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Design, synthesis, and structure-activity relationship of novel and effective apixaban derivatives as FXa inhibitors containing 1, 2, 4-triazole/pyrrole derivatives as P2 binding element

Yong Wang[a], Xiaoqing Sun[a], Di Yang[a], Zhuang Guo[a], Xuxu Fan[a], Minhua Nie[a], Feng Zhang[a], Yue Liu[a], Yue Li[a], Yulin Wang[b], Ping Gong[a], Yajing Liu[a,*]

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Abstract

Four series of novel and potent FXa inhibitors possessing the 1,2,4-triazole moiety and pyrrole moiety as P2 binding element and dihydroimidazole/tetrahydropyrimidine groups as P4 binding element were designed, synthesized, and evaluated for their anticoagulant activity in human and rabbit plasma *in vitro*. Most compounds showed moderate to excellent activity. Compounds **14a**, **16**, **18c**, **26c**, **35a**, and **35b** were further examined for their inhibition activity against human FXa *in vitro* and rat venous thrombosis *in vivo*. The most promising compound **14a**, with an IC_{50} (FXa) value of 0.15 μM and 99% inhibition rate, was identified for further evaluation as an FXa inhibitor.

Keywords: FXa; Anticoagulant activity; Synthesis; Structure-activity relationships.

1. Introduction

Thromboembolic events are a leading cause of mortality worldwide, and play a pivotal role in the pathogenesis of numerous cardiovascular disorders, including unstable angina, venous thromboembolism (VTE) ischemic stroke, acute coronary syndrome (ACS), deep venous thrombosis (DVT), and pulmonary embolism (PE).[1-5] One of the most feasible methods for treating these fatal diseases is to prevent the generation of thrombus.[6] Warfarin and heparin are traditional pharmacotherapies and have been used extensively, however, numerous limitations exist such as monitoring clotting time, bleeding, and interactions with other drugs and foods.[7]

Coagulation enzyme factor Xa (FXa), as the promising target of anticoagulation, is located at the convergence point of the extrinsic and intrinsic coagulation cascade. Over the past decades, several oral, direct and selective FXa inhibitors have been approved or are in clinical research, such as rivaroxaban, apixaban, edoxaban, darexaban, and betrixaban (**Fig. 1**). All of these possess lower risks of bleeding and more specific mechanisms of preventing thrombin generation, instead of affecting platelet function and the level of thrombin.[8-10] On the basis of data obtained for apixaban, we endeavored to create novel scaffolds for further exploration such as decreasing dosages and reducing side effects of bleeding. Thus, a series of derivatives were synthesized as novel FXa inhibitors.

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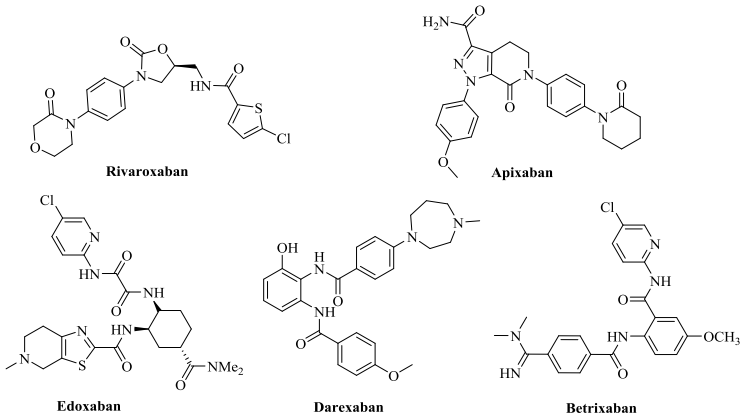


Fig. 1. Structures of oral FXa inhibitors.

The X-ray structure of FXa bound to apixaban (**Fig. 2**) [11] shows that the 4-methoxy group in the S1 pocket is oriented in a planar manner relative to the phenyl P1 moiety. The pendant P4 phenyllactam in the S4 pocket is appropriately positioned in a hydrophobic pocket. The hydrogen bonds between the protein and ligand contributed to its high affinity, involving the pyrazole N-2 atom with Gln192, carbonyl oxygen (scaffold carboxamide) with Gly216, and the pyrazole C-3 carboxamido with Glu146.

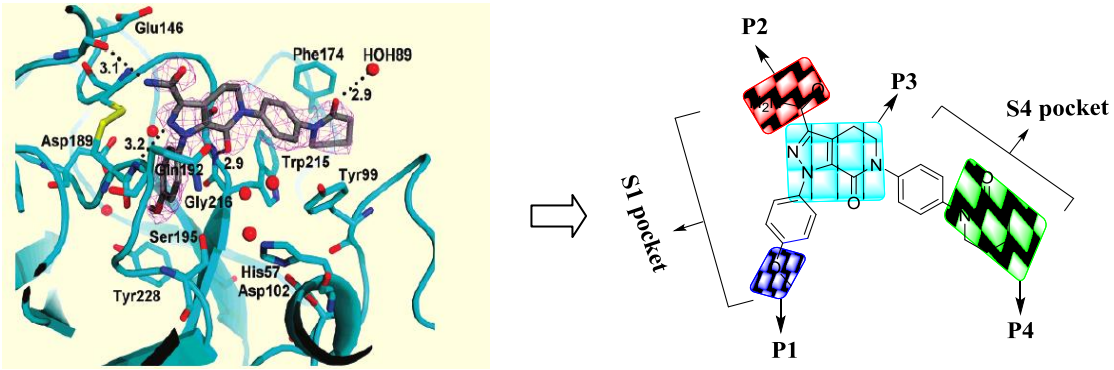


Fig. 2. X-ray structure of FXa bound to apixaban.

According to the structure-activity relationships (SARs), the 1,4,5,6-tetrahydro-7*H*-pyrazolo[3,4-*c*]pyridin-7-one scaffold (P3 moiety) was retained. To occupy the P2 cavity on a larger scale and enhance the hydrogen bonding to Glu146, a series of nitrogenous heterocyclic were introduced into pyrazole C-3 to replace the carboxamido moiety. In the P1 moiety, fluoromethoxy and halogens were introduced into the methoxy group to accommodate the narrow cavity and investigate the influence of the electric effect of the substituents. Furthermore, to increase the hydrophilicity of the lactam moiety (P4), imidazoline and tetrahydropyrimidine groups were incorporated into the phenyl group.

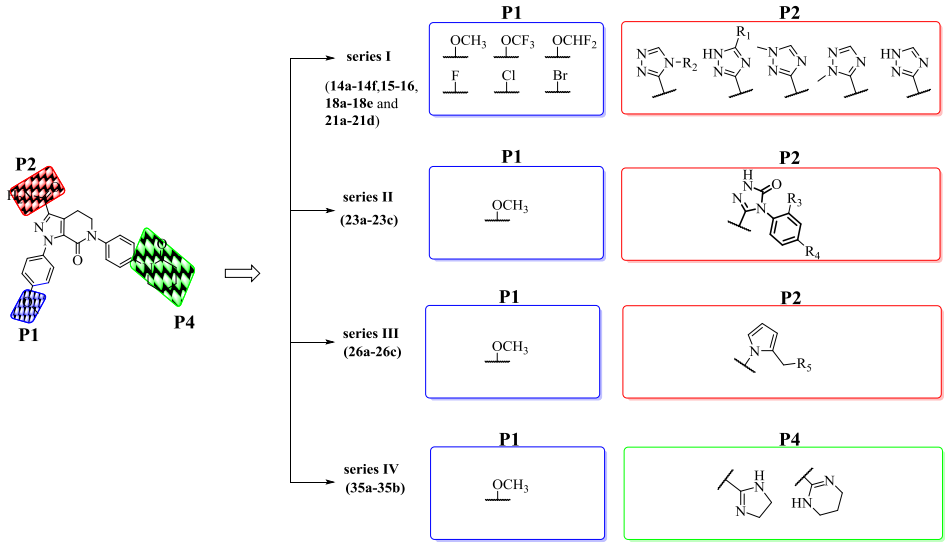


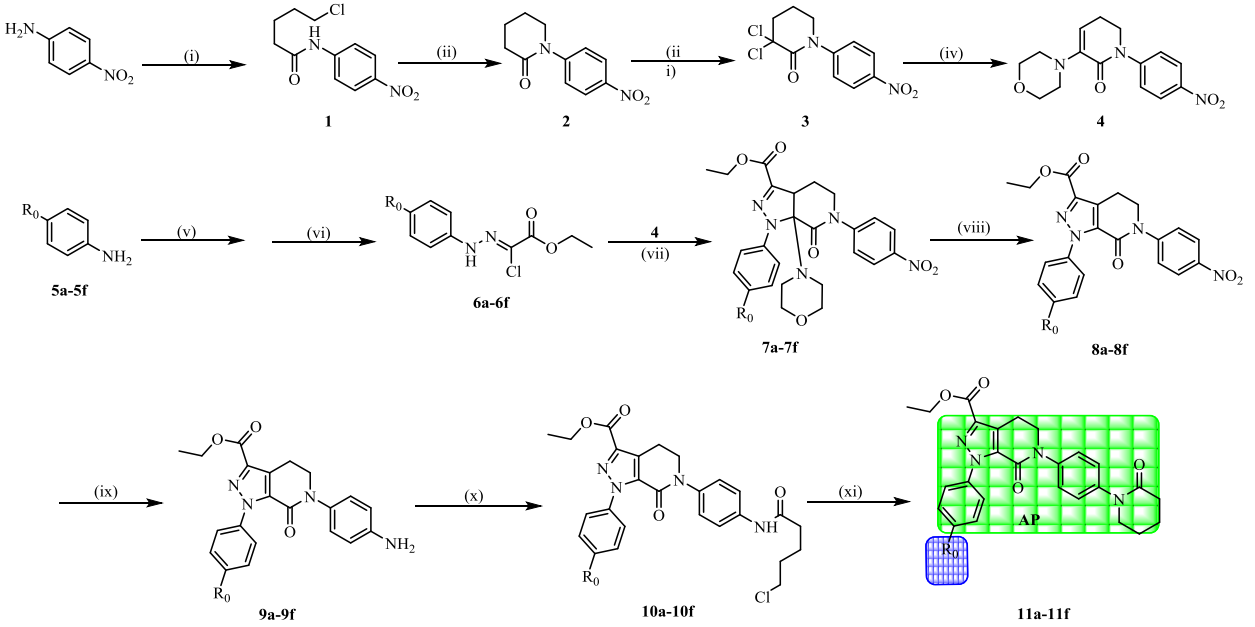
Fig. 3. Structure of the series I~IV target compounds

2. Chemistry

The majority of the target compounds listed in **Fig. 3** were synthetized at variable yields, as shown in **Schemes 1-5**. In the process of synthesis, the most critical steps were the synthesis of key intermediates **11a-11f** and **32a**.

*2.1. Synthesis of intermediates **11a-11f***

The synthesis of intermediates **11a-11f** was achieved using a convenient eleven-step procedure starting from 4-nitroaniline, outlined in **Scheme 1**. The commercially available 4-nitroaniline was reacted with 4-chlorobutanoyl chloride to provide compound **1**, which was treated with potassium carbonate to obtain intermediate **2**. Then, compound **2** was chlorinated with PCl_5 and substituted with morpholine to obtain compound **4**. Different substituted anilines (**5a-5f**) were treated with $NaNO_2$/HCl, and the corresponding diazoniums were condensed with ethyl 2-chloro-3-oxobutanoate in the presence of sodium acetate to provide chlorohydrazones **6a-6f**. Reaction of **6a-6f** with compound **4** using excess triethylamine afforded the cycloadducts **7a-7f**. Compounds **7a-7f** were treated with TFA in dichloromethane to obtain compounds **8a-8f**, respectively. Finally, the cardinal intermediates **11a-11f** were prepared from compounds **8a-8f** through reduction, amidation, and cyclization reactions.



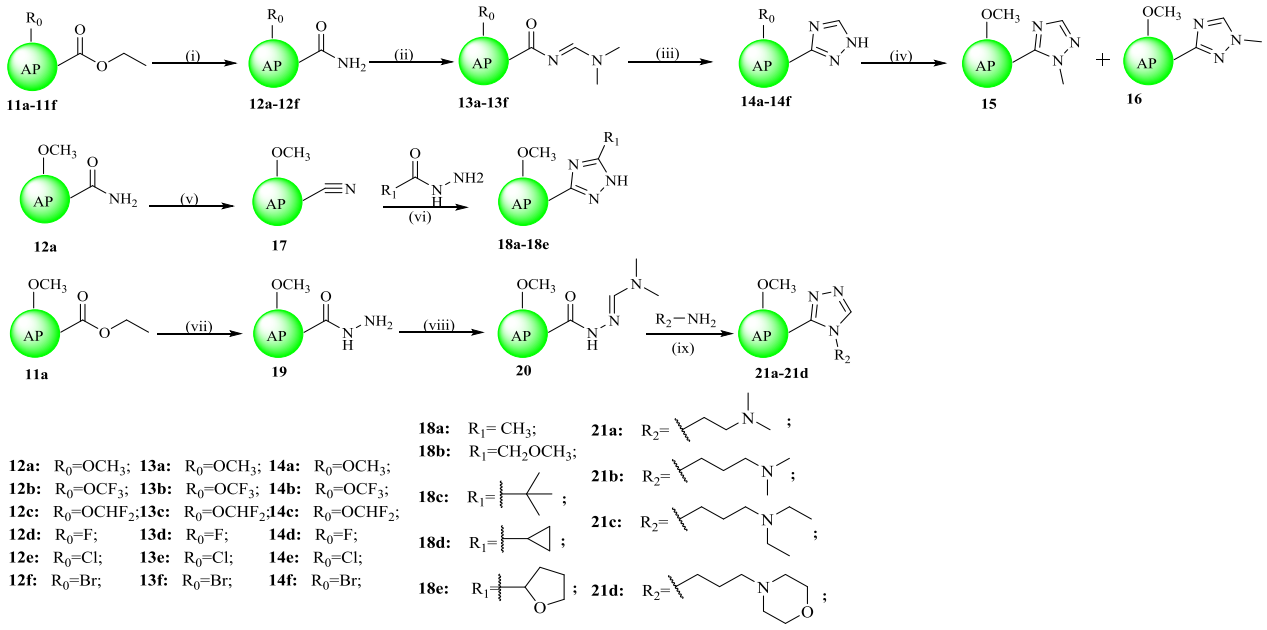
5a: R_0=OCH_3; **6a:** R_0=OCH_3; **7a:** R_0=OCH_3; **8a:** R_0=OCH_3; **9a:** R_0=OCH_3; **10a:** R_0=OCH_3; **11a:** R_0=OCH_3;
5b: R_0=OCF_3; **6b:** R_0=OCF_3; **7b:** R_0=OCF_3; **8b:** R_0=OCF_3; **9b:** R_0=OCF_3; **10b:** R_0=OCF_3; **11b:** R_0=OCF_3;
5c: R_0=$OCHF_2$; **6c:** R_0=$OCHF_2$; **7c:** R_0=$OCHF_2$; **8c:** R_0=$OCHF_2$; **9c:** R_0=$OCHF_2$; **10c:** R_0=$OCHF_2$; **11c:** R_0=$OCHF_2$;
5d: R_0=F; **6d:** R_0=F; **7d:** R_0=F; **8d:** R_0=F; **9d:** R_0=F; **10d:** R_0=F; **11d:** R_0=F;
5e: R_0=Cl; **6e:** R_0=Cl; **7e:** R_0=Cl; **8e:** R_0=Cl; **9e:** R_0=Cl; **10e:** R_0=Cl; **11e:** R_0=Cl;
5f: R_0=Br; **6f:** R_0=Br; **7f:** R_0=Br; **8f:** R_0=Br; **9f:** R_0=Br; **10f:** R_0=Br; **11f:** R_0=Br;

Scheme 1. Reagents and conditions: (i) 4-chlorobutanoyl chloride, THF, 0 ºC, 30 min, r.t., 6 h; (ii) K_2CO_3, DMSO, 80 ºC, 6 h; (iii) $POCl_5$, CH_2Cl_2, 85 ºC, 5 h; (iv) morpholine, 130 ºC, 1.5 h; (v) $NaNO_2$, HCl, H_2O, 0 ºC, 30 min; (vi) ethyl 2-chloro-3-oxobutanoate, sodium acetate, r.t., 6 h; (vii) triethylamine, ethyl acetate, 80 ºC, 6 h; (viii) TFA, CH_2Cl_2, r.t., 1 h; (ix) Fe powder, HCl, NH_4Cl, EtOH/H_2O, reflux, 2.5 h; (x) 4-chlorobutanoyl chloride, THF, 0 ºC, 30 min, r.t., 6 h; (xi) NaH, DMF, 0 ºC, 1 h.

2.2. Synthesis of apixaban derivatives of series I: substituted 1,2,4-triazole

The target compounds of series I were synthesized according to the procedures outlined in **Scheme 2**. Compounds **14a-14f**, **15**, and **16** were synthesized as follows: firstly, the key intermediates **11a-11f** were aminolysized with formamide to provide **12a-12f**. [12] Compounds **13a-13f** were obtained from **12a-12f** in CH_2Cl_2. Next, the target compounds **14a-14f** were available *via* cyclization of **13a-13f** and 80% hydrazine monohydrate. Compounds **14a-14f** were methylated with iodomethane to obtain the target compounds **15** and **16**. [13-15]

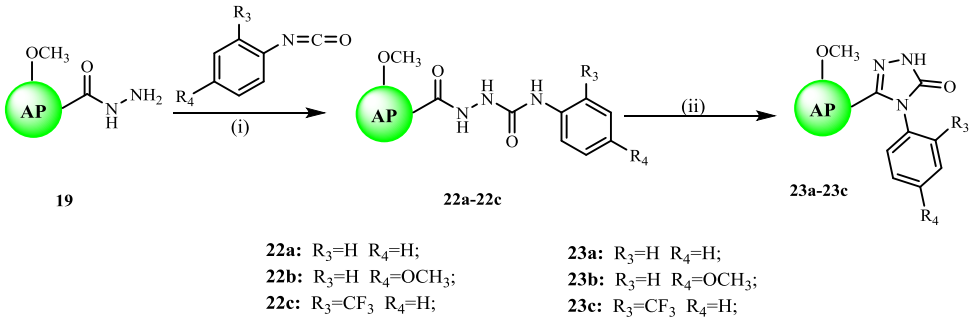
For obtaining the derivatives **18a-18e** and **21a-21d** with substituents at different positions of triazole, two synthetic routes were used; as depicted in **Scheme 2**. The compounds **18a-18e** were obtained by converting compound **17** using different hydrazides followed by dehydration of **12a**.[16-17] The target compounds **21a-21d** were created by a three-step procedure and treatment of compound **11a** with hydrazine hydrate afforded intermediate **19**, which was treated with DMF-DMA to afford compound **20**. The desired compounds **21a-21d** were obtained from **20** and different amines *via* intramolecular cyclization. [18]



Scheme 2. Reagents and conditions: (i) CH_3ONa, $HCONH_2$, DMF, 50 °C, 5 h; (ii) DMF-DMA, CH_2Cl_2, reflux, 3 h; (iii) $NH_2NH_2{\cdot}H_2O$, acetic acid, reflux, 3 h; (iv) CH_3I, NaH, DMF, 40 °C, 5 h; (v) triethylamine, trifluoroacetic anhydride, CH_2Cl_2, reflux, 3 h; (vi) K_2CO_3, hydrazides, n-butyl alcohol, reflux, 20 h; (vii) $NH_2NH_2{\cdot}H_2O$, reflux, 4 h; (viii) DMF-DMA, CH_2Cl_2, reflux, 4 h; (ix) R_2NH_2, acetic acid, reflux, 10 h.

2.3. Synthesis of apixaban of series II:N-4-substituted-1,2,4-triazolin-3-one derivatives

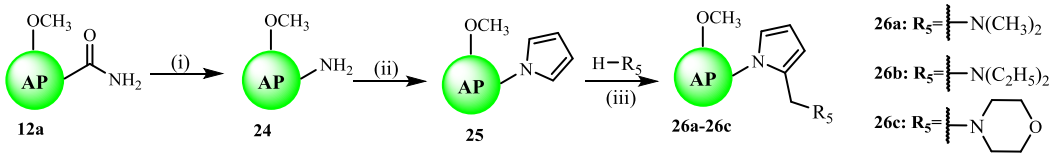
The desired compounds **23a-23c** were synthesized as outlined in **Scheme 3**. Treatment of compound **19** with substituted phenyl isocyanates in anhydrous tetrahydrofuran afforded intermediates **22a–22c**, which were treated with anhydrous potassium carbonate in dimethyl sulfoxide (DMSO) to afford target compounds **23a–23c** *via* intramolecular cyclization .[19]



Scheme 3. Reagents and conditions: (i) substituted phenyl isocyanates, tetrahydrofuran, 50 °C, 3 h; (ii) potassium carbonate, dimethyl sulfoxide, 80 °C, 6 h.

2.4. Synthesis of apixaban derivatives of series III: pyrrole derivatives

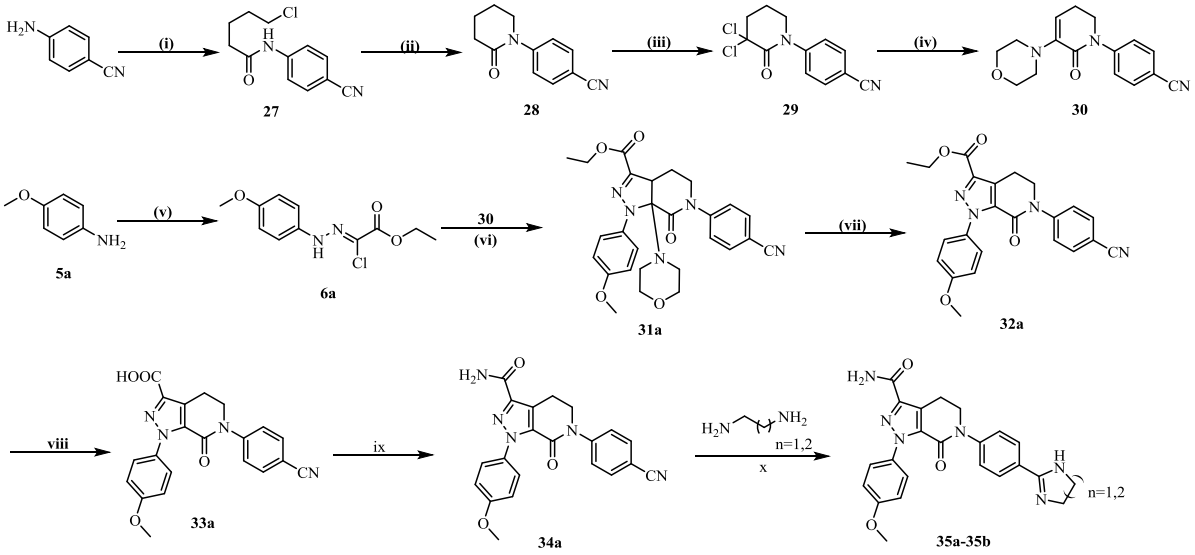
The preparation of compounds **26a-26c** is illustrated in **Scheme 4**. Firstly, amino derivative **24** was obtained by the Hofmann rearrangement of **12a** under basic conditions in the presence of iodobenzene diacetate.[20] Then, treatment of **24** with 2,5-dimethoxytetrahydrofuran and *p*-toluenesulfonic acid in tetrahydrofuran afforded compound **25**.[21] Finally, the target compounds **26a-26c** were obtained *via* the Mannich reaction of **25**, formaldehyde, and appropriate secondary amines.



Scheme 4. Reagents and conditions: (i) KOH, MeOH, r.t., 10 min, $PhI(OAc)_2$, dioxane, reflux, 6-10 h; (ii) 2,5-dimethoxytetrahydrofuran, *p*-toluenesulfonic, tetrahydrofuran, 60 °C, 1 h; (iii) HCHO, HR_5, HOAc, r.t. 30 min.

2.5. Synthesis of apixaban derivatives of series IV: dihydroimidazole/tetrahydropyrimidine groups in P4

The synthesis of the intermediates **27-30**, **31a**, and **32a** is shown in **Scheme 1**. As described in **Scheme 5**, the target compounds **35a-35b** were prepared from **32a** through hydrolysis, ammoniation, and cyclization with corresponding diamines. [22]



Scheme 5. Reagents and conditions: (i) 4-chlorobutanoyl chloride, THF, 0 °C, 30 min, r.t., 6 h; (ii) K_2CO_3, DMSO, 80 °C, 6 h; (iii) $POCl_5$, CH_2Cl_2, 85 °C, 5 h; (iv) morpholine, 130 °C, 1.5 h; (v) $NaNO_2$, HCl, H_2O, 0 °C, 30 min, 2-chloro-3-oxobutanoate, sodium acetate, r.t., 6 h; (vi) triethylamine, ethyl acetate, 80 °C, 6 h; (vii) TFA, CH_2Cl_2, r.t., 1 h; (viii) Sodium hydroxide, methanol/water (5:1 v/v), reflux, 3h; (ix) triethylamine, ethyl chlorocarbonate, ammonia solution rt, 3 h; (x) ethylenediamine or trimethylenediamine, sulfur, 130 °C, 0.5 h.

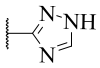
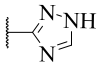
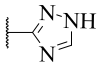
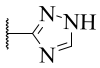
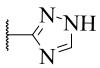
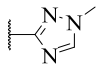
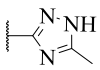
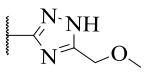
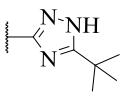
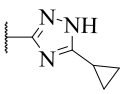
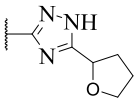
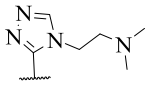
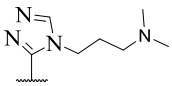
3. Results and discussion

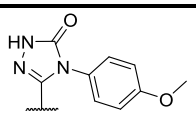
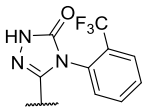
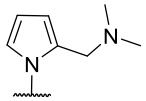
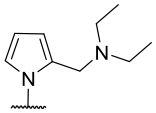
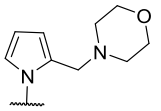
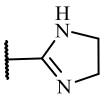
3.1. In vitro anticoagulant activity

All target compounds were evaluated for their anticoagulant activity by using human and rabbit plasma *in vitro*. The results were expressed as prothrombin time (PT) and activated partial thromboplastin time (APTT). PT measures the effect of a compound on the extrinsic pathway of coagulation, whereas APTT represents the effect on the intrinsic pathway. The results were expressed as EC_{2x} values and are summarized in **Table 1**. The EC_{2x} values in **Table 1** are the average of at least three independent experiments.

Table 1 Anticoagulant activity of series I-IV.

Compd.	P2	P1	P4	EC_{2x}(μM)	
				PT[a](human/rabbit)	APTT[a](human/rabbit)
14a				**0.5/0.7**	**0.8/1.2**

14b				5.5/6.4	10.5/12.0
14c				3.6/4.0	6.6/7.8
14d				1.8/2.8	4.5/5.1
14e				4.3/4.2	8.4/9.9
14f				6.4/7.9	11.3/10.2
15				3.3/4.1	10.0/15.1
16				**0.8/0.9**	**1.9/3.0**
18a				1.6/1.9	3.7/3.2
18b				2.4/3.0	4.1/6.0
18c				**1.0/1.4**	**2.3/4.1**
18d				3.5/4.9	5.1/6.0
18e				3.9/5.0	6.3/7.9
21a				3.9/4.4	7.0/8.0
21b				4.5/5.7	9.0/10.1
21c				5.0/6.1	10.0/11.1
21d				5.5/7.5	10.5/15.0
23a				12.9/13.3	20.3/22.1

23b				14.8/9.2	21.3/19.2
23c				16.8/17.9	30.1/32.1
26a				1.5/1.9	3.5/3.2
26b				1.6/2.1	3.7/3.9
26c				**1.1/1.7**	**2.4/3.9**
35a				**0.9/0.7**	**0.9/1.0**
35b				**0.9/1.1**	**1.1/2.0**
Apixaban[b]				**0.8/0.6**	**0.9/1.4**

[a] Concentration of the compound required to double the clotting time in the PT assay using human/rabbit plasma.

[b] Used as a positive control.

As shown in **Table 1**, most of the target compounds exhibited moderate to excellent activity, with the EC_{2x} value from 30 to 0.5 μM, wherein the activity of compounds **14a** (PT = 0.5, APTT = 0.8), **16** (PT = 0.8, APTT = 1.9), **18c** (PT = 1.0, APTT = 2.3), **26c** (PT = 1.1, APTT = 2.4), **35a** (PT = 0.9, APTT = 0.9), and **35b** (PT = 0.9, APTT = 1.1) was comparable to that of apixaban (PT = 0.8, APTT = 0.9). Particularly, compound **14a** was the most potent candidate for further research in these series. Furthermore, the anticoagulant activity in rabbit and human plasma was correlative.

During the investigation of the C-3 pyrazole position, as illustrated in **Table 1**, **14a** (PT = 0.5, APTT = 0.8) with unsubstituted 1,2,4-triazole showed superior anticoagulant activity. Unfortunately, different electron-withdrawing groups (EWGs) in the methoxyl position decreased the activity obviously. The order of anticoagulant activity was $-OCH_3$ (**14a**) > -F (**14d**) > $-OCHF_2$ (**14c**) > -Cl (**14e**) > $-OCF_3$ (**14b**) > -Br (**14f**), suggesting the importance of methoxyl as an electron-donating group (EDG) in the P1 ligand.

Upon comparing the isomers **15** and **16**, significant differences were observed between 2-N-methyl and 1-N-methyl in anticoagulant activity. By switching from the N-methyl to C-methyl position, **18a** was produced by introducing 5-C-methyl into 1,2,4-triazole. The activity of **18a** was between that of compounds **15** and **16** (**16** > **18a** > **15**). Subsequently, a series of derivatives **18b-18e** were synthesized with different substituents at 5-C. They showed a decrease in anticoagulant potency except for compound **18c** with *t*-butyl (PT = 1.0, APTT = 2.3), indicating that the 5-C-substituted EDG and steric effects were factors that could not be neglected.

Compounds **21a-21d** were also synthetized to increase the hydrophilicity or solubility by adding an alkaline

hydrophilic group. All of them displayed decreased potency. For increasing hydrogen bond action, 5-oxo-substituted 1,2,4-triazole with 4-N-phenyl was introduced into the C-3 pyrazole to obtain compounds **23a**, **23b**, and **23c**. The pharmacological data indicated that the activity was lost completely (PT > 12, APTT > 20). A plausible explanation for this is that the steric hindrance produced by the introduction of large groups influenced the activity.

Further studies were performed to examine the effect of different nitrogenous heterocyclics on the C-3 pyrazole. Compounds **26a**, **26b**, and **26c** were synthetized with alkaline-substituted pyrrole moieties. The biological data showed that compounds **26c** (PT = 1.1, APTT = 2.4), **26a** (PT = 1.5, APTT = 3.5), and **26b** (PT = 1.6, APTT = 3.7) possessed high anticoagulant activity.

As illustrated in **Table 1**, the introduction of dihydroimidazol and tetrahydropyrimidine groups into the hydrophobic pocket (P4 region) could improve the anticoagulant activity, such as compounds **35a** (PT = 0.9, APTT = 0.9) and **35b** (PT = 0.9, APTT = 1.1). More studies on the P4 moiety are under way.

The SARs based on the EC_{2x} values in **Table 1** showed that the hydrogen bond action and size of the nitrogenous heterocyclic in P2 and the lipophilic region in P4 were responsible for the anticoagulant activity.

3.2. In vitro FXa enzymatic assays and in vivo antithrombotic effect

Based on the anticoagulant potency, as shown in **Table 1**, the six tested compounds **14a**, **16**, **18c**, **26c**, **35a**, and **35b** showed excellent anticoagulant activity. Therefore, these compounds, at their IC_{50} values, were tested against human FXa *in vitro* and rat venous thrombosis *in vivo*, as listed in **Table 2**. The fitting curve, indicating the percent of inhibition, and the curve detailing the IC_{50} values of **14a** and **35b** are summarized in **Fig. 4** and **Fig. 5**, respectively. Wherein compound **14a** displays the most potent activity against human FXa with an IC_{50} value of 0.15 μM and 99% inhibition rate in the rat venous thrombosis test, which is superior to that of apixaban.

Table 2 *In vivo* FXa inhibition and antithrombotic effect.

Compd.	IC_{50} on FXa (μM)[a]	Thrombus weight (mg)	Inhibition rate (%)
14a	0.15	0.07±0.3	99
16	2.40	1.6±0.3	65
18c	0.85	1.4±1.2	70
26c	2.40	1.9±1.0	57
35a	1.99	1.5±0.2	66
35b	0.35	0.8±0.3	82
Apixaban[b]	0.23	0.2±0.2	96
model		4.4±3.9	

[a] Inhibitory activity against human FXa. IC_{50}values shown were the mean of duplicate measurements.

[b] Used as positive control.

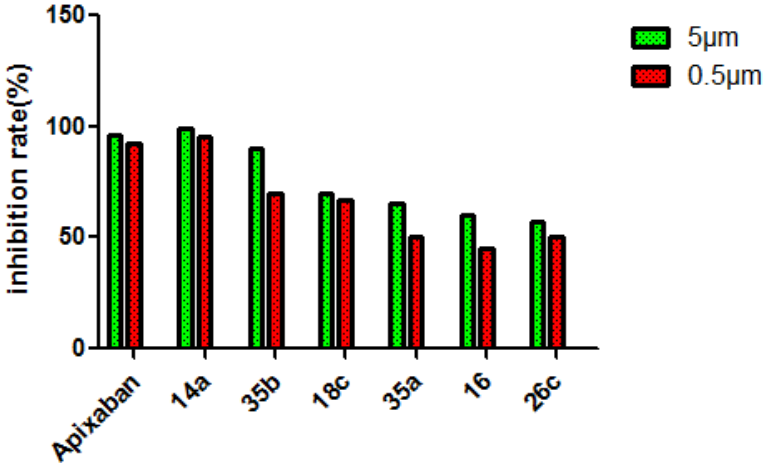


Fig. 4. Preliminary screening of seven compounds against human FXa

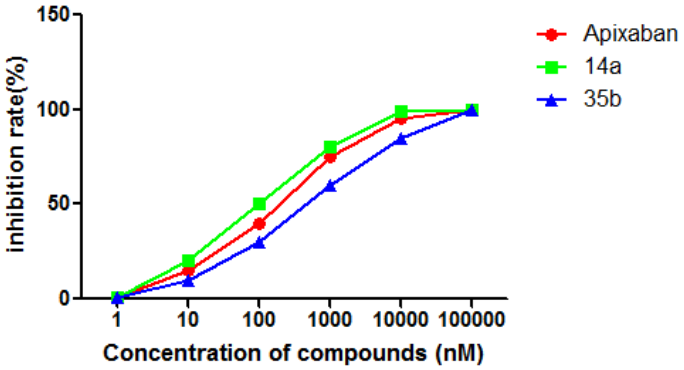


Fig. 5. Determination of the IC_{50} of apixaban, 14a, and 35b against human FXa

3.4. Molecular modeling study

To clarify the binding mode of the compounds, a detailed docking analysis was performed. The co-crystal structure of apixaban with human FXa was selected as the docking model (PDB ID code: 2P16).[11] The docking simulation was conducted using Accelrys DS visualizer 3.0 systems. The image files were generated using Accelrys DS visualizer 4.0 systems. The binding model was exemplified by the interaction of compound **14a** with human FXa. As shown in **Fig. 6**, the model further suggested that the 1-N**H** group of the 1,2,4-triazole linked to C-3 pyrazole formed a H-bond with Glu146. Additionally, the pyrazole N-2 nitrogen atom interacted with the Gln192. The pendant phenyllactam positioned between the Tyr215 and Phe174 occupies the P4 pocket in the FXa hydrophobic cavity.

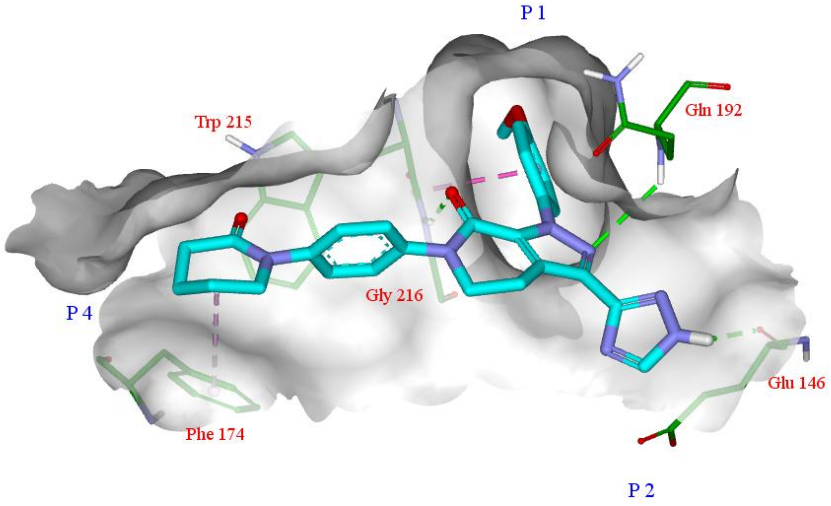


Fig. 6. The human FXa active site in the complex of compound 14a.

Furthermore, some physicochemical properties of the target compounds and apixaban were predicted using free online software (http://www.molinspiration.com/) for their adaptability with Lipinski's rule of five. As shown in **Table 3**, compounds **14a** and **35b** conformed well to the Lipinski's rule of five.[23] Both of them were predicted to possess strong drug resistance.

Table 3 Prediction of physicochemical properties[a] of the target compounds

Compound	miLogP	TPSA	natoms	MW	nON	nOHNH	nviolations	nrotb	volume
Accepted range	< 5	< 140		< 500	< 10	< 5		≤10	
14a	**2.17**	**109.25**	**36**	**483.53**	**10**	**1**	**0**	**5**	**424.36**
16	2.24	98.40	37	497.56	10	0	0	5	441.30
18c	3.98	109.25	40	539.64	10	1	1	6	490.55
26c	3.42	85.08	43	580.69	10	0	1	7	527.99
35a	0.99	114.85	32	430.47	9	3	0	5	376.84
35b	**1.26**	**114.85**	**33**	**444.50**	**9**	**3**	**0**	**5**	**393.64**
Apixaban[b]	1.78	110.77	34	459.51	9	2	0	5	406.55

[a] miLogP: molinspiration predicted Log P; TPSA: topological polar surface area; natoms: no. of atoms; MW: molecular weight; nON: no. of hydrogen bond acceptors; nOHNH: no. of hydrogen bond donors; nviolations: no. of violations; nrotb: no. of rotatable bonds; volume: molar volume.

[b] Used as positive control.

4. Conclusions

In this study, four series of novel potent FXa inhibitors based on apixaban were designed and synthesized. The pharmacological data indicated that several compounds (**14a**, **16**, **18c**, **26c**, **35a**, and **35b**) exhibited moderate to excellent anticoagulant potency in human and rabbit plasma *in vitro*. The SARs, based on the EC_{2x} values shown in **Table 1**, showed that the hydrogen bond action and size of the nitrogenous heterocyclic in P2 and the lipophilic region in P4 were responsible for the anticoagulant activity. Eventually, compound **14a** possessing a novel scaffold was selected for further exploration owing to its pronounced enzymatic anticoagulant activity with an IC_{50} value of 0.15 μM *in vitro* and 99% inhibition rate in rat venous thrombosis test *in vivo*. The docking model indicated that compound **14a** formed a hydrogen bond with Glu146, and 1,2,4-triazole provided a much more suitable size (5-membered triazole ring) accommodating the position of P2 compared with apixaban.

5. Experimental

5.1. Chemistry

Unless otherwise noted, all materials were used without further purification and were obtained from commercial suppliers. Reactions' time and purity of the products were monitored by TLC on FLUKA silica gel aluminum cards (0.2 mm thickness) with fluorescent indicator 254 nm. All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland). Column chromatography was run on silica gel (200–300 mesh) from Qingdao Ocean Chemicals (Qingdao, Shandong, China). Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC–MS (Agilent, Palo Alto, CA, USA). Elemental analysis was determined on a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy). ^{1}H NMR spectra were recorded on Bruker ARX-400, 400 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard.

*5.2. The preparation of the key intermediates **11a-11f***

*5.2.1. Preparation of 5-Chloro-N-(4-nitrophenyl)pentanamide (**1**)*

P-nitroaniline (50.0 g, 0.36 mol) and trimethylamine (100 mL, 0.72 mol) were solubilized in tetrahydrofuran (200 mL), then a solution of 5-chlorovaleryl chloride (70 mL, 0.54 mol) was added drop-wise at 0-5°C. After stirring for 6 h at r.t., the mixture was poured into ice-water (200 mL). The resulting precipitate was filtered, washed with diethyl ether and dried under reduced pressure to yield the title compound as a yellow solid (72.3 g, 77.8%), M.p.: 121.4-123.3°C; MS (ESI) m/z (%): 257.06 $[M+H]^+$.

*5.2.2. Preparation of 1- (4-Nitrophenyl) piperidin-2-one (**2**)*

A mixture of compound **1** (72.3 g, 0.28 mol), K_2CO_3 and DMSO (360 mL) was stirred at 80°C for 6 h. Upon cooling to r.t., the mixture was poured into ice-water (200 mL). The resulting precipitate was filtered, washed with water and dried under reduced pressure to yield the title compound as a yellow powder (55.7 g, 89.6%)，M.p.: 96.4-98.3°C; MS (ESI) m/z (%): 221.08 $[M+H]^+$.

*5.2.3. Preparation of 3,3-Dichloro-1-(4-nitrophenyl)piperidin-2-one **(3)***

A solution of intermediate **2** (55.7 g, 0.25 mol) in dichloromethane (330 mL) was stirred to dissolve, PCl_5 (158.0 g, 0.76 mol) was added and refluxed for 5 h. After cooling to r.t., the reaction mixture was poured into ice-water (300 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The organic phase was washed with water (3 × 100 mL), brine (200 mL). The organic layer was dried with Na_2SO_4, filtered and evaporated in vacuo. The residue was triturated with methanol to give a white powder (50.3 g, 69.0%), M.p.: 170.0-172.1°C; MS (ESI) m/z (%): 289.01 $[M+H]^+$.

*5.2.4. Preparation of 3-Morpholino-1-(4-nitrophenyl)-5,6-dihydropyridin-2(1H)-one **(4)***

A mixture of compound **3** (50.3 g, 0.17mol) and morpholine (200 mL) was stirred at 130°C for 1.5 h , and then cooled to room temperature. The solvent was removed under vacuum, and the precipitate was collected by filtration and washed with water to afford the desired product **4** as a yellow solid (46.3g，87.6%), M.p.: 157.3-160.3°C; MS (ESI) m/z (%): 304.0 $[M+H]^+$, 326.0 $[M+Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (d, J = 3.0 Hz, 1H, Ar-H), 8.18 (d, J = 3.0 Hz, 1H, Ar-H), 7.62 (d, J = 3.0 Hz, 1H, Ar-H), 7.59 (d, J = 3.0 Hz, 1H, Ar-H), 5.77 (t, J = 4.5 Hz, 1H, C = CH-C), 3.79 (t, J = 7.5 Hz, 2H, N-CH_2-C), 3.62-3.59 (m, 4H, O-CH_2-C), 2.73-2.77 (m, 4H, N-CH_2-C), 2.47-2.41(m, 2 H, =C-CH_2-C).

*5.2.5. General procedure for preparation of 2-Chloro-2-(2-(4-substituted phenyl)hydrazono) acetate **6a–6f***

HCl (0.6 mol, 50 mL) was added to a well-stirred solution of 4-substituted phenyl amines **5a-5f** (0.20 mol) in water (100 mL) at r.t.. After the completion of addition, $NaNO_2$ (15.2 g, 0.22 mol) in H_2O (30 mL) was added

dropwise at -5°C. Then the mixture was stirred at 0°C for 30 min. Ethyl 2-chloroacetoacetate (34.5 g, 0.21 mol) and sodium acetate (49.2 g, 0.60 mol) were added to the solution and stirred for 30 min. The reaction mixture was then heated to 25°C for 2 h. The resulting precipitate was filtered, washed with methano and dried under reduced pressure to yield the title compound **6a-6f**.

*5.2.5.1. Ethyl (Z)-2-Chloro-2-(2-(4-methoxyphenyl)hydrazono)acetate (**6a**)*

Light yellow solid; Yield: 85.1%; M.p.: 92.3-94.3°C; MS (ESI) m/z (%): 279.3 $[M+Na]^+$.

*5.2.5.2. Ethyl (Z)-2-Chloro-2-(2-(4-trifluoromethoxyphenyl)hydrazono)acetate (**6b**)*

Light yellow solid; Yield: 80.7%; M.p.: 91.3-93.3°C; MS (ESI) m/z (%): 333.6 $[M+Na]^+$.

*5.2.5.3. Ethyl (Z)-2-Chloro-2-(2-(4-difluoromethoxyphenyl)hydrazono)acetate (**6c**)*

Light yellow solid; Yield: 83.3%; M.p.: 94.1-96.3°C; MS (ESI) m/z (%): 293.2 $[M+H]^+$, 315.2 $[M+Na]^+$.

*5.2.5.4. Ethyl (Z)-2-Chloro-2-(2-(4-fluorophenyl)hydrazono)acetate (**6d**)*

Light yellow solid; Yield: 83.3%; M.p.: 90.3-92.0°C; MS (ESI) m/z (%): 245.1 $[M+H]^+$, 268.0 $[M+Na]^+$.

*5.2.5.5. Ethyl (Z)-2-Chloro-2-(2-(4-chlorophenyl)hydrazono)acetate (**6e**)*

Light yellow solid; Yield: 84.5%; M.p.: 91.5-93.0°C; MS (ESI) m/z (%): 261.2 $[M+H]^+$, 283.1 $[M+Na]^+$.

*5.2.5.6. Ethyl (Z)-2-Chloro-2-(2-(4-bromophenyl)hydrazono)acetate (**6f**)*

Light yellow solid; Yield: 82.5%; M.p.: 91.7-93.9°C; MS (ESI) m/z (%): 305.2$[M+H]^+$.

*5.2.6. General procedure for the preparation of intermediates **7a-7f***

Trimethylamine (32 mL, 0.23 mol) and **6a-6f** (0.18 mol) were added to a solution of intermediate **4** (45.5 g, 0.15 mol) in ethyl acetate (500 mL). The mixture was stirred at 80°C for 6 h. After cooling to r.t., yellow solid was precipitated. The resultant precipitate was filtered and washed with ethyl acetate to afford corresponding compounds ***7a-7f***.

*5.2.6.1. Ethyl1-(4-methoxyphenyl)-7a-morpholino-6-(4-nitrophenyl)-7-oxo-3a, 4, 5, 6, 7, 7a-hexahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**7a**)*

Yellow solid; Yield: 97.5%; M.p.: 175.3-177.3°C; MS (ESI) m/z (%): 524.21 $[M+H]^+$.

*5.2.6.2. Ethyl7a-morpholino-6-(4-nitrophenyl)-7-oxo-1-(4-(trifluoromethoxy) phenyl)-3a,4,5,6,7,7a-hexahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**7b**)*

Yellow solid; Yield: 94.6%; M.p.: 174.4-175.7°C; MS (ESI) m/z (%): 578.18 $[M+H]^+$.

*5.2.6.3. Ethyl1-(4-(difluoromethoxy) phenyl)-7a-morpholino-6-(4-nitrophenyl)-7-oxo-3a,4,5,6,7,7a-hexahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**7c**)*

Yellow solid; Yield: 96.3%; M.p.: 175.8-176.9°C; MS (ESI) m/z (%): 560.19 $[M+H]^+$.

*5.2.6.4. Ethyl1-(4-fluorophenyl)-7a-morpholino-6-(4-nitrophenyl)-7-oxo-3a,4,5,6,7,7a-hexahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**7d**)*

Yellow solid; Yield: 94.1%; M.p.: 173.3-174.3°C; MS (ESI) m/z (%): 512.19 $[M+H]^+$.

*5.2.6.5. Ethyl1-(4-chlorophenyl)-7a-morpholino-6-(4-nitrophenyl)-7-oxo-3a,4,5,6,7,7a-hexahydro-1H-pyrazolo [3,4-c]pyridine-3-carboxylate (**7e**)*

Yellow solid; Yield: 93.0%; M.p.: 175.6-176.3°C; MS (ESI) m/z (%): 528.16 $[M+H]^+$.

*5.2.6.6 Ethyl1-(4-bromophenyl)-7a-morpholino-6-(4-nitrophenyl)-7-oxo-3a,4,5,6,7,7a-hexahydro-1H-pyrazolo [3,4-c]pyridine-3-carboxylate (**7f**)*

Yellow solid; Yield: 94.6%; M.p.: 176.3-177.9°C; MS (ESI) m/z (%): 572.11 $[M+H]^+$.

*5.2.7. General procedure for the preparation of intermediates **8a-8f***

Trifluoroacetic acid (TFA) was added drop-wise in ice-bath to a solution of intermediates **7a-7f** (0.12 mol) in CH_2Cl_2 (650 mL). The resulting solution was stirred at r.t. for 1 h . The reaction mixture was poured into ice-water (300 mL). The organic phase was washed with water (3 × 100 mL), brine (200 mL) and dried with Na_2SO_4, filtered and evaporated in vacuo to get red-brown solid. Then the solid was triturated in ether to afford intermediates **8a-8f**.

*5.2.7.1. Ethyl1-(4-methoxyphenyl)-6-(4-nitrophenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carb oxylate **(8a)***

Light yellow powder; Yield: 62.1%; M.p.: 159.6-161.3°C; MS (ESI) m/z (%): 459.0 $[M+Na]^+$.

*5.2.7.2. Ethyl6-(4-nitrophenyl)-7-oxo-1-(4-(trifluoromethoxy)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo [3,4-c] pyridine-3-carboxylate (**8b**)*

Light yellow powder; Yield: 59.8%; M.p.: 158.5-159.9°C; MS (ESI) m/z (%): 513.0 $[M+Na]^+$.

*5.2.7.3. Ethyl1-(4-(difluoromethoxy)phenyl)-6-(4-nitrophenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c] pyridine-3-carboxylate (**8c**)*

Light yellow powder; Yield: 57.7%; M.p.: 160.6-161.3°C; MS (ESI) m/z (%): 495.0 $[M+Na]^+$.

*5.2.7.4. Ethyl1-(4-fluorophenyl)-6-(4-nitrophenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**8d**)*

Light yellow powder; Yield: 64.4%; M.p.: 160.5-161.2°C; MS (ESI) m/z (%): 446.9 $[M+Na]^+$.

*5.2.7.5. Ethyl1-(4-chlorophenyl)-6-(4-nitrophenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**8e**)*

Light yellow powder; Yield: 63.8%; M.p.: 160.2-162.3°C; MS (ESI) m/z (%): 463.0 $[M+Na]^+$.

*5.2.7.6. Ethyl1-(4-bromophenyl)-6-(4-nitrophenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**8f**)*

Light yellow powder; Yield: 63.0%; M.p.: 161.6-162.3°C; MS (ESI) m/z (%): 506.8 $[M+Na]^+$.

*5.2.8. General procedure for the preparation of intermediates **9a-9f***

A mixture of Fe powder (22.4 g, 0.40 mol), HCl (1.7 mL, 0.02 mol) and ammonium chloride (2.7 g, 0.05 mol) in ethanol (90%, 350 mL) was heated to 80°C with vigorous agitation for 30 min. Then corresponding intermediates **8a-8f** (0.1 mol) were added portion-wise until TLC showed the completion of the reaction. The hot solution was filtered and washed with hot methanol. The filtrate was evaporated to afford **9a-9f**.

*5.2.8.1. Ehyl6-(4-aminophenyl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1H-Pyrazolo[3,4-c] pyridine-3-carboxylate (**9a**)*

White solid; Yield: 80.5%; M.p.: 150.6-152.3°C; MS (ESI) m/z (%): 407.0 $[M+H]^+$, 429.1 $[M+Na]^+$.

*5.2.8.2.Ehyl6-(4-aminophenyl)-7-oxo-1-(4-(trifluoromethoxy)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c] pyridine-3-carboxylate (**9b**)*

White solid; Yield: 75.6%; M.p.: 151.4-153.2°C; MS (ESI) m/z (%): 461.0 $[M+H]^+$, 483.1 $[M+Na]^+$.

*5.2.8.3. Ehyl6-(4-aminophenyl)-1-(4-(difluoromethoxy)phenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c] pyridine-3-carboxylate (**9c**)*

White solid; Yield: 76.4%; M.p.: 151.6-153.3°C; MS (ESI) m/z (%): 443.1 $[M+H]^+$, 465.2 $[M+Na]^+$.

*5.2.8.4. Ehyl6-(4-aminophenyl)-1-(4-fluorophenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carbo xylate (**9d**)*

White solid; Yield: 82.7%; M.p.: 149.6-151.3°C; MS (ESI) m/z (%): 395.0 $[M+H]^+$, 417.1 $[M+Na]^+$.

*5.2.8.5. Ehyl6-(4-aminophenyl)-1-(4-chlorophenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carbo xylate (**9e**)*

White solid; Yield: 77.8%; M.p.: 148.8-150.9°C; MS (ESI) m/z (%): 411.0 $[M+H]^+$, 433.0 $[M+Na]^+$.

*5.2.8.6. Ehyl6-(4-aminophenyl)-1-(4-bromophenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carbo xylate (**9f**)*

White solid; Yield: 82.1%; M.p.: 149.6-151.3°C; MS (ESI) m/z (%): 454.9 $[M+H]^+$, 477.0 $[M+Na]^+$.

*5.2.9. General procedure for the preparation of compounds **10a-10f***

Trimethylamine (19.5 mL, 0.14 mol) and a solution of 5-chlorovalerylchloride (13.5 mL, 0.11 mol) in anhydrous tetrahydrofuran (60 mL) were added to a well-stirred mixture of **9a-9f** (0.07 mol) in anhydrous tetrahydrofuran (120 mL) at 0°C. After the completion of addition, the mixture was warmed to 25°C for 6 h, and poured into ice-water (200 mL). The resulting precipitate was filtered, washed with water and dried under reduced pressure to yield yellow solid. Then the solid was triturated in ether to afford corresponding compounds **10a-10f**.

*5.2.9.1. Ethyl6-(4-(5-chloropentanamido)phenyl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**10a**)*

Gray solid; Yield: 86.7%; M.p.: 139.6-141.3°C; MS (ESI) m/z (%): 547.1 $[M+Na]^+$.

*5.2.9.2. Ethyl6-(4-(5-chloropentanamido)phenyl)-7-oxo-1-(4-(trifluoromethoxy)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**10b**)*

Gray solid; Yield: 84.0%; M.p.: 138.6-139.6°C; MS (ESI) m/z (%): 600.9 $[M+Na]^+$.

*5.2.9.3. Ethyl6-(4-(5-chloropentanamido)phenyl)-1-(4-(difluoromethoxy)phenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**10c**)*

Gray solid; Yield: 83.1%; M.p.: 140.5-142.3°C; MS (ESI) m/z (%): 583.1 $[M+Na]^+$.

*5.2.9.4 Ethyl6-(4-(5-chloropentanamido)phenyl)-1-(4-fluorophenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**10d**)*

Gray solid; Yield: 84.5%; M.p.: 138.1-140.3°C; MS (ESI) m/z (%): 535.2 $[M+Na]^+$.

*5.2.9.5 Ethyl6-(4-(5-chloropentanamido)phenyl)-1-(4-chlorophenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**10e**)*

Gray solid; Yield: 87.1%; M.p.: 138.4-139.7°C; MS (ESI) m/z (%):550.8[M+Na]$^+$.

*5.2.9.6 Ethyl1-(4-bromophenyl)-6-(4-(5-chloropentanamido)phenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**10f**)*

Gray solid; Yield: 86.7%; M.p.: 137.6-138.8°C; MS (ESI) m/z (%): 595.0 [M+Na]$^+$.

*5.2.10. General procedure for the preparation of compounds **11a-11f***

Compounds **10a-10f** (0.05 mol) and NaH (3.6 g, 0.15 mol) were added in DMF (270 mL) at 0°C, and then stirred for 1 h. The reaction mixture was poured into ice-water (200 mL).The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The organic phase was washed with water (3 × 100 mL), brine (200 mL) and dried with Na_2SO_4, filtered and evaporated in vacuo. The residue was triturated with methanol to give the title compounds **11a-11f** as white solid.

*5.2.10.1. Ethyl1-(4-methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**11a**)*

White solid; Yield: 80.3%; M.p.: 126.4-128.3°C; MS (ESI) m/z (%): 489.21 [M+H]$^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, J = 8.9 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 7.24 (d, J =8.4 Hz, 2H), 6.91 (d, J = 8.9 Hz, 2H), 4.46 (q, J = 7.1 Hz, 2H), 4.13 (t, J = 6.7 Hz, 2H), 3.81 (s, 3H),3.62 (t, J = 6.6 Hz 2H), 3.32 (t, J = 6.6 Hz, 2H), 2.66 (t, J = 6.2 Hz, 2H), 1.95-1.92 (m, 4H), 1.43(t, J = 7.1 Hz, 2H); Anal. calcd. for $C_{27}H_{28}N_4O_5$ (%): C, 66.38; H, 5.78; N, 11.47. Found (%): C, 66.36; H, 5.79; N, 11.43.

*5.2.10.2. Ethyl 7-oxo-6-(4-(2-oxopiperidin-1-yl) phenyl)-1-(4-(trifluoromethoxy)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**11b**)*

White solid; Yield: 82.3%; M.p.: 125.6-127.3°C; MS (ESI) m/z (%): 543.18 [M+H] $^+$; Anal. calcd. for $C_{27}H_{25}F_3N_4O_5$ (%): C, 59.78; H, 4.65; N, 10.33. Found (%): C, 59.79; H, 4.68; N, 10.35.

*5.2.10.3. Ethyl 1-(4-(difluoromethoxy) phenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**11c**)*

White solid; Yield: 79.7%; M.p.: 127.6-129.4°C; MS (ESI) m/z (%): 523.19 [M+H]$^+$; Anal. calcd. for $C_{27}H_{26}F_2N_4O_5$ (%): C, 61.83; H, 5.00; N, 10.68. Found (%): C, 61.86; H, 5.02; N, 10.69.

*5.2.10.4 Ethyl1-(4-fluorophenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl) phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**11d**)*

White solid; Yield: 76.0%; M.p.: 128.2-129.7°C; MS (ESI) m/z (%): 477.19 [M+H]$^+$; Anal. calcd. for $C_{26}H_{25}FN_4O_4$ (%): C, 65.54; H, 5.29; N, 11.76. Found (%): C, 65.56; H, 5.27; N, 11.78.

*5.2.10.5 Ethyl1-(4-chlorophenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c] pyridine-3-carboxylate (**11e**)*

White solid; Yield: 81.7%; M.p.: 127.5-129.5°C; MS (ESI) m/z (%): 493.16 [M+H]$^+$; Anal. calcd. for $C_{26}H_{25}ClN_4O_4$ (%): C, 63.35; H, 5.11; N, 11.37. Found (%): C, 63.37; H, 5.13; N, 11.39.

5.2.10.6 Ethyl1-(4-bromophenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]

*pyridine-3-carboxylate (**11f**)*

White solid; Yield: 81.7%; M.p.: 125.6-127.3°C; MS (ESI) m/z (%): 537.11 $[M+H]^+$; Anal. calcd. for $C_{26}H_{25}BrN_4O_4$ (%): C, 58.11; H, 4.69; N, 10.43. Found (%): C, 58.14; H, 4.71; N, 10.44.

5.3. Synthesis of Apixaban derivatives of series I

*5.3.1. General procedure for the preparation of intermediates **12a-12f***

A mixture of CH_3ONa (4.10 g, 0.075 mol), methanamide (15.8 g, 0.35 mol), intermediates **11a-11f** (0.05 mol) and DMF (100 mL) was stirred at 50°C for 4 h. After being cooled to r.t., the reaction mixture was poured into ice-water. The precipitate was filtered and dried to afford products **12a-12f**.

*5.3.1.1. 1-(4-Methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo [3,4-c]pyridine-3-carboxamide (**12a**)*

White solid; yield: 76.2%; M.p.: 177.4-178.3°C; MS (ESI) m/z (%): 482.2 $[M+Na]^+$.

*5.3.1.2. 7-Oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-1-(4-(trifluoromethoxy)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo [3,4-c]pyridine-3-carboxamide (**12b**)*

White solid; yield: 76.4%; M.p.: 176.5-178.6°C; MS (ESI) m/z (%): 436.2 $[M+Na]^+$.

*5.3.1.3. 1-(4-(Difluoromethoxy)phenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo [3,4-c]pyridine-3-carboxamide (**12c**)*

White solid; yield: 78.8%; M.p.: 177.3-178.3°C; MS (ESI) m/z (%): 518.2 $[M+Na]^+$.

*5.3.1.4. 1-(4-Fluorophenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo [3,4-c]pyridine-3-carboxamide (**12d**)*

White solid; yield: 80.5%; M.p.: 176.8-178.9°C; MS (ESI) m/z (%): 470.2 $[M+Na]^+$.

*5.3.1.5. 1-(4-Chlorophenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo [3,4-c]pyridine-3-carboxamide(**12e**)*

White solid; yield: 79.9%; M.p.: 177.8-179.3°C; MS (ESI) m/z (%): 486.2 $[M+Na]^+$.

*5.3.1.6. 1-(4-Bromophenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo [3,4-c]pyridine-3-carboxamide (**12f**)*

White solid; yield: 77.3%; M.p.: 178.4-179.2°C; MS (ESI) m/z (%): 530.0 $[M+Na]^+$.

*5.3.2. General procedure for the preparation of intermediates **13a–13f***

DMF-DMA (8.0 mL, 0.06 mol) was added to a solution of **12a-12f** (4.59 g, 0.01 mol) in CH_2Cl_2 (80.0 mL). The reaction was heated to 40°C for 3 h. After cooling to r.t., the resultant solid **13a-13f** were collected by concentrating under vacuum.

*5.3.2.1. (E)-N-((Dimethylamino)methylene)-1-(4-methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7 -tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide (**13a**)*

Light yellow solid; yield: 96.1%; M.p.: 178.6-179.9°C; MS (ESI) m/z (%): 515.2 $[M+H]^+$.

*5.3.2.2. (E)-N-((Dimethylamino)methylene)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-1-(4-(trifluoromethoxy) phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide (**13b**)*

Light yellow solid; yield: 98.7%; M.p.: 178.3-180.3°C; MS (ESI) m/z (%): 569.2 $[M+H]^+$.

*5.3.2.3. (E)-1-(4-(Difluoromethoxy)phenyl)-N-((dimethylamino)methylene)-7-oxo-6-(4-(2-oxopiperidin-1-yl) phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide (**13c**)*

Light yellow solid; yield: 99.8%; M.p.: 179.4-180.6°C; MS (ESI) m/z (%): 551.2 $[M+H]^+$.

*5.3.2.4. (E)-N-((Dimethylamino)methylene)-1-(4-fluorophenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide (**13d**)*

Light yellow solid; yield: 97.0%; M.p.: 179.1-181.8°C; MS (ESI) m/z (%): 503.2 $[M+H]^+$.

*5.3.2.5. (E)-1-(4-Dhlorophenyl)-N-((dimethylamino)methylene)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide (**13e**)*

Light yellow solid; yield: 95.9%; M.p.: 179.9-181.7°C; MS (ESI) m/z (%): 519.2 $[M+H]^+$.

*5.3.2.6. (E)-1-(4-Bromophenyl)-N-((dimethylamino)methylene)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide (**13f**)*

Light yellow solid; yield: 93.0%; M.p.: 180.4-181.9°C; MS (ESI) m/z (%): 562.1 $[M+H]^+$.

*5.3.3. General procedure for the preparation of compounds **14a–14f***

A solution of intermediates **13a-13f** (1.54 g, 3.0 mmol) and hydrazine hydrate (1.5 g, 30.0 mmol) in glacial acetic acid (20.0 mL) was refluxed on an oil-bath for approximate 3 h. After cooling to ambient temperature, the contents were concentrated under reduced pressure, and the residue solution was adjusted to pH 9 with saturated sodium carbonate solution. The resulting precipitate was filtered, washed with ether, and dried under vacuum to afford the title compounds **14a-14f** .

*5.3.3.1. 1-(4-Methoxyphenyl)-6-(4-(2-oxopiperidin-1-yl)phenyl)-3-(1H-1,2,4-triazol-3-yl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one (**14a**)*

White solid; yield: 82.8%; M.p.: 176.1-178.2°C; MS (ESI) m/z (%): 505.7 $[M+Na]^+$; ^{1}H NMR (400 MHz, DMSO) δ 8.46 (s, 1H), 7.52 (d, *J* = 8.9 Hz, 2H), 7.37 (d, *J* = 8.7 Hz, 2H), 7.28 (d, *J* = 8.7 Hz, 2H), 7.01 (d, *J* = 9.0 Hz, 2H), 4.11 (t, *J* = 6.5 Hz, 2H), 3.81 (s, 3H), 3.59 (t, *J* = 5.4 Hz, 2H), 3.29 (t, *J* = 6.5 Hz, 2H), 2.39 (t, *J* = 6.2 Hz, 2H), 1.99-1.75 (m, 4H); ^{13}C NMR (101 MHz, DMSO) δ 169.34, 159.43, 157.21, 141.83, 140.36, 133.22, 133.03, 127.16, 126.79, 126.50, 123.68, 113.86, 55.92, 51.46, 51.30, 33.06, 23.46, 21.68, 21.36. Anal. calcd. for $C_{26}H_{25}N_7O_3$ (%): C, 64.58; H, 5.21; N, 20.28. Found (%): C, 64.61; H, 5.24; N, 20.30.

*5.3.3.2. 6-(4-(2-Oxopiperidin-1-yl)phenyl)-3-(1H-1,2,4-triazol-3-yl)-1-(4-(trifluoromethoxy)phenyl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one (**14b**)*

White solid; yield: 74.5%; M.p.: 174.4-176.5°C; MS (ESI) m/z (%): 560.0 $[M+Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.10 (s, 1H), 7.67 (d, *J* = 8.9 Hz, 2H), 7.33 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.6 Hz, 2H), 7.22 (d, *J* = 8.6 Hz, 2H), 4.10 (t, *J* = 6.5 Hz, 2H), 3.63-3.60 (m, 2H), 3.34 (t, *J* = 6.4 Hz, 2H), 2.60-2.57 (m, 2H), 1.95-1.93 (m, 4H); Anal. calcd. for $C_{26}H_{22}F_3N_7O_3$ (%): C, 58.10; H, 4.13; N, 18.24. Found (%): C, 58.13; H, 4.16; N, 18.30.

*5.3.3.3. 1-(4-(Difluoromethoxy)phenyl)-6-(4-(2-oxopiperidin-1-yl)phenyl)-3-(1H-1,2,4-triazol-3-yl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one **(14c)***

White solid; yield: 70.6%; M.p.: 174.3-176.7°C; MS (ESI) m/z (%): 542.0 $[M+Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.11 (s, 1H), 7.60 (d, *J* = 8.6 Hz, 2H), 7.33 (d, *J* = 8.7 Hz, 2H), 7.26 (d, *J* = 8.2 Hz, 2H), 7.11 (d, *J* = 8.6 Hz, 2H), 6.69-6.32 (s, 1H), 4.11 (t, *J* = 6.5 Hz, 2H), 3.62-3.60 (m, 2H), 3.34 (t, *J* = 6.4 Hz, 2H), 2.60-2.57 (m, 2H),

1.96-1.94 (m, 4H); Anal. calcd. for $C_{26}H_{23}F_2N_7O_3$ (%): C, 60.11; H, 4.46; N, 18.87. Found (%): C, 60.14; H, 4.48; N, 18.89.

5.3.3.4. 1-(4-Fluorophenyl)-6-(4-(2-oxopiperidin-1-yl)phenyl)-3-(1H-1,2,4-triazol-3-yl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one ***(14d)***

White solid; yield: 84.9%; M.p.: 186.5-188.7°C; MS (ESI) m/z (%): 493.9 $[M+Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.59 (dd, *J* = 8.8, 4.7 Hz, 2H), 7.34 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.6 Hz, 3H), 7.09 (t, *J* = 8.6 Hz, 2H), 4.13 (t, *J* = 6.5 Hz, 2H), 3.63-3.60 (m, 2H), 3.37 (t, *J* = 6.5 Hz, 2H), 2.60-2.57 (m, 2H), 1.96-1.94 (m, 4H); Anal. calcd. for $C_{25}H_{22}FN_7O_2$ (%): C, 63.69; H, 4.70; N, 20.80. Found (%): C, 63.71; H, 4.73; N, 20.82.

5.3.3.5. 1-(4-Chlorophenyl)-6-(4-(2-oxopiperidin-1-yl)phenyl)-3-(1H-1,2,4-triazol-3-yl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one ***(14e)***

White solid; yield: 82.1%; M.p.: 188.4-190.3°C; MS (ESI) m/z (%): 512.3 $[M+Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.57 (d, *J* = 8.7 Hz, 2H), 7.38–7.32 (m, 4H), 7.26（d, *J* = 8.7 Hz, 2H), 4.13 (t, *J* = 6.6 Hz, 2H), 3.63-3.60 (m, 2H), 3.36 (t, *J* = 6.6 Hz, 2H), 2.60-2.57 (m, 2H), 1.96-1.94 (m, 4H); Anal. calcd. for $C_{25}H_{22}ClN_7O_2$ (%): C, 61.54; H, 4.54; N, 20.09. Found (%): C, 61.57; H, 4.57; N, 20.11.

5.3.3.6. 1-(4-Bromophenyl)-6-(4-(2-oxopiperidin-1-yl)phenyl)-3-(1H-1,2,4-triazol-3-yl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one ***(14f)***

White solid; yield: 91.6%; M.p.: 185.5-187.2°C; MS (ESI) m/z (%): 554.1 $[M+Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.72 (d, *J* = 8.4 Hz, 0.7H), 7.54-7.49 (m, 2.6H), 7.38 (d, *J* = 8.6 Hz, 0.7H), 7.34 (d, *J* = 8.6 Hz, 2H), 7.27 (d, *J* = 8.5 Hz, 2H), 4.14 (t, *J* = 6.6 Hz, 2H), 3.63-3.61 (m, 2H), 3.37 (t, *J* = 6.6 Hz, 2H), 2.60–2.57 (m, 2H), 1.96-1.94 (m, 4H); Anal. calcd. for $C_{25}H_{22}BrN_7O_2$ (%): C, 56.40; H, 4.17; N, 18.42. Found (%): C, 56.44; H, 4.19; N, 18.45.

5.3.4. General procedure for the preparation of intermediates ***15–16***

NaH (0.03 g, 1.2 mmol) was added dropwise to the mixture of **14a** (0.2 g, 0.4 mmol) in dry DMF (9 mL) at 0°C. Then the mixture was warmed to 40°C for 5 h. The solution was adjusted to pH 7 with acetic acid. The reaction mixture was poured into ice-water (10 mL). The aqueous layer was extracted with ethyl acetate (3 ×10 mL). The organic phase was washed with water (3 × 10 mL), brine (20 mL). The organic layer was dried with Na_2SO_4, filtered and evaporated in vacuo to afford white solid. The solid was purified by silica gel column chromatography (eluent, ethyl acetate/MeOH = 80:1 to 50:1) to afford corresponding **15** and **16**.

5.3.4.1. 1-(4-Methoxyphenyl)-3-(1-methyl-1H-1,2,4-triazol-5-yl)-5-(4-(2-oxopiperidin-1-yl)phenyl)-1,5,6,7-tetrahydro-4H-pyrazolo[4,3-c]pyridin-4-one ***(15)***

White solid; Yield: 93.2%; M.p.: 183.3-185.1°C; MS (ESI) m/z (%): 498.5 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.46 (d, *J* = 8.9 Hz, 2H), 7.30 (d, *J* = 8.6 Hz, 2H), 7.19 (d, *J* = 3.6 Hz, 2H), 6.88 (d, *J* = 8.9 Hz, 2H), 4.23 (s, 3H), 4.09 (t, *J* = 7.0 Hz, 2H), 3.76 (s, 3H), 3.56 (t, *J* = 17.4 Hz, 2H), 3.36 (t, *J* = 6.6 Hz, 2H), 2.49 (t, *J* = 5.8 Hz, 2H), 1.85 (m, *J* = 13.0 Hz, 4H); Anal. calcd. for $C_{27}H_{27}N_7O_3$ (%): C, 65.18; H, 5.47; N, 19.71. Found (%): C, 65.20; H, 5.49; N, 19.74.

5.3.4.2. 1-(4-Methoxyphenyl)-3-(1-methyl-1H-1,2,4-triazol-3-yl)-5-(4-(2-oxopiperidin-1-yl)phenyl)-1,5,6,7-tetrahydro-4H-pyrrolo[3,4-c]pyridin-4-one ***(16)***

White solid; Yield: 93.2%; M.p.: 184.2-186.1°C; MS (ESI) m/z (%): 498.5 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (s, 1H), 7.50 (d, *J* = 8.9 Hz, 2H), 7.37 (d, *J* = 8.7 Hz, 2H), 7.28 (d, *J* = 8.7 Hz, 2H), 7.00 (d, *J* = 9.0 Hz,

2H), 4.10 (t, *J* = 6.5 Hz, 2H), 3.95 (s, 3H), 3.81 (s, 3H), 3.60 (t, *J* = 5.5 Hz, 2H), 3.27 (t, *J* = 6.5 Hz, 2H), 2.39 (t, *J* = 6.2 Hz, 2H), 1.92-1.78 (m, 4H); ^{13}C NMR (101 MHz, DMSO) δ 169.31, 159.31, 157.27, 156.72, 145.84, 141.81, 140.41, 140.17, 133.32, 132.75, 126.99, 126.78, 126.48, 123.68, 113.83, 55.90, 51.44, 51.30, 36.48, 33.06, 23.47, 21.83, 21.37. Anal. calcd. for $C_{27}H_{27}N_7O_3$ (%): C, 65.18; H, 5.47; N, 19.71. Found (%): C, 65.21; H, 5.46; N, 19.73.

5.3.5. Preparation of 1-(4-Methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carbonitrile ***17***

Triethylamine (0.13 mol, 18.1 mL) was added to a solution of **12a** (15.0 g, 33.0 mmol) in DCM (225.0 mL) at 30°C. After 30 min, trifluoroacetic anhydride (0.13 mol, 18.3 mL) was added dropwise to the reaction and stirred for 2 h. The organic layer was washed with water and filtered to give a solid. The residue was crystallized with methanol to afford the title compound **17** (10.5 g, 72.9%), M.p.: 171.3-173.6 °C； MS (ESI) m/z (%): 435.3 $[M+H]^+$.

5.3.6. General procedure for the preparation of compounds ***18a-18e***

A mixture of **17** (1.0 g, 2.3 mmol), K_2CO_3 (0.3 g, 2.3 mmol) and hydrazide (4.5 mmol) in n-butanol (10 mL) was refluxed for 20-30 h. The reaction mixture was poured into ice-water (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 ×10 mL). The organic phase was washed with water (3 × 10 mL), brine (20 mL). The organic layer was dried with Na_2SO_4, filtered and evaporated in vacuo to give the title compounds **18a-18e**.

5.3.6.1. *1-(4-Methoxyphenyl)-3-(5-methyl-1H-1,2,4-triazol-3-yl)-6-(4-(2-oxopiperidin-1-yl)phenyl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one (****18a****)*

Light yellow solid; Yield: 43.7%; M.p.: 172.2-174.5°C; MS (ESI) m/z (%): 520.2 $[M+Na]^+$; ^{1}H NMR(400 MHz, $CDCl_3$) *δ* 7.51 (d, *J* = 8.9 Hz, 2H), 7.33 (d, *J* = 8.6 Hz, 2H), 7.23 (d, *J* = 8.6 Hz, 2H), 6.89 (d, *J* = 8.9 Hz, 2H), 4.09 (t, *J* = 6.5 Hz, 2H), 3.80 (s, 3H), 3.62-3.59 (m, 2H), 3.31 (t, *J* = 6.5 Hz, 2H), 2.59-2.56 (m, 2H), 2.41 (s, 3H), 1.95-1.93 (m, 4H); Anal. calcd. for $C_{27}H_{27}N_7O_3$ (%): C, 65.18; H, 5.47; N, 19.71. Found (%): C, 65.22; H, 5.44; N, 19.75.

*5.3.6.2. 3-(5-(Methoxymethyl)-1H-1,2,4-triazol-3-yl)-1-(4-methoxyphenyl)-6-(4-(2-oxopiperidin-1-yl)phenyl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one (****18b****)*

Light yellow solid; Yield: 41.2%; M.p.: 164.4-166.3°C; MS (ESI) m/z (%): 549.5 $[M+Na]^+$, 526.1 $[M-H]^-$, 562.1 $[M+Cl]^-$; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.50 (d, *J* = 8.9 Hz, 2H), 7.34 (d, *J* = 8.7 Hz, 2H), 7.25 (d, *J* = 8.7 Hz, 2H), 6.89 (d, *J* = 8.9 Hz, 2H), 4.65 (s, 2H), 4.12 (t, *J* = 6.6 Hz, 2H), 3.80 (s, 3H), 3.61-3.58 (m, 2H), 3.48 (s, 3H), 3.38 (t, *J* = 6.6 Hz, 2H), 2.58-2.55 (m, 2H), 1.94-1.92 (m, 4H); Anal. calcd. for $C_{28}H_{29}N_7O_4$ (%): C, 63.74; H, 5.54; N, 18.58. Found (%): C, 63.76; H, 5.56; N, 18.57.

*5.3.6.3. 3-(5-(Tert-butyl)-1H-1,2,4-triazol-3-yl)-1-(4-methoxyphenyl)-6-(4-(2-oxopiperidin-1-yl)phenyl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one (****18c****)*

Light yellow solid; Yield: 40.3%; M.p.: 172.6-174.4°C; MS (ESI) m/z (%): 562.2 $[M+Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.49 (d, *J* = 8.7 Hz, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.24 (d, *J* = 8.4，2H), 6.90 (d, *J* = 8.7 Hz, 2H), 4.13 (t, *J* = 6.5 Hz, 2H), 3.80 (s, 3H), 3.60 (s, 2H), 3.39 (t, *J* = 6.4 Hz, 2H), 2.56 (s, 2H), 1.93 (s, 4H), 1.44 (s, 9H); Anal. calcd. for $C_{30}H_{33}N_7O_3$ (%): C, 66.77; H, 6.16; N, 18.17. Found (%): C, 66.79; H, 6.19; N, 18.19.

5.3.6.4. 3-(5-Cyclopropyl-1H-1,2,4-triazol-3-yl)-1-(4-methoxyphenyl)-6-(4-(2-oxopiperidin-1-yl)phenyl)-1,4,5,6-

*tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one (**18d**)*

Light yellow solid; Yield: 41.6%; M.p.: 170.4-172.1°C; MS (ESI) m/z (%): 546.1 $[M+Na]^+$, 562.0 $[M+K]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 8.6 Hz, 2H), 6.89 (d, J = 8.7 Hz, 2H), 4.10 (t, J = 5.8 Hz, 2H), 3.79 (s, 3H), 3.60 (s, 2H), 3.38-3.31 (m, 2H), 2.56 (s, 2H), 1.94 (s, 4H), 1.25 (s, 1H), 1.09-1.08 (m, 2H), 0.98-0.96 (m, 2H); Anal. calcd. for $C_{29}H_{29}N_7O_3$ (%): C, 66.52; H, 5.58; N, 18.73. Found (%): C, 66.54; H, 5.60; N, 18.74.

*5.3.6.5. 1-(4-Methoxyphenyl)-6-(4-(2-oxopiperidin-1-yl)phenyl)-3-(5-(tetrahydrofuran-2-yl)-1H-1, 2, 4-triazol-3-yl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one (**18e**)*

Light yellow solid; Yield: 39.3%; M.p.: 169.4-171.7°C; MS (ESI) m/z (%): 553.6 $[M+H]^+$, 575.6 $[M+Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, J = 8.9 Hz, 2H), 7.35 (d, J = 8.7 Hz, 2H), 7.24 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 8.9 Hz, 2H), 5.18 (dd, J = 7.4, 6.0 Hz, 1H), 4.13 (t, J = 6.6 Hz, 2H), 4.08 (dd, J = 14.7, 6.8 Hz, 1H), 3.96 (dd, J = 15.0, 7.1 Hz, 1H), 3.80 (s, 3H), 3.61-3.58 (m, 2H), 3.38 (dd, J = 7.0, 5.4 Hz, 2H), 2.57-2.54 (m, 2H), 2.45-2.38 (m, 1H), 2.35-2.27 (m, 1H), 2.04 (dd, J = 14.0, 7.0 Hz, 2H), 1.94-1.91 (m, 4H); Anal. calcd. for $C_{30}H_{31}N_7O_4$ (%): C, 65.09; H, 5.64; N, 17.71. Found (%): C, 65.10; H, 5.66; N, 17.74.

5.3.7. *Preparation of 1-(4-Methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carbohydrazide (**19**)*

A solution of **11a** (10.0 g, 20.0 mmol) in hydrazine hydrate (60.0 mL) was stirred at 80°C for 3.5 h. The hydrazine hydrate was distilled. Solid was filtered and washed with water to afford **19** (7.8 g, 80.3%), M.p.: 168.3-170.6 °C; MS (ESI) m/z (%): 497.2 $[M+Na]^+$.

*5.3.8. Preparation of (Z)-N'-(1-(4-methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carbonyl)-N,N-dimethylformohydrazonamide (**20**)*

DMF-DMA (0.1 mol, 14.0 mL) was added to a stirring solution of **19** (8.3 g, 18.0 mmol) in DCM (160 mL) and heated to reflux for 4 h. The reaction mixture was poured into ice-water (150 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The organic phase was washed with water (3 × 50 mL), brine (50 mL) and dried with Na_2SO_4, filtered and evaporated in vacuo.The yellow residue was purified by silica gel column chromatography to give a white solid (7.5 g, 81.0%), M.p.: 169.3-171.4°C; MS (ESI) m/z (%): 530.24 $[M+H]^+$.

*5.3.9. General procedure for the preparation of compounds **21a-21d***

Compound **20** (0.3 g, 0.57 mmol) was dissolved with acetic acid (6 mL). Then appropriate amine (3 mL) was added dropwise to the solution and heated to 90 °C for 10 h. The solution was poured into ice-water (5 mL), and then adjusted to pH 9 with saturated sodium carbonate. The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was washed with water (3 × 10 mL), brine (20 mL) and dried with Na_2SO_4, filtered and evaporated in vacuo to give the title compounds **21a-21d**.

*5.3.9.1. 3-(4-(2-(Dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)-1-(4-methoxyphenyl)-6-(4-(2-oxopiperidin-1-yl) phenyl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one (**21a**)*

Light yellow solid; Yield: 60.1%; M.p.: 164.3-166.4°C; MS (ESI) m/z (%): 555.1 $[M+H]^+$, 577.2, 578.1 $[M+Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.50 (d, J = 8.9 Hz, 2H), 7.37 (d, J = 8.6 Hz, 2H), 7.26 (d, J = 8.6 Hz, 2H), 6.94 (d, J = 8.9 Hz, 2H), 4.60 (t, J = 6.5 Hz, 2H), 4.17 (t, J = 6.6 Hz, 2H), 3.83 (s, 3H), 3.62-3.59 (m, 2H), 3.50 (t, J = 6.6 Hz, 2H), 2.77 (t, J = 6.4 Hz, 2H), 2.57-2.54 (m, 2H), 2.28 (s, 6H), 1.94-1.93 (m, 4H); Anal. calcd. for $C_{30}H_{34}N_8O_3$ (%): C, 64.96; H, 6.18; N, 20.20. Found (%): C, 64.99; H, 6.17; N, 20.23.

*5.3.9.2. 3-(4-(3-(Dimethylamino)propyl)-4H-1,2,4-triazol-3-yl)-1-(4-methoxyphenyl)-6-(4-(2-oxopiperidin-1-yl) phenyl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one (**21b**)*

Light yellow solid; Yield: 55.6%; M.p.: 160.5-162.8°C; MS (ESI) m/z (%): 568.0 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.51 (d, *J* = 8.9 Hz, 2H), 7.37 (d, *J* = 8.7 Hz, 2H), 7.26 (d, *J* = 8.7 Hz, 2H), 6.94 (d, *J* = 8.9 Hz, 2H), 4.54 (t, *J* = 6.8 Hz, 2H), 4.17 (t, *J* = 6.6 Hz, 2H), 3.83 (s, 3H), 3.62-3.59 (m, 2H), 3.50 (t, *J* = 6.7 Hz, 2H), 2.56-2.55 (m, 2H), 2.27 (t, *J* = 6.7 Hz, 2H), 2.19 (s, 6H), 2.05-2.01 (m, 2H), 1.94-1.93 (m, 4H); Anal. calcd. for $C_{31}H_{36}N_8O_3$ (%): C, 65.47; H, 6.38; N, 19.70. Found (%): C, 65.49; H, 6.40; N, 19.73.

*5.3.9.3. 3-(4-(3-(Diethylamino)propyl)-4H-1,2,4-triazol-3-yl)-1-(4-methoxyphenyl)-6-(4-(2-oxopiperidin-1-yl) phenyl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one (**21c**)*

Light yellow solid; Yield: 58.9%; M.p.: 161.4-163.7°C; MS (ESI) m/z (%): 596.3 $[M+H]^+$; ^{1}H NMR(400 MHz, $CDCl_3$) δ 8.27(s, 1H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.7 Hz, 2H), 6.94 (d, *J* = 8.9 Hz, 2H), 4.53 (t, *J* = 7.0 Hz, 2H), 4.17 (t, *J* = 6.6 Hz, 2H), 3.83 (s, 3H), 3.62-3.59 (m, 2H), 3.50 (t, *J* = 6.6 Hz, 2H), 2.61-2.46 (m, 8H), 2.11-2.04 (m, 2H), 1.95-1.93 (m, 4H), 0.98 (t, *J* = 7.1 Hz, 6H); Anal. calcd. for $C_{33}H_{40}N_8O_3$ (%): C, 66.42; H, 6.76; N, 18.78. Found (%): C, 66.44; H, 6.78; N, 18.80.

*5.3.9.4. 1-(4-Methoxyphenyl)-3-(4-(3-morpholinopropyl)-4H-1,2,4-triazol-3-yl)-6-(4-(2-oxopiperidin-1-yl)phenyl) -1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one (**21d**)*

Light yellow solid; Yield: 57.5%; M.p.: 156.3-158.6°C; MS (ESI) m/z (%): 610.9 $[M+H]^+$, 632.8 $[M+Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.49 (d, *J* = 8.9 Hz, 2H), 7.37 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.7 Hz, 2H), 6.94 (d, *J* = 8.9 Hz, 2H), 4.54 (t, *J* = 6.8 Hz, 2H), 4.17 (t, *J* = 6.6 Hz, 2H), 3.83 (s, 3H), 3.66-3.57 (m, 6H), 3.49 (t, *J* = 6.6 Hz, 2H), 2.57-2.54 (m, 2H), 2.40-2.31 (m, 6H), 2.10-2.03 (m, 2H), 1.94-1.93 (m, 4H); Anal. calcd. for $C_{33}H_{38}N_8O_4$ (%): C, 64.90; H, 6.27; N, 18.35. Found (%): C, 64.93; H, 6.29; N, 18.37.

5.4. The preparation of Apixaban derivatives of series II: 3-*substituted-1,2,4-triazolin-5-one derivatives **23a-23c***

*5.4.1 General procedure for preparation of **22a–22c***

A solution of substituted phenyl isocyanates (0.25 g, 0.0021 mol) was added to a solution of **19** (1.0 g, 0.0019 mol) in THF (10.0 mL). The resulting reaction mixture was heated to 50°C for 3 h. After cooling to r.t, the precipitate was collected by filtration, washed with diethyl ether and dried to afford **22a-22c**.

*5.4.1.1. 2-(1-(4-Methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c] pyridine-3-carbonyl)-N-phenylhydrazine-1-carboxamide (**22a**)*

White solid; Yield: 90%; M.p.: 176.3-178.2°C; MS (ESI) m/z (%): 594.2 $[M+H]^+$

*5.4.1.2. N-(4-Methoxyphenyl)-2-(1-(4-methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro -1H-pyrazolo[3,4-c]pyridine-3-carbonyl)hydrazine-1-carboxamide (**22b**)*

White solid; Yield: 90%; M.p.: 177.3-179.5°C; MS (ESI) m/z (%): 624.2 $[M+H]^+$

*5.4.1.3. 2-(1-(4-Methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c] pyridine-3-carbonyl)-N-(2-(trifluoromethyl)phenyl)hydrazine-1-carboxamide (**22c**)*

White solid; Yield: 90%; M.p.: 175.6-177.7°C; MS (ESI) m/z (%): 662.2 $[M+Na]^+$

*5.4.2 General procedure for preparation of **23a–23c***

K_2CO_3 (0.3 g, 2.4 mmol) was added to a solution of **22a-22c** (1.7 mmol) in DMSO (6 mL). The reaction mixture was stirred at 80°C for 6 h. After cooling to r.t., the mixture was poured into water with vigorously

stirring. The resulting precipitate was filtered, washed with water and dried under vacuum to afford **23a–23c**.

*5.4.2.1. 1-(4-Methoxy-phenyl)-3-(5-oxo-4-phenyl-4,5-dihydro-1H-[1,2,4]triazol-3-yl)-6-[4-(2-oxo-piperidin-1-yl)-phenyl]-1,4,5,6-tetrahydro-pyrazolo[3,4-c]pyridin-7-one (**23a**)*

White solid; Yield: 90%; M.p.: 184.2-190.6°C; MS (ESI) m/z (%): 598.1 $[M+Na]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 10.27 (s, 1H, triazol-H), 7.83 (d, J = 8.8 Hz, 2H, Ar-H), 7.58 (d, J = 8.9 Hz, 2H, Ar-H), 7.35-7.27 (m, 5H, Ar-H), 7.09 (d, J = 8.8 Hz, 2H, Ar-H), 7.03 (d, J = 8.8 Hz, 2H, Ar-H), 4.09 (t, J = 6.6 Hz, 2H, pyrazolo pyridine N-CH_2), 3.82 (s, 3H, OCH_3), 3.60 (t, J = 5.5 Hz, 2H, piperidone N-CH_2), 3.27 (t, J = 6.5 Hz, 2H, pyrazolo pyridine=C-CH_2), 2.39 (t, J = 6.2 Hz, 2H, OC-CH_2), 1.85–1.75 (m, 4H, CH_2CH_2); Anal. calcd. for $C_{32}H_{29}N_7O_4$ (%): C, 66.77; H, 5.08; N, 17.03. Found (%): C, 66.79; H, 5.10; N, 17.04.

*5.4.2.2. 1-(4-Methoxy-phenyl)-3-[4-(4-methoxy-phenyl)-5-oxo-4,5-dihydro-1H-[1,2,4]triazol-3-yl]-6-[4-(2-oxo-piperidin-1-yl)-phenyl]-1,4,5,6-tetrahydro-pyrazolo[3,4-c]pyridin-7-one(**23b**)*

White solid; Yield: 92%; M.p.: 186.2-194.5°C; MS (ESI) m/z (%): 605.6 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 10.23 (s, 1H, triazol-H), 7.57 (d, J = 8.9 Hz, 2H, Ar-H), 7.45 (d, J = 8.2 Hz, 2H, Ar-H), 7.36 (d, J = 8.6 Hz, 2H, Ar-H), 7.28 (d, J = 8.7 Hz, 2H, Ar-H), 7.22 (d, J = 8.1 Hz, 2H, Ar-H), 7.02 (d, J = 8.9 Hz, 2H, Ar-H), 4.09 (t, J = 6.5 Hz, 2H, pyrazolo pyridine N-CH_2), 3.81 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.59 (t, J = 5.3 Hz, 2H, piperidone N-CH_2), 3.26 (t, J = 6.5 Hz, 2H, pyrazolo pyridine=C-CH_2), 2.39 (t, J= 6.1 Hz, 2H, OC-CH_2), 1.84-1.75(m, 4H,CH_2CH_2); Anal. calcd. for $C_{33}H_{31}N_7O_5$ (%): C, 65.44; H, 5.16; N, 16.19. Found (%): C, 65.46; H, 5.15; N, 16.18.

*5.4.2.3. 1-(4-Methoxy-phenyl)-6-[4-(2-oxo-piperidin-1-yl)-phenyl]-3-[5-oxo-4-(2-trifluoromethyl-phenyl)-4,5-dihydro-1H-[1,2,4]triazol-3-yl]-1,4,5,6-tetrahydro-pyrazolo[3,4-c]pyridin-7-one(**23c**)*

White solid; Yield: 89%; M.p.: 186.4-194.1°C; MS (ESI) m/z (%): 644.1 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 9.85 (s, 1H, triazol-H), 7.88-7.70 (m, 3H, Ar-H), 7.56 (d, J = 8.9 Hz, 2H, Ar-H), 7.49 (s, 1H, Ar-H), 7.36 (d, J = 8.7 Hz, 2H, Ar-H), 7.29 (d, J = 8.7 Hz, 2H, Ar-H), 7.04 (d, J = 9.0 Hz, 2H, Ar-H), 4.09 (t, J = 6.5 Hz, 2H, pyrazolo pyridine N-CH_2), 3.81 (s, 3H, OCH_3), 3.59 (t, J = 5.5 Hz, 2H, piperidone N-CH_2), 3.24 (t, J = 6.5 Hz, 2H, pyrazolo pyridine=C-CH_2), 2.39 (t, J = 6.3 Hz, 2H, OC-CH_2), 1.85-1.75(m, 4H, CH_2CH_2); Anal. calcd. for $C_{33}H_{28}F_3N_7O_4$ (%): C, 61.58; H, 4.39; N, 15.23. Found (%): C, 61.59; H, 4.36; N, 15.25.

*5.5. The preparation of Apixaban derivatives of series III: pyrazole derivatives **26a-26c***

*5.5.1. Preparation of 3-Amino-1-(4-methoxyphenyl)-6-(4-(2-oxopiperidin-1-yl)phenyl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one (**24**)*

A mixture of **12a** (3.0 g, 6 mmol), Potassium hydroxide (0.9 g, 16.0 mmol), iodobenzene diacetate (4.2 g, 15.0 mmol) and methanol (60 mL) was stirred at r.t. for 5 h. After evaporating in vacuum, the residue was dissolved in a solution of H_2O (30.0 mL), dioxane (60.0 mL) and NaOH (0.8 g, 0.02 mol). The mixture was heated to 100°C for 6 h. The residue was treated with water 10 mL, extracted with CH_2Cl_2 (3 × 10 mL), washed with brine (2 × 10 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to afford **24** (2.3 g, 62.4%), M.p.: 178.4-179.9°C; MS (ESI) m/z (%): 432.3 $[M+H]^+$.

*5.5.2. Preparation of 1-(4-Methoxyphenyl)-6-(4-(2-oxopiperidin-1-yl)phenyl)-3-(1H-pyrrol-1-yl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one (**25**)*

24 (1.0 g, 2.8 mmol) and catalytic amount of *p*-toluenesulfonic acid (0.01g, 0.06mmol) were added to a solution of 2,5-dimethoxytetrahydrofuran (0.34 mL, 2.3 mmol) in THF. The mixture was heated to 60°C for 1 h. After cooling to r.t., the solution was exacted by CH_2Cl_2. The organic layer was washed by water and then dried

under vacuum to yield **25** (0.73 g, 70.0%), M.p.: 178.6-180.3°C; MS (ESI) m/z (%): 504.1 $[M+Na]^+$.

*5.5.3. General procedure for preparation of **26a–26c**.*

A mixture of corresponding secondary amines (5.2 mmol), 37% formaldehyde solution (0.2 g, 2.2 mmol) and **25** (1 g, 2.0 mmol) was added in a sequence to a well-stirred solution of acetic acid (20.0 mL) at r.t.. The mixture was stirred for 1 h. The residue was poured into water and adjusted pH to 8 by 10% NaOH in ice bath. The separated solid was collected by filtration and the residue was dried to afford **26a–26c**.

*5.5.3.1. 3-(2-((Dimethylamino)methyl)-1H-pyrrol-1-yl)-1-(4-methoxyphenyl)-6-(4-(2-oxopiperidin-1-yl)phenyl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one (**26a**)*

White solid; Yield: 67%; M.p.: 178.4-179.6°C; MS (ESI) m/z (%): 539.2 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, *J* = 8.9 Hz, 2H), 7.35 (d, *J* = 8.7 Hz, 2H), 7.25 (d, *J* =8.7 Hz 2H), 6.92 (d, *J* =8.9 Hz, 2H), 6.88 (dd, *J* = 2.5, 1.8 Hz, 1H), 6.32 (d, 1.8 Hz, 1H), 6.29 (d, *J* = 2.5 Hz, 1H), 4.11 (t, *J* = 6.5 Hz, 2H), 3.81 (s, 3H), 3.76 (s, 2H), 3.59 (t, *J* = 5.3 Hz, 2H), 3.00 (t, *J* =5.3 Hz, 2H), 2.55 (t, *J* = 5.5 Hz, 2H), 2.28 (s, 6H), 1.95-1.92 (m, 4H); Anal. calcd. for $C_{31}H_{34}N_6O_3$ (%): C, 69.12; H, 6.36; N, 15.60. Found (%): C, 69.14; H, 6.38; N, 15.61.

*5.5.3.2. 3-(2-((Diethylamino)methyl)-1H-pyrrol-1-yl)-1-(4-methoxyphenyl)-6-(4-(2-oxopiperidin-1-yl)phenyl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one (**26b**)*

White solid; Yield: 65%; M.p.: 179.1-180.6°C; MS (ESI) m/z (%): 567.2 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, *J* = 8.9 Hz, 2H), 7.35 (d, *J* = 8.7 Hz, 2H), 7.25 (d, *J* =8.7 Hz, 2H), 6.91 (d, *J* = 8.9 Hz, 2H), 6.86 (dd, *J* = 2.9, 1.6 Hz 1H), 6.34 (d, *J* =1.6 Hz 1H), 6.28 (d, *J* = 2.9 Hz, 1H), 4.12 (t, *J* = 6.5 Hz, 2H), 3.91 (s, 2H), 3.81 (s, 3H), 3.60 (t, *J* = 6.5 Hz ,2H), 2.97 (t, *J* = 6.5 Hz, 2H), 2.58 (d, *J* = 6.4 Hz, 4H), 2.55 (t, *J* = 3.1 Hz, 2H), 1.94-1.92 (m, 4H), 0.98 (t, *J* =6.4 Hz, 6H); Anal. calcd. for $C_{33}H_{38}N_6O_3$ (%): C, 69.94; H, 6.76; N, 14.83. Found (%): C, 69.96; H, 6.75; N, 14.84.

*5.5.3.3. 1-(4-Methoxyphenyl)-3-(2-(morpholinomethyl)-1H-pyrrol-1-yl)-6-(4-(2-oxopiperidin-1-yl)phenyl)-1,4,5,6-tetrahydro-7H-pyrazolo[3,4-c]pyridin-7-one (**26c**)*

Light yellow solid; Yield: 65%; M.p.: 178.7-181.9°C; MS (ESI) m/z (%): 581.5 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7. 49 (d, *J* = 9.0 Hz, 2H), 7.35(d, *J* = 8.6 Hz ,2H), 7.28 (d, *J* =8.5 Hz 2H), 7.26 (dd, *J* = 2.4,1.8 Hz, 1H), 6.92 (d, *J* = 9.0 Hz, 2H), 6.88 (d, *J* = 1.8 Hz, 1H), 6.28 (d, *J* = 2.4 Hz ,1H), 4.12 (t, *J* = 6.5 Hz, 2H), 3.81 (s, 3H), 3.71 (m,6H), 3.61 (t, *J* = 6.5 Hz ,2H), 3.00 (t, *J* = 6.5 Hz, 2H), 2.56 (t, *J* = 5.2 Hz, 4H), 2.46 (t, *J* = 5.5 Hz, 2H), 1.95-1.92 (m, 4H); Anal. calcd. for $C_{33}H_{36}N_6O_4$ (%): C, 68.26; H, 6.25; N, 14.47. Found (%): C, 68.27; H, 6.28; N, 14.45.

*5.6. Synthesis of Apixaban derivatives of series IV (compounds **35a-35b**)*

*5.6.1. Procedure for the preparation of compound **32a***

The preparation of the intermediate **32a** was similar to **8a**, so their synthetic methods was not be listed here.

*5.6.1.1. 5-Chloro-N-(4-cyanophenyl)pentanamide (**27**)*

White solid. M.p.: 121.7-123.4°C; MS (ESI) m/z (%): 237.07 $[M+H]^+$.

*5.6.1.2. 4-(2-Oxopiperidin-1-yl)benzonitrile (**28**)*

White solid. M.p.: 101.4-103.3°C; MS (ESI) m/z (%): 201.09 $[M+H]^+$.

*5.6.1.3. 4-(3,3-Dichloro-2-oxopiperidin-1-yl)benzonitrile (**29**)*

White solid. M.p.: 171.4-173.3°C; MS (ESI) m/z (%):269.02 $[M+H]^+$.

*5.6.1.4. 4-(5-Morpholino-6-oxo-3,6-dihydropyridin-1(2H)-yl)benzonitrile (**30**)*

White solid. M.p.: 157.1-159.3°C; MS (ESI) m/z (%): 284.13 $[M+H]^+$.

*5.6.1.5. Ethyl6-(4-Cyanophenyl)-1-(4-methoxyphenyl)-7a-morpholino-7-oxo-3a,4,5,6,7,7a-hexahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**31a**)*

Yellow solid. M.p.: 176.4-178.3°C; MS (ESI) m/z (%): 504.22 $[M+H]^+$.

*5.6.1.6. Ethyl6-(4-Cyanophenyl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (**32a**)*

Yellow solid. M.p.: 165.5-167.3°C; MS (ESI) m/z (%): 417.15 $[M+H]^+$.

*5.6.2. Procedure for the preparation of compound **33a***

A mixture of **32a** (1.0 g, 2.4 mmol) , NaOH (0.29 g, 7.3 mmol) in the solution of methanol and water (methanol : water = 5 : 1) was stirred at 60°C for 3 h. The solvent was removed under vacuum, and water (20 mL) was added. The solution was adjusted to pH 4-5 with 3N hydrochloric acid, filtered and dried to afford a white solid.

*5.6.2.1. 6-(4-Cyanophenyl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylic acid (**33a**)*

White solid; M.p.: 168.3-170.3°C; MS (ESI) m/z (%): 387.12 $[M-H]^-$.

*5.6.3. Procedure for the preparation of compound **34a***

TEA (1.4 mL) and ethyl chloroformate (1 mL) were added to the solution of compound **33a** (2.7 g, 7.7 mmol) in THF (5 mL) at 15°C. The mixture was warmed to r.t. for 3 h. Then NH_4OH (15 mL) was added to the reaction and stirred for another 2 h. The mixture was then poured into water (30 mL) and stirred for 30 min. The precipitate was filtered, washed with water and dried to give the title compound **34a**.

*5.6.3.1. 6-(4-Cyanophenyl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide (**34a**)*

White solid; M.p.: 170.3-172.3°C; MS (ESI) m/z (%): 388.13 $[M+H]^+$.

*5.6.4. General procedure for the preparation of compounds **35a-35b***

A stirring mixture of an appropriate **34a** (0.2 g, 0.5 mmol), ethylenediamine or propanediamine (1 mL) and S (0.13 mmol) was refluxed for 0.5 h. After cool to r.t., water was added to the solution and stirred for 30 min. The precipitate was filtered to give the corresponding **35a-35b** as a white solid.

*5.6.4.1. 6-(4-(4,5-Dihydro-1H-imidazol-2-yl)phenyl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide (**35a**)*

White solid; M.p.: 170.3-172.5°C; MS (ESI) m/z (%): 431.18 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.79 (s, 2H), 8.07 (d, J = 8.8 Hz, 2H), 7.76 (d, 2H), 7.63 (d, J = 8.8 Hz, 2H), 7.49 (d, J = 5.7 Hz, 2H), 6.99 (d, J = 9.0 Hz, 2H), 4.14 (t, J = 6.5 Hz, 2H), 3.96 (d, 2H), 3.97-3.93 (d, 2H), 3.78 (s, 3H), 3.20 (t, J = 6.4 Hz, 2H); ^{13}C NMR (101 MHz, DMSO) δ 163.63, 163.50, 159.62, 156.96, 144.13, 141.97, 133.40, 133.03, 128.47, 127.73, 127.34, 125.75, 125.56, 113.88, 55.73, 50.96, 21.30, 14.10. Anal. calcd. for $C_{23}H_{22}N_6O_3$ (%): C, 64.18; H, 5.15; N, 19.52. Found

(%): C, 64.19; H, 5.17; N, 19.54.

*5.6.4.2. 1-(4-Methoxyphenyl)-7-oxo-6-(4-(1,4,5,6-tetrahydropyrimidin-2-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide (**35b**)*

White solid; M.p.: 171.2-173.1°C; MS (ESI) m/z (%): 445.19 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 2H), 7.81 (d, J = 8.6 Hz, 2H), 7.76 (s, 1H), 7.60 (d, J = 8.6 Hz, 2H), 7.51 (d, J = 7.4 Hz, 2H), 7.01 (d, J = 9.0 Hz, 2H), 4.13 (t, J = 6.5 Hz, 2H), 3.81 (s, 3H), 3.50 (t, 2H), 3.45 (t, J = 5.7 Hz, 2H), 3.23 (t, J = 6.5 Hz, 2H), 1.97 (m, J = 5.2 Hz, 2H); Anal. calcd. for $C_{24}H_{24}N_6O_3$ (%): C, 64.85; H, 5.44; N, 18.91. Found (%): C, 64.86; H, 5.47; N, 18.90.

5.7 Pharmacology

5.7.1. In vitro coagulation assays

PT and APTT were measured using commercially available kits. Blood was obtained from healthy human volunteers or rabbits and anticoagulated with 3.8% sodium citrate. Plasma was obtained after centrifugation at 2000 g for 10 min. An initial stock solution of the inhibitor was prepared in DMSO. Subsequent dilutions were done. Clotting time was determined using the control plasma and plasma containing five to seven different concentrations of the inhibitor. PT measurement was performed in a temperature-controlled automated coagulation device (SysmexCA50, Dade-Behring) using a Thromborel-S (Dade Behring) kit according to the reagent instructions. Determinations at each plasma concentration were done in duplicate. [24]

5.7.2. In vitro human FXa inhibition assays

The potent inhibitory activity of six test compounds against human FXa was evaluated by using chromogenic substrate in 96-well microtiter plate. The enzymatic action of human factor Xa was measured using the conversion of a chromogenic substrate specific for FXa. FXa cleaved *p*-nitroaniline from the chromogenic substrate.

Table 4 Setting up reactions for human FXa inhibiton assay

Mix step(10 μL)	Sample	Negative Control	Positive Control
Compounds	5 μL	5 μL 10% DMSO/buffer	5 μL 10% DMSO/buffer
Human FXa	5 μL	0 μL	5 μL
Seal plate and incubate at 25ºC 10 min			
Enzyme step(40 μL)			
Tris-buffer	35 μL	40 μL	35 μL
Pefachrome FXa	5 μL	5 μL	5 μL
Seal plate and incubate 20 min at 25ºC			

5.7.3 Data Analysis

Determination of the inhibition rate of compounds was as follows:

Inhibition (%) = (ODpositive[a] -ODsample[b])/(ODpositive-ODnegative[c]) × 100%

[a] ODpositive: absorbance of positive wells (FXa + Substrate); [b] ODsample: absorbance of compound wells (FXa + Substrate +Compound); [c] ODnegative: absorbance of negative wells (Buffer + Substrate)

The IC_{50} curves were generated along with the IC_{50} values by using GraphPad Prism 5.0 software.

The IC_{50} value was obtained by plotting the anti-FXa activity against the inhibitor concentration.

5.7.4. In vivo experimental vein thrombosis

Vein thrombosis was induced according to Reyers *et al*.[25] with modifications. Briefly, the animals were anesthetized with pentobarbital (40 mg/kg, i.p.) and the abdomen was opened. The venacava inferior was carefully separated from surrounding tissues and ligated tightly with a cotton thread just below the left renal vein. The

abdomen was then closed with a double layer of sutures. After 2 h, the abdomen was reopened, and the vena cava was dissected longitudinally and the formed thrombus was removed. The obtained thrombi were kept at 37 °C for 24 h and after this time, their dry weight was measured.

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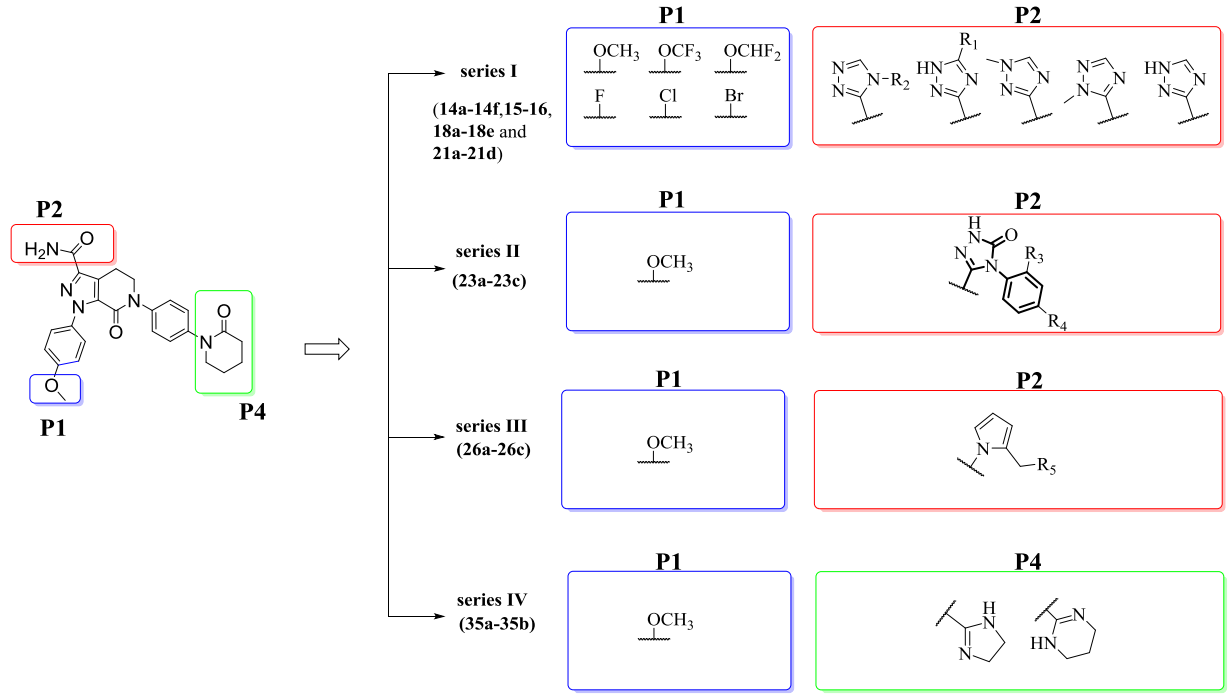
Graphical abstract



Four series of Apixaban derivatives containing 1, 2, 4-triazole/ pyrrole moietys as P2 binding element and dihydroimidazole/tetrahydropyrimidine groups as P4 binding element were designed, synthesized and evaluated for their biological activity.