ = 7.2 Hz, 2H, –$CH_2$–), 3.72 (s, 3H, –$OCH_3$), 4.09 (t, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.16 (q, $J$ = 7.2 Hz, 2H, –$OCH_2$–), 4.21 (q, 2H, –$NCH_2$–), 4.56 (d, 2H, –$NCH_2$–), 5.51 (brs, H, –NH–), 6.73 (m, 1H, Ar-H), 6.83 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.01 (m, 1H, Ar-H), 7.16 (m, 1H, Ar-H), 7.27 (m, 1H, Ar-H), 7.35 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.58 (m, 1H, Ar-H), 7.69 (m, 1H, Ar-H). HRMS (ESI) *m/z*: 544.2319 $[M+H]^+$; calcd. for $C_{30}H_{30}FN_5O_4$ (M+H) 544.2355.

*3-[{2-[(4-Cyano-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(3-methoxy-phenyl)-amino]-propionic acid ethyl ester (**12d**)*
Yield: 55%; m.p. 181–183°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.22 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 1.36 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 2.72 (t, $J$ = 7.2 Hz, 2H, –$CH_2$–), 3.70 (s, 3H, –$OCH_3$), 4.09 (t, 2H, –$NCH_2$), 4.13 (q, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.22 (q, $J$ = 7.2 Hz, 2H, –$OCH_2$–), 4.52 (d, 2H, –$NCH_2$–), 5.50 (brs, H, –NH–), 6.64 (d, $J$ = 8.5 Hz, 2H, Ar-H), 6.81 (m, 1H, Ar-H), 7.12 (m, 1H, Ar-H), 7.21 (m, 1H, Ar-H), 7.23 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.27 (m, 1H, Ar-H), 7.33 (m, 1H, Ar-H), 7.70 (m, 1H, Ar-H). HRMS (ESI) *m/z*: 544.2343 $[M+H]^+$; calcd. for $C_{30}H_{30}FN_5O_4$ (M+H) 544.2355.

*3-[{2-[(4-Cyano-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(3-fluoro-phenyl)-amino]-propionic acid ethyl ester (**12e**)*
Yield: 60%; m.p. 170–172°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.23 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 1.40 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 2.73 (t, $J$ = 7.2 Hz, 2H, –$CH_2$–), 4.10 (t, 2H, –$NCH_2$), 4.16 (q, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.24 (q, $J$ = 7.2 Hz, 2H, –$OCH_2$–), 4.56 (d, 2H, –$NCH_2$–), 5.57 (brs, H, –NH–), 6.84 (d, $J$ = 8.5 Hz, 2H, Ar-H), 6.89 (m, 1H, Ar-H), 7.18 (m, 1H, Ar-H), 7.25 (m, 1H, Ar-H), 7.31 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.34 (m, 1H, Ar-H), 7.42 (m, 1H, Ar-H), 7.68 (m, 1H, Ar-H). HRMS (ESI) *m/z*: 532.2155 $[M+H]^+$; calcd. for $C_{29}H_{27}F_2N_5O_3$ (M+H) 532.2155.

*3-[{2-[(4-Cyano-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(4-methoxy-phenyl)-amino]-propionic acid ethyl ester (**12f**)*
Yield: 57%; m.p. 150–151°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.23 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 1.39 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 2.72 (t, $J$ = 7.2 Hz, 2H, –$CH_2$–), 3.72 (s, 3H, –$OCH_3$), 4.09 (t, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.16 (q, $J$ = 7.2 Hz, 2H, –$OCH_2$–), 4.21 (q, 2H, –$NCH_2$–), 4.56 (d, 2H, –$NCH_2$–), 5.50 (brs, H, –NH–), 6.73 (m, 1H, Ar-H), 6.83 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.01 (m, 1H, Ar-H), 7.16 (m, 1H, Ar-H), 7.35 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.58 (m, 1H, Ar-H), 7.69 (m, 1H, Ar-H), 7.93 (m, 1H, Ar-H). HRMS (ESI) *m/z*: 544.2387 $[M+H]^+$; calcd. for $C_{30}H_{30}FN_5O_4$ (M+H) 544.2355.

*3-[{2-[(4-Cyano-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(4-chloro-phenyl)-amino]-propionic acid ethyl ester (**12g**)*
Yield: 58%; m.p. 173–174°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.24 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 1.42 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 2.73 (t, $J$ = 7.2 Hz, 2H, –$CH_2$–), 4.11 (t, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.18 (q, $J$ = 7.2 Hz, 2H, –$OCH_2$–), 4.23 (q, 2H, –$NCH_2$–), 4.58 (d, 2H, –$NCH_2$–), 5.52 (brs, H, –NH–), 6.85 (m, 1H, Ar-H), 7.05 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.19 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.20 (m, 1H, Ar-H), 7.28 (m, 1H, Ar-H), 7.34 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.69 (m, 1H, Ar-H). HRMS (ESI) *m/z*: 548.1899 $[M+H]^+$; calcd. for $C_{29}H_{27}F_2N_5O_3$ (M+H) 548.1859.

*3-({2-[(4-Cyano-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-pyridin-2-yl-amino)-propionic acid ethyl ester (**12h**)*
Yield: 60%; m.p. 195–196°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.21 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 1.37 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 2.8 (t, $J$ = 7.2 Hz, 2H, –$CH_2$–), 4.06 (t, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.15 (q, $J$ = 7.2 Hz, 2H, –$OCH_2$–), 4.42 (q, 2H, –$NCH_2$–), 4.56 (d, 2H, –$NCH_2$–), 5.68 (brs, H, –NH–), 6.74 (m, 1H, Ar-H), 6.84 (m, 1H, Ar-H), 7.00 (m, 1H, Py-H), 7.13 (m, 1H, Py-H), 7.21 (m, 1H, Ar-H), 7.30 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.35 (m, 1H, Py-H), 7.70 (m, 1H, Ar-H), 8.43 (m, 1H, Py-H). HRMS (ESI) *m/z*: 515.2238 $[M+H]^+$; calcd. for $C_{28}H_{27}FN_6O_3$ (M+H) 515.2201.

*3-[{2-[(4-Cyano-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(5-methyl-pyridin-2-yl)-amino]-propionic acid ethyl ester (**12i**)*
Yield: 59%; m.p. 170–172°C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 1.23 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 1.40 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 2.24 (s, 3H, –$CH_3$), 2.79 (t, $J$ = 7.2 Hz, 2H, –$CH_2$–), 4.08 (t, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.18 (q, $J$ = 7.2 Hz, 2H, –$OCH_2$–), 4.39 (q, 2H, –$NCH_2$–), 4.58 (d, 2H, –$NCH_2$–), 5.59 (brs, H, –NH–), 6.66 (m, 1H, Ar-H), 6.85 (m, 1H, Ar-H), 7.17 (m, 1H, Py-H), 7.18 (m, 1H, Ar-H), 7.25 (m, 1H, Ar-H), 7.34 (m, 1H, Py-H), 7.36 (m, 1H, Ar-H), 7.71 (m, 1H, Ar-H), 8.26 (m, 1H, Py-H). HRMS (ESI) *m/z*: 529.2360 $[M+H]^+$; calcd. for $C_{29}H_{29}FN_6O_3$ (M+H) 529.2358.

*General procedure for the synthesis of compounds **13a–i***
To a solution of compounds **12a–i** (10 mmol) in anhydrous ethanol (40 mL), hydroxylamine hydrochloride (20 mmol) and triethylamine (20 mmol) were added with stirring. The mixture was heated at 80°C until the completion of the reaction. The reaction mixture was concentrated to remove the solvent. Then ammonium formate (2.14 g), Pd/C (1.07 g) and acetic acid (20 mL) were added and the reaction mixture was stirred at 120°C for 5 h under $N_2$. The mixture was filtered, concentrated under reduced pressure, and purified by column chromatography using DCM/MeOH = (20:1–10:1) as eluent to afford **13a–i**.

*3-({2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-phenyl-amino)-propionic acid ethyl ester (**13a**)*
Yield: 56%; m.p. 170–172°C; $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 1.14 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 1.23 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 2.60 (t, $J$ = 7.2 Hz, 2H, –$CH_2$–), 4.00 (t, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.10 (q, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.28 (q, $J$ = 7.2 Hz, 2H, –$OCH_2$–), 4.68 (d, 2H, –$NCH_2$–), 7.05 (m, 1H, Ar-H), 7.13 (m, 1H, Ar-H), 7.18 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.19 (m, 1H, Ar-H), 7.25 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.37 (m, 1H, Ar-H), 7.49 (m, 1H, Ar-H), 7.51 (m, 1H, Ar-H), 7.62 (m, 1H, Ar-H). HRMS (ESI) *m/z*: 531.2533 $[M+H]^+$; calcd. for $C_{29}H_{31}FN_6O_3$ (M+H) 531.2514.

*3-({2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-o-tolyl-amino)-propionic acid ethyl ester (**13b**)*
Yield: 52%; m.p. 146–147°C; $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 1.14 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 1.24 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 2.14 (s, 3H, –$CH_3$), 2.65 (t, $J$ = 7.2 Hz, 2H, –$CH_2$–), 3.99 (t, $J$ = 7.0 Hz, 2H, –$NCH_2$–), 4.03 (q, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.28 (q, $J$ = 7.2 Hz, 2H, –$OCH_2$–), 4.68 (d, 2H, –$NCH_2$–), 7.04 (m, 1H, Ar-H), 7.13 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.18 (m, 1H, Ar-H), 7.22 (m, 1H, Ar-H), 7.36 (m, 1H, Ar-H), 7.43 (m, 1H, Ar-H), 7.54 (m, 1H, Ar-H), 7.66 (m, 1H, Ar-H), 7.74 (m, 1H, Ar-H). HRMS (ESI) *m/z*: 545.2731 $[M+H]^+$; calcd. for $C_{30}H_{33}FN_6O_3$ (M+H) 545.2671.

*3-[{2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(2-methoxy-phenyl)-amino]-propionic acid ethyl ester (**13c**)*
Yield: 54%; m.p. 162–163°C; $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 1.13 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 1.22 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 2.59 (t, $J$ = 7.2 Hz, 2H, –$CH_2$–), 3.71 (s, 3H, –$OCH_3$), 3.97 (t, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.02 (q, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.28 (q, $J$ = 7.2 Hz, 2H, –$OCH_2$–), 4.68 (d, 2H, –$NCH_2$–), 6.80 (m, 1H, Ar-H), 6.94 (m, 1H, Ar-H), 7.04 (m, 1H, Ar-H), 7.16 (m, 1H, Ar-H), 7.35 (m, 1H, Ar-H), 7.45 (m, 1H, Ar-H), 7.51 (m, 1H, Ar-H), 7.62 (m, 1H, Ar-H), 7.67 (m, 1H, Ar-H), 7.73 (m, 1H, Ar-H). HRMS (ESI) *m/z*: 561.2598 $[M+H]^+$; calcd. for $C_{30}H_{33}FN_6O_4$ (M+H) 561.2620.

*3-[{2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(3-methoxy-phenyl)-amino]-propionic acid ethyl ester (**13d**)*
Yield: 56%; m.p. 175–176°C; $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 1.14 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 1.24 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 2.61 (t, $J$ = 7.2 Hz, 2H, –$CH_2$–), 3.64 (s, 3H, –$OCH_3$), 3.99 (t, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.09 (q, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.29 (q, $J$ = 7.2 Hz, 2H, –$OCH_2$–), 4.69 (d, 2H, –$NCH_2$–), 6.71 (d, $J$ = 8.5 Hz, 2H, Ar-H), 6.83 (m, 1H, Ar-H), 7.06 (m, 1H, Ar-H), 7.12 (m, 1H, Ar-H), 7.20 (m, 1H, Ar-H), 7.24 (m, 1H, Ar-H), 7.40 (m, 1H, Ar-H), 7.53 (m, 1H, Ar-H), 7.65 (m, 1H, Ar-H), 8.52 (s, 2H, N-H), 11.43 (s, 2H, N-H). HRMS (ESI) *m/z*: 561.2590 $[M+H]^+$; calcd. for $C_{30}H_{33}FN_6O_4$ (M+H) 561.2620.

*3-[{2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(3-fluoro-phenyl)-amino]-propionic acid ethyl ester (**13e**)*
Yield: 53%; m.p. 195–196°C; $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 1.14 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 1.24 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 2.62 (t, $J$ = 7.2 Hz, 2H, –$CH_2$–), 4.00 (t, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.11 (q, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.30 (q, $J$ = 7.2 Hz, 2H, –$OCH_2$–), 4.70 (d, 2H, –$NCH_2$–), 6.99 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.05 (m, 1H, Ar-H), 7.18 (m, 1H, Ar-H), 7.22 (m, 1H, Ar-H), 7.26 (m, 1H, Ar-H), 7.42 (m, 1H, Ar-H), 7.53 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.64 (m, 1H, Ar-H). HRMS (ESI) *m/z*: 549.2386 $[M+H]^+$; calcd. for $C_{29}H_{30}F_2N_6O_3$ (M+H) 549.2420.

*3-[{2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(4-methoxy-phenyl)-amino]-propionic acid ethyl ester (**13f**)*
Yield: 55%; m.p. 130–131°C; $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 1.15 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 1.24 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 2.58 (t, $J$ = 7.2 Hz, 2H, –$CH_2$–), 3.66 (s, 3H, –$OCH_3$), 4.00 (t, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.04 (q, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.28 (q, $J$ = 7.2 Hz, 2H, –$OCH_2$–), 4.69 (d, 2H, –$NCH_2$–), 6.81 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.06 (m, 1H, Ar-H), 7.10 (m, 1H, Ar-H), 7.15 (m, 1H, Ar-H), 7.19 (m, 1H, Ar-H), 7.38 (m, 1H, Ar-H), 7.50 (d, $J$ = 8.5 Hz, 2H, Ar-H), 7.61 (m, 1H, Ar-H). HRMS (ESI) *m/z*: 561.2661 $[M+H]^+$; calcd. for $C_{30}H_{33}FN_6O_4$ (M+H) 561.2620.

*3-((4-Chloro-phenyl)-{2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-amino)-propionic acid ethyl ester (**13g**)*
Yield: 56%; m.p. 153–155°C; $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 1.13 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 1.23 (t, $J$ = 7.2 Hz, 3H, –$CH_3$), 2.60 (t, $J$ = 7.2 Hz, 2H, –$CH_2$–), 3.98 (t, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.08 (q, $J$ = 7.2 Hz, 2H, –$NCH_2$–), 4.29 (q, $J$ = 7.2 Hz, 2H, –$OCH_2$–), 4.69 (d, 2H, –$NCH_2$–), 7.05 (m, 1H, Ar-H), 7.17 (d, $J$ = 8.5 Hz, 2H, Ar-H),

7.21 (m, 1H, Ar-H), 7.22 (m, 1H, Ar-H), 7.30 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.41 (m, 1H, Ar-H), 7.50 (m, 1H, Ar-H), 7.61 (m, 1H, Ar-H), 8.45 (s, 2H, N-H), 11.31 (s, 2H, N-H). HRMS (ESI) *m/z*: 565.2182 $[M+H]^+$; calcd. for $C_{29}H_{30}ClFN_6O_3$ (M+H) 565.2125.

*3-({2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-pyridin-2-yl-amino)-propionic acid ethyl ester (**13h**)*
Yield: 57%; m.p. 202–203°C; $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 1.12 (t, $J = 7.2$ Hz, 3H, $-CH_3$), 1.24 (t, $J = 7.2$ Hz, 3H, $-CH_3$), 2.69 (t, $J = 7.2$ Hz, 2H, $-CH_2-$), 3.97 (t, $J = 7.2$ Hz, 2H, $-NCH_2-$), 4.23 (q, $J = 7.2$ Hz, 2H, $-NCH_2-$), 4.31 (q, $J = 7.2$ Hz, 2H, $-OCH_2-$), 4.71 (d, 2H, $-NCH_2-$), 6.93 (m, 1H, Ar-H), 7.06 (m, 1H, Ar-H), 7.13 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.42 (m, 1H, Ar-H), 7.50 (m, 1H, Ar-H), 7.54 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.63 (m, 1H, Ar-H), 8.40 (m, 1H, Ar-H). HRMS (ESI) *m/z*: 532.2511 $[M+H]^+$; calcd. for $C_{28}H_{30}FN_7O_3$ (M+H) 532.2467.

*3-[{2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(5-methyl-pyridin-2-yl)-amino]-propionic acid ethyl ester (**13i**)*
Yield: 52%; m.p. 180–181°C; $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 1.12 (t, $J = 7.2$ Hz, 3H, $-CH_3$), 1.24 (t, $J = 7.2$ Hz, 3H, $-CH_3$), 2.18 (s, 3H, $-CH_3$), 2.65 (t, $J = 7.2$ Hz, 2H, $-CH_2-$), 3.98 (t, $J = 7.2$ Hz, 2H, $-NCH_2-$), 4.18 (q, $J = 7.2$ Hz, 2H, $-NCH_2-$), 4.31 (q, $J = 7.2$ Hz, 2H, $-OCH_2-$), 4.70 (d, 2H, $-NCH_2-$), 6.84 (m, 1H, Ar-H), 7.06 (m, 1H, Ar-H), 7.15 (m, 1H, Ar-H), 7.42 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.49 (m, 1H, Ar-H), 7.52 (m, 1H, Ar-H), 7.63 (m, 1H, Ar-H), 8.23 (m, 1H, Ar-H). HRMS (ESI) *m/z*: 546.2641 $[M+H]^+$; calcd. for $C_{29}H_{32}FN_7O_3$ (M+H) 546.2623.

*General procedure for the synthesis of the target compounds **14a–i***
Compounds **13a–i** (2 mmol) were added to a solution of sodium hydroxide (0.24 g, 6.0 mmol) in water/ethanol (30 mL, 2:1 v/v) and kept stirring at room temperature for 2 h. The mixture was then diluted with 50 mL of water and neutralized with acetic acid. The precipitate was isolated and washed with water to afford the zwitterionic title compounds **14a–i**.

*3-({2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-phenyl-amino)-propionic acid (**14a**)*
Yield: 73%; m.p. 240–242°C; $^1$H NMR (DMSO-$d_6 + {}^2$HCl, 500 MHz) $\delta$: 1.33 (t, 3H, $-CH_3$), 2.53 (t, $J = 7.2$ Hz, 2H, $-CH_2$), 4.04 (t, $J = 7.2$ Hz, 2H, $-CH_2-$), 4.48 (q, $J = 7.2$ Hz, 2H, $-CH_2$), 5.07 (d, $J = 7.2$ Hz, 2H, $-CH_2$), 7.07 (m, 1H, Ar-H), 7.15 (m, 1H, Ar-H), 7.23 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.24 (m, 1H, Ar-H), 7.45 (m, 1H, Ar-H), 7.64 (m, 1H, Ar-H), 7.68 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.81 (m, 1H, Ar-H), 7.87 (m, 1H, Ar-H), 9.01 (s, 2H, N-H), 9.27 (s, 2H, N-H). $^{13}$C NMR (DMSO-$d_6 + {}^2$HCl, 125 MHz) $\delta$: 172.25, 168.88, 163.32, 152.47, 151.00, 148.61, 140.27, 133.49, 132.46, 130.26, 130.03, 129.07, 126.06, 125.45, 116.46, 116.26, 114.68, 114.60, 114.47, 113.82, 112.53, 111.72, 4.67, 40.50, 38.23, 31.96, 13.88. HRMS (ESI) *m/z*: 503.2175 $[M+H]^+$; calcd. for $C_{27}H_{27}FN_6O_3$ (M+H) 503.2201.

*3-({2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-o-tolyl-amino)-propionic acid (**14b**)*
Yield: 75%; m.p. 237–239°C; $^1$H NMR (DMSO-$d_6 + {}^2$HCl, 500 MHz) $\delta$: 1.33 (t, 3H, $-CH_3$), 2.15 (s, 3H, $-CH_3$), 2.61 (t, $J = 7.2$ Hz, 2H, $-CH_2$), 4.23 (t, $J = 7.2$ Hz, 2H, $-CH_2-$), 4.47 (q, $J = 7.2$ Hz, 2H, $-CH_2$), 5.06 (d, $J = 7.2$ Hz, 2H, $-CH_2$), 7.06 (m, 1H, Ar-H), 7.11 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.13 (m, 1H, Ar-H), 7.25 (m, 1H, Ar-H), 7.46 (m, 1H, Ar-H), 7.62 (m, 1H, Ar-H), 7.65 (m, 1H, Ar-H), 7.84 (d, $J = 8.5$ Hz, 2H, Ar-H), 9.01 (s, 2H, N-H), 9.28 (s, 2H, N-H). $^{13}$C NMR (DMSO-$d_6 + {}^2$HCl, 125 MHz) $\delta$: 172.48, 168.39, 163.56, 152.28, 148.62, 140.80, 140.41, 140.29, 134.80, 134.11, 132.21, 131.34, 129.67, 128.10, 126.98, 114.69, 113.90, 112.30, 111.69, 45.23, 40.41, 38.21, 31.77, 17.46, 13.92. HRMS (ESI) *m/z*: 517.2359 $[M+H]^+$; calcd. for $C_{28}H_{29}FN_6O_3$ (M+H) 517.2358.

*3-[{2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(2-methoxy-phenyl)-amino]-propionic acid (**14c**)*
Yield: 80%; m.p. 228–229°C; $^1$H NMR (DMSO-$d_6 + {}^2$HCl, 500 MHz) $\delta$: 1.33 (t, 3H, $-CH_3$), 2.61 (t, $J = 7.2$ Hz, 2H, $-CH_2$), 3.72 (s, 3H, $-OCH_3$), 4.09 (t, $J = 7.2$ Hz, 2H, $-CH_2-$), 4.47 (q, $J = 7.2$ Hz, 2H, $-CH_2$), 5.07 (d, $J = 7.2$ Hz, 2H, $-CH_2$), 6.81 (m, 1H, Ar-H), 6.93 (m, 1H, Ar-H), 7.08 (m, 1H, Ar-H), 7.15 (m, 1H, Ar-H), 7.22 (m, 1H, Ar-H), 7.46 (m, 1H, Ar-H), 7.67 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.84 (d, $J = 8.5$ Hz, 2H, Ar-H), 9.03 (s, 2H, N-H), 9.26 (s, 2H, N-H). $^{13}$C NMR (DMSO-$d_6 + {}^2$HCl, 125 MHz) $\delta$: 172.56, 169.19, 163.52, 154.25, 152.07, 148.62, 140.43, 134.34, 132.20, 130.70, 129.77, 129.44, 129.21, 126.04, 125.26, 120.84, 119.59, 114.70, 113.42, 112.35, 112.14, 111.69, 55.64, 45.00, 40.35, 38.26, 31.89, 13.93. HRMS (ESI) *m/z*: 533.2323 $[M+H]^+$; calcd. for $C_{28}H_{29}FN_6O_4$ (M+H) 533.2307.

*3-[{2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(3-methoxy-phenyl)-amino]-propionic acid (**14d**)*
Yield: 71%; m.p. 232–234°C; $^1$H NMR (DMSO-$d_6 + {}^2$HCl, 500 MHz) $\delta$: 1.35 (t, 3H, $-CH_3$), 2.55 (t, $J = 7.2$ Hz, 2H, $-CH_2$), 3.64 (s, 3H, $-OCH_3$), 4.03 (t, $J = 7.2$ Hz, 2H, $-CH_2-$), 4.50 (q, $J = 7.2$ Hz, 2H, $-CH_2-$), 5.09 (d, $J = 7.2$ Hz, 2H, $-CH_2$), 6.72 (d, $J = 8.5$ Hz, 2H, Ar-H), 6.92 (m, 1H, Ar-H), 7.10 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.51 (m, 1H, Ar-H), 7.66 (m, 1H, Ar-H), 7.72 (m, 1H, Ar-H), 7.82 (m, 1H, Ar-H), 7.87 (m, 1H, Ar-H), 9.03 (s, 2H, N-H), 9.28 (s, 2H, N-H). $^{13}$C NMR (DMSO-$d_6 + {}^2$HCl, 125 MHz) $\delta$: 172.53, 168.22, 163.57, 159.65, 152.19, 151.01, 148.62, 140.40, 134.42, 132.16, 129.97, 129.29, 126.06, 125.79, 120.46, 114.70, 114.49, 114.27, 113.81, 112.74, 112.33, 111.66, 55.23, 46.13, 40.38, 38.17, 32.07, 13.96. HRMS (ESI) *m/z*: 533.2314 $[M+H]^+$; calcd. for $C_{28}H_{29}FN_6O_4$ (M+H) 533.2307.

*3-[{2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(3-fluoro-phenyl)-amino]-propionic acid (**14e**)*
Yield: 75%; m.p. 236–237°C; $^1$H NMR (DMSO-$d_6 + {}^2$HCl, 500 MHz) $\delta$: 1.36 (t, 3H, $-CH_3$), 2.57 (t, $J = 7.2$ Hz, 2H, $-CH_2$), 4.06 (t, $J = 7.2$ Hz, 2H, $-CH_2-$), 4.50 (q, $J = 7.2$ Hz, 2H, $-CH_2-$),

5.00 (d, $J=7.2$ Hz, 2H, $-CH_2$), 7.02 (m, 1H, Ar-H), 7.09 (d, $J=8.5$ Hz, 2H, Ar-H), 7.29 (d, $J=8.5$ Hz, 2H, Ar-H), 7.49 (m, 1H, Ar-H), 7.67 (m, 1H, Ar-H), 7.73 (m, 1H, Ar-H), 7.81 (m, 1H, Ar-H), 7.92 (m, 1H, Ar-H), 8.99 (s, 2H, N-H), 9.24 (s, 2H, N-H). $^{13}$CNMR (DMSO-$d_6$ + $^2$HCl, 125 MHz) $\delta$: 172.50, 168.95, 163.12, 160.68, 152.36, 150.84, 148.46, 133.74, 132.12, 130.72, 130.63, 124.64, 124.42, 116.57, 115.23, 115.00, 114.51, 114.31, 113.96, 113.75, 111.57, 111.23, 48.48, 46.18, 36.61, 32.07, 14.28. HRMS (ESI) *m/z*: 521.2093 $[M+H]^+$; calcd. for $C_{27}H_{26}F_2N_6O_3$ (M+H) 521.2107.

*3-[{2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(4-methoxy-phenyl)-amino]-propionic acid (**14f**)*
Yield: 79%; m.p. 220–221°C; $^1$H NMR (DMSO-$d_6$ + $^2$HCl, 500 MHz) $\delta$: 1.34 (t, 3H, $-CH_3$), 2.72 (t, $J=7.2$ Hz, 2H, $-CH_2$), 3.63 (s, 3H, $-OCH_3$), 3.99 (t, $J=7.2$ Hz, 2H, $-CH_2$–), 4.49 (q, $J=7.2$ Hz, 2H, $-CH_2$), 5.12 (d, $J=7.2$ Hz, 2H, $-CH_2$), 6.79 (d, $J=8.5$ Hz, 2H, Ar-H), 6.93 (m, 1H, Ar-H), 7.08 (m, 1H, Ar-H), 7.15 (m, 1H, Ar-H), 7.43 (m, 1H, Ar-H), 7.66 (d, $J=8.5$ Hz, 2H, Ar-H), 7.83 (d, $J=8.5$ Hz, 2H, Ar-H), 9.01 (s, 2H, N-H), 9.26 (s, 2H, N-H). $^{13}$C NMR (DMSO-$d_6$ + $^2$HCl, 125 MHz) $\delta$: 172.53, 172.03, 168.27, 163.56, 157.75, 152.09, 151.01, 148.62, 140.39, 140, 27, 134.58, 132.01, 129.42, 129.30, 126.05, 122.18, 114.75.114.68, 114.44, 113.69, 112.25, 111.64, 55.23, 46.19, 40.37, 38.14, 31.98, 15.93. HRMS (ESI) *m/z*: 533.2292 $[M+H]^+$; calcd. for $C_{28}H_{29}FN_6O_4$ (M+H) 533.2307.

*3-[{2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(4-chloro-phenyl)-amino]-propionic acid (**14g**)*
Yield: 76%; m.p. 235–237°C; $^1$H NMR (DMSO-$d_6$ + $^2$HCl, 500 MHz) $\delta$: 1.36 (t, 3H, $-CH_3$), 2.55 (t, $J=7.2$ Hz, 2H, $-CH_2$), 4.04 (t, $J=7.2$ Hz, 2H, $-CH_2$–), 4.50 (q, $J=7.2$ Hz, 2H, $-CH_2$–), 5.10 (d, $J=7.2$ Hz, 2H, $-CH_2$), 7.09 (m, 1H, Ar-H), 7.24 (m, 1H, Ar-H), 7.31 (m, 1H, Ar-H), 7.47 (m, 1H, Ar-H), 7.65 (m, 1H, Ar-H), 7.71 (m, 1H, Ar-H), 7.81 (d, $J=8.5$ Hz, 2H, Ar-H), 7.90 (d, $J=8.5$ Hz, 2H, Ar-H), 9.07 (s, 2H, N-H), 9.32 (s, 2H, N-H). $^{13}$C NMR (DMSO-$d_6$ + $^2$HCl, 125 MHz) $\delta$: 172.51, 172.02, 169.34, 163.46, 152.34, 152.22, 150.74, 148.36, 141.98, 131.02, 129.77, 129.23, 129.15, 127.95, 126.02, 124.02, 117.82, 114.42, 114.21, 111.51, 110.50, 48.47, 46.20, 32.08, 21.01, 14.48. HRMS (ESI) *m/z*: 537.1879 $[M+H]^+$; calcd. for $C_{27}H_{26}ClFN_6O_3$ (M+H) 537.1812.

*3-({2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-pyridin-2-yl-amino)-propionic acid (**14h**)*
Yield: 77%; m.p. 264–266°C; $^1$H NMR (DMSO-$d_6$ + $^2$HCl, 500 MHz) $\delta$: 1.37 (t, 3H, $-CH_3$), 2.63 (t, $J=7.2$ Hz, 2H, $-CH_2$), 4.17 (t, $J=7.2$ Hz, 2H, $-CH_2$–), 4.45 (q, $J=7.2$ Hz, 2H, $-CH_2$–), 5.11 (d, $J=7.2$ Hz, 2H, $-CH_2$), 7.10 (m, 1H, Ar-H), 7.21 (d, $J=8.5$ Hz, 2H, Ar-H), 7.44 (m, 1H, Ar-H), 7.65 (m, 1H, Ar-H), 7.70 (d, $J=8.5$ Hz, 2H, Ar-H), 7.85 (m, 1H, Ar-H), 7.91 (m, 1H, Ar-H), 8.36 (m, 1H, Ar-H), 9.03 (s, 2H, N-H), 9.26 (s, 2H, N-H). $^{13}$C NMR (DMSO-$d_6$ + $^2$HCl, 125 MHz) $\delta$: 172.44, 168.76, 163.54, 155.17, 152.51, 150.99, 148.73, 148.60, 140.36, 138.59, 133.79, 132.86, 126.04, 125.56, 121.96, 114.66, 114.58, 114.46, 112.33, 111.68, 44.59, 40.27, 36.31, 32.58, 14.03. HRMS (ESI) *m/z*: 504.2208 $[M+H]^+$; calcd. for $C_{26}H_{26}FN_7O_3$ (M+H) 504.2154.

*3-[{2-[(4-Carbamimidoyl-2-fluoro-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(5-methyl-pyridin-2-yl)-amino]-propionic acid (**14i**)*
Yield: 78%; m.p. 251–253°C; $^1$H NMR (DMSO-$d_6$ + $^2$HCl, 500 MHz) $\delta$: 1.36 (t, 3H, $-CH_3$), 2.20 (s, 3H, $-CH_3$), 2.61 (t, $J=7.2$ Hz, 2H, $-CH_2$), 4.12 (t, $J=7.2$ Hz, 2H, $-CH_2$–), 4.52 (q, $J=7.2$ Hz, 2H, $-CH_2$–), 5.14 (d, $J=7.2$ Hz, 2H, $-CH_2$), 7.10 (m, 1H, Ar-H), 7.19 (m, 1H, Ar-H), 7.45 (m, 1H, Ar-H), 7.61 (m, 1H, Ar-H), 7.68 (m, 1H, Ar-H), 7.72 (m, 1H, Ar-H), 7.82 (m, 1H, Ar-H), 7.90 (m, 1H, Ar-H), 8.24 (m, 1H, Ar-H), 9.03 (s, 2H, N-H), 9.27 (s, 2H, N-H). $^{13}$C NMR (DMSO-$d_6$ + $^2$HCl, 125 MHz) $\delta$: 172.44, 168.51, 163.54, 152.47, 151.02, 148.63, 148.13, 140.40, 139.62, 133.99, 132.64, 131.69, 129.64, 126.07, 125.70, 121.84, 114.73, 114.66, 114.48, 112.54, 111.69, 44.77, 40.42, 38.23, 32.47, 17.24, 13.99. HRMS (ESI) *m/z*: 518.2368 $[M+H]^+$; calcd. for $C_{27}H_{28}FN_7O_3$ (M+H) 518.2310.

## Thrombin assay

To a solution of lyophilized human thrombin (5.4 μg/mL), which was purified from human blood, the test compounds dissolved in DMSO were added and preincubated for 10 min at 37°C. After that, Ac-FVR-AMC (5 μM), a specific fluorogenic thrombin substrate, was added to above system. DMSO was used as a negative control in the assay. We detected the dynamic changes of 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: excitation wavelength, 355 nm; 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 as an activity indicator.

The concentration that induced a 50% inhibition of thrombin activity ($IC_{50}$) was calculated. All measurements were performed in duplicate; the mean values of both determinations are presented.

## Molecular modeling

The molecular modeling was performed using the package Sybyl-X 2.0 (Tripos, Inc., USA). Three-dimensional structures of all compounds involved in this study were optimized using Tripos force field and Gasteiger–Huckel charges. The structural energy minimization was terminated when using Powell gradient algorithm with a convergence criterion of 0.005 kcal/(mol Å) was reached and a maximum of 10000 iterations. The crystal structure of thrombin receptor complex was retrieved from the RCSB Protein Data Bank (PDB entry code: 1KTS). The ligands were docked in the corresponding protein's binding site by an empirical scoring function and a patented search

engine in Surflex-Dock. Before the docking process, the natural ligand was extracted, the other natural ligands and water molecules were removed from the crystal structure. Subsequently, the protein was prepared by using the Biopolymer module implemented. The polar hydrogen atoms were added, and Gasteiger–Huckel charges were assigned to protein atoms. Other parameters were established by default in the software. The automated docking manner was applied in the present work. Surflex-Dock total scores, which were expressed in −log10 (Kd) units to represent binding affinities.

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