European Journal of Medicinal Chemistry 126 (2017) 799–809

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Novel dabigatran derivatives with a fluorine atom at the C-2 position of the terminal benzene ring: Design, synthesis and anticoagulant activity evaluation

Haoran Yang, Qianqian Liu, Xiaodong Gao, Yujie Ren*, Yonghong Gao

College of Chemical and Environmental Engineering, Shanghai Institute of Technology, Shanghai, China

ARTICLE INFO

Article history:
Received 13 June 2016
Received in revised form
27 September 2016
Accepted 6 December 2016
Available online 8 December 2016

Keywords:
Anticoagulant activity
Fluorinated dabigatran derivatives
Molecular docking
Synthesis

ABSTRACT

This manuscript describes the preparation of dabigatran derivatives and their inhibitory potentials toward human thrombin. Among the tested compounds, **7c**, **7k**, **7m** and **7o**, with IC_{50} values of 1.54, 0.84, 1.18 and 1.42 nM, exhibited comparable inhibitory activity to dabigatran (IC_{50} = 1.20 nM). The in vivo anti-thrombotic activity of compounds **7c** and **7o** in SD rats was studied. Results showed that intravenously administering the two compounds significantly inhibited the growth of thrombus with an inhibition rate of (84.24 ± 1.53)% and (84.57 ± 0.45)%, which were comparable to that of dabigatran (85.07 ± 0.61)%. Furthermore, the docking simulation of active compounds (**7k** and **7m**) provided a potential binding model. Results indicated that these compounds could be further investigated to determine their anticoagulant activities.

1. Introduction

The introduction of a fluorine atom or a fluorine-containing group to a small-molecule drug is an important strategy for transforming the chemical properties of drugs. Organofluorine affects nearly all physical adsorption, distribution, metabolism and excretion properties of a lead compound [1]. Therefore, fluorine substitution is commonly used in contemporary medicinal chemistry to improve metabolic stability, bioavailability and protein-ligand interactions [2–5]. The hydrogen atom in aromatic rings is replaced by fluorine to significantly slow down the oxidative metabolic step of a given drug by liver enzymes in Cytochrome P450 and evidently influence the progress of hydrolytic metabolism [6]. For example, a hydrogen atom in uracil is substituted with fluorine to obtain 5-fluorouracil, which was first synthesized in 1957. 5-fluorouracil exhibits high anticancer activity by improving the lipophilicity and biological permeability of the drug [7]. Since the first approval by the American Food and Drug Administration of fluorine-containing drug called fludrocortisone in 1955, nearly 150 fluorinated molecules reached the market, including anti-AIDS (emtricitabine and efavirenz), antitumor (gefitinib and fulvestrant), antidepressant (fluoxetine) and cardiovascular drugs (atorvastatin and pitavastatin) [6–8]. In 2010, fluorinated drugs comprised approximately 20% of administered drugs. This proportion increased from 20% to roughly 30% for all new approved drugs (excluding biopharmaceutical products) in recent years [8–10]. Therefore, introducing fluorine or fluorine-containing group to drug molecules has theoretical significance and practical value in developing new drugs.

In 2008, dabigatran etexilate (Pradaxa; Boehringer Ingelheim) was approved by the European Medicines Agency to prevent venous thromboembolism (VTE) in adult patients undergoing elective total hip or knee replacement, which broke the monopoly of warfarin in the anticoagulant drug market [11–13]. Dabigatran etexilate, which is the first direct thrombin inhibitor, is hydrolysed by carboxylesterase in vivo and converted to its active dabigatran after oral administration of the drug (Fig. 1). Dabigatran binds to the active site of both free and clot-bound thrombin to prevent the formation of insoluble fibrinogen and fibrin clots. Unlike other anticoagulants, dabigatran etexilate has several advantages, such as a wide therapeutic window, a fixed dose without monitoring and less drug-drug interactions [14–17]. However, dabigatran etexilate presents the risk of bleeding at high does [18], and its bioavailability is only 6.5% [19,20]. Thus, the structure of dabigatran could be modified by fluorine to identify active candidate compounds with comparable or superior anthrombin activity to further study their

* Corresponding author. College of Chemical and Environmental Engineering, Shanghai Institute of Technology, 100 Haiquan Road, Shanghai 201418, China.
E-mail address: clab@sit.edu.cn (Y. Ren).

http://dx.doi.org/10.1016/j.ejmech.2016.12.015
0223-5234/

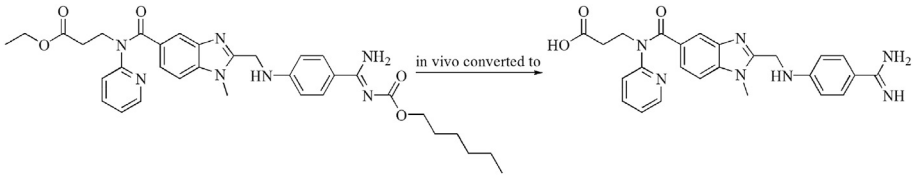

Fig. 1. Structures of dabigatran etexilate and dabigatran.

bioavailability or the risk of bleeding. This coagulant can be potentially utilised in developing a new thrombin inhibitor.

In this study, a series of new fluorinated dabigatran derivatives were designed by introducing a fluorine atom to the C-2 position of the terminal benzene ring and a hydrophobic group was introduced to the benzene ring or pyridine ring to replace the pyridine ring according to preliminary results of our laboratory experiment [21–23] (Fig. 2). The activity of the designed compounds was predicted by molecular docking and QSAR model, and fifteen new compounds were selected to synthesis and evaluate their thrombin inhibitory activity in vitro. The purification method of the key intermediate product was studied in synthesising the target compounds. Moreover, molecular docking was adopted to study the binding model of active compounds. This study also expanded the screening substrate spectrum of anti-clotting drug to identify effective candidate compounds.

2. Results and discussions

2.1. Chemistry

The synthetic method for fluorinated dabigatran derivatives is shown in Scheme 1. Compounds **1** and **2** were mixed in water and stirred at 100 °C for 8 h to obtain compound **3**. According to the literature, compounds **4a**–**o** were synthesized. In the synthesis of compounds **5a**–**o**, CDI was used as coupling reagent to obtain intermediate compounds **5a**–**o**. However, this coupling reagent could only be used under strictly anhydrous and anaerobic conditions, thereby limiting its wide application. Thus, we chose the convenient coupling reagents EDCI and HOBt for the synthesis of intermediate compounds **5a**–**o**. Cyano compounds **5a**–**o** were converted into the corresponding amidino compounds **6a**–**o** by treatment with an ethanol solution of hydroxylamine and then by Pd/C reduction under a N_2 atmosphere. The target compounds **7a**–**o** were obtained from compounds **6a**–**o** by hydrolysis.

The purification method of compounds **5a**–**o** was explored in synthesising the main intermediate compounds **5a**–**o**. Recrystallization by anhydrous ethanol was employed instead of column chromatography to simplify the process, reduce cost and obtain the main intermediate compounds **5a**–**o** with high purity (more than 96%).

2.2. Biological evaluation

2.2.1. Inhibition rate tests

Thrombin is a specific serine protease, which plays a key role in the blood coagulation cascade [24,25]. The inhibition rate against thrombin of all target compounds **7a**–**o** was evaluated at 1 μg/mL (Fig. 3). The results showed that all the compounds, except **7b**, **7d**, **7g** and **7i**, demonstrated high inhibition rates (more than 80%) at 1 μg/mL concentration.

2.2.2. Inhibitory activity in vitro

According to the results of the inhibition rate, eleven compounds, **7a**, **7c**, **7e**, **7f**, **7h** and **7j**–**o**, which exhibited high inhibition rates (>80%), were selected to evaluate for their anticoagulant activities against thrombin in vitro (IC_{50}) with dabigatran as the reference compound. The results are presented in Table 1. All the tested compounds showed considerable inhibitory activity against thrombin. Compounds **7k** and **7m** with IC_{50} values of 0.84 nM and 1.18 nM, respectively, exhibited better inhibitory activity to dabigatran (IC_{50} = 1.20 nM).

Table 1 indicates that the inhibitory activities of the compounds followed the order **7k** > **7m** > Dabigatran > **7o** > **7c** > **7a** > **7j** > **7n** > **7e** > **7h** > **7f** > **7l**. This result suggested that introducing different hydrophobic groups in section A of these compounds affected biological activity. Unlike dabigatran, introducing two fluorine atoms in the phenyl ring (**7k** and **7m**), except for compound **7l**, increases its efficacy against thrombin, which was probably attributed the characteristics of the fluorine atom. The result indicated that introducing two fluorine atoms in the phenyl ring with an interval improves biological activity. Fig. 3 shows that compounds **7b**, **7d**, **7g** and **7i** exhibited an inhibition rate lower than 50%, which indicates that the methyl group at the para position on the phenyl ring inhibited biological activity. However, compound **7j** exerted considerable inhibitory activity with fluorine at the same position on the phenyl ring. This result probably occurred because fluorine is smaller than the methyl group, which enhanced the binding force and improved biological activity. Moreover, introducing the methyl group at the meta position (**7a**, **7c**, **7e**, **7h**, **7j** and **7n**) could promote anticoagulant activity.

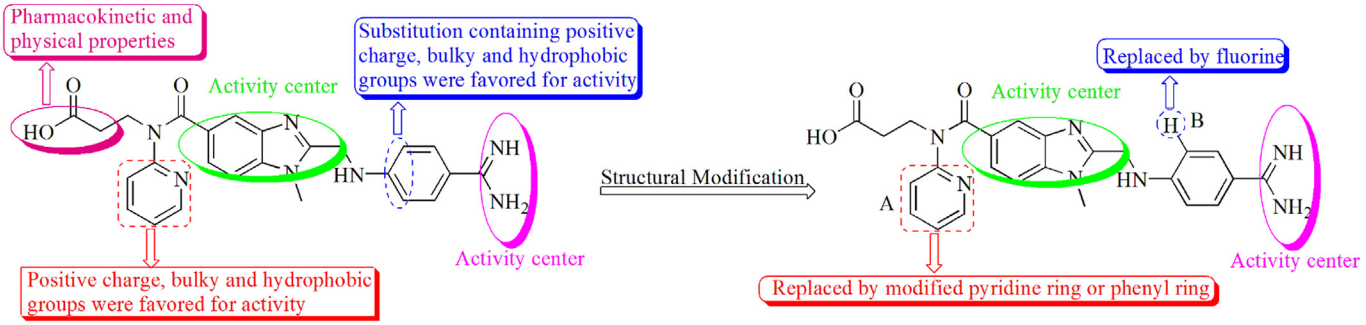

Fig. 2. Design strategies of fluorinated dabigatran derivatives.