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Biarylmethoxy isonipecotanilides as potent and selective inhibitors of blood coagulation factor Xa

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ABSTRACT

New chloro-substituted biarylmethoxyphenyl piperidine-4-carboxamides were synthesized and assayed in vitro as inhibitors of the blood coagulation enzymes factor Xa (fXa) and thrombin. An investigation of effects of the amidine and isopropyl groups attached at the piperidine nitrogen and 5-(halogenoaryl)isoxazol-3-yl groups as biaryl substituents led us to identify new compounds which proved to be selective fXa inhibitors, with inhibition constants in the low nanomolar range. The most potent compound **21e**, that incorporates 2-Cl-thiophen-5-yl group as the P1 motif and 1-isopropylpiperidine P4 group, inhibited fXa with K_i value of 0.3 nM and very high selectivity over thrombin and some other tested serine proteases, achieving moderate levels of anticoagulant activity in the low micromolar range, as assessed by the prothrombin time clotting assay (PT_2 = 3.30 μM). Based on reliable docking simulations, molecular modeling provided a rationale for interpreting structure–activity relationships. The predicted binding modes highlighted the structural requirements for addressing the subsites S1 and S4 of the fXa enzyme.

1. Introduction

Among the novel antithrombotics in development (Arepally and Ortel, 2006; Krynetskiy and McDonnell, 2007), small-molecule orally available inhibitors of proteases in the blood coagulation cascade, mainly factor Xa (fXa) and thrombin, have shown improved efficacy and lesser side effects, such as bleeding, associated with the currently used therapies (warfarin, heparins) (Mackman, 2008; Weitz and Linkins, 2007), which require continuous monitoring for accurate dosing. Due to its position at the confluence of the intrinsic and extrinsic pathways of the coagulation cascade, fXa has emerged as an attractive target for the development of potent and safer anticoagulant drugs. Together with fVa and calcium ions on a phospholipid surface, fXa forms the prothrombinase complex, which is responsible for the conversion of prothrombin to thrombin, the final effector of coagulation. Despite initial efforts in the discovery of oral anticoagulant drugs focused on the development of small-molecule direct inhibitors of thrombin (i.e., the oral DTIs), recently accumulated evidence has suggested that early inhibition in the coagulation cascade at the level of fXa may have greater antithrombotic potential (Ansell, 2007). Indeed, fXa inhibitors do not affect the existing levels of thrombin, allowing the small amounts of remaining thrombin after fXa inhibition to activate thrombin receptors and preserve primary haemostatic functions (Leadley, 2001). Preclinical studies suggest that selective inhibitors of fXa may possess a wider therapeutic index than DTIs (Wong et al., 2009). Recent reviews described the various chemotypes that were employed in the evolution of fXa inhibitors (de Candia et al., 2009a; Pinto et al., 2010).

The availability of numerous fXa crystallographic structures, paved the road to the use of structure-based drug design and modeling techniques that have been successfully employed to develop new oral fXa inhibitors (Maignan and Mikol, 2001). These techniques have been critical in achieving both enzyme selectivity and oral bioavailability. FXa contains a serine protease domain in a trypsin-like closed β-barrel fold encompassing the catalytic triad Ser195–His57–Asp102 and two essential subsites, S1 and S4. The most potent inhibitors adopt L-shaped binding conformations, orienting two almost orthogonal P1 and P4 groups to fill the S1 and S4 pockets in the enzyme binding site.

Early fXa inhibitors brought as primary anchoring points benzamidine (e.g., otamixaban **1**; Fig. 1), naphtylamidine or other basic groups, which in the protonated form interact with Asp189 at the bottom of the S1 pocket, whereas the P4 aromatic moiety is

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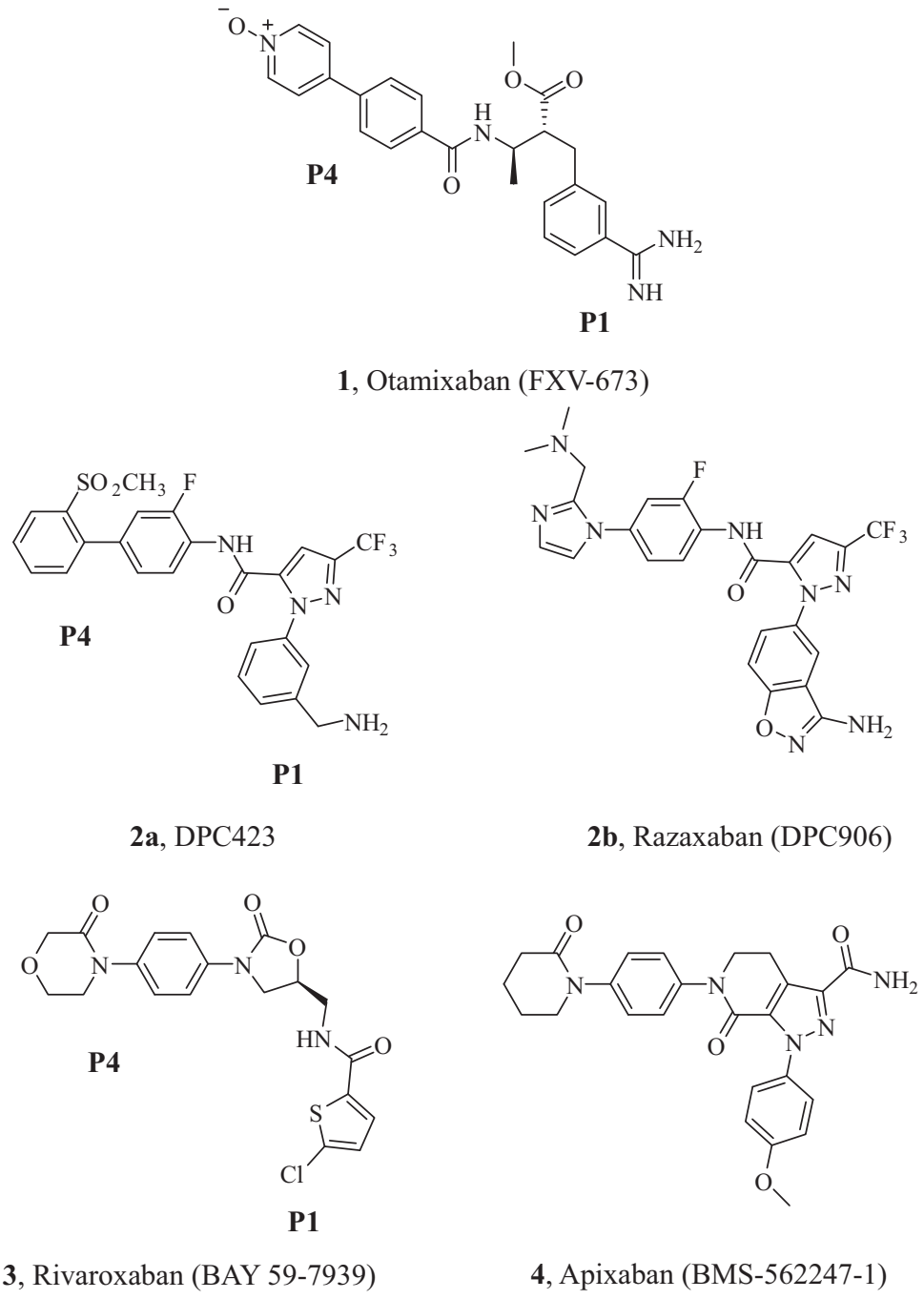

Fig. 1. Structures of five representative factor Xa inhibitors – the so-called xabans – bearing as the P1 interacting moiety a basic benzamidine group (otamixaban), less basic amidine isosters (DPC423, razaxaban), and neutral substituted aryl groups (rivaroxaban, apixaban).

embedded into the S4 site, that is an aromatic box lined by the aromatic side chains of Tyr99, Phe174 and Trp215 (Al-Obeidi and Ostrem, 1999). Due to the limited oral bioavailability often associated with the first generation amidine-based fXa inhibitors, efforts were made to replace the amidine group with less basic, such as in DPC423 (**2a**) that was the first oral clinical candidate (Pinto et al., 2001), or nonpolar neutral groups (Lam et al., 2003). These efforts enabled the discovery of second-generation of fXa inhibitors with improved PK properties. They include compounds bearing either less basic amidine isosters, such as razaxaban **2b** (Quan et al., 2005), or neutral P1 substituents, such as rivaroxaban **3** (Roehrig et al., 2005) and apixaban **4** (Pinto et al., 2007) (Fig. 1).

The chlorothiophene moiety in rivaroxaban is buried inside the S1 pocket, with chlorine pointing towards the center of the Tyr228 aromatic ring. The gain in binding energy due to this hydrophobic contact, that is the so-called chloro binding mode, balances the lack of electrostatic/H-bond interactions between amidine and Asp189. Interestingly, it has been shown that even in the presence of a benzamidine plus a chloroaryl or chloroheteroaryl group, fXa-selective inhibitors engage the enzyme binding site by orienting the neutral group into the S1 pocket (Lumma et al., 1998). A similar binding pose has been reported for apixaban **4**, with the 4-methoxyphenyl substituent occupying the same space of the chlorothiophene P1 moiety.