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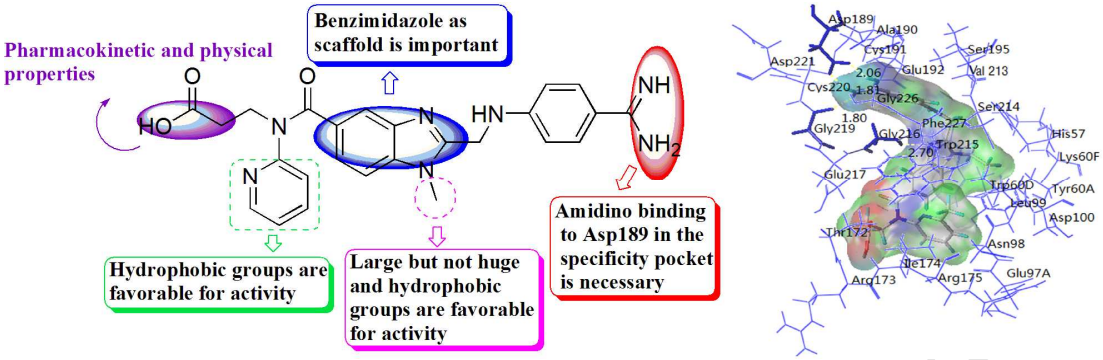

A class of N-ethyl dabigatran derivatives was designed, predicted, synthesized and evaluated anticoagulant activity. Compound **9p** which exhibited better inhibitory activity than dabigatran could as a potential thrombin inhibitor.

Design, synthesis, anticoagulant activity evaluation and molecular docking studies of a class of N-ethyl dabigatran derivatives

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Abstract A class of N-ethyl dabigatran derivatives was designed based on pharmacological strategies for inhibition of thrombin activity and the structure-activity relationship studies of the previous dabigatran derivatives. Activities of these novel compounds were predicted based on CoMFA model, and most of the compounds had comparable predicted activity with dabigatran. All of screened compounds were synthesized and characterized by ^{1}H NMR, ^{13}C NMR and HRMS. Subsequently, these compounds were evaluated inhibitory activity on thrombin. Among these compounds, **9a–9e**, **9h**, **9l–9n** and **9p** exhibited comparable inhibitory activity to dabigatran (IC_{50} = 1.20 nM), additionally, compound **9p** (IC_{50} = 0.96 nM) exhibited better inhibitory activity than dabigatran. Moreover, compound **9p** also exhibited a fairly good inhibitory activity for arteriovenous thrombosis with inhibition rate of (85.35 ± 0.72) %, which was comparable to that of dabigatran (85.07 ± 0.61) %. These results, along with related molecular docking studies, could provide an important basis for further development of compound **9p** as a potent thrombin inhibitor.

Key Words: N-methyl dabigatran derivatives / synthesis / anticoagulant activity / molecular docking

1. Introduction

Atrial fibrillation (AF) increases the risks of stroke and death [1, 2], patients with AF have a nearly 5-fold increase in stroke risk [3]. The prevalence of AF is increasing significantly worldwide, and AF affects approximately 1%–2% of the world population [4–7]. Improving treatment for AF is therefore a key health care focus. The main clinical impact of AF is increased the risk of stroke, accordingly, anticoagulant is employed in the prevention of stroke or systemic embolism (SE) for patients with AF and co-existing heart failure (HF) [8, 9].

Traditionally, prophylactic treatment has been based on vitamin K antagonists (VKAs), and it had been used for more than 60 years for their known effectiveness in preventing thromboembolic events [10, 11]. Managing AF by using VKA therapy has several disadvantages, such as monitoring required and the high risk of hemorrhages, thus, VKA therapy is infrequently used to treat AF cases [12]. Recently, novel oral anticoagulants (NOACs) have been developed for stroke prevention. Compared with traditionally anticoagulants, NOACs have better specificity, more rapid onset of action, and shorter half-lives [13], Examples of NOACs are dabigatran, rivaroxaban, and apixaban; these have potential for use as stroke prevention treatments for patients with nonvalvular AF [14,15].

Particularly, dabigatran is a novel synthetic anticoagulant drug belonging to the class of reversible direct thrombin inhibitors (DTIs). It is the prodrug of dabigatran etexilate, and has a high specificity for thrombin [16]. This drug is superior to other anticoagulants because of the following practical advantages: predictable anticoagulant effect, oral administration, no need for monitoring, and few drug interactions [17, 18]. Dabigatran etexilate has recently been approved in the United State and Europe Union for stroke prevention in patients with AF [19–22]. Safety and efficiency are the major concerns in using anticoagulant drugs in clinical practice. However, the bioavailability after oral administration of dabigatran etexilate is 7.2% [23], and it also has a risk of life-threatening hemorrhage. In the RE-LY trial, the major bleeding incidence of life-threatening hemorrhage increased from 2.71% to

3.11% when dosage for patients treated with from 110mg to 150 mg [24]. A new dabigatran derivative that has better efficacy may contribute to decrease side effects because drug dosage would be reduced, and has high-hydrophobicity may contribute to enhance the banding force between drugs and target enzyme [25].

A previous study shows that dabigatran enhances clot lysis mainly via two mechanisms, as follows: reduction of plasmin generation and alteration of the viscoelastic properties of the clot. Both effects are depended on the ability of the drug to reduce thrombin generation and activity [17]. Effects are based on the combination of drugs and target enzyme. We studied the pharmacological strategies for inhibition of thrombin activity and the structure-activity relationship of the previous dabigatran derivatives (Fig.1) [25–30]. The benzamidine moiety binds to Asp 189 in the specificity pocket, which is necessary for the inhibition of thrombin activity. The benzimidazole ring as scaffold forms p-p stacking interactions with the Trp60D, which is crucial for the thrombin protein binding. Carboxylate group is needed for pharmacokinetics, and it does not interfere with the thrombin binding site. The N-methyl group occupies the P-pocket and the pyridine ring occupies the D-pocket. The two sections interact with the protein mainly due to the hydrophobic characteristics of the groups. Therefore, N-ethyl-substituted N-methyl occupies the P-pocket and may favor activity. Aryl-substituted pyridine or methyl substituted on these ring could increase hydrophobicity, which may also be favor activity.

We designed a class of N-ethyl dabigatran derivatives. All compounds' scores were calculated via molecular docking. Activities were predicted based on a molecular model, and these compounds were synthesized and evaluated for inhibitory activities. We expected to find dabigatran derivatives that have higher inhibition activities than dabigatran, and such derivatives are worth studying in the future.

2. Results and discussion

2.1 Docking scores and activity prediction

Molecular models, such as comparative molecular field analysis (CoMFA), were established on the analysis of three-dimensional quantitative structure-activity

relationship (3D-QSAR) of dabigatran in our previous work [28]. All designed compounds were scored via molecular docking. Based on the CoMFA model, activities of these novel compounds were predicted. The docking result and the predicted result showed that all designed compounds obtained higher scores than dabigatran, and most of the compounds had comparable activity with dabigatran. Additionally, predicted activity values of those *p*-position methyl substituted compounds were lower than the others.

2.2 Chemistry

The synthetic route of N-ethyl dabigatran derivatives is shown in Scheme 1. Compounds **9a–9p** were synthesized using corresponding amines (**1a–1p**) according to the literature [31]. In the synthesis of compound 5, the author used Zn/NH_4Cl as reductant, and THF/H_2O as solvent in this reaction. This method not only needed a long duration for the reaction but also had high cost. Moreover, purification was done by column chromatography. Zinc powder is an effective and cheap reductant and is widely used in the nitroreduction reaction. Justin J. Maresh et al. used Zn/HCl for nitroreduction [32]. Takehito Tsukinoki et al. used zinc to reduce nitroarenes in various conditions, such as NH_4Cl/MeOH [33]. After a number of attempts, we adopted Zn as a reducing agent and AcOH/H_2O as a solvent in this paper. This method needed only 1 hour. Using this method not only saved energy but also reduced cost. We studied the reaction process, and compounds **4a–4p**，AcOH and H_2O could constitute a homogeneous system. Zinc powder can be ionized well in acetic acid and has high reducibility in an acidic environment. The reaction is exothermic. The temperature of the reaction system can reach up to 60 °C–70 °C, and this temperature range was favorable to the reaction. Therefore, the reaction was performed well, and thereby contributing to the subsequent purification. Many experiments were performed. Furthermore, we found that compounds **5a–5p** could be purified through hyperacoustic-aided crystal by using ethyl acetate and petroleum ether solvents. We obtained high purity (97%–99%) products with this method. The purification of later products benefited from this method, and it might be an excellent method for

application in other syntheses.

2.3 Inhibition activity evaluation

2.3.1 Inhibition rate tests

To confirm the activity of these synthesized compounds, we first conducted inhibition rate tests. The thrombin inhibition rates of compounds **9a–9p** were tested at a concentration of 1 μg/mL and are exhibited in Fig. 2.

Fig. 2 shows that all the compounds had high inhibition rates (>90%), thereby demonstrating that these compounds inhibited thrombin. Additionally, we studied dates of the inhibition rate. We found that there was little difference between **9a**, **9b**, **9h**, **9l**, **9m**, and **9p** (*o*-position methyl substituted) and **9d**, **9f**, **9i**, **9k**, and **9o** (*p*-position methyl substituted). The inhibition rates of the former were a little higher than those of the later.

2.3.2 Inhibitory activity in vitro

To save resources and improve efficiency, combining this result with the result of the predicted IC_{50}, we selected 11 compounds as representatives to evaluate their inhibitory activity in vitro, and the IC_{50} values were exhibited in Table 1.

As shown in Table 1, compounds **9a–9e**, **9h**, **9l–9n**, and **9p** had comparable inhibition activities with dabigatran. Furthermore, compound **9p** had better inhibitory activity compared with dabigatran, but compound **9f** exhibited worse inhibitory activity than the others. Additionally, inhibitory activities of compounds **9b**, **9h**, and **9p** (*o*-position methyl substituted) were better than compounds **9d**, **9f,** and **9h** (*p*-position methyl substituted). These results were consistent with the predicted IC_{50} and the inhibition rate tests in Fig. 2, possibly because the hydrophobic and electron-donating group at *o*-position is favorable for activity, but not favorable for activity at *p*-position.

2.3.3 Inhibitory activity in vivo

According to the analysis of inhibitory activity in vitro, compounds **9b** and **9p** were selected to evaluate their effects on blood clotting in rats. The results of inhibitory

activity in vivo were exhibited in Table 2.

As shown in Table 2, compounds **9b** and **9p** could inhibit the embolus weight of arteriovenous thrombosis effectively. Moreover, compound **9p** exhibited a fairly good inhibitory activity for arteriovenous thrombosis with inhibition rate of (85.35 ± 0.72) %, which was comparable to that of dabigatran (85.07 ± 0.61) %.

2.4 Molecular docking study

Molecular docking could simulate the bonding form between molecule and target enzymes [34–37]. To understand the recognition processes of these derivatives, we studied the molecular modeling between dabigatran and the promising compound **9p** and the thrombin protein, and performed it in Surflex-Dock (Sybyl 2.0). The X-ray crystal structure of dabigatran (PDB code: 1KTS) was retrieved from the RCSB Protein Data Bank. The docking results are exhibited in Figs. 3 and 4.

As shown in Fig. 3, the original ligand was redocked to validate the docking reliability. Their binding modes are basically similar. The benzimidazole ring of the two compounds is located in the same pocket. The pyridine ring of the redocked ligand is also positioned in D-pocket, except slight rotation of bonds. After validating the docking reliability, the compound **9p** with highest activity was selected for exploring the probable binding conformation. It bonded with the protein compactly, and the binding modes were basically similar with dabigatran.

As shown in Fig. 4, compared with dabigatran, compound **9p** also formed hydrogen bonds with Asp189 (the most important specific binding sites). The benzimidazole ring forms p-p stacking interactions with the Trp60D residue. N-ethyl group occupies the P-pocket, and the 6-position methyl substituted pyridine ring occupies the D-pocket tightly. Van der Waals forces were formed between the target compound and important residues (such as: Asp221, Cys220, Ala190, Cys191, Glu217, Arg173, Ile174, Arg175, Glu97A, Asn98, Asp100, Leu99, Leu97A, Tyr60A, Lys60F, His57, Phe227, Trp215, Val213, Ser195 and Glu192). Additionally, compound **9p** has a high amount of residues around the N-ethyl group (Trp215 and Lys60F) and around the 6-methyl substituted pyridine ring (Arg175 and Asp100). Moreover, the distance

between the two groups and residues is nearer than that between the two groups and dabigatran. Compound **9p** has stronger binding affinity than dabigatran.

3. Conclusion

Activities of 16 novel designed compounds were predicted via molecular models. All compounds were synthesized and characterized by ^{1}H NMR, ^{13}C NMR, and HRMS. Zinc powder was used as reductant, and AcOH/H_2O was used as solvent for the synthesis of **5a–5p**. Furthermore, compounds **5a**–**5p** were purified through hyperacoustic-aided crystal by using ethyl acetate and petroleum ether. These not only improved efficiency but also reduced the cost. Subsequently, the synthesized compounds were evaluated for inhibitory activity on thrombin. Compounds **9a–9e**, **9h**, **9l–9n** and **9p** had comparable inhibition activity to dabigatran, and compound **9p** exhibited better inhibitory activity than dabigatran in vitro. Additionally, compound **9p** also exhibited a fairly good inhibitory activity for arteriovenous thrombosis in vivo, which was comparable to that of dabigatran. These results, along with related molecular docking studies, demonstrated that the promising compound **9p** could be a potential thrombin inhibitor for further study.

4. Experimental

4.1 Materials and methods

All reagents were A.R. grade and purchased from Shanghai Chemical Reagent Company in China. Melting points were measured on WRS-1B and were uncorrected. NMR spectra were carried out using a Brucker Avance 500 or 400MHz MHz NMR spectrometer using TMS as internal reference. The HRMS spectra were recorded on a SolariX-70FT-MS Bruker spectrometer. The reactions were monitored by thin-layer chromatography (TLC). TLC was performed on silica gel plates (GF254) with visualisation of components by UV light (254 nm) or exposure to I_2. The intermediates 3–5 were purified through hyperacoustic forced crystal by SY7200-D ultrasonic cleaner. Purity were measured on HPLC (P230 Ⅱ).

*4.2 Synthesis of the compounds **9a–9p***

*4.2.1 General procedure for the synthesis of compounds **2a–2p***

Aromatic amine derivatives **(1a**–**1p)** (5.0g, 45mmol), ethyl acrylate (68mmol) and T_fOH 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 via reduced-pressure distillation. The residue was purified by column chromatography on silica gel eluted with PE/EtOAc = (10:1–5:1). Compounds **2a**–**2p** were obtained. Yield: 75%–85%.

*4.2.2 General procedure for the synthesis of compounds **4a–4p***

A mixture of 4-chloro-3-nitrobenzoic acid (25 mmol) and ethylamine solution (0.1mol) was stirred at 70 ℃ for 5h. cooled to room temperature, the resulting solution was poured into water and acidized to pH 4–5 with AcOH . Filtering and drying, we obtained yellow solid 4-substituted-3-nitrobenzoic acid .Yield 99%.

In a mixture of 4-substituted-3-nitrobenzoic acid (24mmol) and DCM (50 mL), containing N,N-dimethylforma- mide (DMF),thionyl chloride (36 mmol) was slowly dropping added at reflux temperature for 2 h. Cooled to room temperature, the reaction mixture was concentrated to near dryness by vacuum distillation. It obtained oily residue **3**. Compounds **2a**–**2p** (28 mmol) were dissolved in DCM (30 mL) containing triethylamine (Et_3N) (35 mmol). Subsequently, it was dropping added in a DCM (40 mL) solution of the oily residue **3** at room temperature, and then the mixture was stirred for 1–3h. After the completion of reaction, the reaction mixture was extracted with H_2O/DCM in the separatory funnel. The DCM layer was concentrated by vacuum distillation, it obtained yellow oily residue **(4a–4p).**

*4.2.3 General procedure for the synthesis of compounds **5a–5p***

Compounds **4a–4p** (20 mmol) were dissolved by a mixture solvent of AcOH/H_2O (25/25mL). Subsequently, Zn (80mmol) was added and stired at room temperature for 1h. Then the mixture was filtered, extracted (DCM 3*50 mL) and concentrated by vacuum distillation. The residue was dissolved in EtOAc(1–3ml) and were forced

crystal by adding PE (1~3ml) in ultrasonic. Filtered and dried, white and high-purity solid (**5a–5p**) were obtained.

*4.2.3.1 3-[(3-Amino-4-ethylamino-benzoyl)-phenyl-amino]-propionic acid ethyl ester (**5a**).* Yield: 85.2%; m.p. 195–196□; HPLC: 99.3% ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.28 (d, *J* = 8.0 Hz, 1H), 7.24 (s, 1H), 7.15 (t, *J* = 7.3 Hz, 1H), 7.08 (d, *J* = 7.8 Hz, 2H), 6.86 (d, *J* = 1.4 Hz, 1H), 6.73 (dd, *J* = 8.2, 1.3 Hz, 1H), 6.31 (d, *J* = 8.3 Hz, 1H), 4.20 (t, *J* = 7.4 Hz, 2H), 4.07 (q, *J* = 7.1 Hz, 2H), 3.09 (q, *J* = 7.1 Hz, 2H), 2.71 (t, *J* = 7.4 Hz, 2H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.22 (t, *J* = 7.1 Hz, 3H).

*4.2.3.2 3-[(3-Amino-4-ethylamino-benzoyl)-o-tolyl-amino]-propionic acid ethyl ester (**5b**).*Yield: 86.3%; m.p. 133–135□; HPLC: 97.3% ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.14 (d, *J* = 9.1 Hz, 3H), 7.07 (d, *J* = 6.0 Hz, 1H), 6.86 (s, 1H), 6.67 (d, *J* = 7.3 Hz, 1H), 6.28 (d, *J* = 7.6 Hz, 1H), 4.38 – 4.30 (m, 1H), 4.07 (q, *J* = 7.1 Hz, 2H), 3.88 – 3.81 (m, 1H), 3.08 (q, *J* = 6.4 Hz, 2H), 2.76 – 2.71 (m, 2H), 2.20 (s, 3H), 1.27 –1.21(m, 6H).

*4.2.3.3 3-[(3-Amino-4-ethylamino-benzoyl)-m-tolyl-amino]-propionic acid ethyl ester (**5c**).*Yield: 83.7%; m.p. 148–149□; HPLC: 97.0% ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.11 (t, *J* = 7.7 Hz, 1H), 6.97 (d, *J* = 7.5 Hz, 1H), 6.93 (s, 1H), 6.89 (s, 1H), 6.83 (d, *J* = 7.7 Hz, 1H), 6.73 (d, *J* = 8.3 Hz, 1H), 6.31 (d, *J* = 8.3 Hz, 1H), 4.17 (t, *J* = 7.4 Hz, 2H), 4.07 (q, *J* = 7.1 Hz, 2H), 3.09 (q, *J* = 7.1 Hz, 2H), 2.71 (t, *J* = 7.4 Hz, 2H), 2.29 (s, 3H), 1.24 (dt, *J* = 17.1, 7.1 Hz, 6H).

*4.2.3.4 3-[(3-Amino-4-ethylamino-benzoyl)-p-tolyl-amino]-propionic acid ethyl ester (**5d**).* Yield: 86.5%; m.p. 133–136□; HPLC: 98.6% ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.02 (d, *J* = 8.1 Hz, 2H), 6.93 (d, *J* = 8.2 Hz, 2H), 6.84 (d, *J* = 1.8 Hz, 1H), 6.71 (dd, J = 8.3, 1.8 Hz, 1H), 6.29 (d, *J* = 8.3 Hz, 1H), 4.14 (t, *J* = 7.4 Hz, 2H), 4.04 (q, *J* = 7.1 Hz, 2H), 3.06 (q, *J* = 7.1 Hz, 2H), 2.67 (t, *J* = 7.4 Hz, 2H), 2.27 (s, 3H), 1.21 (dt, *J* = 14.6, 7.1 Hz, 6H).

*4.2.3.5 3-[(3-Amino-4-ethylamino-benzoyl)-(2,3-dimethyl-phenyl)-amino]-propionic acid ethyl ester (**5e**).* Yield: 83.3%; m.p. 119–120□; HPLC: 97.7% ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.99 (d, *J* = 10.8 Hz, 2H), 6.86 (d, *J* = 7.1 Hz, 2H), 6.60 (d, *J* = 8.1 Hz, 1H), 6.24 (d, *J* = 8.0 Hz, 1H), 4.39 – 4.29 (m, 1H), 4.04 (q, *J* = 7.1 Hz, 2H), 3.78 – 3.68 (m, 1H), 3.05 (q, *J* = 7.1 Hz, 2H), 2.78 – 2.61 (m, 2H), 2.22 (s, 3H), 2.09 (s, 3H), 1.24 – 1.21 (m, 3H), 1.19 (t, *J* = 5.3 Hz, 3H).

*4.2.3.6 3-[(3-Amino-4-ethylamino-benzoyl)-(2,4-dimethyl-phenyl)-amino]-propionic acid ethyl ester (**5f**).* Yield: 86.1%; m.p. 121–122□; HPLC: 99.0% ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.97 (d, *J* = 8.1 Hz, 3H), 6.92 (s, 1H), 6.68 (d, *J* = 8.1 Hz, 1H), 6.29 (d, *J* = 8.2 Hz, 1H), 4.37 – 4.29 (m, 1H), 4.09 (q, *J* = 7.1 Hz, 2H), 3.86 – 3.79 (m, 1H), 3.08 (q, *J* = 6.8 Hz, 2H), 2.76 – 2.71 (m, 2H), 2.29 (s, 3H), 2.16 (s, 3H), 2.09 (s, 3H), 1.31 – 1.18 (m, 6H).

*4.2.3.7 3-[(3-Amino-4-ethylamino-benzoyl)-(2,5-dimethyl-phenyl)-amino]-propionic acid ethyl ester (**5g**).* Yield: 81.2%; m.p. 124–126□; HPLC: 98.8% ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.99 (d, *J* = 7.2 Hz, 1H), 6.92 (d, *J* = 7.8 Hz, 1H), 6.87 (d, *J* = 5.2 Hz, 2H), 6.64 (d, *J* = 7.1 Hz, 1H), 6.24 (d, *J* = 8.0 Hz, 1H), 4.30 – 4.19 (m, 1H), 4.05 (q, *J* = 7.1 Hz, 2H), 3.89 – 3.78 (m, 1H), 3.05 (q, *J* = 6.9 Hz, 2H), 2.70 (t, *J* = 7.4 Hz, 2H), 2.24 (s, 3H), 2.08 (s, 3H), 1.21 (dt, *J* = 14.4, 7.3 Hz, 6H).

*4.2.3.8 3-[(3-Amino-4-ethylamino-benzoyl)-(2,6-dimethyl-phenyl)-amino]-propionic acid ethyl ester (**5h**).* Yield: 78.4%; m.p. 151–153□; HPLC: 97.2% ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.07 – 7.03 (m, 1H), 6.99 (d, *J* = 7.2 Hz, 2H), 6.83 (d, *J* = 1.8 Hz, 1H), 6.57 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.22 (s, 1H), 4.05 (q, *J* = 7.1 Hz, 2H), 4.00 – 3.94 (m, 2H), 3.03 (q, *J* = 7.1 Hz, 2H), 2.80 – 2.73 (m, 2H), 2.17 (s, 6H), 1.20 (dt, *J* = 7.1, 5.4 Hz, 6H).

4.2.3.9 3-[(3-Amino-4-ethylamino-benzoyl)-(3,4-dimethyl-phenyl)-amino]-propionic

*acid ethyl ester(**5i**).* Yield: 81.7%; m.p. 126–129□; HPLC: 97.1% ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.95 (d, *J* = 7.9 Hz, 1H), 6.89 – 6.83 (m, 2H), 6.75 – 6.70 (m, 2H), 6.29 (d, *J* = 8.3 Hz, 1H), 4.12 (t, *J* = 7.5 Hz, 2H), 4.05 (q, *J* = 7.1 Hz, 2H), 3.07 (q, *J* = 7.1 Hz, 2H), 2.66 (t, *J* = 7.5 Hz, 2H), 2.17 (s, 3H), 2.16 (s, 3H), 1.21 (dt, *J* = 14.4, 7.1 Hz, 6H).

*4.2.3.10 3-[(3-Amino-4-ethylamino-benzoyl)-(3,5-dimethyl-phenyl)-amino]-ropionic acid ethyl ester (**5j**).* Yield: 88.2%; m.p. 134–136□; HPLC: 96.8% ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.89 (d, *J* = 1.8 Hz, 1H), 6.77 (s, 1H), 6.72 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.66 (s, 2H), 6.29 (d, *J* = 8.3 Hz, 1H), 4.14 – 4.09 (m, 2H), 4.05 (q, *J* = 7.1 Hz, 2H), 3.07 (q, *J* = 7.1 Hz, 2H), 2.70 – 2.64 (m, 2H), 2.20 (s, 6H), 1.22 (dt, *J* = 14.2, 7.1 Hz, 6H).

*4.2.3.11 3-[(3-Amino-4-ethylamino-benzoyl)-(3-fluoro-4-methyl-phenyl)-amino]-propionic acid ethyl ester (**5k**).* Yield: 80.3%; m.p. 135–136□; HPLC: 97.7% ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.00 (t, *J* = 8.4 Hz, 1H), 6.85 (d, *J* = 1.9 Hz, 1H), 6.77 (dd, *J* = 10.5, 1.8 Hz, 1H), 6.75 – 6.70 (m, 2H), 6.32 (d, *J* = 8.3 Hz, 1H), 4.12 (t, *J* = 7.3 Hz, 2H), 4.06 (q, *J* = 7.1 Hz, 2H), 3.09 (q, *J* = 7.1 Hz, 2H), 2.67 (t, *J* = 7.3 Hz, 2H), 2.20 (s, 3H), 1.23 (dt, *J* = 16.5, 7.1 Hz, 6H).

*4.2.3.12 3-[(3-Amino-4-ethylamino-benzoyl)-(4-fluoro-3-methyl-phenyl)-amino]-propionic acid ethyl ester(**5l**).* Yield: 81.5%; m.p. 144–145□; HPLC: 97.3% ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.92 (dd, *J* = 6.6, 1.8 Hz, 1H), 6.86 (d, *J* = 1.8 Hz, 1H), 6.84 (s, 1H), 6.83 – 6.79 (m, 1H), 6.67 (dd, *J* = 8.2, 1.7 Hz, 1H), 6.30 (d, *J* = 8.3 Hz, 1H), 4.11 (t, *J* = 7.4 Hz, 2H), 4.06 (q, *J* = 7.1 Hz, 2H), 3.08 (q, *J* = 7.2 Hz, 2H), 2.67 (t, *J* = 7.4 Hz, 2H), 2.19 (s, 3H), 1.22 (dt, *J* = 15.5, 7.1 Hz, 6H).

*4.2.3.13 3-[(3-Amino-4-ethylamino-benzoyl)-(2,4-difluoro-phenyl)-amino]- propionic acid ethyl ester(**5m**).* Yield: 84.6%; m.p. 113–114□; HPLC: 98.0% ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.17–7.12 (m5, 1H), 6.88 (d, *J* = 1.9 Hz, 1H), 6.81 (t, *J* = 8.2

Hz, 2H), 6.72 (dd, *J* = 8.2, 1.4 Hz, 1H), 6.34 (d, *J* = 8.3 Hz, 1H), 4.10 (q, *J* = 7.1 Hz, 4H), 3.11 (q, *J* = 7.1 Hz, 2H), 2.73 (s, 2H), 2.09 (s, 1H), 1.28 (t, *J* = 5.2 Hz, 3H), 1.24 (t, *J* = 5.2 Hz, 3H).

*4.2.3.14 3-[(3-Amino-4-ethylamino-benzoyl)-(4-methyl-pyridin-2-yl)-amino]-ropionic acid ethyl ester (**5n**).* Yield: 82.2%; m.p. 112–113□; HPLC: 97.3% ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.13 (d, *J* = 5.1 Hz, 1H), 6.74 (d, *J* = 6.7 Hz, 2H), 6.51 (d, *J* = 11.2 Hz, 2H), 6.12 (d, *J* = 8.4 Hz, 1H), 4.15 (t, *J* = 7.2 Hz, 2H), 3.89 (q, *J* = 7.1 Hz, 2H), 2.89 (q, *J* = 7.0 Hz, 2H), 2.57 (t, *J* = 7.2 Hz, 2H), 1.99 (s, 3H), 1.03 (t, *J* = 7.1 Hz, 6H).

*4.2.3.15 3-[(3-Amino-4-ethylamino-benzoyl)-(5-methyl-pyridin-2-yl)-amino]-ropionic acid ethyl ester (**5o**).* Yield: 84.6%; m.p. 114–115□; HPLC: 97.1% ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 8.27 (s, 1H), 7.22 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.86 (d, *J* = 1.8 Hz, 1H), 6.74 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.63 (d, *J* = 8.1 Hz, 1H), 6.35 (d, *J* = 8.3 Hz, 1H), 4.35 (t, *J* = 7.4 Hz, 2H), 4.07 (q, *J* = 7.1 Hz, 2H), 3.12 (q, *J* = 7.1 Hz, 2H), 2.75 (t, *J* = 7.4 Hz, 2H), 2.28 (s, 3H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.22 (t, *J* = 7.1 Hz, 3H).

*4.2.3.16 3-[(3-Amino-4-ethylamino-benzoyl)-(6-methyl-pyridin-2-yl)-amino]-ropionic acid ethyl ester (**5p**).* Yield: 80.2%; m.p. 119–120□; HPLC: 98.2% ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.32 (d, *J* = 2.3 Hz, 1H), 6.91 (d, *J* = 7.1 Hz, 2H), 6.77 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.52 (d, *J* = 7.9 Hz, 1H), 6.37 (d, *J* = 8.3 Hz, 1H), 4.41 (t, *J* = 7.3 Hz, 2H), 4.10 (q, *J* = 7.1 Hz, 2H), 3.14 (q, *J* = 7.1 Hz, 2H), 2.79 (t, *J* = 7.3 Hz, 2H), 2.58 (s, 3H), 2.14 (s, 1H), 1.30 (t, *J* = 7.2 Hz, 3H), 1.25 (t, *J* = 7.2 Hz, 3H).

*4.2.4 Procedure for the synthesis of common compound **6***

A mixture of aminobenzyl cyanide (0.1mol) and bromoacetic acid (0.15mol) was stirred at 100 ℃ in water for 5 h. cooled to room temperature, solid separated. We obtained white solid (4-cyano-2-substituted-phenylamino)-acetic acid (**6**), yield 80–87%.

*4.2.5 General procedure for the synthesis of compounds **7a–7p***

A mixture of (4-cyano-2-substituted-phenylamino)-acetic acid **(6)** (15 mmol), HOBt (15 mmol), EDCI (15 mmol) and 4 mL DMF was dissolved in 30 mL tetrahydrofuran (THF), and the mixture was stirred under ice-cooling for 30 min, then, a THF (40 mL) solution of the **5a–5p** (13mmol) was slowly added at room temperature, the mixture was stirred for 10 h. After the completion of reaction, the solvent THF was removed by vacuum distillation. Then the mixture was extracted with DCM (3*50 mL) and concentrated by vacuum distillation. The residue was added in AcOH (50 mL) and heated to reflux for 2.5 h. After the completion of reaction, the solvent AcOH was removed by vacuum distillation. Then, ammonia ($NH_3 \cdot H_2O$) was added and let pH to 8~9, the mixture was extracted with DCM (3*50 mL) and the DCM layer was dried over anhydrous Na_2SO_4, and concentrated by vacuum distillation. The residue was chromatographed (silicagel, dichloromethane/methanol=100:1) to obtain compounds. Finally, the compounds were dissolved in EtOAc (5ml) and were forced crystal by adding PE (1–3ml) in ultrasonic cleaner. Filtered and dried, white and high-purity solid (**7a–7p**) were obtained.

*4.2.5.1 3-({2-[(4-Cyano-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-phenyl-amino)-propionic acid ethyl ester (**7a**).* Yield: 81.5%; m.p. 195–196□; ^{1}H NMR (501 MHz, $CDCl_3$) δ (ppm) 7.70 (s, 1H), 7.48 (d, *J* = 8.6 Hz, 2H), 7.36 (dd, *J* = 8.5, 1.1 Hz, 1H), 7.22 (t, *J* = 7.7 Hz, 2H), 7.16 (d, *J* = 8.5 Hz, 1H), 7.13 (t, *J* = 7.5 Hz, 1H), 7.10 (d, *J* = 7.5 Hz, 2H), 6.73 (d, *J* = 8.6 Hz, 2H), 5.47 (t, *J* = 3.9 Hz, 1H), 4.50 (d, *J* = 4.5 Hz, 2H), 4.27 (t, *J* = 7.3 Hz, 2H), 4.15 (q, *J* = 7.3 Hz, 2H), 4.09 (q, *J* = 7.2 Hz, 2H), 2.75 (t, *J* = 7.3 Hz, 2H), 1.40 (t, *J* = 7.3 Hz, 3H), 1.23 (t, *J* = 7.1 Hz, 3H).

*4.2.5.2 3-({2-[(4-Cyano-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-o-tolyl-amino)-propionic acid ethyl ester (**7b**).* Yield: 83.7%; m.p. 175 –

177□; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm)7.63 (s, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.37 (d, *J* = 8.6 Hz, 1H), 7.17 – 7.07 (m, 5H), 6.73 (d, *J* = 8.4 Hz, 2H), 5.44 (s, 1H), 4.48 (d, *J* = 4.0 Hz, 2H), 4.17 – 4.12 (m, 2H), 4.09 (q, *J* = 7.0 Hz, 2H), 2.85 – 2.71 (m, 2H), 2.26 (s, 3H), 1.68 (s, 1H), 1.40 (t, *J* = 7.1 Hz, 3H), 1.24 (t, *J* = 7.0 Hz, 3H).

*4.2.5.3 3-({2-[(4-Cyano-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-m-tolyl-amino)-propionic acid ethyl ester (**7c**).* Yield:83.1%; m.p. 187–188□; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.72 (s, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.16 (d, *J* = 8.5 Hz, 1H), 7.07 (t, *J* = 7.7 Hz, 1H), 6.96 (s, 1H), 6.94 (d, *J* = 7.7 Hz, 1H), 6.83 (d, *J* = 7.9 Hz, 1H), 6.73 (d, *J* = 8.5 Hz, 2H), 5.48 (s, 1H), 4.50 (d, *J* = 4.4 Hz, 2H), 4.25 (t, *J* = 7.4 Hz, 2H), 4.15 (q, *J* = 7.2 Hz, 2H), 4.10 (q, *J* = 7.2 Hz, 2H), 2.74 (t, *J* = 7.4 Hz, 2H), 2.26 (s, 3H), 1.40 (t, *J* = 7.3 Hz, 3H), 1.24 (t, *J* = 7.1 Hz, 3H).

*4.2.5.4 3-({2-[(4-Cyano-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-p-tolyl-amino)-propionic acid ethyl ester (**7d**).*Yield: 82.8%; m.p. 158–160□; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.69 (s, 1H), 7.47 (d, *J* = 8.5 Hz, 2H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.15 (d, *J* = 8.5 Hz, 1H), 7.01 (d, *J* = 8.0 Hz, 2H), 6.97 (d, *J* = 7.9 Hz, 2H), 6.73 (d, *J* = 8.5 Hz, 2H), 5.51 (s, 1H), 4.49 (d, *J* = 4.3 Hz, 2H), 4.23 (t, *J* = 7.3 Hz, 2H), 4.15 (q, *J* = 7.2 Hz, 2H), 4.09 (q, *J* = 7.1 Hz, 2H), 2.73 (t, *J* = 7.3 Hz, 2H), 2.25 (s, 3H), 1.40 (t, *J* = 7.2 Hz, 3H), 1.23 (t, *J* = 7.1 Hz, 3H).

*4.2.5.5 3-[{2-[(4-Cyano-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(2,3-dimethyl-phenyl)-amino]-propionic acid ethyl ester (**7e**).* Yield: 87.6%; m.p. 150–151□; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.63 (s, 1H), 7.46 (d, J = 8.5 Hz, 2H), 7.32 (d, J = 8.5 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H), 6.99 (d, J = 7.2 Hz, 1H), 6.95 (t, J = 7.6 Hz, 1H), 6.89 (d, J = 7.5 Hz, 1H), 6.72 (d, J = 8.5 Hz, 2H), 5.51 (s, 1H), 4.47 (d, J = 4.1 Hz, 2H), 4.11–4.15 (m, 2H), 4.10 – 4.05 (m, 2H), 2.67–2.84 (m, 2H), 2.20 (s, 3H), 2.18 (s, 3H), 1.38 (t, J = 7.2 Hz, 3H), 1.23 (t, J = 7.1 Hz, 3H).

*4.2.5.6 3-[{2-[(4-Cyano-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(2,4-dimethyl-phenyl)-amino]-propionic acid ethyl ester (**7f**).* Yield: 85.4%; m.p. 155–157□; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.64 (s, 1H), 7.46 (d, *J* = 6.8 Hz, 2H), 7.35 (d, *J* = 7.8 Hz, 1H), 7.13 (d, *J* = 7.5 Hz, 1H), 6.96 (d, *J* = 7.9 Hz, 1H), 6.92 (s, 1H), 6.87 (d, *J* = 7.6 Hz, 1H), 6.72 (d, *J* = 8.3 Hz, 2H), 5.50 (s, 1H), 4.48 (d, *J* = 3.5 Hz, 2H), 4.46 – 4.40 (m, 1H), 4.17 – 4.11 (m, 2H), 4.11 – 4.06 (m, 2H), 3.79–3.85 (m, *J* = 1H), 2.84 – 2.68 (m, 2H), 2.22 (s, 3H), 2.20 (s, 3H), 1.39 (t, *J* = 5.8 Hz, 3H), 1.23 (t, *J* = 7.1 Hz, 3H).

*4.2.5.7 3-[{2-[(4-Cyano-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(2,5-dimethyl-phenyl)-amino]-propionic acid ethyl ester (**7g**).* Yield: 84.5%; m.p. 163–165□; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.65 (s, 1H), 7.47 (d, *J* = 8.3 Hz, 2H), 7.36 (d, *J* = 9.0 Hz, 1H), 7.14 (d, *J* = 8.9 Hz, 1H), 6.98 (d, *J* = 7.5 Hz, 1H), 6.91 (d, *J* = 5.1 Hz, 2H), 6.73 (d, *J* = 8.6 Hz, 2H), 5.49 (s, 1H), 4.48 (d, *J* = 4.1 Hz, 2H), 4.43 – 4.34 (m, 1H), 4.14 (q, *J* = 6.4 Hz, 2H), 4.11 – 4.06 (m, 2H), 3.94 – 3.86 (m, 1H), 2.78 (t, *J* = 6.3 Hz, 2H), 2.23 (s, 3H), 2.16 (s, 3H), 1.39 (t, *J* = 7.3 Hz, 3H), 1.24 (t, *J* = 7.0 Hz, 3H).

*4.2.5.8 3-[{2-[(4-Cyano-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(2,6-dimethyl-phenyl)-amino]-propionic acid ethyl ester (**7h**).* Yield: 83.3%; m.p. 184–185□; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.58 (s, 1H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 7.08 – 7.03 (m, 1H), 6.99 (d, *J* = 7.5 Hz, 2H), 6.72 (d, *J* = 8.4 Hz, 2H), 5.50 (s, 1H), 4.47 (d, *J* = 4.5 Hz, 2H), 4.17 – 4.12 (m, 2H), 4.11 – 4.06 (m, 4H), 2.84 (t, *J* = 7.5 Hz,2H), 2.25 (s, 6H), 1.39 (t, *J* = 7.3 Hz, 3H), 1.24 (t, *J* = 7.1 Hz, 3H).

*4.2.5.9 3-[{2-[(4-Cyano-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(3,4-dimethyl-phenyl)-amino]-propionic acid ethyl ester (**7i**).* Yield: 85.5%; m.p. 149–151□; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.71 (s, 1H), 7.46 (d, *J* = 8.6 Hz, 2H), 7.37 (dd, *J* = 8.6, 0.7 Hz, 1H), 7.15 (d, *J* = 8.6 Hz, 1H), 6.92 (d, *J* = 7.9 Hz,

2H), 6.72 – 6.75 (m,3H), 5.51 (s, 1H), 4.49 (d, *J* = 4.4 Hz, 2H), 4.21 (t, *J* = 7.5 Hz, 2H), 4.15 (q, *J* = 7.2 Hz, 2H), 4.09 (q, *J* = 7.1 Hz, 2H), 2.72 (t, *J* = 7.4 Hz, 2H), 2.15 (s, 6H), 1.40 (t, *J* = 7.2 Hz, 3H), 1.23 (t, *J* = 7.1 Hz, 3H).

*4.2.5.10 3-[{2-[(4-Cyano-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(3,5-dimethyl-phenyl)-amino]-propionic acid ethyl ester (**7j**).* Yield: 86.1%; m.p. 163–166□; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.74 (s, 1H), 7.49 (d, *J* = 8.6 Hz, 2H), 7.38 (dd, *J* = 8.5, 1.2 Hz, 1H), 7.18 (d, *J* = 8.4 Hz, 1H), 6.76 (d, *J* = 4.9 Hz, 2H), 6.73 (s, 1H), 6.70 (s, 2H), 5.47 (t, *J* = 4.3 Hz, 1H), 4.51 (d, *J* = 4.4 Hz, 2H), 4.22 (t, *J* = 7.5 Hz, 2H), 4.17 (q, *J* = 7.2 Hz, 2H), 4.10 (q, *J* = 7.1 Hz, 2H), 2.74 (t, *J* = 7.5 Hz, 2H), 2.18 (s, 6H), 1.68 (s, 3H), 1.41 (t, *J* = 7.3 Hz, 3H), 1.24 (t, *J* = 7.2 Hz, 3H).

*4.2.5.11 3-[{2-[(4-Cyano-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(3-fluoro-4-methyl-phenyl)-amino]-propionic acid ethyl ester (**7k**).* Yield: 85.8%; m.p. 167–168□; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.69 (s, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.38 (d, *J* = 8.4 Hz, 1H), 7.20 (d, *J* = 8.5 Hz, 1H), 7.00 (t, *J* = 8.2 Hz, 1H), 6.81 – 6.76 (m, 2H), 6.74 (d, *J* = 8.4 Hz, 2H), 5.48 (t, *J* = 3.7 Hz, 1H), 4.51 (d, *J* = 4.5 Hz, 2H), 4.22 (t, *J* = 7.2 Hz, 2H), 4.17 (q, *J* = 6.9 Hz, 2H), 4.11 (q, *J* = 7.0 Hz, 2H), 2.73 (t, *J* = 7.2 Hz, 2H), 2.17 (s, 3H), 1.42 (t, *J* = 7.2 Hz, 3H), 1.24 (t, *J* = 7.2 Hz, 3H).

*4.2.5.12 3-[{2-[(4-Cyano-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(4-fluoro-3-methyl-phenyl)-amino]-propionic acid ethyl ester (**7l**).* Yield: 87.6%; m.p. 185–186□; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.68 (s, 1H), 7.47 (d, *J* = 8.5 Hz, 2H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.18 (d, *J* = 8.5 Hz, 1H), 6.98 (d, *J* = 5.8 Hz, 1H), 6.82 (s, 1H), 6.79 (d, *J* = 8.7 Hz, 1H), 6.74 (d, *J* = 8.6 Hz, 2H), 5.50 (t, *J* = 4.4 Hz, 1H), 4.51 (d, *J* = 4.4 Hz, 2H), 4.21 (t, *J* = 7.3 Hz, 2H), 4.17 (q, *J* = 7.5 Hz, 2H), 4.10 (q, *J* = 7.1 Hz, 2H), 2.72 (t, *J* = 7.2 Hz, 2H), 2.17 (s, 3H), 1.41 (t, *J* = 7.2 Hz, 3H), 1.24 (t, *J* = 7.1 Hz, 3H).

*4.2.5.13 3-[{2-[(4-Cyano-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(2,4-difluoro-phenyl)-amino]-propionic acid ethyl ester (**7m**).* Yield: 82.2%; m.p. 148–149□; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.67 (s, 1H), 7.50 (d, *J* = 8.7 Hz, 2H), 7.38 (d, *J* = 7.8 Hz, 1H), 7.22 (d, *J* = 8.7 Hz, 1H), 7.20 – 7.16 (m, 1H), 6.75 (d, *J* = 8.6 Hz, 4H), 4.52 (d, *J* = 4.5 Hz, 2H), 4.16 – 4.20 (m, 4H), 4.11 (q, *J* = 7.2 Hz, 2H), 2.77 (d, *J* = 40.7 Hz, 2H), 1.65 (s, 2H), 1.43 (t, *J* = 7.2 Hz, 3H), 1.25 (t, *J* = 7.1 Hz, 3H).

*4.2.5.14 3-[{2-[(4-Cyano-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(4-methyl-pyridin-2-yl)-amino]-propionic acid ethyl ester (**7n**).* Yield: 76%; m.p. 167–169□; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 8.27 (d, *J* = 5.0 Hz, 1H), 7.75 (s, 1H), 7.48 (d, *J* = 8.5 Hz, 2H), 7.37 (d, *J* = 7.8 Hz, 1H), 7.19 (d, *J* = 8.5 Hz, 1H), 6.85 (d, *J* = 5.1 Hz, 1H), 6.75 (d, *J* = 8.7 Hz, 2H), 6.66 (s, 1H), 5.52 (t, *J* = 4.6 Hz, 1H), 4.52 (d, *J* = 4.4 Hz, 2H), 4.40 (t, *J* = 7.3 Hz, 2H), 4.18 (q, *J* = 7.1 Hz, 2H), 4.10 (q, *J* = 7.1 Hz, 2H), 2.81 (t, *J* = 7.3 Hz, 2H), 2.11 (s, 3H), 1.75 (s, 1H), 1.42 (t, *J* = 7.2 Hz, 3H), 1.23 (t, *J* = 7.1 Hz, 3H).

*4.2.5.15 3-[{2-[(4-Cyano-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(5-methyl-pyridin-2-yl)-amino]-propionic acid ethyl ester (**7o**).* Yield: 71%; m.p. 172–174□; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 8.25 (s, 1H), 7.70 (s, 1H), 7.45 (d, *J* = 8.7 Hz, 2H), 7.33 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.19 – 7.14 (m, 2H), 6.73 (d, *J* = 8.6 Hz, 2H), 6.64 (d, *J* = 8.0 Hz, 1H), 4.51 (d, *J* = 4.5 Hz, 2H), 4.39 (t, *J* = 7.4 Hz, 2H), 4.16 (q, *J* = 7.2 Hz, 2H), 4.08 (q, *J* = 7.2 Hz, 2H), 2.79 (t, *J* = 7.3 Hz, 2H), 2.24 (s, 3H), 1.40 (t, *J* = 7.2 Hz, 3H), 1.22 (t, *J* = 7.1 Hz, 3H).

*4.2.5.16 3-[{2-[(4-Cyano-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole-5-carbonyl}-(6-methyl-pyridin-2-yl)-amino]-propionic acid ethyl ester (**7p**).* Yield: 70.8%; m.p. 157–158□; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.72 (s, 1H), 7.46 (d, *J* = 8.6 Hz, 2H), 7.33 (d, *J* = 8.5 Hz, 1H), 7.21 (t, *J* = 7.7 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 6.86 (d, *J* = 7.6 Hz, 1H), 6.74 (d, *J* = 8.6 Hz, 2H), 6.47 (d, *J* = 7.8 Hz, 1H), 5.55

(t, *J* = 4.0 Hz, 1H), 4.51 (d, *J* = 4.5 Hz, 2H), 4.44 (t, *J* = 7.3 Hz, 2H), 4.16 (q, *J* = 7.2 Hz, 2H), 4.09 (q, *J* = 7.1 Hz, 2H), 2.81 (t, *J* = 7.3 Hz, 2H), 2.52 (s, 3H), 1.83 (s, 1H), 1.40 (t, *J* = 7.2 Hz, 3H), 1.23 (t, *J* = 7.1 Hz, 3H).

*4.2.6 General procedure for the synthesis of compounds **8a–8p***

A mixture of compounds **(7a–7p)** (5.7 mmol), hydroxylamine hydrochloride (24 mmol) and triethylamine (24mmol) were dissolved in 30 mL ethanol (EtOH), and the mixture was heated at reflux temperature for 3 h. The solvent was distilled off. In a acetic acid (AcOH) (20 mL) solution of the residue, 10% Pd/C (24mmol) and ammonium formate (34 mmol) were added to, and the mixture was stirred at reflux temperature under nitrogen atmosphere for 5 h. The reaction mixture was filtered and the filtrate was concentrated through vacuum distillation. The residue was chromatographed (silica gel, dichloromethane/methanol = 7:1) to obtain solid or oil compounds. Finally, the compounds were dissolved in EtOAc (5ml) and were forced crystal by adding PE (1~3ml) in ultrasonic. Filtered and dried, white and high-purity solid (**8a–8p**) were obtained.

*4.2.6.1 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-phenyl-amino)-propionic acid ethyl ester (**8a**).* Yield: 71.6%, m.p. 183–185□; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 7.62 (d, *J* = 8.7 Hz, 2H), 7.47 (s, 1H), 7.39 (d, *J* = 8.5 Hz, 1H), 7.25 (t, *J* = 7.6 Hz, 2H), 7.19 (t, *J* = 9.8 Hz, 3H), 7.14 (t, *J* = 7.4 Hz, 1H), 6.85 (d, *J* = 8.7 Hz, 2H), 4.62 (d, *J* = 5.4 Hz, 2H), 4.25 (q, *J* = 7.0 Hz, 2H), 4.10 (t, *J* = 7.1 Hz, 2H), 3.99 (q, *J* = 7.1 Hz, 2H), 2.61 (t, *J* = 7.2 Hz, 2H), 1.71 (s, 2H), 1.24 (t, *J* = 7.1 Hz, 3H), 1.14 (t, *J* = 7.1 Hz, 3H).

*4.2.6.2 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-o-tolyl-amino)-propionic acid ethyl ester (**8b**).* Yield: 77.3%; m.p. 200–201□; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm)7.61 (d, *J* = 8.4 Hz, 2H), 7.39 (d, *J* = 11.4 Hz, 2H), 7.29 (s, 1H), 7.23 (t, *J* = 7.4 Hz, 2H), 7.13 (d, *J* = 8.7 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 4.61 (d, *J* = 4.4 Hz, 2H), 4.23 (d, *J* = 6.8 Hz, 2H), 3.99 (q, *J*

= 7.0 Hz, 2H), 3.74 – 3.66 (m, 2H), 2.64 (t, *J* = 7.5 Hz, 2H), 2.14 (s, 3H), 1.79 (s, 3H), 1.23 (t, *J* = 6.7 Hz, 3H), 1.14 (t, *J* = 7.0 Hz, 3H), 0.94 (t, *J* = 7.2 Hz, 1H).

*4.2.6.3 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-m-tolyl-amino)-propionic acid ethyl ester (**8c**).* Yield: 71.5%; m.p. 199–200□; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 7.61 (d, *J* = 8.6 Hz, 2H), 7.50 (s, 1H), 7.41 (d, *J* = 8.4 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 7.11 (s, 1H), 7.08 (d, *J* = 7.8 Hz, 1H), 6.96 (d, *J* = 7.6 Hz, 1H), 6.88 (d, *J* = 7.8 Hz, 1H), 6.84 (d, *J* = 8.6 Hz, 2H), 4.62 (d, *J* = 5.3 Hz, 2H), 4.25 (q, *J* = 7.0 Hz, 2H), 4.07 (t, *J* = 7.2 Hz, 2H), 4.00 (q, *J* = 14.2, 7.2 Hz, 2H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.21 (s, 3H), 1.24 (t, *J* = 7.1 Hz, 3H), 1.14 (t, *J* = 7.1 Hz, 3H).

*4.2.6.4 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-p-tolyl-amino)-propionic acid ethyl ester (**8d**).* Yield: 76.6%; m.p. 200–201□; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 7.62 (d, *J* = 8.7 Hz, 2H), 7.48 (s, 1H), 7.40 (d, *J* = 8.5 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.08 – 7.03 (m, 4H), 6.85 (d, *J* = 8.7 Hz, 2H), 4.62 (d, *J* = 5.3 Hz, 2H), 4.25 (q, *J* = 7.2 Hz, 2H), 4.05 (t, *J* = 7.1 Hz, 2H), 4.00 (q, *J* = 7.1 Hz, 2H), 2.58 (t, *J* = 7.1 Hz, 2H), 2.19 (s, 3H), 1.24 (t, *J* = 7.1 Hz, 3H), 1.14 (t, *J* = 7.1 Hz, 3H).

*4.2.6.5 3-[{2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(2,3-dimethyl-phenyl)-amino]-propionic acid ethyl ester (**8e**).* Yield: 74.8%; m.p. 200–202□; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 7.60 (d, *J* = 8.5 Hz, 2H), 7.41 (s, 1H), 7.38 (d, *J* = 8.5 Hz, 1H), 7.19 (d, *J* = 8.7 Hz, 1H), 7.03 – 6.96 (m, 3H), 6.83 (d, *J* = 8.3 Hz, 2H), 4.61 (d, *J* = 5.1 Hz, 2H), 4.23 (q, *J* = 7.4 Hz, 2H), 3.99 (q, *J* = 7.0 Hz, 2H), 2.70 – 2.53 (m, 2H), 2.15 (s, 3H), 2.10 (s, 3H), 1.23 (t, *J* = 6.8 Hz, 3H), 1.14 (t, *J* = 7.0 Hz, 3H), 0.94 (t, *J* = 7.1 Hz, 1H).

*4.2.6.6 3-[{2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(2,4-dimethyl-phenyl)-amino]-propionic acid ethyl ester (**8f**).* Yield:

78.1%; m.p. 200–202□; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 7.62 (d, *J* = 8.5 Hz, 2H), 7.41 (s, 1H), 7.40 (d, *J* = 8.9 Hz, 1H), 7.23 (d, *J* = 7.4 Hz, 1H), 7.11 (d, *J* = 7.8 Hz, 1H), 6.95 (s, 0H), 6.93 (d, *J* = 8.1 Hz, 1H), 6.84 (d, *J* = 8.2 Hz, 2H), 4.61 (d, *J* = 4.7 Hz, 1H), 4.27 – 4.22 (m, 1H), 4.00 (q, *J* = 7.0 Hz, 1H), 3.67 – 3.63 (m, 1H), 2.62 (t, *J* = 7.7 Hz, 1H), 2.16 (s, 1H), 2.10 (s, 1H), 1.24 (t, *J* = 6.5 Hz, 2H), 1.14 (t, *J* = 6.8 Hz, 2H).

*4.2.6.7 3-[{2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(2,5-dimethyl-phenyl)-amino]-propionic acid ethyl ester (**8g**).* Yield: 73.3%; m.p. 183–184□; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 7.61 (d, *J* = 7.6 Hz, 2H), 7.43 (s, 1H), 7.40 (d, *J* = 8.3 Hz, 1H), 7.25 (d, *J* = 8.6 Hz, 1H), 7.12 (s, 1H), 7.00 (d, *J* = 6.4 Hz, 1H), 6.93 (d, *J* = 7.3 Hz, 1H), 6.84 (d, *J* = 8.3 Hz, 2H), 4.61 (d, *J* = 3.8 Hz, 2H), 4.25 – 4.21 (m, 2H), 4.00 (q, *J* = 6.7Hz, 2H), 3.74 (m, 2H), 2.64 (t, *J* = 8.9 Hz, 2H), 2.20 (s, 3H), 2.04 (s, 3H), 1.23 (t, *J* = 5.7 Hz, 3H), 1.14 (t, *J* = 6.9 Hz, 3H), 0.94 (t, *J* = 7.1 Hz, 1H).

*4.2.6.8 3-[{2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(2,6-dimethyl-phenyl)-amino]-propionic acid ethyl ester (**8h**).* Yield: 72.6%; m.p. 196–199□; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 7.61 (d, *J* = 8.7 Hz, 2H), 7.40 (d, *J* = 8.5 Hz, 1H), 7.31 (s, 1H), 7.24 (d, *J* = 8.5 Hz, 1H), 7.07 – 7.04 (m, 1H), 7.03 (d, *J* = 5.5 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 4.61 (d, *J* = 5.1 Hz, 2H), 4.24 (q, *J* = 6.9 Hz, 2H), 3.99 (q, *J* = 7.1 Hz, 2H), 3.92 (t, *J* = 7.3 Hz, 2H), 2.70 (t, *J* = 7.4 Hz, 2H), 2.18 (s, 6H), 1.23 (t, *J* = 7.0 Hz, 3H), 1.14 (t, *J* = 7.1 Hz, 3H).

*4.2.6.9 3-[{2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(3,4-dimethyl-phenyl)-amino]-propionic acid ethyl ester (**8i**).* Yield: 75%; m.p. 198–199□; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 7.61 (d, *J* = 8.6 Hz, 2H), 7.51 (s, 1H), 7.41 (d, *J* = 8.4 Hz, 1H), 7.23 (d, *J* = 8.3 Hz, 1H), 7.07 (s, 1H), 6.96 (d, *J* = 7.9 Hz, 1H), 6.85 (d, *J* = 8.7 Hz, 2H), 6.81 (d, *J* = 7.8 Hz, 1H), 4.62 (d, *J* = 5.2 Hz, 2H), 4.25 (q, *J* = 7.0 Hz, 2H), 4.06 – 4.02 (m, 2H), 4.01 – 3.97 (m, 2H), 2.58 (t, *J*

= 7.2 Hz, 2H), 2.12 (s, 3H), 2.10 (s, 3H), 1.24 (t, *J* = 7.0 Hz, 3H), 1.14 (t, *J* = 7.1 Hz, 3H).

*4.2.6.10 3-[{2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(3,5-dimethyl-phenyl)-amino]-propionic acid ethyl ester (**8j**).* Yield: 76.7%; m.p. 200–204□; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 7.63 (d, *J* = 8.7 Hz, 2H), 7.52 (s, 1H), 7.42 (d, *J* = 8.5 Hz, 1H), 7.24 (d, *J* = 8.3 Hz, 1H), 6.86 (d, *J* = 8.8 Hz, 2H), 6.81 (s, 2H), 6.79 (s, 1H), 4.64 (d, *J* = 5.5 Hz, 2H), 4.27 (q, *J* = 7.0 Hz, 2H), 4.06 – 4.01 (m, 4H), 4.01 – 3.97 (m, 2H), 2.60 (q, *J* = 7.2 Hz, 6H), 2.13 (s, 6H), 1.24 (t, *J* = 7.1 Hz, 3H), 1.15 (t, *J* = 7.1 Hz, 3H).

*4.2.6.11 3-[{2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(3-fluoro-4-methyl-phenyl)-amino]-propionic acid ethyl ester (**8k**).* Yield: 76.3%; m.p. 213–216□; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 7.64 (d, *J* = 8.7 Hz, 2H), 7.52 (s, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.23 (d, *J* = 8.4 Hz, 1H), 7.14 (s, 1H), 7.11 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 4.64 (d, *J* = 5.3 Hz, 2H), 4.27 (q, *J* = 6.7 Hz, 2H), 4.07 (t, *J* = 7.1 Hz, 2H), 4.00 (q, *J* = 7.2 Hz, 2H), 2.62 – 2.58 (m, 2H), 2.11 (s, 3H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.14 (t, *J* = 7.1 Hz, 3H).

*4.2.6.12 3-[{2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(4-fluoro-3-methyl-phenyl)-amino]-propionic acid ethyl ester (**8l**).*
Yield: 72.1%; m.p. 205–206□; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 7.61 (d, *J* = 8.7 Hz, 2H), 7.51 (s, 1H), 7.42 (d, *J* = 8.3 Hz, 1H), 7.26 (d, *J* = 5.8 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.00 (d, *J* = 8.6 Hz, 1H), 6.97 (s, 1H), 6.85 (d, *J* = 8.6 Hz, 2H), 4.63 (d, *J* = 5.3 Hz, 2H), 4.26 (q, *J* = 6.8 Hz, 2H), 4.05 (t, *J* = 7.0 Hz, 2H), 4.00 (q, *J* = 7.0 Hz, 2H), 2.60 (t, *J* = 7.0 Hz, 2H), 2.13 (s, 3H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.14 (t, *J* = 7.1 Hz, 3H).

*4.2.6.13 3-[{2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(2,4-difluoro-phenyl)-amino]-propionic acid ethyl ester (**8m**).* Yield:

77.8%; m.p. 223–225□; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm)7.62 (d, *J* = 8.5 Hz, 2H), 7.57 (d, *J* = 8.2 Hz, 1H), 7.49 (s, 1H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.32 (s, 1H), 7.21 (d, *J* = 6.7 Hz, 2H), 6.85 (d, *J* = 8.7 Hz, 2H), 4.64 (d, *J* = 4.9 Hz, 2H), 4.26 (q, *J* = 7.0 Hz, 2H), 4.02 (t, *J* = 6.3 Hz, 2H), 4.00 – 3.95 (m, 2H), 2.63 (d, *J* = 10.0 Hz, 2H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.13 (t, *J* = 7.1 Hz, 3H).

*4.2.6.14 3-[{2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(4-methyl-pyridin-2-yl)-amino]-propionic acid ethyl ester (**8n**).* Yield: 70.5%; m.p. 202–205□; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 8.20 (d, *J* = 5.0 Hz, 1H), 7.62 (d, *J* = 8.7 Hz, 2H), 7.50 (s, 1H), 7.43 (d, *J* = 8.5 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 6.98 (d, *J* = 4.9 Hz, 1H), 6.91 (s, 1H), 6.85 (d, *J* = 8.7 Hz, 2H), 4.64 (d, *J* = 5.4 Hz, 2H), 4.28 (q, *J* = 7.0 Hz, 3H), 4.17 (t, *J* = 7.1 Hz, 2H), 3.98 (q, *J* = 7.1 Hz, 2H), 2.67 (t, *J* = 7.2 Hz, 2H), 2.11 (s, 3H), 1.24 (t, *J* = 7.0 Hz, 3H), 1.13 (t, *J* = 7.1 Hz, 3H).

*4.2.6.15 3-[{2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(5-methyl-pyridin-2-yl)-amino]-propionic acid ethyl ester (**8o**).* Yield: 71.3%; m.p. 221–224□; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm)8.23 (s, 1H), 7.62 (d, *J* = 8.7 Hz, 2H), 7.48 (s, 1H), 7.44 (d, *J* = 8.5 Hz, 1H), 7.40 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.18 (d, *J* = 8.4 Hz, 1H), 6.84 (t, *J* = 9.3 Hz, 3H), 4.64 (d, *J* = 5.3 Hz, 2H), 4.27 (q, *J* = 6.7 Hz, 2H), 4.18 (t, *J* = 7.0 Hz, 2H), 3.98 (q, *J* = 7.1 Hz, 2H), 2.66 (t, *J* = 7.1 Hz, 2H), 2.19 (s, 3H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.13 (t, *J* = 7.1 Hz, 3H).

*4.2.6.16 3-[{2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(6-methyl-pyridin-2-yl)-amino]-propionic acid ethyl ester (**8p**).* Yield: 68.2%; m.p. 200–202□; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 7.64 (d, *J* = 8.7 Hz, 2H), 7.48 (s, 1H), 7.44 (d, *J* = 8.5 Hz, 1H), 7.40 (d, *J* = 4.6 Hz, 1H), 7.16 (d, *J* = 8.2 Hz, 1H), 6.99 (d, *J* = 7.6 Hz, 1H), 6.86 (d, *J* = 8.8 Hz, 2H), 6.62 (d, *J* = 8.0 Hz, 1H), 4.65 (d, *J* = 5.4 Hz, 2H), 4.28 (q, *J* = 7.0Hz, 2H), 4.21 (t, *J* = 7.0 Hz, 2H), 3.98 (q, *J*=7.0Hz, 2H), 2.67 (t, *J* = 7.1 Hz, 2H), 2.42 (s, 3H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.12 (t, *J*

= 7.1 Hz, 3H).

*4.2.7 General procedure for the synthesis of compounds **9a–9p***

Compounds **8a–8p** (2 mmol) were dissolved in ethanol/H_2O (5/5mL), and a solution of sodium hydroxide (6 mmol) were added, and keeping stirred at room temperature for 30 min. The mixture was acidized with acetic acid to pH = 6–7 and it precipitated white solid. Filtered, washed with water and methanol and dried, compounds **9a–9p** were obtained.

*4.2.7.1 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-phenyl-amino)-propionic acid (**9a**).* White solid, yield: 90.2%; m.p. 244–246□; HPLC: 98.3% ^{1}H NMR (400 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 8.96 (s, 1H), 8.71 (s, 1H), 7.68 (d, *J* = 8.8 Hz, 2H), 7.58 (d, *J* = 11.1 Hz, 2H), 7.31 (d, *J* = 8.7 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.22 (m, 3H), 7.15 (t, *J* = 7.0 Hz, 1H), 6.88 (d, *J* = 8.9 Hz, 2H), 4.79 (s, 2H), 4.35 (q, *J* = 6.7 Hz, 2H), 4.06 (t, *J* = 7.5 Hz, 2H), 3.16 (s, 2H), 2.56 (t, *J* = 7.5 Hz, 2H), 1.28 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 173.03, 170.29, 164.74, 153.36, 152.99, 143.76, 135.71, 130.69, 130.18, 129.69, 128.40, 127.13, 123.88, 119.46, 113.87, 113.80, 113.30, 112.21, 110.23, 48.99, 46.86, 39.06, 32.64, 15.20. HRMS (ESI) m/z Calcd for $C_{27}H_{28}O_6N_3$ (M+H): 485.22229, Fond: 458.23193 $[M+H]^+$.

*4.2.7.2 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoImidazole -5-carbonyl}-o-tolyl-amino)-propionic acid (**9b**).* Yield: 90.3%; m.p. 259–260□; HPLC: 98.6% ^{1}H NMR (400 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 8.91 (s, 1H), 8.66 (s, 1H), 7.66 (d, *J* = 8.7 Hz, 2H), 7.47 (s, 1H), 7.40 (d, *J* = 11.7 Hz, 2H), 7.25 (t, *J* = 9.6 Hz, 2H), 7.13 (d, *J* = 5.9 Hz, 2H), 6.86 (d, *J* = 8.7 Hz, 2H), 4.63 (s, 2H), 4.23 (t, *J* = 12.9 Hz, 2H), 3.82 – 3.56 (m, 2H), 2.59 (dd, *J* = 11.8, 7.6 Hz, 2H), 2.14 (s, 3H), 1.23 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 173.05, 170.34, 164.71, 153.37, 152.97, 142.24, 135.88, 135.22, 131.70, 130.40, 130.18, 129.99, 128.14, 127.37, 123.46, 119.11, 113.72, 113.27, 112.15, 110.04, 56.46, 45.97, 38.99,

32.38, 18.01, 15.23. HRMS (ESI) m/z Calcd for $C_{28}H_{30}O_6N_3$ (M+H): 499.23794, Fond: 499.24786 $[M+H]^+$.

*4.2.7.3 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoImidazole -5-carbonyl}-m-tolyl-amino)-propionic acid (**9c**).* Yield: 93.3%; m.p. 252–253□; HPLC: 99.2% ^{1}H NMR (400 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 8.93 (s, 1H), 8.69 (s, 1H), 7.67 (d, *J* = 8.6 Hz, 2H), 7.53 (s, 1H), 7.46 (d, *J* = 7.3 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 1H), 7.13 (s, 1H), 7.09 (t, *J* = 7.7 Hz, 1H), 6.96 (d, *J* = 7.6 Hz, 1H), 6.88 (t, *J* = 9.0 Hz, 3H), 4.69 (s, 2H), 4.29 (q, *J* = 6.6 Hz, 2H), 4.03 (t, *J* = 7.5 Hz, 2H), 2.55 (t, *J* = 7.6 Hz, 2H), 2.21 (s, 3H), 1.25 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 172.96, 169.30, 164.62, 153.01, 152.64, 141.31, 138.84, 134.11, 133.09, 130.92, 130.28, 129.79, 127.81, 126.59, 125.51, 115.06, 114.87, 113.70, 112.62, 112.43, 56.43, 45.93, 32.03, 20.44, 14.80, 14.55. HRMS (ESI) m/z Calcd for $C_{28}H_{30}O_6N_3$ (M+H): 499.23794, Fond: 499.24750 $[M+H]^+$.

*4.2.7.4 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-p-tolyl-amino)-propionic acid (**9d**).* Yield: 88.7%; m.p. 246–247□; HPLC: 97.6% ^{1}H NMR (400 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 8.91 (s, 1H), 8.65 (s, 1H), 7.66 (d, *J* = 8.8 Hz, 2H), 7.50 (s, 1H), 7.42 (d, *J* = 8.3 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 7.11 – 7.02 (m, 4H), 6.87 (d, *J* = 8.9 Hz, 2H), 4.66 (s, 2H), 4.27 (q, *J* = 6.8 Hz, 2H), 4.02 (t, *J* = 7.5 Hz, 2H), 2.57 – 2.52 (m, 2H), 2.19 (s, 3H), 1.24 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 173.08, 170.26, 164.72, 154.88, 153.26, 152.97, 141.05, 139.17, 136.43, 135.45, 131.11, 130.32, 130.19, 128.21, 124.04, 119.04, 113.88, 113.32, 112.23, 110.43, 46.87, 32.57, 21.50, 20.89, 15.53, 15.16. HRMS (ESI) m/z Calcd for $C_{28}H_{30}O_6N_3$ (M+H): 499.23794, Fond: 499.24792 $[M+H]^+$.

*4.2.7.5 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(2,3-dimethyl-phenyl)-amino)-propionic acid (**9e**).* Yield: 87.6%; m.p. 255–256□; HPLC: 97.7% ^{1}H NMR (400 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 8.97 (s, 1H),

8.74 (s, 1H), 7.68 (d, *J* = 8.6 Hz, 2H), 7.52 (d, *J* = 8.3 Hz, 1H), 7.49 (s, 1H), 7.26 (d, *J* = 8.5 Hz, 1H), 7.00 (d, *J* = 6.7 Hz, 2H), 6.97 (d, *J* = 7.6 Hz, 1H), 6.87 (d, *J* = 8.8 Hz, 2H), 4.74 (s, 2H), 4.35 – 4.28 (m, 2H), 2.63 (m, 2H), 2.13 (d, J = 13.0 Hz, 6H), 1.26 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 173.05, 170.02, 164.71, 153.19, 152.99, 143.49, 139.23, 135.19, 131.43, 130.20, 129.38, 128.53, 127.96, 125.80, 124.29, 118.62, 114.02, 113.31, 112.27, 110.67, 56.46, 46.91, 32.59, 21.27, 18.90, 15.08. HRMS (ESI) m/z Calcd for $C_{29}H_{32}O_6N_3$ (M+H): 513.25359, Fond: 513.26341$[M+H]^+$.

*4.2.7.6 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(2,4-dimethyl-phenyl)-amino)-propionic acid (**9f**).* Yield: 91.7%; m.p. 250–251□; HPLC: 97.3% ^{1}H NMR (400 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 8.94 (s, 1H), 8.71 (s, 1H), 7.67 (d, *J* = 8.7 Hz, 2H), 7.48 (d, *J* = 12.0 Hz, 2H), 7.27 (d, *J* = 8.3 Hz, 1H), 7.14 (d, *J* = 7.8 Hz, 1H), 6.94 (d, *J* = 10.9 Hz, 2H), 6.87 (d, *J* = 8.8 Hz, 2H), 4.70 (s, 2H), 4.36 – 4.25 (m, 2H), 3.71 – 3.56 (m, 2H), 2.54–2.59 (m, 2H), 2.13 (d, *J* = 23.1 Hz, 6H), 1.26 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 173.09, 170.36, 164.78, 153.27, 152.96, 139.57, 137.34, 135.56, 134.84, 132.23, 130.94, 130.18, 129.77, 127.99, 123.68, 118.61, 113.93, 113.31, 112.23, 110.31, 45.98, 32.34, 21.50, 20.88, 17.91, 15.16. HRMS (ESI) m/z Calcd for $C_{29}H_{32}O_6N_3$ (M+H): 513.25359, Fond: 513.26259 $[M+H]^+$.

*4.2.7.7 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(2,5-dimethyl-phenyl)-amino)-propionic acid (**9g**).* Yield: 89.3%; m.p. 248–249□; HPLC: 98.0% ^{1}H NMR (400 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 8.89 (s, 1H), 8.70 (s, 1H), 7.65 (d, *J* = 8.7 Hz, 2H), 7.44 (s, 1H), 7.40 (d, *J* = 8.5 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 1H), 7.15 (s, 1H), 7.00 (d, *J* = 7.8 Hz, 1H), 6.93 (d, *J* = 7.6 Hz, 1H), 6.86 (d, *J* = 8.7 Hz, 2H), 4.62 (s, 2H), 4.34 – 4.20 (m, 2H), 4.20 – 4.10 (m, 1H), 3.75 – 3.66 (m, 1H), 2.59 (t, *J* = 7.0 Hz, 2H), 2.20 (s, 3H), 2.03 (s, 3H), 1.23 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 173.10, 170.32, 164.72, 153.44, 152.96, 142.17, 140.79, 136.62, 136.14, 131.94, 131.44, 130.17, 128.85, 123.42,

119.50, 113.62, 113.29, 112.13, 109.83, 46.31, 38.88, 32.40, 20.85, 17.60, 15.29. HRMS (ESI) m/z Calcd for $C_{29}H_{32}O_6N_3$ (M+H): 513.25359, Fond: 513.26344 $[M+H]^+$.

*4.2.7.8 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole 5-carbonyl}-(2,6-dimethyl-phenyl)-amino)-propionic acid (**9h**).* Yield: 81.3%; m.p. 250–253□; HPLC: 96.6% ^{1}H NMR (400 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 8.90 (s, 1H), 8.64 (s, 1H), 7.65 (d, *J* = 8.9 Hz, 2H), 7.41 (s, 1H), 7.31 (d, *J* = 1.0 Hz, 1H), 7.25 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.04 (q, *J* = 4.7 Hz, 3H), 6.85 (d, *J* = 8.9 Hz, 2H), 4.63 (s, 2H), 4.25 (q, *J* = 6.9 Hz, 2H), 3.87 (t, *J* =8.0Hz,2H), 2.65 (t, *J* =7.8Hz, 2H), 2.18 (s, 6H), 1.23 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 172.98, 169.92, 164.80, 164.73, 153.24, 153.13, 141.33, 135.68, 135.58, 130.70, 130.33, 130.20, 129.53, 128.28, 123.63, 117.82, 113.97, 113.32, 112.25, 110.47, 46.28, 32.36, 18.77, 18.34, 15.46, 15.10. HRMS (ESI) m/z Calcd for $C_{29}H_{32}O_6N_3$ (M+H): 513.25359, Fond: 513.26362 $[M+H]^+$.

*4.2.7.9 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(3,4-dimethyl-phenyl)-amino)-propionic acid (**9i**).* Yield: 88.4%; m.p. 247–248□; HPLC: 97.2% ^{1}H NMR (400 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 8.97 (s, 1H), 8.73 (s, 1H), 7.69 (d, *J* = 8.8 Hz, 2H), 7.60 (s, 2H), 7.34 (d, *J* = 8.6 Hz, 1H), 7.11 (d, *J* = 1.8 Hz, 1H), 6.97 (d, *J* = 8.1 Hz, 1H), 6.89 (d, *J* = 8.9 Hz, 2H), 6.83 (dd, *J* = 7.9, 1.6 Hz, 1H), 4.80 (s, 2H), 4.36 (q, *J* = 6.8 Hz, 2H), 4.00 (t, *J* = 7.5 Hz, 2H), 2.54 (d, *J* = 7.7 Hz, 2H), 2.12 (s, 3H), 2.10 (s, 3H), 1.29 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 172.97, 169.20, 164.82, 164.74, 153.01, 152.73, 140.69, 137.94, 135.62, 133.94, 133.45, 130.59, 130.27, 128.94, 126.10, 125.71, 116.00, 114.85, 113.54, 112.57, 112.14, 56.45, 46.89, 32.53, 19.81, 19.26, 18.87, 14.68. HRMS (ESI) m/z Calcd for $C_{29}H_{32}O_6N_3$ (M+H): 513.25359, Fond: 513.25989 $[M+H]^+$.

4.2.7.10 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole

*-5-carbonyl}-(3,5-dimethyl-phenyl)-amino)-propionic acid (**9j**).* Yield: 92.1%; m.p. 256–257□; HPLC: 98.1% ^{1}H NMR (400 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 8.96 (s, 1H), 8.73 (s, 1H), 7.68 (d, *J* = 8.8 Hz, 2H), 7.59 (m, 2H), 7.33 (d, *J* = 8.4 Hz, 1H), 6.89 (d, *J* = 8.9 Hz, 2H), 6.85 (s, 2H), 6.80 (s, 1H), 4.77 (s, 2H), 4.35 (q, *J* =6.5 Hz, 2H),3.98(t, *J* =7.4 Hz, 2H), 2.54 (t, *J* =7.6 Hz,2H), 2.13 (s, 6H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 173.03, 170.23, 164.76, 153.38, 152.92, 143.64, 138.77, 135.79, 130.76, 130.32, 130.18, 128.71, 125.98, 123.79, 119.42, 113.27, 112.20, 110.19, 56.49, 47.05, 39.04, 32.64, 21.17, 19.11, 15.21. HRMS (ESI) m/z Calcd for $C_{29}H_{32}O_6N_3$ (M+H): 513.25359, Fond: 513.26371 $[M+H]^+$.

*4.2.7.11 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(3-fluoro-4-dimethyl-phenyl)-amino)-propionic acid (**9k**).* Yield: 84.7%; m.p. 235–236□; HPLC: 96.4% ^{1}H NMR (400 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 9.00 (s, 1H), 8.76 (s, 1H), 7.69 (t, *J* = 7.1 Hz, 3H), 7.64 (s, 1H), 7.38 (d, *J* = 8.5 Hz, 1H), 7.19 (dd, *J* = 10.8, 1.9 Hz, 1H), 7.14 (t, *J* = 8.5 Hz, 1H), 6.94 (d, *J* = 1.8 Hz, 1H), 6.90 (d, *J* = 9.0 Hz, 2H), 4.86 (s, 2H), 4.40 (q, *J* = 6.9 Hz, 2H), 4.04 (t, *J* = 7.4 Hz, 2H), 2.56 (t, *J* = 7.4 Hz, 2H), 2.11 (s, 3H), 1.32 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 173.10, 170.35, 164.73, 161.85, 159.42, 153.33, 153.10, 142.97, 135.88, 132.14, 130.51, 130.32, 130.18, 124.51, 123.73, 123.19, 123.02, 119.41, 115.15, 114.92, 112.21, 110.35, 46.83, 32.62, 18.97, 15.22, 14.13. HRMS (ESI) m/z Calcd for $C_{28}H_{29}FO_6N_3$ (M+H): 517.22852, Fond: 517.23569 $[M+H]^+$.

*4.2.7.12 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(4-fluoro-3-dimethyl-phenyl)-amino)-propionic acid (**9l**).* Yield: 87.6%; m.p. 250–251□; HPLC: 96.5% ^{1}H NMR (400 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 8.92 (s, 1H), 8.68 (s, 1H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.53 (s, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.28 (d, *J* = 5.7 Hz, 1H), 7.22 (dd, *J* = 8.4, 0.9 Hz, 1H), 6.98 (d, *J* = 7.9 Hz, 2H), 6.87 (d, *J* = 8.9 Hz, 2H), 4.67 (s, 2H), 4.28 (q, *J* = 6.9 Hz, 2H), 4.01 (t, *J* = 7.5 Hz, 2H), 2.55 (t, *J* = 7.5 Hz, 2H), 2.13 (s, 3H), 1.25 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 173.05, 170.05, 164.72, 160.53, 158.11, 153.19, 153.02,

139.49, 135.22, 131.25, 130.20, 128.09, 125.78, 125.60, 124.18, 118.63, 115.98, 115.75, 114.02, 112.28, 110.71, 56.45, 46.90, 32.56, 18.88, 15.08, 14.57. HRMS (ESI) m/z Calcd for $C_{28}H_{29}FO_6N_3$ (M+H): 517.22852, Fond: 517.23790 $[M+H]^+$.

*4.2.7.13 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(2,4-difluoro-phenyl)-amino)-propionic acid (**9m**).* Yield: 83%; m.p. 254–257□; HPLC: 97.7% ^{1}H NMR (400 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 8.93 (s, 1H), 8.68 (s, 1H), 7.67 (d, *J* = 8.9 Hz, 2H), 7.62 (dd, *J* = 8.9, 2.8 Hz, 1H), 7.51 (s, 2H), 7.22 (dd, J = 22.0, 8.6 Hz, 2H), 6.88 (d, *J* = 8.9 Hz, 2H), 4.70 (s, 2H), 4.30 (q, *J* = 6.7 Hz,2H), 3.98 (t, *J* = 6.9 Hz, 2H), 2.72 – 2.52 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 173.09, 171.02, 164.75, 162.57, 162.45, 160.11, 160.00, 153.44, 153.18, 141.16, 136.37, 132.04, 130.15, 129.33, 122.88, 119.36, 112.84, 112.62, 112.15, 110.09, 105.43, 105.17, 104.92, 46.29, 38.88, 32.78, 21.52, 15.28. HRMS (ESI) m/z Calcd for $C_{27}H_{26}F_2O_6N_3$ (M+H): 521.20344, Fond: 521.20994 $[M+H]^+$.

*4.2.7.14 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(4-methyl-pyridin-2-yl)-amino)-propionic acid (**9n**).*Yield: 82.7%; m.p. 236–238□; HPLC: 96.8% ^{1}H NMR (400 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 8.91 (s, 1H), 8.68 (s, 1H), 8.18 (d, *J* = 5.6 Hz, 1H), 7.66 (d, *J* = 8.9 Hz, 2H), 7.52 (d, *J* = 1.1 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.21 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.00 (s, 2H), 6.88 (d, *J* = 8.9 Hz, 2H), 4.68 (s, 2H), 4.30 (q, *J* = 6.9 Hz, 2H), 4.13 (t, *J* = 7.5 Hz, 2H), 2.65 – 2.56 (m, 2H), 2.13 (s, 3H), 1.25 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 172.93, 169.92, 164.70, 156.00, 153.28, 152.92, 150.00, 148.65, 134.54, 132.81, 130.24, 125.01, 123.30, 122.57, 117.05, 114.52, 113.37, 112.44, 111.75, 56.43, 45.26, 33.03, 20.83, 18.91, 14.83. HRMS (ESI) m/z Calcd for $C_{27}H_{29}O_7N_3$ (M+H): 500.23319, Fond: 500.24048 $[M+H]^+$.

*4.2.7.15 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(5-methyl-pyridin-2-yl)-amino)-propionic acid (**9o**).* Yield: 86.5%; m.p.

238–241□; HPLC: 96.2% ^{1}H NMR (400 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 8.97 (s, 1H), 8.74 (s, 1H), 8.21 (d, *J* = 2.3 Hz, 1H), 7.69 (d, *J* = 8.8 Hz, 2H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.56 (s, 1H), 7.45 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.27 (d, *J* = 9.0 Hz, 1H), 6.96 (d, *J* = 8.1 Hz, 1H), 6.89 (d, *J* = 8.9 Hz, 2H), 4.80 (s, 2H), 4.37 (q, *J* = 6.9 Hz, 2H), 4.14 (t, *J* = 7.4 Hz, 2H), 2.60 (t, *J* = 7.5 Hz, 2H), 2.19 (s, 3H), 1.30 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 173.04, 170.58, 164.72, 154.03, 153.35, 153.22, 149.19, 139.03, 136.26, 131.26, 130.50, 130.33, 130.17, 123.64, 122.06, 119.57, 113.73, 113.30, 112.19, 110.36, 56.47, 45.00, 33.13, 18.87, 17.68, 15.25. HRMS (ESI) m/z Calcd for $C_{27}H_{29}O_7N_3$ (M+H): 500.23319, Fond: 500.23995 $[M+H]^+$.

*4.2.7.16 3-({2-[(4-Carbamimidoyl-phenylamino)-methyl]-1-ethyl-1H-benzoimidazole -5-carbonyl}-(6-methyl-pyridin-2-yl)-amino)-propionic acid (**9p**).* Yield: 81.5%; m.p. 237–240□; HPLC: 95.3% ^{1}H NMR (400 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 8.95 (s, 1H), 8.71 (s, 1H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.55 (d, *J* = 8.3 Hz, 2H), 7.45 (t, *J* = 7.5 Hz, 1H), 7.23 (d, *J* = 8.2 Hz, 1H), 7.01 (d, *J* = 7.5 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 2H), 6.72 (d, *J* = 8.0 Hz, 1H), 4.75 (s, 2H), 4.34 (d, *J* = 6.7 Hz, 2H), 4.17 (t, *J* = 7.1 Hz, 2H), 2.62 (t, *J* = 7.2 Hz, 2H), 2.40 (s, 3H), 1.28 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6+^{2}HCl) δ (ppm) 173.03, 170.37, 164.78, 164.70, 157.96, 155.43, 153.23, 138.65, 135.89, 130.98, 130.32, 130.19, 123.93, 121.24, 119.67, 119.01, 113.90, 113.28, 112.23, 110.64, 44.75, 33.21, 24.28, 15.15. HRMS (ESI) m/z Calcd for $C_{27}H_{29}O_7N_3$ (M+H): 500.23319, Fond: 500.24104 $[M+H]^+$.

4.3 Anticoagulant assay

4.3.1 Inhibitory activity on thrombin in vitro

The test compounds dissolved in DMSO were added to a solution of lyophilized human thrombin (5.4 mg/mL), and preincubated for 10 min at 37°C. Subsequently, Ac-FVR-AMC (5 μ M), a specific fluorogenic thrombin substrate was added to it. DMSO was used as a negative control in the assay. The dynamic changes of fluorescence intensity were detected by 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 thrombin inhibitor dabigatran was used as positive control. Instrument settings included: excitation wavelength, 355 nm; emission wavelength, 460 nm. Each well was measured 20 times every 20s for 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.

4.3.2 Inhibitory activity on rat arteriovenous thrombosis in vivo

The rats were anesthetised and fixed in supine position. The neck skin was incised. The left carotid artery and right external carotid vein were separated, and connected through a bypass pipe. A surgical thread was set in this pipe. Various subjects were injected in the tail vein. Blood flow was opened for 15 min immediately. Then, the thread was removed and weighed. The wet weight of the thrombus was calculated by subtracting the weight of the surgical thread. The average value and standard deviation between the experimental groups of thrombus wet weights were calculated.

Inhibition rate of rat arteriovenous thrombosis sample (%) = [control group (thromboembolism weight) – sample group (thromboembolism weight)]/control group (thromboembolism weight) × 100%.

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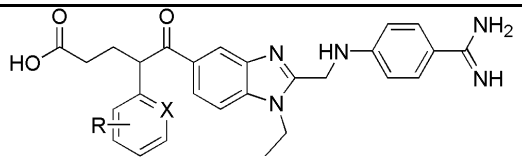
Table 1 The inhibitory activities on thrombin of selected compounds

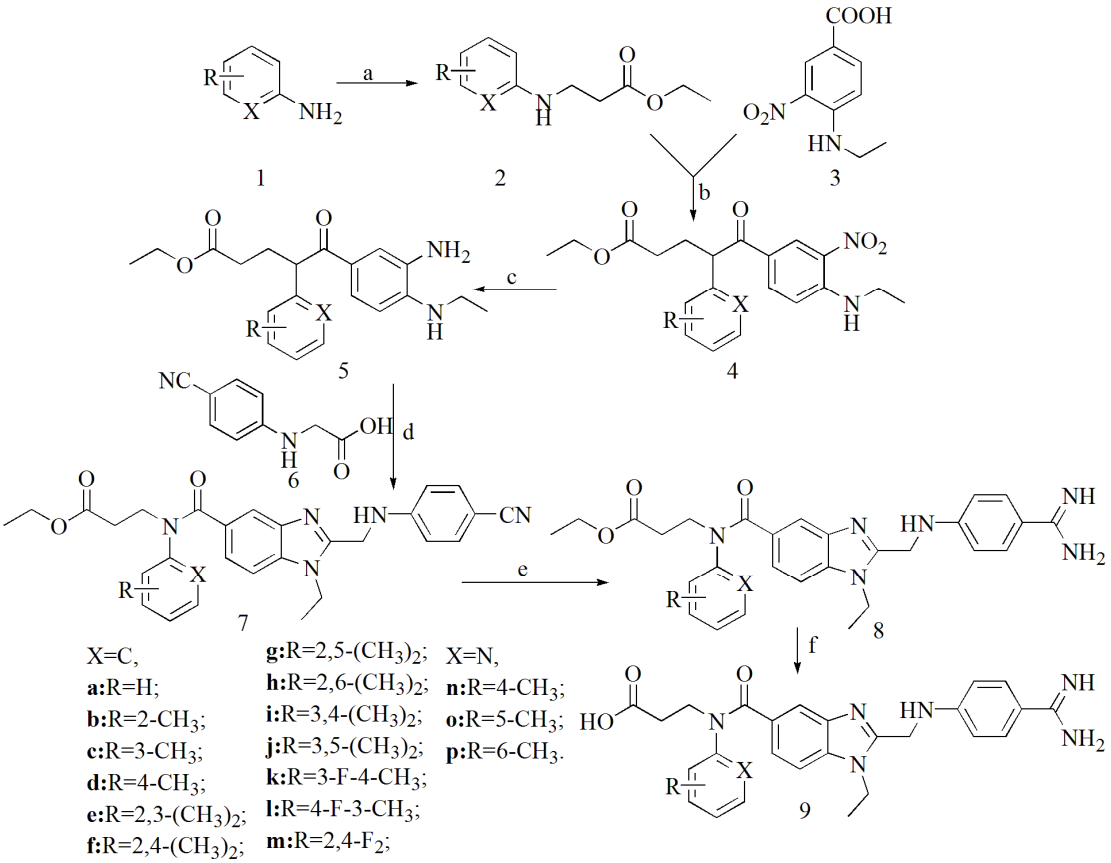


Compound	X	R	Mean IC_{50} ± SD（nM）
9a	C	H	4.69 ± 0.11
9b	C	2–CH_3	3.11 ± 0.21
9c	C	3–CH_3	2.13 ± 0.44
9d	C	4–CH_3	4.98 ± 1.99
9e	C	2,3–$(CH_3)_2$	6.59 ± 0.94
9f	C	2,4–$(CH_3)_2$	16.45 ± 1.72
9h	C	2,6–$(CH_3)_2$	3.10 ± 0.37
9l	C	4–F–3–CH_3	6.27 ± 0.72
9m	C	2,4–F_2	4.23 ± 0.76
9n	N	4–CH_3	1.62 ± 0.24
9p	N	6–CH_3	0.92 ± 0.07
Dabigatran	N	H	1.20 ± 0.09

Table 2 The inhibitory activity on rat arteriovenous thrombosis

Compound	Concentration (mg/mL)	Thromboembolic weight (mg)	The inhibition rate (%)
Control	—	42.04 ± 5.09	0.00 ± 12.11
Dabigatran	0.5	6.28 ± 0.26**	85.07 ± 0.61**
9b	0.5	9.28 ± 1.13**	77.93 ± 2.69**
9p	0.5	6.16 ± 0.30**	85.35 ± 0.72**

**: $p<0.01$ versus the control group.



Scheme 1 The synthesis routes of N-ethyl dabigatran derivatives. Reagents and conditions: (a) T_fOH, 100℃, 16h; (b) DCM, Et_3N, r.t, 1–3h; (c) Zn, AcOH/H_2O, rt, 1h; (d) EDCI, HOBt; THF/DMF, rt, 10h; (e) $NH_2OH\cdot HCl$, Et_3N, EtOH, 80℃, 3h; $HCOONH_4$, Pd/C, AcOH, N_2, 120℃, 5h; (f) NaOH, EtOH/H_2O, rt, 30 min; AcOH, pH=5–6.

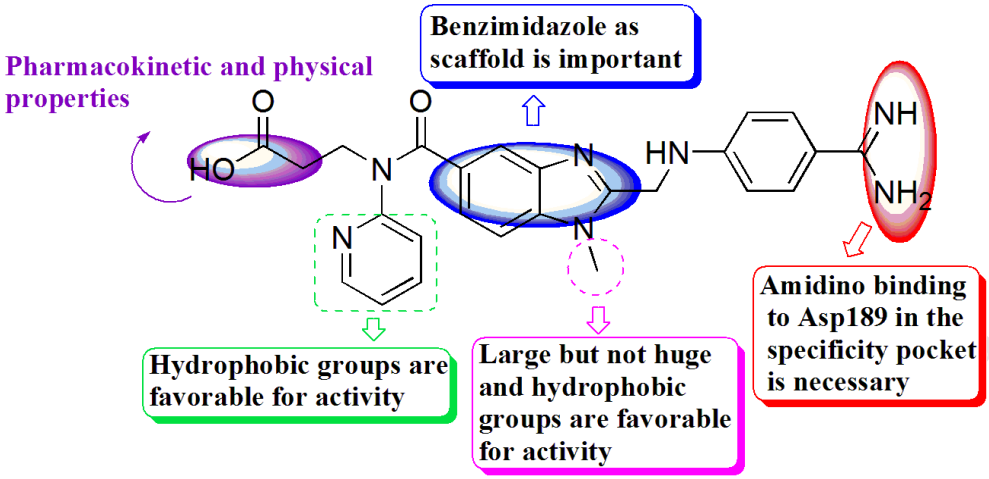


Fig.1 The structure-activity relationships of dabigatran for inhibitory activity on thrombin

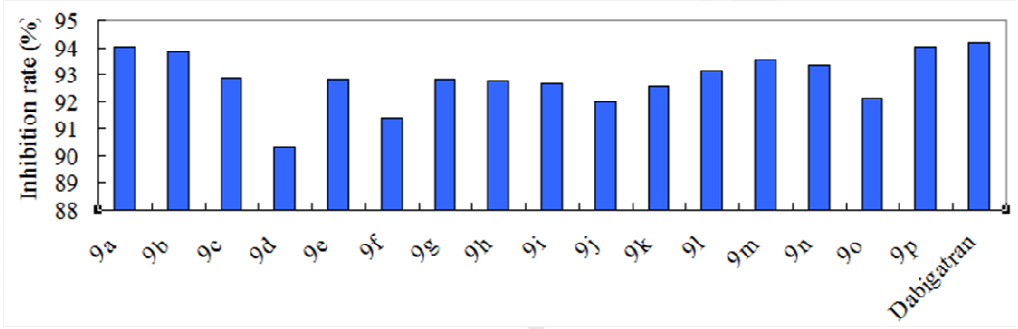


Fig 2 The thrombin inhibition rate of compounds **9a–9p**

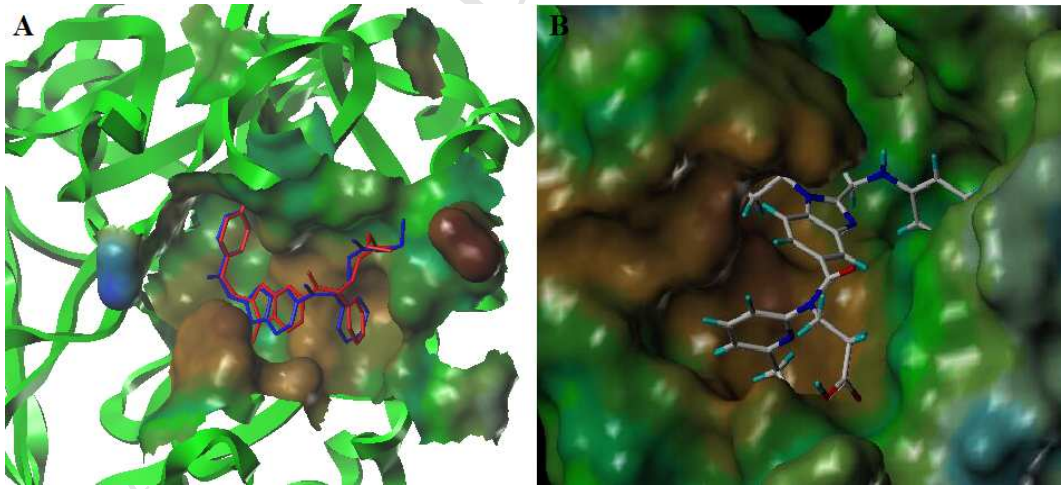


Fig. 3 View of the cognate ligand (red) and re-docking result (blue) in the docked complex by superimposing the coordinates of protein together (A). Molecular surface depicted by lipophilic potential of the compound **9p** at the active site of inhibitor–thrombin complexes (B).

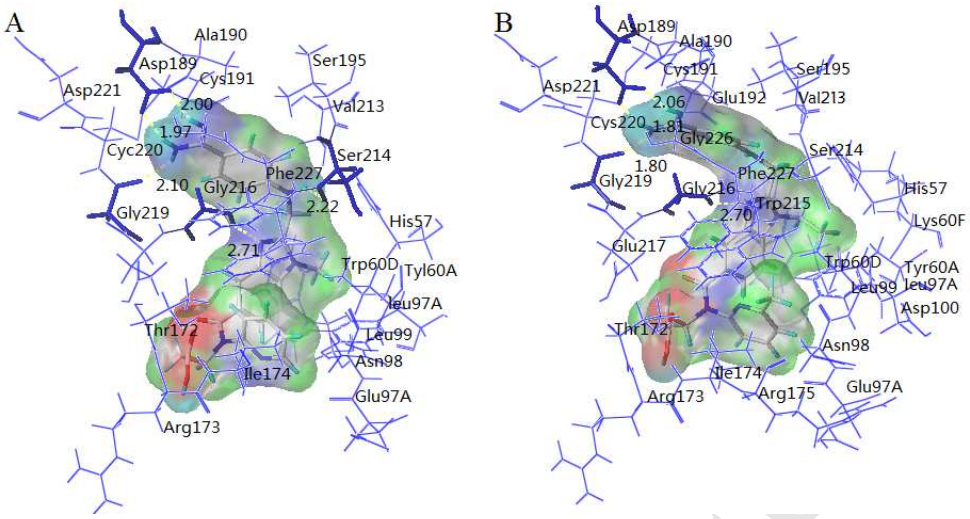


Fig. 4 Docking result of dabigatran (A) and compound **9p** (B) into the binding site of the protein (PDB code: 1KTS). Hydrogen bonds are shown as yellow dashed lines, with distance unit of Å.

Highlights

- Sixteen N-ethyl dabigatran derivatives were designed and synthesized.
- All designed compounds were predicted activities via molecular model.
- Hyperacoustic-aided crystal as a novel purification method was found in synthesis.
- Compound **9p** exhibited better inhibitory activity on thrombin than dabigatran.
- Binding form between compound **9p** with protein was simulated via molecular docking.