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Molecular design, synthesis and anticoagulant activity evaluation of fluorinated dabigatran analogues

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ABSTRACT

In the present study, a series of unreported fluorinated dabigatran analogues, which were based on the structural scaffold of dabigatran, were designed by computer-aided simulation. Fifteen fluorinated dabigatran analogues were screened and synthesized. All target compounds were characterized by ^{1}H NMR, ^{13}C NMR, ^{19}F NMR and HRMS. According to the preliminary screening results of inhibition ratio, eleven analogues (inhibition ratio >90%) were evaluated for antithrombin activity in vitro (IC_{50}). The test results expressed that all the analogues showed effective inhibitory activities against thrombin. Especially, compounds **8f**, **8k** and **8o**, with IC_{50} values of 1.81, 3.21 and 2.16 nM, respectively, showed remarkable anticoagulant activities which were in the range of reference drug dabigatran (IC_{50} = 1.23 nM). Moreover, compounds **8k** and **8o** were developed to investigate their anticoagulant activities in vivo. In those part, compound **8o** exhibited a fairly strong inhibitory action for arteriovenous thrombosis with inhibition ratio of 84.66%, which was comparable with that of dabigatran (85.07%). Docking simulations demonstrated that these compounds could act as candidates for further development of novel anticoagulant drugs.

1. Introduction

Cardiovascular diseases (CVDs), commonly referred to as thromboembolic diseases, are the leading causes of morbidity and mortality in the contemporary world.[1] Fatal CVDs, include deep venous thrombosis (DVT), pulmonary embolism (PE), ischemic stroke, and myocardial infarction.[2–4] Thrombin, which is a multifunctional serine effector protease, exerts an important effect on the processes of CVDs.[5] Thrombin exhibits procoagulant and anticoagulant properties in the blood coagulation cascade.[6] It is the final key mediator in coagulation cascade that proteolytically cleaves fibrinogen, thereby releasing fibrinopeptides to generate fibrin; polymerization follows to form a hemostatic plug.[7] It also affects arterial thrombosis via activation of the protease-activating receptor PAR1.[8] The platelets are activated, and the conversion of fibrinogen to fibrin is catalyzed by thrombin to promote the stability of blood clots and cause blood coagulation.[9,10] Therefore, thrombin inhibitors can effectively cure thrombosis. To date, anticoagulant drugs include indirect thrombin inhibitors (heparin, vitamin K antagonists, Coumadin) and direct thrombin inhibitors (DTIs) (Argatroban,[11] Ximelagatran,[12] Melagatran[13]) (Fig. 1). DTIs, an emerging class of anticoagulants, focus on the disadvantages of indirect thrombin inhibitors[14–17] and, indeed, do show great advantages that cannot only inhibit thrombin but also inhibit the activity of thrombin-mediated factors V, VIII, and XII, and fibrinogen. Furthermore, DTIs also inhibit platelet aggregation and exert anti-inflammatory effects, indicating their broad application for the clinical treatment of thrombotic-related diseases.[18] However, these drugs are accompanied with complications during the treatment process, such as bleeding risk, hepatotoxicity, and dose-dependent risk of hemorrhage.[19] In this context, further investigations are required to develop better compounds for anticoagulant therapy.

Dabigatran etexilate (Fig. 2a), which broke the earlier 50 year monopoly of the anticoagulant drug market, is reportedly a potent and reversible inhibitor of thrombin as the first oral DTI.[20,21] This inhibitor has many advantages, including fixed dose, no requirement for blood coagulation monitoring, and reduced propensity for drug–drug interactions.[22–25] After oral administration, the prodrug is hydrolyzed, absorbed, and converted to dabigatran (Fig. 2b) through esterase-catalyzed hydrolysis in plasma and liver.[26] Dabigatran competitively binds to the active site of both free and clot-bound thrombin, thereby effectively preventing the formation of insoluble fibrinogen and fibrin clots. Dabigatran was approved by the EMA of European Union in 2008 and was approved for its listing in US by the FDA in 2010 for stroke prevention in patients with atrial fibrillation (AF), thrombosis prevention after

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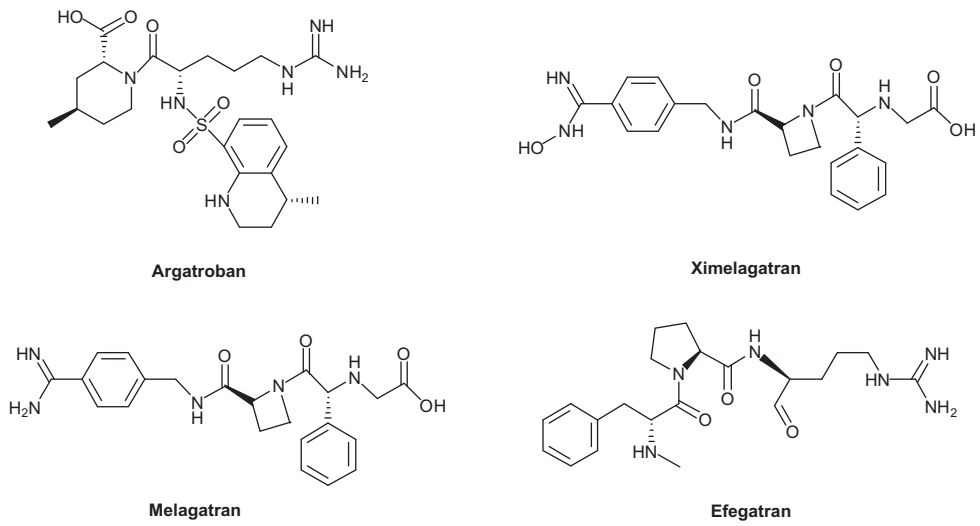

Figure 1. Structures of some direct thrombin inhibitors.

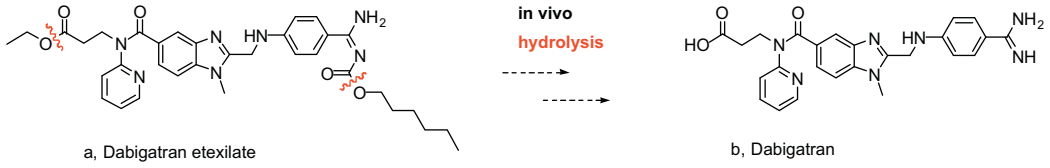

Figure 2. Structures of dabigatran etexilate and dabigatran.

orthopaedic hip and knee surgery.[27] Hauel[16] reported that the structure of the benzamidine ring of dabigatran interacts closely with the residues of the thrombin active site cleft and forms a two-tooth salt bridge with carboxylic ester of Asp189; moreover, the pyridine ring of dabigatran is not essential for bioactivity. Hence, *N*-methyl is highly suitable for the P-pocket, which is located in the active site of the enzyme, and benzamidine is bound to the D-pocket via hydrophobic interaction. Guo-qiang Lin[28] successfully replaced the pyridine ring with the benzene ring in dabigatran etexilate through chemical reactions to obtain a non-peptide thrombin inhibitor. Thus, the structure of dabigatran may be modified by changing the pyridine ring for exploring more effective thrombin inhibitors.

Fluorine, a highly active element, is widely used in drugs with high electronegativity and small size. Currently, the use of fluorinated drugs in clinical treatment is attracting considerable interest.[29–33] With the distinct properties in particular sites of certain drugs, fluorination can enhance receptor–ligand interactions, improve metabolic stability, accelerate bioavailability, prolong the effects in the body, and improve various physical properties.[34–37] Kirk summarized the introduction of fluorine atoms to drugs for the treatment of CVDs. For example, oxidation at specific sites and strategic substitution of fluorine in azetidinone (Fig. 3a) are used to obtain ezetimibe (Fig. 3b), which is attributed to the improved metabolic oxidation of the drug.[30,38] Müller[39] showed various direct fluorine–protein binding interactions in thrombin inhibitors. Thus, designing and synthesizing new thrombin inhibitor derivatives through fluorine-containing structural modifications are significant approaches. Consequently, we hoped to introduce fluorine to dabigatran as an improvement for exploring novel thrombin inhibitors.

Benzimidazole derivatives with diverse substituent groups at particular locations demonstrate different inhibitory activities.[40–43] They cannot only closely combine with the thrombin active site to exert anticoagulant activity but also bind to a branch of amidinofenylalanine as false arginine.[44] The displacement of the pyridine ring by the modified phenyl ring, ethyl group substitution at N-1 of benzimidazole and fluorine at the C-2 position of the terminal benzene ring in the dabigatran structure showed good anticoagulant activity against thrombin.[45] In this case, to combine the benefits of both dabigatran and fluorine, we optimized dabigatran by replacing the pyridine ring with modified pyridine or modified phenyl ring, changing its N-substituent with ethyl, and introducing fluorine at the C-2 position of its terminal phenyl (Fig. 4A). This optimization reaction was based on the structural scaffold of dabigatran. During this work, a series of novel fluorinated dabigatran analogues were designed and screened by the molecular modeling techniques, such as 3D-QSAR and Topomer CoMFA model, such as 3D-QSAR and Topomer CoMFA model, which were concerned with the theoretical calculations about thrombin inhibitors (Dabigatran derivatives) (Fig. 4B).[46] Among these analogues, two modified pyridine ring analogues were designed for comparison. Therefore, 15 analogues were synthesized via a modified procedure and preliminarily screened for the inhibition ratio. Subsequently, 11 analogues were selected to evaluate their in vitro activities against thrombin, and the predicted PIC_{50} values were obtained simultaneously. In particular, compounds **8f**, **8k** and **8o** showed strong activities, which were at the same level of dabigatran. To rationalize the values of IC_{50}, molecular docking was used to study the possible binding modes of inhibitors at the active site of thrombin protein.

2. Results and discussion

2.1. Screening of novel fluorinated dabigatran analogues

Computer aided drug design (CADD) approaches, for saving resources and expediting schedule of medicinal development, was used to offer a deeper insight into inhibitor's structure or