Allosteric inhibitors of thrombin

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Thrombotic disorders such as deep vein thrombosis and venous thrombosis are one of the leading causes of death in the modern society. It is estimated that the mortality may reach 23.6 million by the end of 2030.[1, 2] 1 in 1000 people suffers from thrombotic disorder. Treatment of thrombotic diseases heavily relied on heparin and coumarin from the last five decades. Recently, hirudin, argatroban and dabigatran were approved for few thrombotic conditions. However, all of these drugs are associated with serious life-threatening side effects and need medical supervision for patients on therapy.[3] Despite enormous effort and advancement in understanding the coagulation cascade, no significant progress has been made. Thrombin is the most important and highly targeted protease in the coagulation cascade. Structurally, thrombin displays multiple distinct ligand-binding sites that can modulate its activity and interactions with other molecules in the blood. These sites include the active site, the anion-binding exosites I and II, and the sodium binding site. Enormous efforts are made to design thrombin inhibitors that have focused almost exclusively on sterically blocking the substrate's access to the active site through a competitive process. Despite the discovery of several hundreds of inhibitors, no candidate is yet approved for clinical use. This owes to lack of significant *in-vivo* activity and high toxicity. Recently, efforts have been made to design the allosteric inhibitors of thrombin to selectively regulate the coagulation function. Thrombin is highly modulated by allosteric sites (exosite I & II). These sites offer the avenue to allosterically modulate the function of thrombin. However, no small molecule was reported to regulate the coagulation function of thrombin via these sites due to the shallow and the highly charged nature of these exosites.

Recently, a class of inhibitors was reported by *Sidhu et al* which selectively inhibits the coagulation activity of thrombin by binding to exosite II.[4-9] These were benzofuran-based scaffold derived from β-5 linkage present in naturally occurring lignin polymer. The focused libraries of benzofuran-based monomers and dimers were synthesized and determined to inhibit thrombin in low micromolar range. The Michaelis-Menten kinetic studies reveal the allosteric nature of these inhibitors.[4, 5] Competitive inhibition studies with various known ligands of thrombin confirm that these inhibitors explore the region of exosite II of thrombin. The binding site is located on the edge of exosite II and close to outer rim of active site. Site-directed mutagenesis studies revealed that Arg173 is an important residue involved in binding and mediating the anti-coagulation effect of these inhibitors.[8] Selectivity studies were performed against a panel of closely related proteases advocates to identify the selective inhibitors for thrombin. Comparison of crystal structures of thrombin and factor Xa shows significant differences in electronic features at corresponding site in factor Xa and thrombin, which explains the selective behavior of these inhibitors. A proposed binding mode was identified using *in-silico* techniques, which was able to explain majority of the SAR studies. This proposed binding model can be effectively used to design new inhibitors with improved inhibition potency.[7, 8]

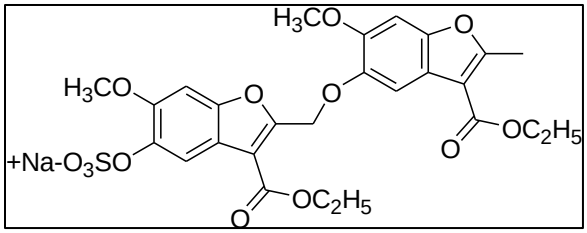

Figure 1: Structure of most potent inhibitor

Designing allosteric inhibitors for thrombin opens the new avenue to discover newer generation of inhibitors with fewer side effects. This mode of inhibition is advantageous due to partial inhibition of thrombin activity and ease of designing the antidote. However, significant improvements are still required in terms of potency and pharmacokinetic profile.

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