Chemical Characteristics of Direct FXa Inhibitors Registered for Atrial Fibrillation

Alexandre Bridoux1*, Michel Meyer Samama2,3 and Shaker A Mousa1

1Pharmaceutical Research Institute, USA
2Biomnis Laboratories R&D, France
3Hospital Group Broca-Cauchin-Hôtel-Dieu, France

Abstract

This review publication highlights chemical characteristics of several direct factor Xa inhibitors that have been recently approved for Atrial Fibrillation (AFIB) and prevention and treatment of Venous Thromboembolic (VTE) disorders. Our goal is to help clinicians clarify the differences between these inhibitors by presenting the chemist and clinician points of view. A summary of the development phase (between the years 2000 to 2010) of the factor Xa inhibitors by the major pharmaceutical companies is reported followed by a focus on some important structure-activity relationships and pharmacological properties of Apixaban, Rivaroxaban, and Edoxaban. To go beyond what is found in the literature, we discuss and rationalize clinical pharmacological properties by reviewing the chemical characteristics of each molecule together with their pharmacological effects.

Keywords: Direct factor Xa inhibitor; Anticoagulant; Apixaban; Rivaroxaban; Edoxaban

Introduction

The risk of stroke is about five times greater in people with Atrial Fibrillation (AFIB) because blood can pool in the atria (the heart's two small upper chambers) and form blood clots. Venous Thromboembolism (VTE) is characterized as a blood clot that induces the occlusion of a vein [1]. It has been a common cause of morbidity and mortality following total hip replacement [2] and total knee replacement surgeries [3], with Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE) being VTEs [4]. DVT is characterized as a blood clot localized in the deep veins of the human locomotor apparatus. PE could be the result of a blood clot originating in these large veins or elsewhere that has formed, broken off, and then travelled to the point of obstruction. Therefore, prevention of DVT has been determined to be the best strategy to decrease the morbidity and mortality of PE [5] and stroke [6].

Several of the clinical situations resulting in the highest risk of VTE, such as those associated with major orthopedic or other surgeries or hospitalization for acute medical illnesses, have been transitory; in consequence, guidelines recommend short-term VTE prophylaxis, which has most often been achieved with parenteral anticoagulants [7,8]. These drugs may also be prescribed to keep blood clots from forming in AFIB. These agents have included heparin [9] and Low Molecular Weight Heparins (LMWH) [10], which carries a low but serious risk of thrombocytopenia. By their very nature, parenteral agents are not likely to be used properly by patients after hospital discharge compared with simple per os regimens. Thus, an unmet medical need for safe and effective agents that could be administered per os exists [11].

These observations justified the new concept of isolating, characterizing, and inhibiting the protease factor X in the activated state as a step forward in this research field. In the middle of the blood coagulation process, factor X associates as a complex to the already formed Factor VIIa-Tissue Factor complex to form the protease activated factor X (FXa), which acts at the convergence zone of the coagulation pathways. Not all patients undergoing orthopedic surgery currently receive appropriate thromboprophylaxis because surgeons are concerned about the bleeding risks of available agents [12]. In 2001, Fondaparinux was approved as a parenteral indirect FXa inhibitor for short-term VTE prophylaxis because it inhibited FXa by interacting with antithrombin [11]. Further research and development work on oral FXa inhibitors yielded to the discovery and advancement of per-os, direct-acting FXa inhibitors that progressed to clinical trials [12]. The purpose of this review is to consider some interesting developments in the field, to consider the structure-activity...
The Development of Direct Anti-FXa Strategy

The FXa L-shaped binding site has been reported to have the S1 and S4 pockets at the edge positions in the protease (Figure 1) [13]. S1 was described to be a deep, largely hydrophobic pocket bearing the Asp189 and Tyr228 residues. S4 was described to be a highly hydrophobic pocket bearing the aryl-binding Tyr99, Phe174, and Trp215 amino acids, just as in the serine proteases. Other features include His57, Asp102, and Ser195, which were found to play a catalytic role and the β-strand region that covers Trp215 (S4 region) (Figure 1) [14].

To discover new direct FXa inhibitors based on small molecule structures, an initial strategy was to design compounds with functional groups that could interact strongly and invariably with the edging pockets (S1, S4) of the catalytic site and thus stabilize the ligand–protease interactions, thereby preventing the enzyme from any catalytic action [15]. Tested compounds were from the Max Planck Institute for Biochemistry and were bis-substituted derivatives of Na-benzenesulfonyl-glycyl-phenylalanine (1 and 2) [16]. Compound 1 showed micromolar activity with selectivity for FXa, whereas 2 had submicromolar activity on FXa and lost selectivity only by a difference of an ethyl ester function. Then, the first hits that were active in the nanomolar range were discovered at Rhone-Poulenc Rorer (3, IC₅₀=30.0 nM) [17], Berlex Biosciences Company (4, Kᵢ=0.66 nM) [18], and the DuPont Merck Pharmaceutical Company (5, Kᵢ=9.0 nM) [19]. The design of these compounds was simple: The edging functions (amide, guanidinium, and dimethoxy) that could interact with the S1 and S4 sites were separated by a long-enough, mostly lipophilic linker. Table 1 summarizes the first potent FXa direct inhibitors.

These analogues successfully showed a powerful inhibition of FXa (Table 1) [20]; nevertheless, selectivity for this protease over others playing a major role in the coagulation cascade was still questionable [21]. These other proteases are different from FXa because their tertiary structure is similar to that of trypsin, a digestive enzyme in the Gastrointestinal (GI) tract. Thus selectivity for FXa over trypsin could have counted positively in the assays for a general selectivity over trypsin-like enzymes [22].

A second group of FXa inhibitors was developed by pharmaceutical companies (Table 2). Structure-based design led researchers at DuPont Merck Pharmaceuticals to put forward the theory of replacing the 4-amidino moiety of one of their leads with a neutral o-phenylsulfonyamide group to improve permeability. This afforded one of the first known monobasic FXa inhibitors (6, Kᵢ=0.52 nM), which also exhibited enhanced potency and selectivity in comparison to compound 5 [23]. The biaryl moiety (shown in red in Table 2) of 6 was designed to localize in between the top and bottom of the aryl binding domain in the S4 pocket (Tyr99, Phe174, and Trp215, Figure 1). Other molecules such as 7 (a derivative of 4) [24], or 8 [25], or 10 [25], which resulted from the modification of one or the other edge groups of 6, and 9 [26], (that could be seen as a parent structure of Edoxaban, an approved anti-Xa drug), were released by other companies. In response, DuPont Merck Pharmaceuticals released the anti-Xa molecule, compound 11 (Kᵢ (FXa)=13 pM and selective) [27].

In the following years, High Throughput Screening (HTS) [28], a new drug-discovery process that leverages automation to quickly assay the biological or biochemical activity of a large number of drug-like compounds, was applied to screen a variety of 5- and 6-membered ring scaffolds bearing vicinal substitution. The technique confirmed that the pyrrole and heterocyclic derivatives, N-linked templates, were the ones to use in designing lead structures [29]. This effort later led to the discovery of 16 (SN429, Kᵢ (FXa)=0.013 nM) by Bristol-Myers Squibb (BMS), which had taken over DuPont Pharmaceuticals by that time [30]. The mean dose needed for 16 to divide by half the thrombus weight in the rabbit arteriovenous shunt model was 0.02 µmol/kg/h by iv infusion [27]. Nevertheless, 16 did not reach clinical trials. Meanwhile, different companies released many other potent molecules carrying either the benzylamine group (13, 14, 15) or the amidine moiety (15, 17, 18, 19), none of which reached clinical trials [31-33]. Indeed, BMS moved forward as the seeded player with three new compounds (13, 14, 15), which showed enhanced oral bioavailability and selectivity, by giving up solely on potency [34,35]. The strategy that was successful was to modulate the basicty of the edge groups of the part of the molecules that locate in the S1 pocket. The development phase was the discovery of the first orally bioavailable molecule, 23 (DPC423, Table 3) [27], which was also shown to be potent on the protease and in clotting assays (Kᵢ (FXa)=0.15 nM; aPTT × 2=4.86 μM) and selective (thrombin Kᵢ=6000 nM, trypsin Kᵢ=60 nM) [22]. Following the leader were Aventis Pharmaceuticals (12) [36] and GlaxoSmithKline (20) [37], who released some of the first potent, selective, and bioavailable anti-Xa candidates carrying the 2-chloro thiophene group (Table 2), which was later incorporated in other synthetic Factor-Xa inhibitors [38].

Table 3 summarizes potent FXa direct inhibitors that reached clinical trials. As early as 1999, Rhone-Poulenc Rorer decided to enter two sulfonamidopyrrolidinone derivatives (21 and 22) in clinical trials because they possessed the sought-after profile [39,40]. Since the discovery of 11 [27], the strategy of BMS was to keep the pyrazole...
as the pivot part of their molecules. This paid off because in addition to compound 23 they submitted compounds 24 (Razaxaban, Phase 2) [27, 41] and 25 (Apixaban, Phase 3) to clinical trials [42]. Apixaban inhibited free FXa as well as thrombus-associated FXa and FXa within the prothrombinase complex and did not require antithrombin III to inhibit FXa. By inhibiting FXa, Apixaban reduced directly tissue factor-induced thrombin generation and indirectly thrombin-mediated platelet aggregation, suggesting that it could prevent and treat both venous as well as arterial thrombosis [43]. In addition, Apixaban also effectively inhibited the growth of an intravascular thrombus that was formed before the closure of a shunt to induce thrombus development and indirectly thrombin-mediated platelet aggregation, suggesting that it could prevent and treat both venous as well as arterial thrombosis [43]. In addition, Apixaban also effectively inhibited the growth of an intravascular thrombus that was formed before the closure of a shunt to induce thrombus development and indirectly thrombin-mediated platelet aggregation, suggesting that it could prevent and treat both venous as well as arterial thrombosis [43]. In addition, Apixaban also effectively inhibited the growth of an intravascular thrombus that was formed before the closure of a shunt to induce thrombus development and indirectly thrombin-mediated platelet aggregation, suggesting that it could prevent and treat both venous as well as arterial thrombosis [43].

**Structure-Activity Relationships of Selected Anti-Xa Compounds**

Many reports have discussed the structure-activity relationships of Apixaban, Rivaroxaban, and Edoxaban (Table 4) [48]. By blocking the action of FXa, these agents could inhibit the initiation step of the cell-mediated hemostasis, thereby having an impact on coagulation. These compounds were designed to be small and rather lipophilic molecules and to follow Lipinski’s rule of five, which predicts the good pharmacokinetic profiles of drugs in the human body. In preclinical studies, they were reported to function as direct factor Xa inhibitor, which is representative of an extremely powerful means of inhibition [49]. Apixaban had the best fit in terms of steric bulk: Occupation of space, hydrophobicity, and Van der Waals interactions. Here, in addition to the description of the chemical characteristics of those anticoagulants, the presence of the chlorine atom on the thiophene edge group of Rivaroxaban (compound 26, Table 3) and on the pyridine edge group of Edoxaban (compound 27, Table 3) will receive particular attention.

**On the presence of the chlorine in Rivaroxaban and in Edoxaban**

Chlorine is a halogen element that is isolated as a heavy greenish-
Table 2: Potent anti-Xa inhibitors which did not reach clinical trials, listed in chronological order of release.

<table>
<thead>
<tr>
<th></th>
<th>Chemical family</th>
<th>Company</th>
<th>Ki (FXa) (nM)</th>
<th>Selectivity for Factor Xa</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Isoxazoline derivatives</td>
<td>DuPont Pharmaceuticals Company</td>
<td>0.52</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>Meta-substituted bis(amidines)</td>
<td>Rhone-Poulenc Rorer</td>
<td>13.0</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>Amidinonindole derivatives</td>
<td>DuPont Pharmaceuticals Company</td>
<td>0.32</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>N2-Aroylanthranilamide derivatives</td>
<td>Eli Lilly &amp; Company</td>
<td>11.5</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>Benoxazinone analogues</td>
<td>DuPont Pharmaceuticals Company</td>
<td>3.0</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>Pyrazole benzylamine analogues</td>
<td>DuPont Pharmaceuticals Company</td>
<td>13.0 (pM)</td>
<td>Yes</td>
</tr>
<tr>
<td>12</td>
<td>Azaindole derivatives</td>
<td>Aventis Pharmaceuticals</td>
<td>0.66</td>
<td>Yes and orally bioavailable</td>
</tr>
<tr>
<td>#</td>
<td>Structure</td>
<td>Name</td>
<td>Company</td>
<td>IC50</td>
</tr>
<tr>
<td>----</td>
<td>-----------</td>
<td>------</td>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>13</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>1-[2-(Aminomethyl) phenyl]pyrazole derivatives</td>
<td>Bristol-Myers Squibb</td>
<td>3.8</td>
</tr>
<tr>
<td>14</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>1-[2-(Aminomethyl) phenyl]pyrazole derivatives</td>
<td>Bristol-Myers Squibb</td>
<td>0.91</td>
</tr>
<tr>
<td>15</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>Guanidine/benzamidine mimics</td>
<td>Bristol-Myers Squibb</td>
<td>0.33</td>
</tr>
<tr>
<td>16</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>Guanidine/benzamidine mimics</td>
<td>Bristol-Myers Squibb</td>
<td>13.0 (pM)</td>
</tr>
<tr>
<td>17</td>
<td><img src="image5.png" alt="Structure" /></td>
<td>Benzamidine with pyruvic acid substitution</td>
<td>Ajinomoto Company Inc.</td>
<td>3.0</td>
</tr>
<tr>
<td>18</td>
<td><img src="image6.png" alt="Structure" /></td>
<td>Dihydroquinoloxin-2-yl benzamidine derivatives</td>
<td>Pfizer</td>
<td>0.83</td>
</tr>
<tr>
<td>19</td>
<td><img src="image7.png" alt="Structure" /></td>
<td>Tetrahydroisoquinoline derivatives</td>
<td>Japan Tobacco Inc.</td>
<td>30.0</td>
</tr>
<tr>
<td>20</td>
<td><img src="image8.png" alt="Structure" /></td>
<td>N-[(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]-sulfonamides</td>
<td>Glaxo SmithKline</td>
<td>4.0</td>
</tr>
</tbody>
</table>
Table 3: Molecules that reached clinical trials, listed in chronological order of release. All molecules are selective for Factor Xa and are orally bioavailable.

<table>
<thead>
<tr>
<th>21</th>
<th>NH2</th>
<th>NH2</th>
<th>Sulfonamidopyrrolidinone derivatives</th>
<th>Rhone-Poulenc Rorer</th>
<th>Ki = 2.0 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>NH2</td>
<td>OCH3</td>
<td>3-Amino-2-pyridinone derivatives</td>
<td>Rhone-Poulenc Rorer</td>
<td>Ki = 7.0 nM</td>
</tr>
<tr>
<td>23</td>
<td>NH2</td>
<td>OCH3</td>
<td>Pyrazole benzylamine analogues</td>
<td>DuPont Pharmaceuticals Company</td>
<td>Ki = 5.0 pM</td>
</tr>
<tr>
<td>24</td>
<td>H3CSO2NH</td>
<td>H3CSO2NH</td>
<td>Aminobenzisoxazoly pyrazole derivatives (Razaxaban)</td>
<td>Bristol-Myers Squibb</td>
<td>Ki = 19.0 pM</td>
</tr>
<tr>
<td>25</td>
<td>H3CSO2NH</td>
<td>H3CSO2NH</td>
<td>Apixaban</td>
<td>Bristol-Myers Squibb, Pfizer</td>
<td>Ki = 0.08 nM</td>
</tr>
<tr>
<td>26</td>
<td>BAY 59-7939 (Rivaroxaban)</td>
<td>BAY 59-7939 (Rivaroxaban)</td>
<td>Bayer HealthCare AG</td>
<td>IC50 = 0.7 nM</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Edoxaban</td>
<td>Edoxaban</td>
<td>Daiichi Sankyo</td>
<td>Ki = 0.56 nM</td>
<td></td>
</tr>
</tbody>
</table>
yellow diatomic gas of pungent odor and is used especially as a bleach, oxidizing agent, and disinfectant in water purification. The role of the Rivaroxaban chlorine on the thiophene group, also named as strong cytotoxic agents [52]. For these reasons, the chlorine atom in Rivaroxaban and Edoxaban should be replaced by a bioisostere functional group, i.e., a group of atoms having similar biological effects rather than chemical properties, which at least should not disable the molecules.

Structure-activity relationships of Apixaban

Apixaban has been indicated for the prevention of VTE in adult patients who have undergone elective hip or knee replacement surgery; for the prevention of stroke and systemic embolism in adult patients who have undergone elective hip or knee replacement surgery; for the prevention of stroke and systemic embolism in adult

### Table 4: Pharmacological properties of Apixaban, Rivaroxaban, and Edoxaban.

<table>
<thead>
<tr>
<th></th>
<th>Apixaban</th>
<th>Rivaroxaban</th>
<th>Edoxaban</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase II</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dosage (study, compared to warfarin)</td>
<td>5 mg twice a day (ARISTOTLE registration)</td>
<td>20 mg once daily (ROCKET AF), 10 mg o. d. (registration)</td>
<td>30 mg or 60 mg once daily (ENGAGE AF-TIMI 48)</td>
</tr>
<tr>
<td>Dose adjusted according to weight and age</td>
<td>No</td>
<td>No</td>
<td>Yes, to treat patients with renal impairment and/or low body weight, or those taking strong P-glycoprotein inhibitors</td>
</tr>
<tr>
<td>Coagulation monitoring/HR</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Phase I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Route of administration</td>
<td>Oral</td>
<td>Oral</td>
<td>Oral</td>
</tr>
<tr>
<td>Mean half-life, (h, t1/2)</td>
<td>15-Aug</td>
<td>11-Jul</td>
<td>11-Sep</td>
</tr>
<tr>
<td>Tmax, (h)</td>
<td>4-Mar</td>
<td>4-Feb</td>
<td>2-Jan</td>
</tr>
<tr>
<td>Potential for drug-drug interaction</td>
<td>CYP3A4 P-GP inhibit</td>
<td>CYP3A4 P-GP inhibit</td>
<td>CYP3A4 P-GP inhibit</td>
</tr>
<tr>
<td>Renal excretion, %</td>
<td>25</td>
<td>66</td>
<td>35</td>
</tr>
<tr>
<td>Kon (M⁻¹ s⁻¹); Koff (s)</td>
<td>Kon= 2 × 10⁻²</td>
<td>Kon=1.7 10⁻² (very fast); Koff=5 10⁻³</td>
<td></td>
</tr>
<tr>
<td>Inhibition of Prothrombinase</td>
<td>Non-competitive</td>
<td>I₅₀=2.1 nmol/l</td>
<td>Ki=2.98 nmol/l</td>
</tr>
<tr>
<td>Drug or food interaction</td>
<td>Clarithromycin</td>
<td>Ritonavir Ketokonazole</td>
<td>No</td>
</tr>
<tr>
<td><strong>Preclinical tests</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MW (Da)</td>
<td>459.5</td>
<td>435.8</td>
<td>548</td>
</tr>
<tr>
<td>In vitro activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Fxa Ki (nM)</td>
<td>0.08</td>
<td>0.4</td>
<td>0.56</td>
</tr>
<tr>
<td>- cloting assays (µM)</td>
<td>PT2x=3.8; aPTT2x=5.1</td>
<td>PT2x=0.23; aPTT2x=0.69</td>
<td>PT2x=0.26; aPTT2x=0.51</td>
</tr>
<tr>
<td>Preclinical efficacy/bleeding studies</td>
<td>thrombosis and bleeding models (rabbit, rat)</td>
<td>thrombosis and bleeding models (rabbit, rat)</td>
<td>thrombosis and bleeding models (rabbit, rat)</td>
</tr>
<tr>
<td>Oral bioavailability (%)</td>
<td>51 (chimpanzee), 88 (dog), 34 (rat)</td>
<td>57-66 (rat), 60-88 (dog), 66 (human)</td>
<td>50 (monkeys), 61.8 (human)</td>
</tr>
<tr>
<td>Volume of distribution (L/kg)</td>
<td>0.17 (chimpanzee), 0.28 (dog), 0.31 (rat), 0.3 (human)</td>
<td>0.3 (rat), 0.4 (dog)</td>
<td>0.4 (rat), 0.3 (dog), 10 (human)</td>
</tr>
<tr>
<td>Protein binding (%)</td>
<td>95 (chimpanzee), 92 (dog), 96 (rat), 87 (human)</td>
<td>99 (rat), 90 (dog), 92-95 (human)</td>
<td>40-59 (human)</td>
</tr>
<tr>
<td>Systemic clearance (L/h/kg)</td>
<td>0.018 (chimpanzee), 0.032 (dog), 0.026 (rat), 0.015 (human)</td>
<td>0.4 (rat), 0.3 (dog), 10 (human)</td>
<td>12.7 (human)</td>
</tr>
<tr>
<td>Elimination (% of dose)</td>
<td>fecal 54%, renal 15% (mouse, rat, rabbit, dog); fecal 46%, renal 25-28% (human)</td>
<td>fecal 67%, renal 25% (rat); fecal 43%, renal 52% (dog); fecal 33%; renal 33% (human)</td>
<td>fecal 62.2% (human)</td>
</tr>
<tr>
<td>Mechanism of action</td>
<td>Selective direct Factor Xa inhibitor</td>
<td>Selective direct Factor Xa inhibitor</td>
<td>Competitive direct Factor Xa inhibitor</td>
</tr>
<tr>
<td>Reversible inhibitor</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
| **Table 5:** Kᵢ, clotting assays and kᵣ, measurements for Apixaban, Rivaroxaban, and Edoxaban.

<table>
<thead>
<tr>
<th></th>
<th>Apixaban</th>
<th>Rivaroxaban</th>
<th>Edoxaban</th>
</tr>
</thead>
<tbody>
<tr>
<td>in vitro activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FXa Kᵢ (nM)</td>
<td>0.08</td>
<td>0.4</td>
<td>0.56</td>
</tr>
<tr>
<td>Clotting assays (µM)</td>
<td>PT2x=3.8</td>
<td>0.23</td>
<td>0.26</td>
</tr>
<tr>
<td>aPTT2x</td>
<td>5.1</td>
<td>0.69</td>
<td>0.51</td>
</tr>
<tr>
<td>kᵣ (M⁻¹ s⁻¹)</td>
<td>2 × 10⁻²</td>
<td>1.7 10⁻² (very fast)</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

For any class of compounds, engineers should be aware that using a chlorine atom is not advantageous. Indeed, Rivaroxaban should be considered as a dual inhibitor because in the past chlorine-bearing molecules have shown a high potency to interact with DNA and to act as strong cytotoxic agents [52]. For these reasons, the chlorine atom in Rivaroxaban and Edoxaban should be replaced by a bioisostere functional group, i.e., a group of atoms having similar biological effects rather than chemical properties, which at least should not disable the molecules.
patients with nonvalvular AFIB and with one or more risk factors such as prior stroke or Transient Ischemic Attack (TIA), age ≥ 75 years, hypertension, diabetes mellitus, and symptomatic heart failure (NYHA Class ≥ II) [53]. It has been presented as a film-coated tablet containing 2.5 mg of Apixaban [54]. The marketed core tablet has included microcrystalline cellulose and anhydrous lactose, croscarmellose sodium, magnesium stearate as a lubricant, and sodium laurilsulphate as a wetting agent. The film coating has contained hypromellose, lactose monohydrate, yellow iron oxide, triacetin, and titanium dioxide. It showed polymorphism however, and only one form was consistently produced by the reported synthetic process [53].

Diazotization of 33 followed by condensation with ethyl 2-chloroacetoacetate afforded 34. Treatment of 34 with compound 35 in the presence of excess base, followed by treatment with a strong acid afforded intermediate 36. Subsequent treatment of 36 with 8-valerolactam under Ullmann conditions followed by aminolysis afforded Apixaban (25) in an overall 10.1% yield (Scheme 2).

Synthesized Apixaban appeared as a white to pale yellow [44], non-hygroscopic crystalline powder. It was characterized with a Molecular Weight (MW)=459.5 Da (Table 4), 9 heteroatoms, 1 H-bond donor (NH$_2$) and 5 H-bond acceptors (N, O). It was a non-ionisable compound, and its partition coefficient at 24°C in the n-octanol/water buffer system, a technique that stimulates the biological membrane of cells, was 44.7 (a high value) at pH 7.4. It was highly soluble for doses up to 10 mg/mL and was a low permeable compound since the fraction of oral dose absorbed was <90%. Thus, as per the Biopharmaceutical Classification System (BCS), it has been classified as a class III (high solubility/low permeability) compound [53]. Apixaban was reported as a planar, fixed 5-membered ring (a pyrazole) fused to a flexible 6-membered ring (piperidin-2-one) to which were connected a part that fits in the S1 pocket and another that fits in the S4 pocket (Figure 2 and 3). Indeed, the planar pyrazole ring replaced a flexible ring (an isoxazoline, discovered with HTS as a potent feature for direct anti-Xa) in order to improve the IC$_{50}$. This central chemical system made

\[
\text{Scheme 1: A) Reported chemical reaction involving the union between a chlorothiophene (28) molecule and a phenol derivative (29) [50,51]. B) Extrapolation to the possible union with Tyr228 (31) by Rivaroxaban (26).}
\]

\[
\text{Scheme 2: Synthesis of Apixaban (25).}
\]
a link between the part that fills the S1 and S4 pockets. The part of
the molecule that fits in the S1 pocket was made hydrophobic, which
resulted in the improvement of the PK (pharmacokinetic) profile and
the oral absorption, and to slight reduction of the IC_{50} of the
drug (Figure 2 and 3). The selectivity of Apixaban for FXa over Trypsin
was rationalized in 2010 (Figure 4) [49]. Indeed, the amino acid at the 190
position, which is an Alanine in the FXa active site instead of a Ser190
in Trypsin, makes the S1 pocket larger. Thus selectivity could have
been reached if this difference in size was considered. Apixaban is
selective because it benefits from a OCH_{3} group on the aryl (Figure 2
and 4), whose spherical atomic volume allows a good fit in the
S1 pocket of FXa but not in the pocket of Trypsin. Figure 4B shows a
schematic explanation for why Ser190 does not tolerate the insertion
of OCH_{3} (O, red sphere; CH_{3}, green sphere) because their spherical
atomic volumes are not allowed to overlap. Originally, just an amide
was used to link the pyrazole to the part that fits into the S4 pocket. It
was then changed to the present piperidinone ring in order to avoid
metabolism into aniline of the entire part that fits in the S4 pocket.
The attached group then showed a planar aromatic aryl ring bonded
to another piperidinone. This more flexible feature came in place of
a rigid biaryl moiety and was shown to significantly improve the PK
profile of the compound [45].

One of the most important features in Apixaban for its potency as
per os drug was the incorporation of the amide (CONH_{2}) substituent
(in red on Figure 2) [55]. This substituent definitely solved the
solubility issue and also strongly helped to decrease the Kd by
anchoring the compound in the active site with strong H-bonds with
the surrounding Phe174, Gly216, Arg143, and Gln192 (Figure 3).

Apixaban has been reported to get biotransformed into two metabolites in humans: O-demethyl Apixaban (37, minor product,
possibly by Breast Cancer Resistance Protein (BCRP)), or
P-Glycoprotein (P-GP) and 4-hydroxy Apixaban (38, major product,
by CYP3A4/5). Compound 37 was found to get biotransformed
by SULT1A1 in O-demethyl Apixaban sulphate (39) (Scheme 3).
Apixaban also showed no significant inhibition or induction to
important P450 enzymes [56]. The dual mechanism of excretion was
reported to occur via the kidneys (26% to 28% containing Apixaban
and inactive metabolites), and via the fecal-biliary route (46%). Due
to the multiple routes of Apixaban clearance, the possible interactions
with CYP3A4/5 inhibitors were minor [56].

**Structure-activity relationships of Rivaroxaban**

Rivaroxaban has been indicated as 10 mg for the prevention of
VTE in adult patients undergoing elective hip or knee replacement
surgery; and as 15 mg or 20 mg for the prevention of stroke and
systemic embolism in adult patients with nonvalvular AFIB with one
or more risk factors such as congestive heart failure, hypertension, age
≥ 75 years, diabetes mellitus, and prior stroke or transient ischemic
attack [57].

Rivaroxaban has been proposed for market as a film-coated
tablet for a direct release. Other ingredients in the core tablet have
included microcrystalline cellulose and lactose monohydrate,
croscarmellose sodium, hypromellose 5 cp, magnesium stearate,
and sodium laurilsulphate. The film coating has contained iron (III)
oxide, hydroxypropyl methylcellulose 15 cp, polyethylene glycol,
and Titanium (IV) oxide. It showed polymorphism, but only the
thermodynamically stable polymorphism has been used for testing
and commercial purposes [57].

Rivaroxaban was identified as a compound of interest with HTS.
Enantiomerically pure Rivaroxaban, i.e., one of the two in a pair of
chemical compounds whose molecular structures have a non-
superimposable mirror-image relationship to each other, was obtained
from a linear synthesis sequence (Scheme 4). Starting from compound
40, bearing a free amino group, a condensation released Baeyer’s
strain from the epoxide of the enantiopure epoxypropylphthalimide
to generate compound 41. Activation of 41 with a catalytic amount of
dimethylaminopyridine and then with a carbonyldimidazole solution
afforded the central oxazolidinone ring, compound 42. The amino
and commercial purposes [57].
into Rivaroxaban (26).

Compound 26 was characterized with a MW=435.8 Da (Table 4), 9 heteroatoms, 1 H-bond donor (NH) and 6 H-bond acceptors (N, O). It was practically insoluble in buffered aqueous solutions; nevertheless, its particles could have been dissolved by being crushed to micron size. The partition coefficient in n-octanol/water was 1.5. These low solubility and high permeability properties allowed the compound to get a position in the class II of the BCS. Rivaroxaban has been made as an asymmetric compound and is also shorter in length than Apixaban. The S configuration was the active enantiomer. The five-membered ring was incorporated to link the S1 and S4 features that were optimally connected on its positions 1 and 3 (Figure 5).

In this molecule, the feature that was designed to fit the S1 pocket, the chlorothiophene, was shown to be crucial for the potency of the compound because the chlorine should induce stabilization in the S1 pocket by interacting with the nearby Tyr228. The feature designed to fit into the S4 pocket could only stabilize the interaction of the compound by a strong H-bond with Gly229 and showed a minor impact on the potency at inhibiting FXa (Figure 5 and 6) [45].

In the human body, Rivaroxaban has been detected to be metabolized by liver enzymes, principally cytochrome P450 3A4, and also by cytochrome-independent mechanisms. No adverse effects were detected from the generated metabolites. The dual mechanism of excretion was shown to be partitioned as follows: approximately 66% of the dose was excreted via the kidneys, in roughly equal proportions of Rivaroxaban and inactive metabolites; the remainder was excreted by the fecal-biliary route. Intestinal excretion of Rivaroxaban appeared to be mediated, at least in part, by P-GP because potent P-GP inhibitors increased its plasma concentrations [58].

**Structure-activity relationships of Edoxaban**

Edoxaban has been presented as tablet containing 15 mg or 30 mg of Edoxaban (DU-176b) for oral use. No data on polymorphism was reported [59].

Synthesized Edoxaban was characterized with a MW=548.0 Da (Table 4), 12 heteroatoms, 3 H-bond donors (NH), and 8 H-bond acceptors (N, O, S). No data on solubility and permeability were reported. Edoxaban uses a flexible 6-membered ring (a cyclohexane) as a linker (Figure 7). In this class of molecule, a central 6-membered ring gave a better IC₅₀ than any other ring size. This molecule could exist as both cis and trans-stereoisomers. The cis-stereoisomer was selected for R&D purposes. Just as in Apixaban, an aide substituent was used to improve the bioavailability of this class of compounds. Moreover, with this substituent, optimal microsomal stability and improved activity in the PT (prothrombin time) assays were obtained. The part that was designed to fit in the S1 pocket was intended to interact in an analogous manner to the one in Rivaroxaban because the bulky chlorine atom was responsible for inducing selectivity (factor >10,000) for serine proteases. The part that was designed to fit in the S4 pocket was mainly responsible for the selectivity against trypsin because the protein S4 pocket could not accommodate such a bulky substituent (Figure 7) [48].

Edoxaban has not been detected to be metabolized by either liver enzymes or microsomes. The observed metabolites, formed through hydrolysis, were compound 44 (elimination of 5-chloropyridin-2-yl-oxamoyl group) and compound 45 (elimination of N, N-dimethylcarbamoyl group) (Scheme 5). Other research has shown
Pharmacological Properties

Table 4 summarizes the pharmacological properties of Apixaban [46,62], Rivaroxaban [63], and Edoxaban. The clinical pharmacology profile of Apixaban has been characterized based on the results of 26 pharmacology studies. The genuine Apixaban rapidly reacted with Factor Xa ($K_{in}$=2 × 10$^7$ M$^{-1}$s$^{-1}$), was a powerful inhibitor ($K_i$=0.3 nM at 37°C), and was bioavailable in chimpanzees and dogs. With a half-life of 8 h to 15 h, Apixaban reached a maximum concentration 3 h to 4 h after dose administration. The absolute bioavailability of orally administered Apixaban was approximately 52%. Protein binding in serum taken from humans following Apixaban administration (Apixaban concentrations from 0.068 µg/mL to 0.22 µg/mL) was around 93% [64].

To support the current, proposed indication in orthopedic surgery, four additional studies were conducted to demonstrate the efficacy of Apixaban relative to Enoxaparin: One phase II, dose-ranging study in subjects undergoing total knee replacement surgery (CV185010); two phase III, pivotal studies in patients undergoing total hip replacement and total knee replacement (CV185035 and CV185047, respectively); and a supportive phase III study in patients undergoing total knee replacement (CV185034). Six ongoing phase II randomized, controlled Apixaban study have been started to evaluate efficacy and safety in other indications (prevention of stroke and systemic embolism in AFIB, VTE prevention, secondary prevention of acute coronary syndrome, and VTE prevention in subjects with acute medical illness) [64]. In some studies, however, the bleeding risk was higher than with Enoxaparin; thus only results in cardiological applications would free this product from doubts on safety profile [65]. Recently completed, the ARISTOTLE trial (funded by BMS and Pfizer) randomized approximately 18,000 patients to warfarin and dose-adjusted Rivaroxaban during those trials. Nevertheless, there has been a trend for a higher risk of bleeding in treated groups with Rivaroxaban (dose =10 mg) during those trials. Rivaroxaban was shown to be better than the LMWH Enoxaparin during the phase III clinical trials for safety and efficacy. Clinical efficacy was assessed in the RECORD 1 and RECORD 2 trials in total knee replacement surgery, and the RECORD 3 trial in total knee replacement surgery, which were randomized, double-blind trials with both North American and European populations [57]. Rivaroxaban was approved for stroke prevention in non-valvular AFIB, orthopedic surgery, general treatment of DVT, and prevention of recurrence [67].

Edoxaban has been shown to be competitively potent in vitro ($K_i$ (FXa)=0.56 nM) with a maximum inhibition obtained 1½ h after oral administration. In terms of bioactivity, the antithrombotic effect was prolonged for up to 5 h, and the anticoagulant effect correlated linearly with the plasma concentration [47,59]. Edoxaban has been self-developed by Daiichi-Sankyo in USA, Europe, and Asian countries. It showed a similar safety profile as warfarin in patients with AFIB and validated in phase II studies [47]. Because it showed a reduced risk of bleeding, it received a local Japanese marketing approval for 15 mg and 30 mg tablets to prevent VTE after major orthopedic surgery [47]. The ENGAGE AF-TIMI 48 clinical phase III study funded by Daiichi Sankyo was completed, comparing Edoxaban to warfarin for the prevention of stroke and systemic embolism in patients with AFIB. Edoxaban was approved by the US FDA in January, 2015 for the prevention of stroke risk in non-valvular AFIB and for the treatment of DVT and PE following 5 to 10 days of initial therapy with a parenteral anticoagulant. It will be marketed as SAVAYSA™ [70].

Discussion

ADME values showed Apixaban and Rivaroxaban are equivalent in routes of elimination and potential drug-drug interactions despite that no biotransforms occurred because of cytochrome P enzymes or NADPH in human liver microses [60]. No serious adverse effects were detected from these metabolites. During the research on elimination pathways, Edoxaban was found mostly unchanged (>75% total dose), with modest metabolism (<25% total dose) in plasma, urine, and feces. Edoxaban was eliminated through multiple pathways including renal and biliary excretion [61].

Figure 8: Conformations of the lowest total energy of A) Apixaban (25), B) Rivaroxaban (26), and C) Edoxaban (27). Perkin Elmer ChemBio3D Ultra 13.0 software was used to generate the most stable conformations. Apixaban has a rigid L-shape structure that could easily fit in the FXa active site. Rivaroxaban is stable as a Z-shape structure and Edoxaban as a C-shape structure, thus they need energy to turn to the L-shape structure that could fit in the FXa active site.
both forming different metabolites (Table 4). Both are more easily bound by proteins, thus their transport in the body should be much easier. In contrast to Apixaban and Edoxaban, Rivaroxaban binds reversibly to plasma protein with a ratio >90% (Table 4). So, Rivaroxaban should have a better chance to circulate quickly in blood and not be dialyzed. The poly functional nature of Apixaban and Rivaroxaban, i.e., the number of atom groups that could involve interactions may explain this (Figure 2 and 5). In addition, since the measured half-life time of Apixaban (8 h to 15 h) was found to be similar to the half-life time of Rivaroxaban (7 h to 11 h), an adequate once-a-day or twice-a-day low dose, which could be beneficial to avoid the dose-related bleeding risks, were considered for registration (Table 4). In terms of drug or food interaction and dosage, Edoxaban was the most-studied, which proved liability of the data. Rivaroxaban was studied at an interesting low dose but was found to interact with other drugs (Table 4).

Since the year 2000, the main goal in research on new anticoagulants has been to develop anti-FXa agents that could be administered per os with limited risks of bleeding [71,72]. If the main goal of the strategy was to obtain a drug that could be administered per os, then the first step in this direction was to get a true absorption through the intestine. In this regard, all drugs considered herein reached this goal; they also were shown to transit through the GI tract with enhanced chemical stability toward the first pass effect and metabolism [73]. For bleeding risk, they were assessed as neither non-inflammatory nor superior to the LMWH, so the risk should still be taken into account [74,75].

The inhibition of FXa was chosen on purpose to modify some coagulation parameters such as Quick’s prothrombin time (PTx2) and activated partial thromboplastin time (aPTTxx2). When looking at the reported pharmacological data on these properties, some results could not be correlated this way. For example, in the clotting assays, the dose needed for Apixaban to double clotting times was very high (aPTTxx2=5.1 µM) (Table 4), although Apixaban was shown to inhibit FXa with a very low K value (K=0.08 nM) (Table 5). Also, despite Rivaroxaban being more structurally related to Apixaban than to Edoxaban, the activities of Rivaroxaban and Apixaban were very different and the activity of Rivaroxaban (aPTTxx2=0.69 µM) was equivalent to that shown by Edoxaban (aPTTxx2=0.56 µM) (Table 4). No explanation of this observation is available. Meanwhile, Quick’s Time (QT) prolongation was addressed both in vitro and in healthy volunteers for Apixaban and Rivaroxaban. PTx2 value was insignificant for Apixaban and it showed no prolongation of QT interval that could be correlated. PTx2 value was significant for Rivaroxaban and it showed no prolongation of QT interval, this could not be correlated. No explanation of this observation is available. All patients are different and all of them are not always in healthy condition at the time VTPE prophylaxis is needed, which makes things more difficult. Because the clinical studies are sometimes difficult to correlate [76], understanding the differences in chemical characteristics of the new anticoagulants for AFI should help the clinician to distinguish drugs with similar pharmacological profiles and to decide which one to choose for adequate therapy.

Therefore, the purpose of this discussion is to highlight some chemical characteristics of the drugs in order to clarify the understanding of each drug for the physician. Molecular Weight (MW), polarity, lipophilicity; solubility, ionizability, rigidity, and the quantity of H-bond donors and acceptors held by a given compound are 7 relevant drug characteristics that could help to predict if a molecule could become a drug.

First, molecular docking studies (Figure 3 and 7) [49,77], a computer-assisted method used to determine ligand-receptors interactions, helped to understand why those inhibitors act as competitive inhibitors, i.e., once inside the catalytic site, they fill it almost totally and leave no space for another substrate to enter, and thereby the binding of these drugs leaves a biologically inactive supramolecule. This causes ligand structures to ‘freeze’ and to lose vibrational energy, resulting from a periodic motion of the particles of an elastic body, and rotational energy, resulting from the turning of a molecule part about its long axis as if on a pivot. Thus, the rigidity of a molecule should be a matter of utmost concern because the binding of a rigid molecule would lose less energy in comparison to a more flexible one.

Three dimensional representations of the lowest energy drug conformers in the gas phase were calculated (Figure 8, Perkin Elmer ChemBio3D Ultra) and clarified that Apixaban is the most rigid and undergoes little dynamic conformational changes with a lowest energy state of 57.8 kcal/mol, i.e., the optimized atom setting in space. Rivaroxaban has a flexible chlorothiophene arm that takes the stable Z-shaped conformation and has a lowest energy state of 42.0 kcal/mol (Figure 8). It is expected it will be in a different spatial conformation in solution; Edoxaban takes a C-shaped conformation with the S1 and S4 features stacked above one another in the gas phase with a lowest energy state found at 17.4 kcal/mol (Figure 8). Thus, the rigid L-shaped conformation of Apixaban would be the most suitable for a lock and key model of inhibition of FXa. The other two structures should be more flexible and thus more dynamic in solution. This could explain the observed good K and fast K values that were measured (Table 5).

These inhibition and kinetic values reflected the energetically favored insertion and ligand-receptor association observed with Apixaban. For the other two drugs, stabilization in the FXa active site may occur due to the great number of atoms these molecules bear that are strong H-bond donors and acceptors. Indeed, these atoms could fix the fit by displacing water molecules set around the amino acids in the binding site [52]. These other characteristics could help us to better understand the overall low K values of these compounds. With at least one H bond donor and five H bond acceptors, these molecules possess groups that could anchor them on a time-limited manner by interacting non-covalently via the H atoms or the free pairs of electron on the amino acids surrounding the active site (Figure 3 and 7) [56]. Moreover, these molecules have numerous bulky substituent’s (such as aryl and pyridine groups, piperidino) that fill the inside of the S1 and S4 pockets by numerous Van-der-Waals interactions, involving the arrangements of atoms in space that also repel water and thus diminish the local disorganization of atoms. This diminution of the degree of disorder in the FXa catalytic site could contribute to a long-lasting effect of the inactivation of FXa. The supramolecule complex thus could be formed via a multiple binding mode.

With an oral absorption of 66% in human and 86% in dog (Table 4), Rivaroxaban was quickly assessed as bio-available. It is a molecule that has a MW<450 Da, is polar and has a small particle size. These characteristics should make it sufficiently permeable to go through the enterocytes of the GI tract via a paracellular type of absorption, which should proceed through the highly polar tight junctions. (These experimental results were rationalized this way because the
size of the aqueous pores between cells in the GI tract is larger in dog than in human [78].)

Moreover, no explanation of the observation that Apixaban is water soluble but low permeable is available [53]. The low permeability could be rationalized by considering it is bigger (MW>450 Da), has bigger particle size, and that it could be a better substrate to P-GP than to plasma protein binding.

Rivaroxaban could have benefited from the overall good data on bioavailability and potency that were quickly generated for a quick approval; in contrast, approval of Apixaban and Edoxaban faced more administrative difficulties in generating the equivalent amount of data. Nevertheless, some teratogenic effects linked to the uptake of Rivaroxaban were seen at clinically relevant exposure [57], but not for Apixaban or Edoxaban. These were shown to affect mainly the skeleton, heart, and vessels. Because these teratogenic effects cannot be excluded from further consideration, it is reasonable to speculate on the possible role that the chlorine atom on Rivaroxaban could play at inducing these effects by means of a chemical condensation reaction.

Rivaroxaban is structurally related to the clinically used antibiotic Linezolid, which has the potency to inhibit bacterial protein synthesis. An additional concern about Linezolid that has not been addressed yet is the possibility of mitochondrial toxicity leading to a loss of mitochondrial function, which may deregulate physiological apoptosis and probably induce carcinogenesis as a result [79].

Edoxaban offers fewer chemical groups that could interact with biological receptors; this correlates with its less potent Kᵢ value (Table 4). Nevertheless, with 12 heteroatoms and one ionicizable nitrogen atom, Edoxaban could be characterized as bearing the most polar surface of all three structures (Figure 7). This polarity could thus create electrostatic interactions in the active site that are very energizing and that could favor the binding if the FXa binding site was also polar. Its measured Kᵢ value reflects that the binding site should not be so polar (Table 5).

The difference between the high potency of Apixaban on the purified FXa and its unaccounted weaker activity in the clotting time assays was rationalized. The Kᵢ value was found to be very low, and its binding was found to be kinetically controlled (i.e., K₋₋ is very fast) because the molecular recognition between the receptor and the molecule could follow a lock and key model. Indeed, the structure of Apixaban was shown here to take an L-shape. This geometric 3D structure was seen to perfectly fit the active site at the top of the FXa protease. The insertion of the other two molecules, Rivaroxaban and Edoxaban, in the purified FXa protease should be more energy consuming because their structures are less rigid. They were thus less potent on the purified protease, whereas their activity in the clotting time assays was more potent. These different activities were also correlated in regards to the differences in permeability, solubility, and protein binding shown by each molecule. Thus the better chemical profile of Apixaban gave the molecule the lowest Kᵢ value, whereas the better biological profile of Rivaroxaban and Edoxaban gave those compounds better results in the clotting time assays.

Conclusion

In the 21st century, the assurance that the 1980s’ LMWHs were flawed provided great impetus for the discovery of synthetic, small-molecule direct FXa inhibitors. The most potent molecules that were discovered showed a selective best fit in the FXa’s active site and invariably engaged both sites (S1 and S4). These features improved some properties of the original molecules that were discovered in the field of direct anti-Xa but were not tested and yielded to the presently registered drugs. On top of this long-term development lie Apixaban, Rivaroxaban, and Edoxaban. This review focuses on the outcomes between 2000 and 2010; molecules that were disclosed thereafter would be the subject of another publication.

These molecules entered clinical trials with the hope of being registered for many applications. To date, they are mostly recommended for the prevention of first or recurrent stroke in patients with nonvalvular AFIB. These drugs are potent, orally available, and selective FXa inhibitors; nevertheless some data have no rational explanation and some clinical studies are only somewhat comparable. They should be selected only on the basis of the consideration of risk factors, cost, potential for drug interactions, and the hemorrhagic tendency of the patients. Owing to its polyfunctional chemical nature and the lack of a peripheral chlorine atom in its structure, Apixaban seems to be the one to select for patients who are sensitive to side effects and for preventing a blood clot efficiently. Rivaroxaban meets the criteria for long-term treatment with a minimum dose because higher dosing should induce poisonous effects. Despite the presence of a chlorine atom, Edoxaban should be safer but somewhat less potent than Rivaroxaban. As an alternative, researchers could start to consider other targets upstream in the coagulation cascade.

Acknowledgement

Special thanks to Dr. Kelly Keating (Pharmaceutical Research Institute at Albany College of Pharmacy and Health Sciences) for her excellent editing. Thanks to Mrs. Fabienne Bridoux and Mr. Bruno Bridoux for constant help and support. Very special thanks to Mr. Casey Steffen, the creator of the illustration in Figure 1; please visit www.steffenfx.com for other visualizations of emerging discoveries.

References

10. Junqueira DR, Perini E, Penholati RR, CarVALho MG. Unfractionated


44. Pinto DJ, Orwat MJ, Koch S, Rossi KA, Alexander RS, Smallwood A, et al. Discovery of 1-(4-methoxypyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazole[3,4-c]pyridine-3-carboxamide (apixaban, BMS-562247), a highly potent, selective, efficacious, and...


