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Design, synthesis and evaluation of isoxazolo[5,4-*d*]pyrimidin-4(5*H*)-one derivatives as antithrombotic agents

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ABSTRACT

A series of isoxazolo[5,4-*d*]pyrimidin-4(5*H*)-one derivatives have been designed and synthesized as novel antithrombotic agents. The 4-acetoxyl substituted derivative (**6g**) displays very strong FXa inhibitory activity (IC_{50} = 0.013 μM), excellent anticoagulant effect in human plasma (2 × PT = 2.12 μM) and high selectivity to thrombin and trypsin. Docking investigation of **6g** with FXa protein revealed that the pyrimidone ring of **6g** formed a π–π interaction with the phenyl ring of Tyr99, and the carbonyl group in the P1 moiety formed multiple hydrogen bonds to Ser214 and Trp215. These results showed that isoxazolo[5,4-*d*]pyrimidin-4(5*H*)-one is an attractive scaffold for designing novel factor Xa inhibitors and 4-carbonyl substituted phenyl ring could be used as novel S1 binding element.

Thromboembolic disorders, such as stroke, unstable angina, myocardial infarction, deep venous thrombosis (DVT) and pulmonary embolism (PE), are major causes of mortality and morbidity with high incidence in the current society.[1–4] Due to a variety of therapeutic disadvantages of antithrombotic drugs on the market such as bleeding, drug-drug interactions and injection, there is a growing need for the development of new antithrombotic agents with better effectiveness, higher safety profile or greater ease of use.[5,6] Over the past decade, the selective inhibition of specific coagulation enzymes has been considered to be an ideal strategy for the discovery and development of new antithrombotic agents. Among these enzymes, coagulation factors IIa (thrombin, FIIa) and Xa (FXa), both located in the common pathway of coagulation cascade, attracted the greatest attentions.[7] Thrombin has several thrombotic functions including the conversion of fibrinogen into fibrin, the activation of platelets, and the feedback activation of other coagulation factors which results in more thrombin molecules.[8] Thus, inhibition of thrombin can affect a number of physiologically relevant responses. FXa has a more specific role in comparison, which only catalyzes the generation of thrombin. Therefore, inhibition of FXa prevents the activation of prothrombin to thrombin without affecting existing thrombin levels. The remaining thrombin should be sufficient to ensure primary hemostasis, reducing bleeding risk due to no excess thrombin.[9–12] Recent researches have shown that FXa inhibitors cause less bleeding complications.[13] In addition, FXa is located at the upstream of thrombin in the coagulation cascade, which suggests that inhibition of FXa may be more effective in blocking the progression of coagulation than inhibition of thrombin.[14–17]

Over the past decade, enormous efforts have been made to discover and develop novel antithrombotic agents via a direct inhibition of FXa. Several FXa-based drugs such as Rivaroxaban,[18] Apixaban[19] and Edoxaban[20], have been successfully developed (Fig. 1).

The biaryl-substituted isoxazoline derivatives (**I**) were first synthesized and evaluated as potent FXa inhibitors by DuPont Pharmaceuticals Company.[21–24] Subsequent studies proved that the change of carboxamides from 5-position of isoxazoline to 4-position resulted in greater affinity for FXa and the replacement of isoxazoline by a planar isoxazolo core (**II**) could further enhance the affinity[25] (Fig. 2). The lack of chirality of the isoxazole and its high affinity for FXa made it to be an attractive template for further optimization. Pinto et al. optimized the core using other five-membered heterocyclic rings, which led to the discovery of pyrazole core and consequent anticoagulant drug Apixaban containing the bicyclic pyrazole core.[19,26–30]

Although the monocyclic isoxazolo analogues and their deuterogenic bicyclic pyrazole derivatives have showed a good FXa inhibitory activity, the bicyclic isoxazolo scaffold has not been reported. The bicyclic isoxazolo scaffold not only is expected to possess a higher affinity for FXa but also could prevent the degradation of the carboxamido linker, which can generate a mutagenic aniline fragment in vivo.[19,31,32] In this study, we cyclized the carboxamido

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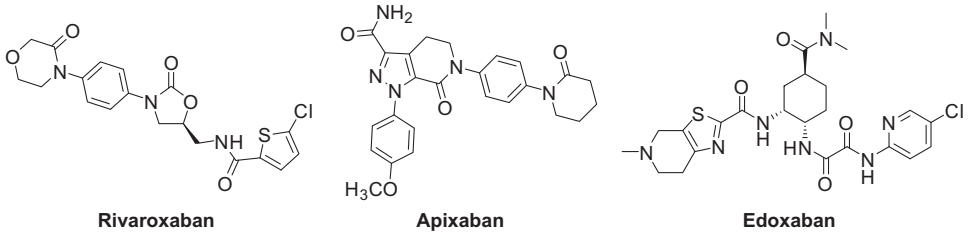


Figure 1. Structures of the FXa-based drugs.

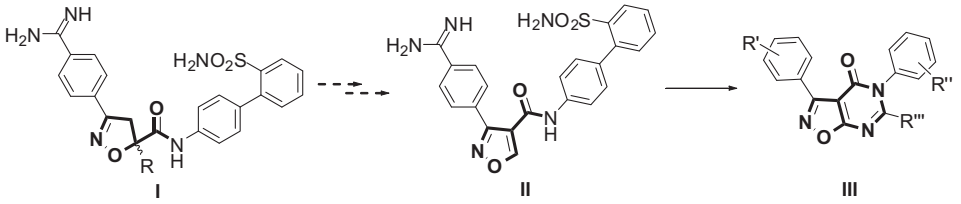
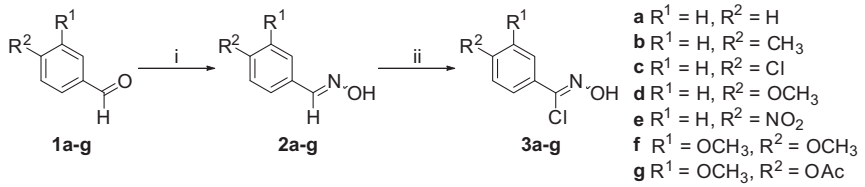
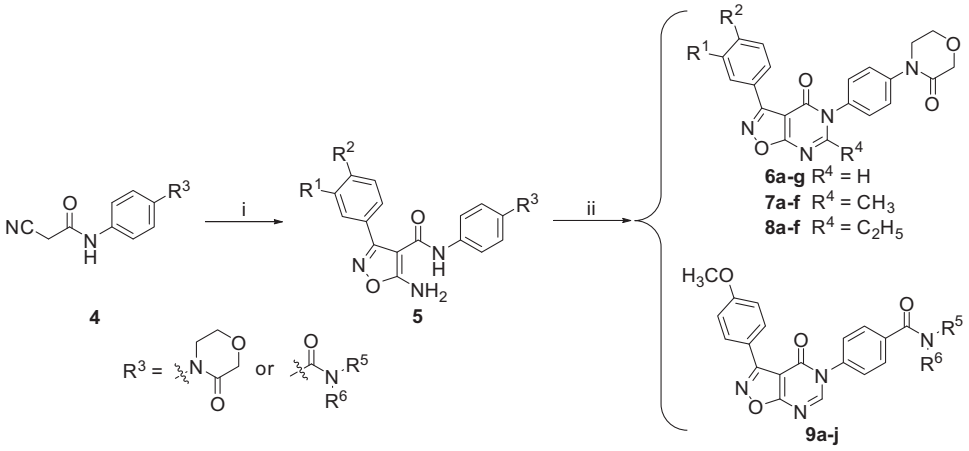


Figure 2. Design of isoxazolo[5,4-*d*]pyrimidin-4(5*H*)-one compounds.



Scheme 1. Syntheses of compounds **3a–g**. Reagents and conditions: (i) hydroxylamine hydrochloride, pyridine, MeOH, rt., 2.5 h. (ii) *N*-Chlorosuccinimide, DMF, 0–35 °C, 4 h.



Scheme 2. Syntheses of compounds **6a–g**, **7a–f**, **8a–f** and **9a–j**. Reagents and conditions: (i) **3a–g**, sodium ethoxide, absolute ethanol, DMF, 40–50 °C, 4 h. (ii) Triethyl orthoformate, triethyl orthoacetate or triethyl orthopropionate in acetic anhydride, refluxed, 6–8 h.

linker of isoxazolo into a pyrimidone ring to form a larger planar isoxazolo[5,4-*d*]pyrimidin-4(5*H*)-one core. The two hydrophobic arms at 3- and 5-position of this core scaffold were used as the P1 and P4 moieties for the interaction with the S1 and S4 pockets of FXa, respectively, to provide an L-shaped molecule (**III**) that is ideal for binding FXa[7] (Fig. 2). In addition, the small neutral substituents that can tolerate the hydrophobic S1 and S4 pockets were introduced and their effects on FXa inhibitory activity were evaluated.

The synthetic route of the hydroxymoyl chlorides (**3a–g**) was outlined in Scheme 1 following a reported method.[33] These compounds were used in the next step without further purification.

The syntheses of 3,5-diphenylisoxazolo[5,4-*d*]pyrimidin-4(5*H*)-one derivatives **6a–g**, **7a–f**, **8a–f** and **9a–j** followed a common reaction pathway (Scheme 2). Their precursors, isoxazole derivatives (**5**), were prepared by the reaction of different 2-cyano-*N*-(4-substituted phenyl)acetamide (**4**) with hydroxymoyl chlorides (**3a–g**). Target compounds (**6a–g**, **7a–f**, **8a–f** and **9a–j**) were synthesized by refluxed the isoxazole compounds (**5**) with triethyl orthoformate, triethyl orthoacetate or triethyl orthopropionate in acetic anhydride, respectively. All target compounds were characterized by requisite spectroscopic techniques like HRMS, NMR and IR.

The FXa inhibitory activities of compounds **6a–g** with different substituent phenyl groups in the P1 moiety were summarized in