Synthetic heparin derivatives as new anticoagulant drugs

Martin de Kort, Rogier C. Buijsman and Constant A.A. van Boeckel

The journey towards a detailed mechanistic understanding of the anticoagulant action of heparin has resulted in synthetic mimetics with improved pharmacodynamic profiles. Inspired by the ternary complex formation of heparin with antithrombin III and thrombin, the active pentasaccharide fondaparinux has been succeeded by several clinical candidates, such as SR123781, that have tailor-made factor Xa and thrombin inhibitory activities combined with less aspecific binding (e.g. binding to platelet factor 4 involved in thrombocytopenia). Novel compounds with both antithrombin III-mediated inhibition of factor Xa and direct thrombin inhibition are emerging. Org42675 is one such compound, balancing dual inhibition of factor Xa and thrombin in one anticoagulant drug, with excellent pharmacokinetic properties and strong inhibitory activity toward clot-bound thrombin.

Heparin was discovered in 1922 in the laboratory of Howell [1]. This sulfated glycosaminoglycan is isolated from porcine intestinal mucosa and is the most widely studied natural anticoagulant. Heparin acquired its name because it is abundant in liver tissues and is composed of a heterogeneous mixture of straight-chain anionic mucopolysaccharides spanning 20–100 monosaccharides (Figure 1a). The varying degree of sulfation and the presence of different 1-4 linked uronic acid and glucosamine disaccharide units gives rise to a complex overall structure [2].

The anticoagulant action of heparin is based on activation of the serine protease inhibitor antithrombin III (ATIII), which accelerates the inhibition of two key proteases in the blood-coagulation cascade – factor Xa (fXa) and thrombin. In the early 1980s, it was determined that a unique pentasaccharide domain in some heparin chains is the minimal sequence required for binding and activation of ATIII [3]. A few years after this structural elucidation, the first active pentasaccharide analogue (**1**, Figure 1b) was synthesized [4–6], laying a basis for R&D programs directed toward two new classes of carbohydrate-based antithrombotics: (i) selective ATIII-mediated inhibitors of fXa; and (ii) dual fXa and thrombin inhibitors.

Clinical aspects

Heparin has been used clinically since 1937 [1] and is a highly effective antithrombotic agent in the prevention and treatment of numerous thromboembolic disorders (http://patients.uptodate.com). Although it offers rapid anticoagulation after subcutaneous administration and is relatively inexpensive, heparin has several limitations. First, heparin is extracted from porcine intestinal mucosa or bovine intestinal or lung tissue, with the potential risk of pathogenic contamination. Since the outbreak of bovine spongiform encephalopathy (or mad cow disease), only porcine derived heparin can be used in the USA and Europe, which poses a threat to the heparin market because of the potential shortage of raw materials. Second, heparin displays a narrow therapeutic window of adequate anticoagulation without bleeding. Third,

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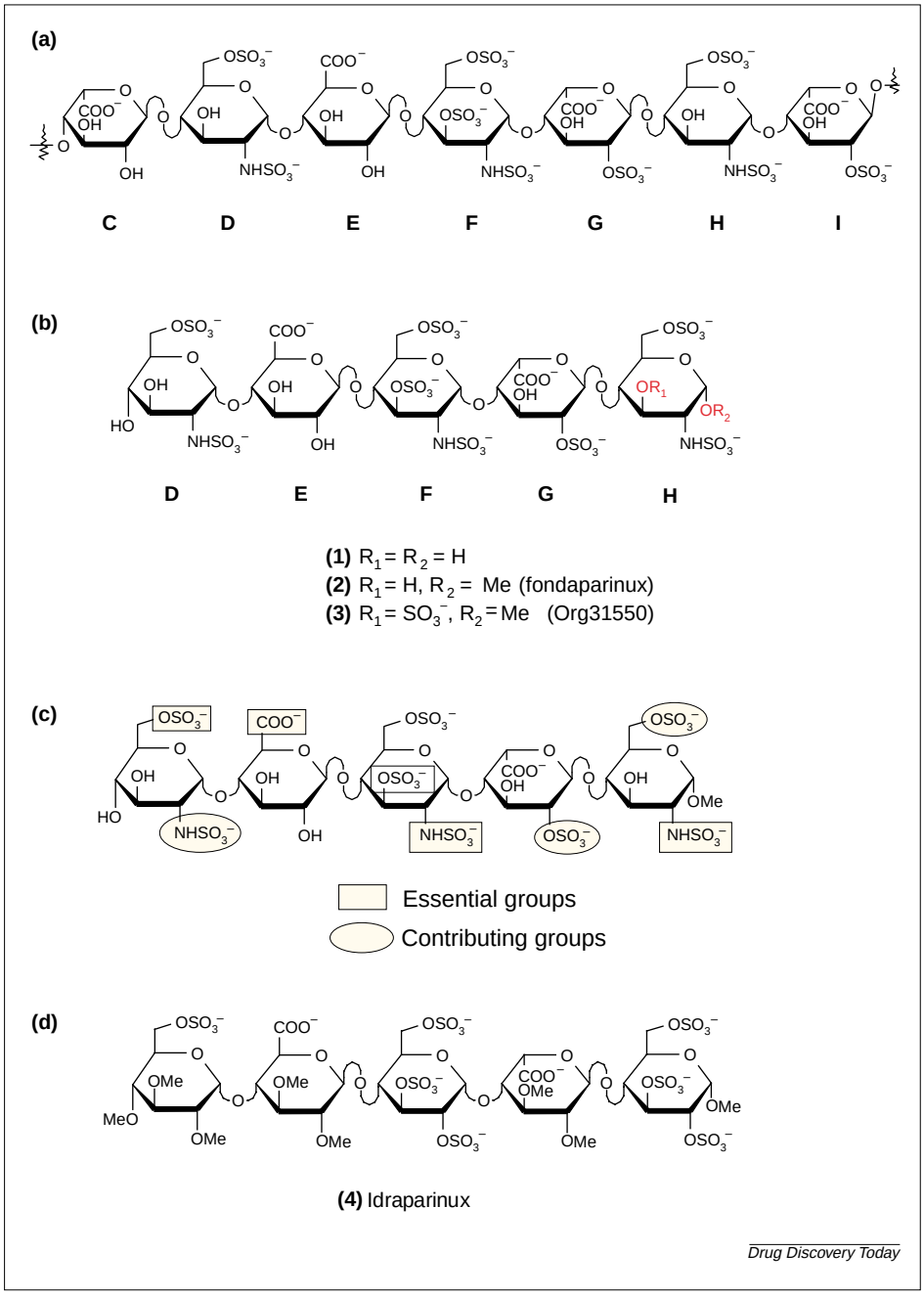

FIGURE 1

Structure of heparin and pentasaccharide fragments. (a) Part of the negatively charged oligosaccharide chain of heparin containing the ATIII-binding pentasaccharide motif. **(b)** The first synthetic ATIII-activating pentasaccharide **1** and the stabilized derivatives **2** (the antithrombotic agent fondaparinux) and a potent derivative **3** (Org31550). **(c)** SAR for the active pentasaccharide illustrating the role of the negatively charged functional groups. Functional groups highlighted in a box are essential for activation of ATIII. **(d)** The simplified pentasaccharide **4** with increased affinity for ATIII exhibiting a circulating $t_{1/2}$ of approximately one week.

it exhibits a highly variable dose-response relationship that requires monitoring by laboratory testing. The variable anticoagulant response is, in part, the result of differences in the bioavailability of subcutaneous heparin and the competitive occupation of binding sites by plasma proteins (other than ATIII and coagulation factors), proteins secreted by platelets [e.g. platelet factor 4 (PF4)] and endothelial cells. Finally, a significant number of patients undergoing prolonged treatment with heparin suffer from heparin-induced thrombocytopenia (HIT; Box 1), leading to severe thrombotic complications, such as myocardial infarction, pulmonary embolism and occlusion of limb arteries.

During the time that the active pentasaccharide sequence of heparin was identified, low-molecular-weight heparins (LMWHs), produced by fragmentation of heparin, were investigated in various clinical trials. Remarkably, despite their higher anti-fXa:antithrombin ratio, LMWHs were efficacious in the prevention of deep venous thrombosis, displaying a better therapeutic window than heparin, which was an incentive to develop the active pentasaccharide as a new drug with an even further improved pharmacological profile.

Selective inhibition of factor Xa

Synthetic pentasaccharide

When Organon and Sanofi-Synthélabo (now Sanofi-Aventis) embarked on a high-risk R&D collaboration, the pentasaccharide **2** [SR90107/Org31540 (fondaparinux); Figure 1b] was selected for further development. Compound **2** is closely related to the natural sequence of heparin, with a stabilizing methyl group at the reducing end of monosaccharide unit H [7]. Fondaparinux binds completely to ATIII, which protects the small pentasaccharide from rapid elimination [half-life ($t_{1/2}$) of 17 h], enabling once-daily administration instead of two or three times daily as is required for the long charged heparins with much shorter half-lives (1–3 h) [8].

In 2001, after a successful clinical development program in the 1990s [7,9], fondaparinux was registered in the USA and Europe as a new antithrombotic drug under the name Arixtra® (GlaxoSmithKline). The use of Arixtra® in major orthopedic surgery led to a decrease in the risk of thrombosis of >50% relative to LMWH [9,10]. In these studies, 2.5 mg/d of Arixtra® was applied, whereas the required dosage of the LMWH enoxaparin is 40 mg once daily or 30 mg twice daily.

Arixtra®, which can be considered a synthetic and selective ultra-LMWH inhibitor of fXa, has gained approval for the treatment of acute deep venous thromboembolic events, as well as extended therapy for up to four weeks to offer continued protection after hospital discharge (www.arixtraus.com).

BOX 1

Patients hit by heparin-induced thrombocytopenia

HIT is a serious side effect observed in approximately 3% of the patients receiving unfractionated heparin (UFH) [63]. HIT occurs typically in the second post-operative week with continued heparin therapy [63] and could result in myocardial infarction, cerebrovascular accidents, limb amputation or death [64]. Of the patients (cardiac surgery) exposed to UFH, ≤50% develop heparin-dependent antibodies [65].

HIT is a hypercoagulability state characterized by elevated thrombin generation and the activation of blood platelets, leading to formation of blood clots primarily composed of aggregated platelets (so-called white clots) [66]. HIT is caused by antibodies formed by the antigenic complex of heparin and PF4. PF4 is released by activated blood platelets and contains many basic amino acids on its surface, which can form strong interactions with UFH (Figure I). In turn, the antibody–heparin–PF4 complex activates blood platelets [67].

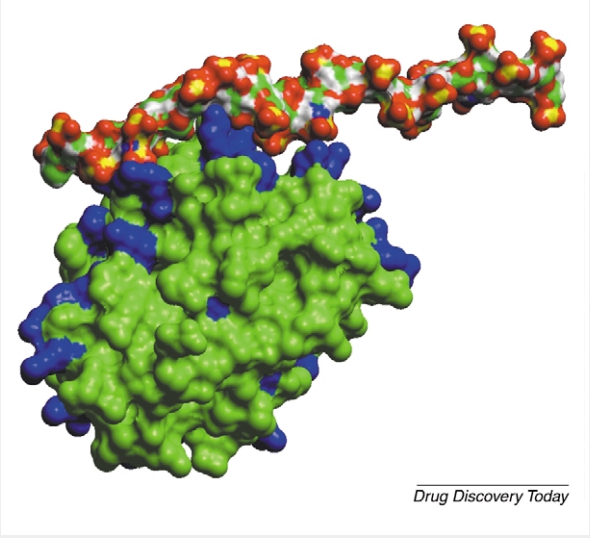

FIGURE I

Model of the interaction of the blood protein platelet factor 4 with a sulfated heparin polysaccharide. The basic residues of PF4 are colored blue.

Once HIT has been diagnosed, it is mandatory to stop heparin therapy immediately. In these cases, LMWHs or direct thrombin inhibitors, such as Refludan® (Berlex; www.refludan.com) or Argatroban (www.argatroban.com) are used to treat any thrombotic complications.

LMWHs are less prone to interact with PF4 than standard heparin [65]. Studies performed thus far have shown that the pentasaccharides, such as fondaparinux (**2**) [48] and idraparinux (**4**) (unpublished results), neither interact with PF4 nor trigger HIT. The design of other heparin-derived oligosaccharides is guided by the observation that the optimal size to form a complex with PF4 is a fragment consisting of 16 monosaccharides [68], whereas the minimal size required for PF4 binding is an octamer [29]. Furthermore, the interaction of heparin analogues with PF4 is facilitated by increasing charge density (higher degree of sulfation) and introduction of conformational flexibility in the backbone of the heparin chain [37].

Moreover, Arixtra® recently received approval for use in patients who are immobilized because of acute illness, such as cardiac insufficiency, acute respiratory disorders, acute infectious disease and/or acute inflammatory disease (www.dailydrugnews.com). Future development is directed towards coronary artery therapy. An interesting clinical aspect of the pentasaccharide is that, although the intrinsic bleeding risk is minimal, recombinant factor VIIa (fVIIa) reverses the anticoagulant effect (as indicated by recent studies in healthy volunteers). This suggests that recombinant fVIIa could be a suitable treatment in cases where serious bleeding complications develop in hospitalized patients [11,12].

Mechanism of action and SAR

The mechanism of ATIII-mediated inhibition of fXa has been systematically studied using the crystal structures of native ATIII and closely related serine protease inhibitors [13,14]. In a detailed model, it was proposed that the interaction of the pentasaccharide with ATIII leads to exposure of its reactive center loop, thus enabling the inhibition of fXa. The cleaved loop, which remains covalently bound to fXa, then reinserts, leading to a release of the pentasaccharide, which explains its catalytic activity. Indeed, this mechanism was subsequently confirmed when crystal structures of the complex and mutants of ATIII [15,16] were elucidated [15,17–19].

An important aspect of studying the SAR of synthetic pentasaccharides was the role of the various negatively charged groups [7]. By altering the sulfation pattern, the anticoagulant activity of the pentasaccharide can be fine-tuned. Initial insights into this phenomenon were obtained when the affinity of fondaparinux (**2**) toward ATIII proved to be twice that of the corresponding *N*-acetylated ATIII-binding domain of porcine heparin (Figure 1a,b). In a systematic approach, the role of the individual groups of the pentasaccharide fondaparinux in ATIII activation was determined (Figure 1c).

Because the interaction of the pentasaccharide with ATIII is highly specific in nature and not merely based on straightforward electrostatic interactions, the design of further analogues of **2** was based on molecular modeling using the postulated interaction model of ATIII with heparin [7,13,14]. As a result, compound **3** (Org31550; Figure 1b) was synthesized. Org31550 contains an extra sulfate group at the 3-*O* position of unit H, therefore binding one order of magnitude stronger to ATIII (compared with **2**) and exhibiting a corresponding enhanced anti-fXa activity [20,21].

In the search for antithrombotic carbohydrates with reduced synthetic complexity and tailor-made pharmacological properties, attention was directed to a novel class of 'non-glycosaminoglycan' analogues. The most striking representative of this class of structurally simplified analogues, in which the hydroxy groups are methylated and the *N*-sulfate groups are replaced by *O*-sulfates, is pentasaccharide **4** [SanOrg34006 (idraparinux); Figure 1d]. This analogue of fondaparinux, with a 'pseudo'-alternating sequence, can be prepared from glucose [7] via a synthetic

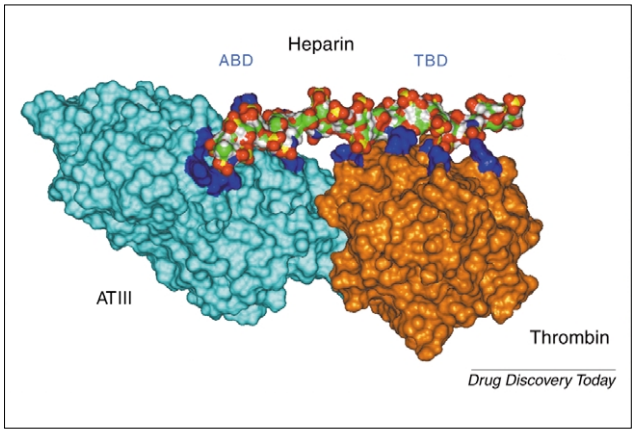

FIGURE 2

A molecular model of the ternary complex of ATIII, thrombin and heparin. Heparin acts as a template to facilitate ATIII-mediated inhibition of thrombin. Heparin clearly forms a bridge of six to eight monosaccharide units that have no interaction with either of the proteins.

route comprising only ~25 steps in a highly convergent manner. Idraparinux (K_d of 1 nM) interacts more strongly with ATIII than fondaparinux (K_d of 50 nM) and idraparinux also exhibits superior anti-fXa activity (2700 U/μmol versus 1200 U/μmol) [22–24]. Idraparinux proved to be particularly promising because of its longer duration of action ($t_{1/2}$ in humans is ~120 h), thus enabling once-weekly administration [25]. In a Phase II dose-ranging study (PERSIST), the prevention of venous thromboembolic events (VTE) at a dose of 2.5 mg once-weekly was at least as effective as existing treatments [26]. Idraparinux is now in late clinical development (Phase III) as a sustained antithrombotic for the prevention of thromboembolic events in patients with atrial fibrillation, and for the prevention and treatment of VTE (comparison with heparin plus vitamin K antagonists).

Indirect thrombin inhibitors

Mimicking the heparin template

Heparin and the active pentasaccharide trigger binding to ATIII and inhibition of serine proteases. However, in the case of thrombin, the heparin-induced conformational change of ATIII is not sufficient to neutralize the enzyme. The unique pentasaccharide domain must be present, but heparin should also act as a negatively charged template to which both ATIII and thrombin bind simultaneously to form a ternary complex (Figure 2) [27]. The interaction between thrombin and the thrombin-binding domain (TBD) of heparin is less specific in nature and weaker by approximately three orders of magnitude than the interaction between ATIII and the pentasaccharide motif [also known as the ATIII-binding domain (ABD)]. Furthermore, investigations of isolated heparin fragments revealed that a sequence of ~18 saccharides is a prerequisite to show inhibitory activity against thrombin [7], which was subsequently refined, using synthesized fragments, to a minimum of a pentadecamer [28–30].

The structural data produced by the initial model of the ternary ATIII–heparin–thrombin complex (Figure 2) [31] clearly revealed that the non-reducing carbohydrate unit (unit D) is oriented in the direction of the reactive center loop of ATIII. Comparison of the thrombin inhibitory activities of two synthetic conjugates (**5** and **6**; Figure 3a) with the ABD and TBD in the two possible arrangements convincingly substantiated the orientation of unit D, as did the recent elucidation of two crystal structures of the ternary ATIII–heparin–thrombin complex [32,33]. In agreement with the proposed mode of action and underlying phenomena, it was identified that, although both **5** and **6** catalyze the inhibition of fXa, only **5** catalyzes thrombin inhibition [34]. Compound **5** is an example of a new tailor-made antithrombotic designed by adjusting the ABD (anti-fXa activity and $t_{1/2}$ in circulation), the spacer moiety and the TBD (antithrombin activity) in a modular and systematic fashion [35,36].

Long heparin fragments with reduced charge

An important outcome of studying the ternary complex formation of heparin with ATIII and thrombin was that the bridge separating the ABD and the TBD – ~eight sugars in length – does not interact with any protein residues (Figure 2) [31–33]. It was reasoned that an oligosaccharide in which a charged ABD and TBD are separated by a neutral domain could deliver an ATIII-mediated fXa–thrombin inhibitor that will not form undesired interactions, particularly with PF4 (Box 1) [30]. Indeed, the conjugate **7** (Figure 3b), which comprises a non-glycosaminoglycan pentasaccharide as the ABD, a spacer of ~50 atoms in length and a simple persulfated maltotrioside moiety as the TBD [34], displays substantial antithrombin activity (140 U/mg versus 160 U/mg for heparin). However, a shortcoming of using a flexible spacer is that, on formation of the ternary complex, the adoption of the proper orientation is accompanied by an unfavorable loss of entropy; in addition, the interaction with PF4 is not completely abolished. The use of a rigid linker with close structural resemblance to the backbone structure of heparin, such as that present in the hexadecasaccharide **8** (SR123781; Figure 3b), not only enhanced the formation of the ternary complex with increased antithrombin activity but also minimized the interaction with PF4. It is interesting to note that, although a hexadecamer heparin fragment is optimal for interaction with PF4, a hexadecamer with carefully chosen ABD and TBD connected via a neutral octasaccharide spacer is devoid of such a side effect [37–40].

SR123781 (**8**) was more potent than heparin and fondaparinux (**2**) in different experimental models for arterial and venous thrombosis and showed high affinity for human ATIII (K_d of 58 nM). After intravenous and subcutaneous administration to rats, rabbits and baboons, SR123781 displayed prolonged anti-fXa and antithrombin activity. It also inhibited thrombus formation in experimental *in vivo*

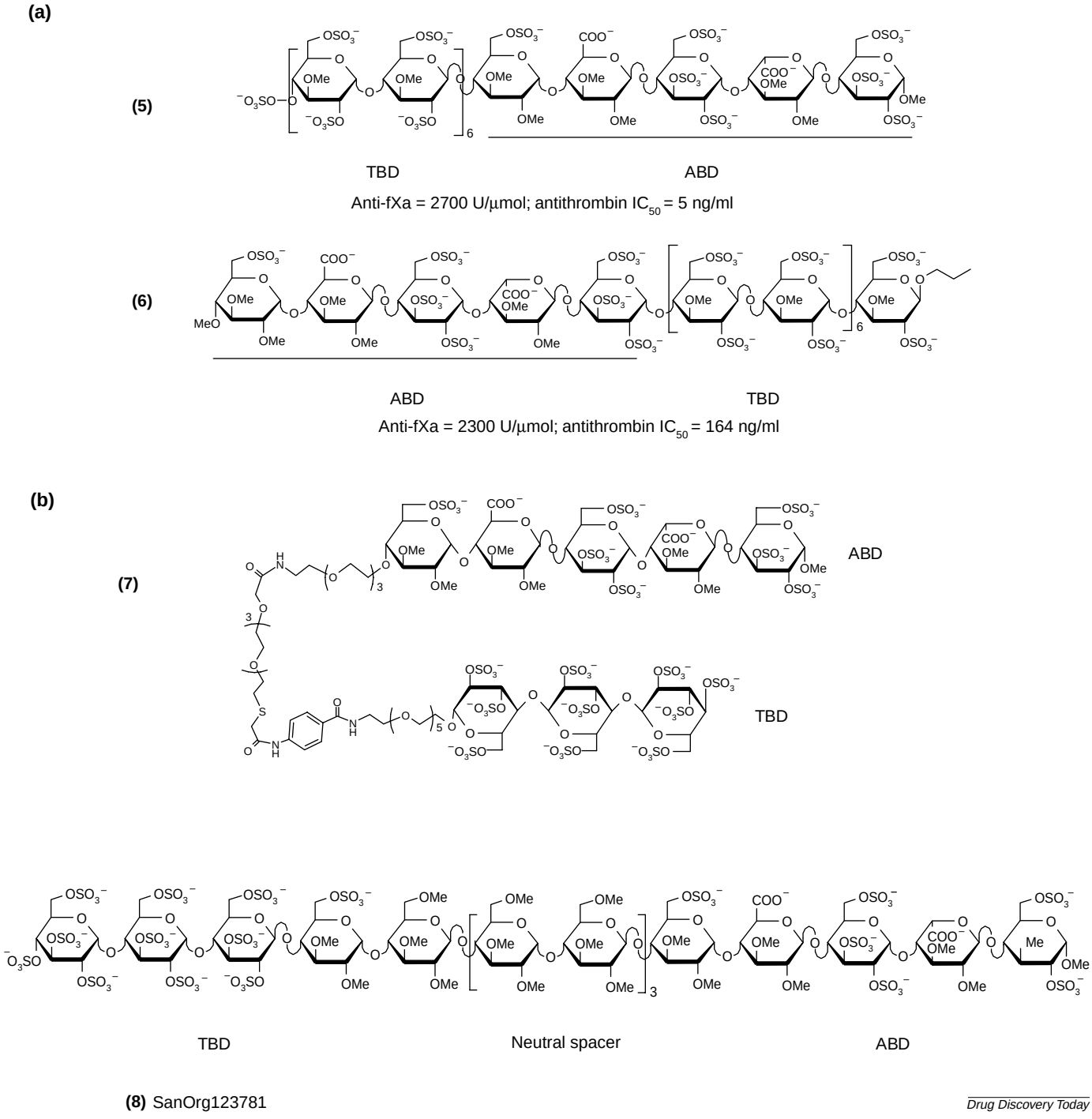

FIGURE 3

Long synthetic heparin fragments. (a) Synthetic fragments **5** and **6** show that the TBD should be positioned at the non-reducing end of the pentasaccharide (ABD). **(b)** Comparison of the flexible conjugate **7** with conjugate **8**, which contains a neutral methylated hexasaccharide, clearly reveals that the use of a rigid spacer results in a significant increase in antithrombotic activity.

models [41] and, compared with standard heparin, had a favorable antithrombotic:bleeding ratio. SR123781 has progressed into clinical development [42].

A dual factor Xa and direct thrombin inhibitor

Designing a dual inhibitor with a mixed profile

Building on experience gained in the field of complex heparin-like pentasaccharides and direct thrombin inhibitors, the preparation of antithrombotics that can directly inhibit thrombin, as well as catalyze the ATIII-mediated inhibition of fXa, were investigated. The concept of such a dual inhibitor in which the benefits of LMWHs and direct thrombin inhibitors (Box 2) are combined is expected to elicit new pharmacological properties. Not

BOX 2

At the heart of thrombin

Blocking the active site of thrombin with small molecule inhibitors is an efficient means of inhibiting the coagulant action of thrombin. Direct thrombin inhibitors have, as expected, a rapid onset of action, affect only the target enzyme and are able to inhibit clot-bound thrombin. In clot-bound thrombin, the heparin-binding site of thrombin, which has a major role in its heparin-induced ATIII-mediated inhibition, is believed to be blocked by fibrin [69]. The inhibition of clot-bound thrombin is of great importance to avoid re-thrombosis after coronary thrombolytic therapy. Another major advantage of the direct inhibitors is their applicability in patients that exhibit HIT (Box 1). Moreover, direct thrombin inhibitors have been clinically demonstrated to be superior over LMWH [70,71] in reducing major cardiac events and are now emerging as replacements for warfarin.

A classical representative of the class of non-electrophilic direct inhibitors of thrombin is α-NAPAP (**11**; Figure II). Although at relatively high concentrations NAPAP itself showed moderate side effects [45], these could be reduced by the introduction of a polar group [72] (e.g. **12**), or a solubilizer, such as a glycoside [43] or a polyethylene glycol [44] moiety. Other direct thrombin inhibitors were developed with improved pharmacokinetic and pharmacodynamic properties, several of which reached the market. Argatroban (**13**) is a selective thrombin inhibitor, which is mainly used in cases of HIT. Argatroban lacks oral bioavailability and is therefore administered intravenously. Recently, Ximelagatran (**14**), a double prodrug of Melagatran, was launched in Europe as the first oral direct thrombin inhibitor under the name Exanta® (AstraZeneca; www.exanta.com). Another important class of direct thrombin inhibitors is represented by the hirudin family. Refludan® (www.refludan.com) and Revasc® (Sanofi-Aventis–Novartis; www.novartispharma.de) are two marketed recombinant hirudins, which originate from the medicinal leech *Hirudo medicinalis* [73]. The C-terminal component of hirudin binds in the fibrinogen recognition exosite of thrombin and its N-terminal part effectively blocks the active site [74]. This bivalent binding motif leads to a highly potent and selective thrombin inhibition (K_i of 230 fM for Revasc®). Lepirudin (marketed as Refludan®) is used for heparin replacement in HIT patients.

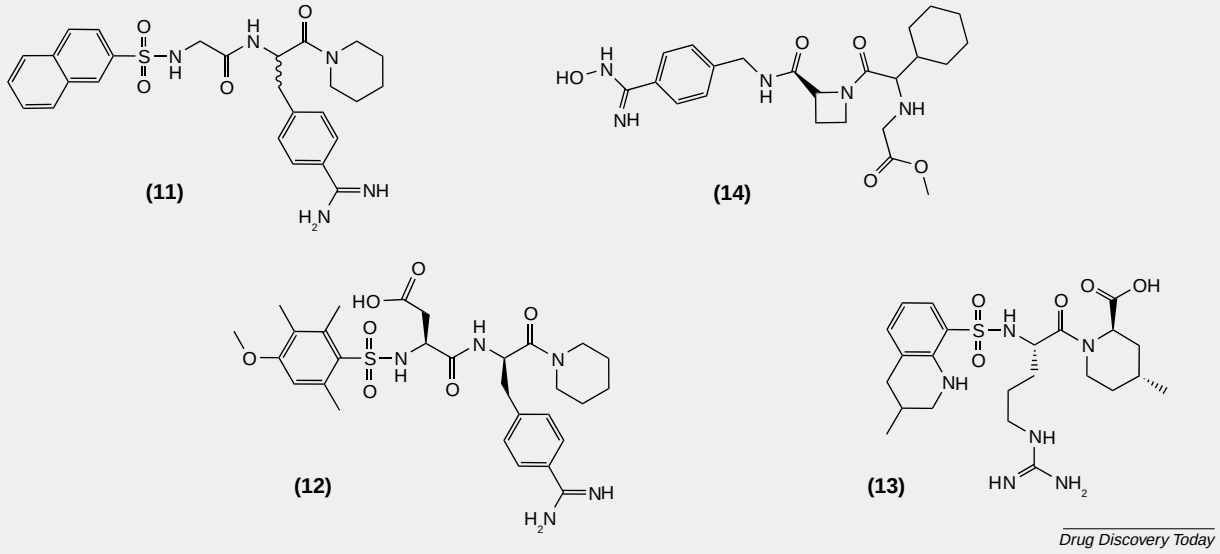

FIGURE II

Examples of potent direct thrombin inhibitors. α-NAPAP **(11)**; an α-NAPAP analog **(12)**; Argatroban **(13)**; and Ximelagatran **(14)**. The amino acid sequence of hirudin as found in Refludan® (X = L) and Revasc® (X = V) is XTYTDCTESGQNLCLCEGSNVCGQGNKCILGSDGEKNQCV TGEGTPKPQSHNDGDFEEIPEEYLQ.

Although an impressive repertoire of direct thrombin inhibitors is at hand [75], a major limitation of these compounds is their inability to prevent thrombin formation [76]. Furthermore, the direct thrombin inhibitors require a careful dosing regimen to avoid plasma level troughs, which could give rise to coagulation-rebound phenomena. However, heparin or heparin-derived ATIII-mediated thrombin inhibitors [29] are able to inhibit the formation and activity of free thrombin, but have limitations in inhibiting clot-bound thrombin [55,56]. Direct thrombin inhibitors, such as Argatroban, are more effective inhibitors of thrombin bound to either a clot or fibrin [76]. Org42675 is a novel dual antithrombotic inhibitor that combines the benefits of ATIII-binding heparins and direct thrombin inhibitors.

only would such a compound be capable of inhibiting newly formed thrombin, but it would also directly block the active site of clot-bound thrombin. Furthermore, the predictable pharmacokinetic behavior of pentasaccharides with high affinity for ATIII should give access to conjugates with tailor-made (prolonged) half-lives.

The first lead conjugate consisted of a suitably derivatized α-NAPAP [α-*N*-(2-naphtalenesulfonyl)-glycyl-D-4-aminophenylalanyl-piperidine] thrombin inhibitor (Box 2) and a heparin-based pentasaccharide. α-NAPAP was chosen on the basis of earlier studies, which showed that the α-carbon of the glycine moiety in NAPAP could be

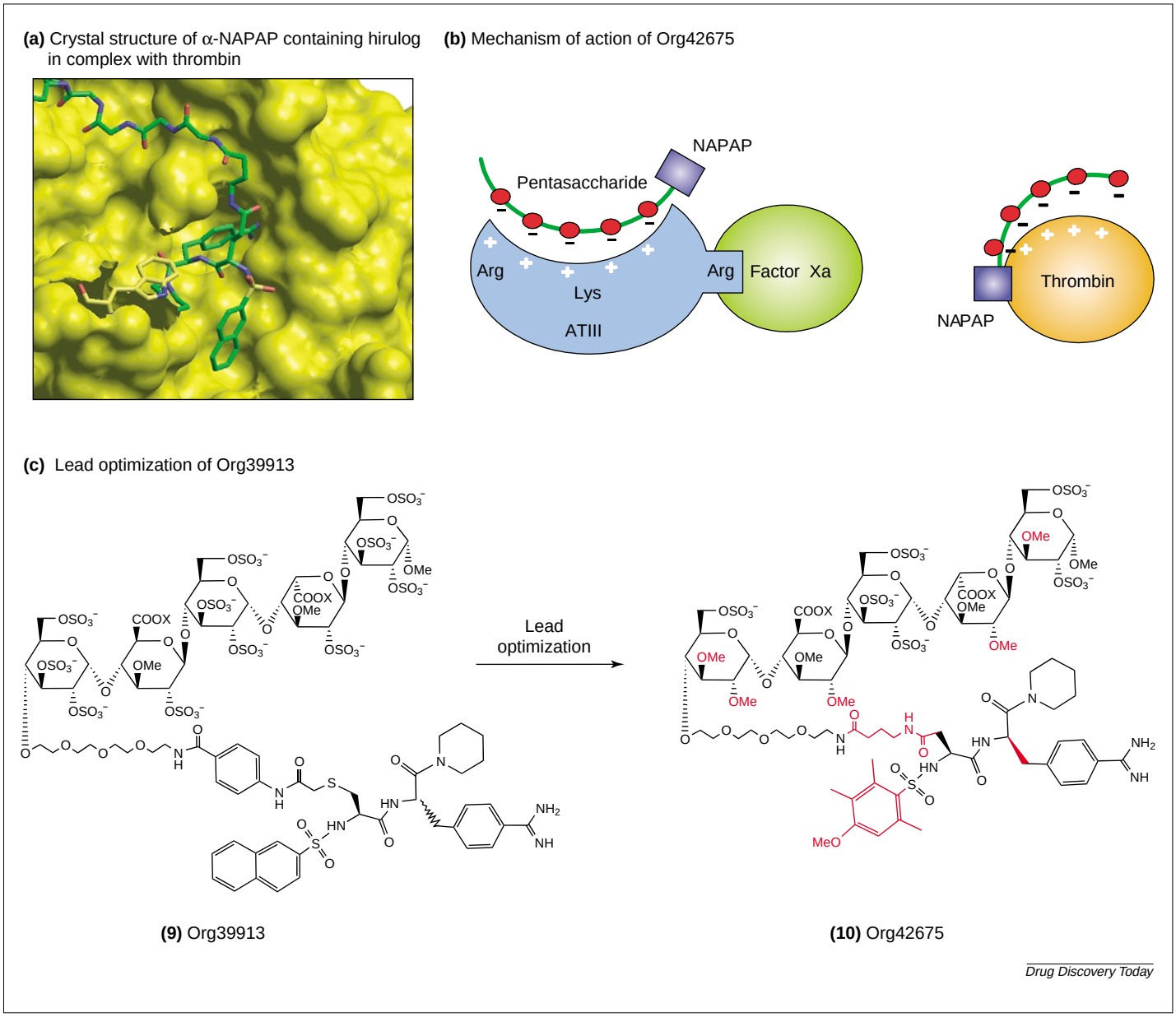

FIGURE 4

Design and mechanism of a dual factor Xa and thrombin inhibitor. (a) Representation of the crystal structure (1QUR) of α-NAPAP containing hirulog (stick presentation) in complex with thrombin (green). **(b)** Mechanism of action of Org42675. The selective inhibition of coagulation cascade protease fXa is the result of binding of the pentasaccharide component to ATIII. In addition, the optimized NAPAP component blocks the active site of thrombin directly. **(c)** Lead optimization of Org39913 (**9**) into Org42675 (**10**). The optimized structural features in Org42675 are depicted in red.

derivatized with glycosides [43] or polyethylene glycol [44] without affecting the inhibitory potency against thrombin but reducing its side effects (decrease in blood pressure) [45]. A recent crystal structure of α-NAPAP containing hirulog in complex with thrombin elegantly illustrates the glycine α-C of NAPAP as a suitable anchor-point for linkage with the ATIII-activating pentasaccharide (Figure 4a) that is oriented in the direction of the solvent [46].

Conjugate **9** (Org39913) displays a unique *in vitro* antithrombotic profile with antithrombin (IC_{50} of 0.35 μM) and ATIII-mediated anti-fXa activity (1300 U/μmol) [47]. An important observation was that the antithrombin activity was similar to its non-conjugated peptidomimetic counterpart (IC_{50} of 0.75 μM), proving the validity of the dual inhibitor concept. The $t_{1/2}$ of Org39913 was dictated by the pentasaccharide and was prolonged compared with NAPAP (~1.5 h for Org39913 versus 9 min for NAPAP in rat). Org39913 was used as a lead compound for further optimization of its two antithrombotic components.

Optimizing antithrombotic components

The antithrombotic properties of the mixed profile dual inhibitor Org39913 (**9**) were optimized in three aspects (Figure 4c). First, the activity of the conjugated direct thrombin inhibitor was improved to balance the two anticoagulant activities. Second, the charge density of the

pentasaccharide was reduced to decrease aspecific protein binding and to fine-tune the desired $t_{1/2}$ and, third, the spacer was replaced.

The outcome of this lead optimization effort was **10** (Org42675), which contains five fewer sulfates than its predecessor Org39913. Additionally, the aromatic linker was replaced by a pharmacologically inert and stable γ-aminobutyric acid moiety. The ATIII-mediated anti-fXa activity of Org42675 (1000 U/μmol) is similar to that of fondaparinux. The activity of the direct thrombin inhibitor was optimized by replacement of the naphthyl sulfonamide moiety and incorporation of the single active enantiomer rather than a mixture, as in Org39913, resulting in a 20-fold rise in direct thrombin inhibitory potency (IC_{50} of 17 nM). When comparing the overall antithrombotic activities of Org42675 to heparin and direct inhibitors of thrombin, it became evident that this novel fXa and direct thrombin dual inhibitor has a distinct anticoagulant profile [48].

Pharmacological profile of Org42675

Scrutinizing the dual inhibitor Org42675 for its antithrombotic properties revealed a novel unprecedented pharmacological profile with complementary thrombin inhibitory activities. In addition to the potent ATIII-mediated inhibition of fXa and the ensuing decline in thrombin generation, Org42675 efficiently blocks the active site of thrombin (Figure 4b). With similar activity to that of Argatroban (**13**), Org42675 inhibits clot formation in overall coagulation tests, such as activated partial thromboplastin time, partial thromboplastin time and thrombin clotting time, underscoring the contribution of the direct thrombin inhibitory component of the compound.

In contrast to heparin, Org42675 exerts potent, dose-dependent inhibitory activity in experimental thrombosis models in rats with thrombi developing under various flow conditions, representing forms of both venous and arterial thrombosis. Compared with fondaparinux, Org42675 is predominantly more potent in the arterial models [48]. It is of particular interest that the $t_{1/2}$ of Org42675 is prolonged (tenfold in rat) compared with the non-conjugated peptidomimetic thrombin inhibitor. In addition, its $t_{1/2}$ is longer than the respective half-lives of fondaparinux, Argatroban and heparin. Org42675 shows complete bioavailability after subcutaneous administration and displays similar area under curve (AUC) values and elimination $t_{1/2}$ for anti-fXa and antithrombin activities, revealing that the dual inhibitor is chemically stable *in vivo* and will be excreted from the body as a single entity.

A known pitfall of cessation of heparin therapy in unstable angina and in patients with coronary syndromes is the rebound generation of thrombin [49–52]. In this respect, recurrent thrombotic occlusions also frequently occur following successful thrombolytic therapy [51–53]. Studies in a rabbit model [48] have shown that, on thrombotic reocclusion following thrombolysis, Org42675 triggers a rapid opening of the occluded vessels, which is in agreement with the observation that direct thrombin inhibitors are capable of enhancing endogenous thrombolysis [54].

Another important drawback of existing therapies is that clot-bound thrombin, which can act as an ongoing source of thrombogenesis at sites of pathological thrombus formation, shows relative resistance towards heparin [55,56]. By contrast, Org42675 inhibits clot-bound thrombin to the same extent as Argatroban (IC_{50} values of 800 nM and 600 nM, respectively), whereas heparin displays an IC_{50} of >50 μM [48].

It has been reported many times that heparin displays, compared with its potent *in vitro* antithrombin activity, a relatively low antithrombotic activity in arterial-type models of thrombosis. This phenomenon is presumably because of the higher sensitivity to neutralization of heparin when platelets are predominantly involved in thrombus formation. Although this neutralization does not occur with fondaparinux, the synthetic pentasaccharide moiety lacks the required complementary inhibition of thrombin. These requirements are united in Org42675, which, in contrast to heparin, does not produce the

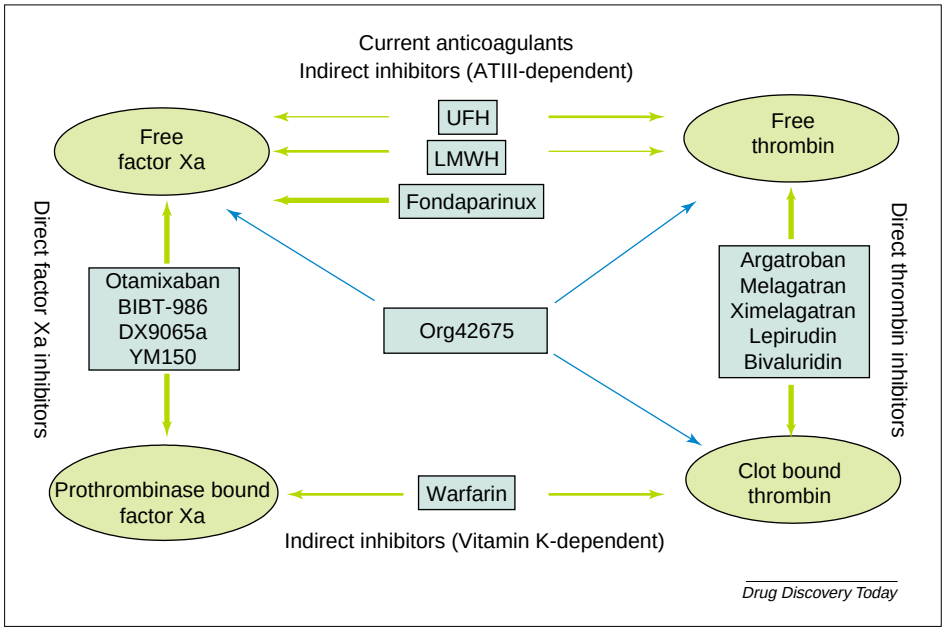

FIGURE 5

Current repertoire of clinically evaluated anticoagulants. Org42675 displays a distinct pharmacological profile among the current repertoire of clinically evaluated anticoagulants, including UFH, LMWH, direct thrombin inhibitors and warfarin. The direct inhibitors of fXa are currently in clinical Phase II. The potent and selective dual inhibitor Org42675 inhibits several essential targets in the coagulation cascade, with potential application in the prevention and effective treatment of thrombosis with a low hemorrhagic risk. Abbreviation: UFH, unfractionated heparin.

typical bell-shaped dose-response of platelet activation in the presence of HIT serum. As a result of the low charge density on the ATIII-binding pentasaccharide moiety and the absence of additional sulfated oligosaccharide domains, Org42675 is only partially neutralized by PF4 and, like fondaparinux, does not cross-react with HIT antibodies.

In summary, several aspects that limit the efficacy of contemporary anticoagulant therapy have been addressed in the design of Org42675. Rational optimization transformed the dual inhibitor lead Org39913 into Org42675, which has a predictable pharmacological profile and balanced ATIII-mediated anti-fXa and direct antithrombin activities. Org42675 is a representative of a novel class of anticoagulants added to the current repertoire (Figure 5), including the heparin family, direct thrombin inhibitors and warfarin. All preclinical data indicate that Org42675 is a potent and selective dual inhibitor that inhibits thrombosis with a low hemorrhagic risk. It has the potential to be effective in a wide therapeutic window for the prevention and treatment of various thrombotic disorders, particularly of arterial origin (e.g. acute coronary syndrome and high risk surgery) with a predictable dose-dependent pharmacokinetic profile.

Conclusion and outlook

Synthetic heparin derivatives

After years of intensive research, many synthetic heparin derivatives have entered the clinical arena. Structure-based approaches gave insight into the mechanism of heparin-induced activation of ATIII and provided access to novel synthetic fragments with higher potency and longer $t_{1/2}$, but without notorious side effects [29,57,58]. R&D activities were challenged with multistep-synthesis of the most complex oligosaccharides prepared to date, both on a laboratory and production scale [29]. Fondaparinux, the first synthetic antithrombotic pentasaccharide, is now available throughout the world as Arixtra®. Other substances, such as idraparinux and SR123781, each with their own pharmacological profile, are in different stages of clinical development.

The most recent searches for novel heparin analogues with even further increased affinity have proven to be a formidable [59], if not insurmountable [60], challenge. Despite a highly detailed crystal structure of the ATIII–pentasaccharide complex being available, it remains a fascinating, but complicated, endeavor to construct more simplified analogues of the unique heparin pentasaccharide domain.

A promising novel dual inhibitor

Not only have (long) synthetic heparin fragments mimicking the anticoagulant action of heparin been generated, other heparin derivatives having an unprecedented mixed profile and tailor-made antithrombin activities have been produced. In one such compound (Org42675), the pharmacological properties of a low molecular weight peptidomimetic are completely altered by conjugation to a suitable pentasaccharide derivative to provide unique pharmacodynamics [48]. Org42675 is a striking example that conjugation of constituents from two different chemical classes can render a highly potent drug that affects two different antithrombotic targets with the same *in vivo* duration of action. The synthetic route to Org42675 comprises ~65 synthetic steps [61,62], a process that has also been successfully scaled-up. Org42675 has considerable potential to be developed as an effective agent for the treatment of venous and arterial thrombotic disorders (once-daily administration by injection) and it is now moving into Phase II clinical trials to obtain proof-of-concept.

Note

Since 1987, Organon has been collaborating with Sanofi-Synthélabo (now Sanofi-Aventis) to develop drugs based on synthetic oligosaccharides for the treatment of atherothrombotic diseases. In January 2004, a revision of the terms of the collaboration was announced and Sanofi-Synthélabo agreed to acquire some of Organon's interests relating to Arixtra®, idraparinux and other oligosaccharides, such as the hexadecasaccharide SR123781. Organon acquired Sanofi-Synthélabo's interest relating to Org42675, and is moving into Phase II clinical trials with this compound. The transfer of remaining rights and development obligations to Sanofi-Synthélabo occurred in exchange for revenues based on future sales from jointly developed antithrombotic products.

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