Bioorganic & Medicinal Chemistry 22 (2014) 6324–6332

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Novel strategy to boost oral anticoagulant activity of blood coagulation enzyme inhibitors based on biotransformation into hydrophilic conjugates

Tsukasa Ishihara [a,*], Yuji Koga [a], Kenichi Mori [a], Keizo Sugasawa [a], Yoshiyuki Iwatsuki [b], Fukushi Hirayama [a]

[a] Drug Discovery Research, Astellas Pharma Inc., 21, Miyukigaoka, Tsukuba-shi, Ibaraki 305-8585, Japan
[b] Pharmacovigilance, Astellas Pharma Inc., 2-5-1, Nihonbashi-Honcho, Chuo-ku, Tokyo 103-8411, Japan

ARTICLE INFO

Article history:
Received 1 September 2014
Revised 26 September 2014
Accepted 30 September 2014
Available online 19 October 2014

Keywords:
Factor Xa
Anticoagulant
Conjugation
Docking study

ABSTRACT

The blood coagulation cascade represents an attractive target for antithrombotic drug development, and recent studies have attempted to identify oral anticoagulants with inhibitory activity for enzymes in this cascade, with particular attention focused on thrombin and factor Xa (fXa) as typical targets. We previously described the discovery of the orally active fXa inhibitor darexaban (**1**) and reported a unique profile that compound **1** rapidly transformed into glucuronide YM-222714 (**2**) after oral administration. Here, we propose a novel strategy towards the discovery of an orally active anticoagulant that is based on the bioconversion of a non-amidine inhibitor into the corresponding conjugate to boost ex vivo anticoagulant activity via an increase in hydrophilicity. Computational molecular modeling was utilized to select a template scaffold and design a substitution point to install a potential functional group for conjugation. This strategy led to the identification of the phenol-derived fXa inhibitor ASP8102 (**14**), which demonstrated highly potent anticoagulant activity after biotransformation into the corresponding glucuronide (**16**) via oral dosing.

1. Introduction

Intravascular clot formation results in thromboembolic diseases including ischemic stroke, deep venous thrombosis, myocardial infarction, unstable angina, and pulmonary embolism, all of which are major causes of morbidity and mortality in the industrialized world. Enzymes in the blood coagulation cascade, such as thrombin and fXa, are attractive targets for the prevention of thrombosis, with numerous efforts devoted to the discovery of inhibitors against these enzymes that can act as orally active anticoagulant agents.

Approaches to the discovery of oral anticoagulant drugs that directly inhibit blood coagulation enzymes are classified into two categories. One involves approaches based on the strategy of amidine prodrugs, represented by the anticoagulants ximelagatran[1] and dabigatran etexilate.[2] An amidine prodrug is bioconverted into the corresponding amidine inhibitor after oral administration, and the amidine group forms a bidentate salt bridge interaction with the carboxylic acid of Asp189 in the S1 site of the enzyme. Although amidine-derived inhibitors demonstrate potent in vivo anticoagulant and antithrombotic activity due to their overall high hydrophilicity, associated with the inhibitor's highly polar amidine group, the hydrophilic and basic profile of the amidine moiety also causes a reduction in membrane permeability and oral bioavailability. Thus, the prodrug strategy seeks to mask the high hydrophilicity and basicity of the amidine unit to improve the inferior profile of amidine-derived inhibitors.

The other methodology involves nonamidine-type inhibitors—eliminating highly hydrophilic and basic amidine groups to improve the oral bioavailability of inhibitors.[3] These efforts have already led to the discovery of the launched coagulants rivaroxaban,[4] apixaban,[5] and edoxaban.[6] These nonamidine-type inhibitors possess a small lipophilic substituent, such as a methoxy or chloro group, which occupies the small hydrophobic pocket formed by the residues Ala190, Val213, and Tyr228 in the bottom of the S1 site of the enzyme, or a less polar surrogate for an amidine group, such as an aromatic amine or benzylamine. However, the removal of the amidine group causes an increase in the overall lipophilicity of the inhibitor, which often leads to a detrimental reduction in anticoagulant potency.[3] Thus, concerted efforts have been devoted to managing both anticoagulant activity and pharmacokinetics profile of orally active anticoagulants.

* Corresponding author. Tel.: +81 29 863 6713; fax: +81 29 854 1519.
E-mail address: tsukasa.ishihara@astellas.com (T. Ishihara).

http://dx.doi.org/10.1016/j.bmc.2014.09.059
0968-0896/

Here, we propose adoption of a third strategy in the discovery of blood coagulation enzyme inhibitors with boosted anticoagulant effects after oral administration, based on non-amidine inhibitors, via bioconversion to corresponding highly hydrophilic conjugates.

2. Strategy

Inhibitors of enzymes in the blood coagulation cascade target the venous and arterial vascular system, therefore their high distribution in the circulation is preferred. Such a property is consistent with a pharmacokinetic profile of low distribution volume, and a general method to decrease volume of distribution is to increase hydrophilicity of a compound.[7] Consequently, an inhibitor with increased hydrophilicity is expected to improve in vivo antithrombotic effect. This concept is consistent with the previous result that found the fXa inhibitor **3**, which contains a combination of highly hydrophilic amidine and carboxylic acid, displays 30-fold higher activity in an in vivo thrombosis model during continuous intravenous infusion despite its equipotency to the non-amidine fXa inhibitor **4** in an in vitro anticoagulant assay (Fig. 1).[8] Pinto et al. also reported the desirability of a pharmacokinetic profile with low distribution volume towards vascular-targeted agents in the discovery process of the launched fXa inhibitor apixaban.[5]

We developed the fXa inhibitor **1** to demonstrate potent anticoagulant effect after oral administration (Fig. 1).[9] Compound **1** was found to be rapidly metabolized when dosed orally, and the predominant metabolite detected in the plasma was the corresponding glucuronide **2**. A comparison of the lipophilicity of the compounds **1** and **2** found that the latter was much more hydrophilic (*c*log*P* values of 2.30 for compound **1** and −1.03 for compound **2**),[10] which led us to consider whether compound **1**'s oral anticoagulant activity was achieved by it being converted into the highly hydrophilic glucuronide **2** in vivo. Biotransformation of a lipophilic amidine prodrug with high membrane permeability into a corresponding hydrophilic amidine inhibitor with high anticoagulant potency is the established methodology for identification of orally active anticoagulants.[1,2] Using this idea as a model, we investigated whether the biotransformation of a lipophilic precursor into the corresponding hydrophilic conjugate could be a new strategy for discovery of oral anticoagulants.

A key step for this strategy is the installation of a 'trigger' at an appropriate position of a suitable chemical scaffold. The trigger refers to a functional group with a potential to act as a substrate for conjugation. A phenolic hydroxyl group is preferable for the trigger owing to two reasons: conjugates of hydroxyl groups are known to be relatively stable compared to those of acyl or amino groups, and phenolic hydroxyl groups have less oxidizability than alcoholic hydroxyl groups. Similar to compound **1**, the launched phenol-derived cholesterol absorption inhibitor ezetimibe was found to rapidly undergo glucuronide conjugation before entering portal plasma via oral dosing.[11] This property indicates a general feature that phenolic hydroxyl groups can be precursors of highly hydrophilic glucuronide conjugates after oral administration.

Computational molecular modeling simulations could be utilized to identify an appropriate scaffold and where to install the triggering phenolic hydroxyl group. Figure 2 shows a proposed binding model of compound **1** and its glucuronide **2** to the active site of fXa. The sugar residue of compound **2** extends into solvent space without disturbing the interaction between the ligand and the enzyme, resulting in fXa inhibitory activity. A novel inhibitor can therefore be designed using computational molecular modeling by selecting a chemical scaffold on which a phenolic hydroxyl group is directed out of the binding site and into the bulk solvent. At the time our study was initiated,[12] only three representative scaffolds, compounds **5** (DPC423),[13] **6**,[14] and **7**,[15] had been reported as non-amidine fXa inhibitors (Fig. 1). Our computational modeling simulation of these three inhibitors and consideration of reactivity for glucuronidation led us to presume the followings:

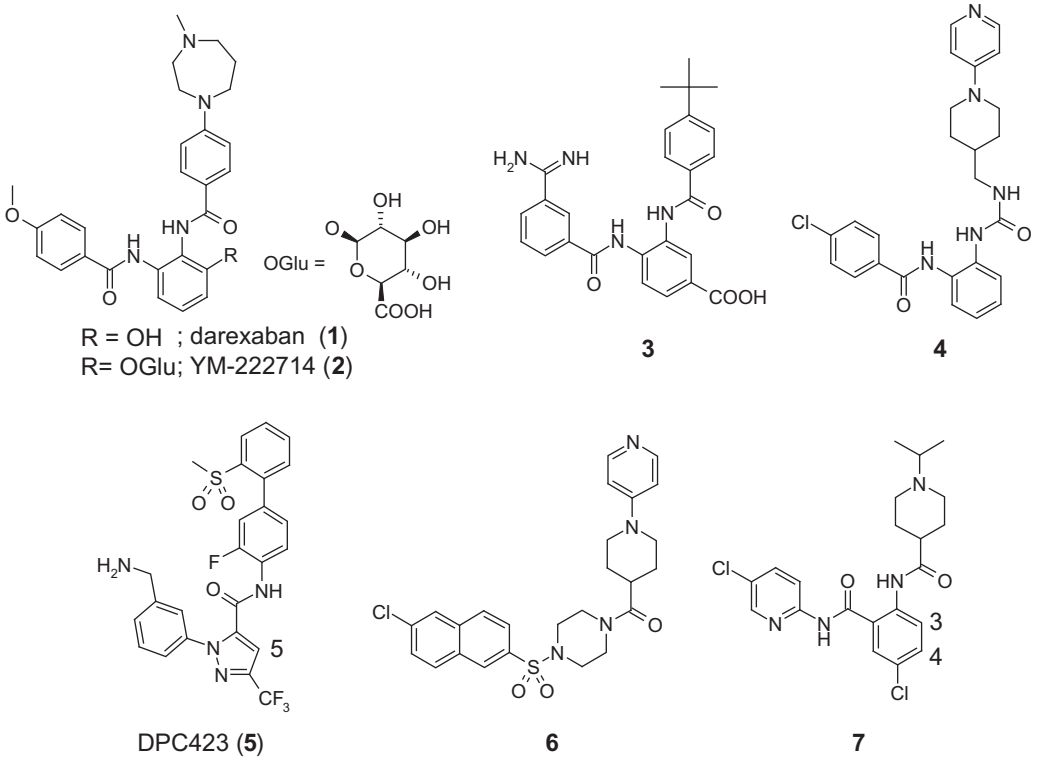

Figure 1. Structures of direct fXa inhibitors.