Heparin/heparan sulphate-based drugs

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Glycosaminoglycans (GAGs) are an untapped source of novel chemical entities and, therefore, offer exciting new opportunities for the development of novel drug molecules because of their unique physical and biological properties. Advances in the functional understanding of GAG–protein interactions are enabling the development of GAG mimetics for use as anti-angiogenic, anti-metastatic, anti-inflammatory, anticoagulant and anti-thrombotic agents. Many anti-thrombotic molecules, such as Fondaparinux and Idraparinux, have been successful in clinical trials, and a new generation of heparin mimetic oligosaccharides and small molecules are currently in different stages of clinical development. In particular, the recent increased activity in the development of new mimetics by altering the composition of sulphated GAGs is very encouraging. This article reviews structurally defined heparin-mimetic oligosaccharides and small molecules currently in development or clinical trials.

Sulphated glycosaminoglycans (GAGs) are glycans found inside the cell and in the extracellular matrix, which act by binding selectively to a variety of proteins and pathogens and are crucially relevant to many disease processes, such as inflammation [1–3], neurodegeneration [4], angiogenesis [5], cardiovascular disorders [6], cancer [7] and infectious diseases [8–10]. Heparin and heparan sulphate (HS) are GAGs consisting of 1–4 linked uronic acid and glucosamine and encompassing varying degrees of sulphation, and they are involved in many of these activities [11]. Heparin is a minor form of the ubiquitous HS, and the anticoagulant activity of pharmaceutical heparin is mainly accounted by fractions containing a pentasaccharide sequence with binding affinity for anti-thrombin (AT) (see below).

The wide range and intricacy of glycan-mediated cellular interactions have turned glycans into novel targets for future drug development [12–14], with drugs already being developed for the treatment of metabolic disorders, cancer and infection. In recent years, there has been a renaissance in the development of carbohydrate-based therapeutics that involve the inhibition of carbohydrate–lectin interactions and carbohydrate-based anticoagulant and AT agents [15]. The pharmacological and therapeutic value of heparin/HS and their mimetics is now recognized because of their ability to bind and cause immobilization and/or activation of a variety of proteins, such as growth factors, chemokines and metalloproteinases [16,17]. Potential strategies based on heparin/HS–protein interactions have recently been described to assist GAG-based drug discovery [18]. GAG-based drugs can act in several ways by activating (agonists) or inactivating (antagonists) protein-based receptors, competing with endogenous GAGs and/or inhibiting GAG biosynthesis. The molecular diversity of heparin/HS interactions has been exploited for the development and clinical progression of GAG mimetics [19].

The anticoagulant market has been very active recently because of the development of new compounds, including indirect factor Xa (FXa) inhibitors (such as Fondaparinux and Idraparinux and its new biotinylated form), direct inhibitors of FXa (such as Rivaroxaban and Apixaban) and direct inhibitors of thrombin (such as Dabigatran) [20,21]. The mechanism of action of these anticoagulants has been reviewed extensively [22,23]. The discovery of the mechanism of binding of heparin to AT and FXa has focused interest on the development of small, structurally defined heparin mimetics with AT activity but with reduced side-effects [24]. This

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article reviews the principles under which recently developed GAG (heparin/HS)-based inhibitors act and gives a description of the different classes of inhibitors and their development as drugs.

Heparin/HS mimetics as anticoagulants

Anticoagulants based on heparin/HS are drugs of choice in the therapy and prophylaxis of thromboembolic diseases [25]. Structural and functional studies have shown that a unique pentasaccharide (sometimes referred to as AGA*IA or DEFGH), GlcNAc/NS6S → GlcA → GlcNS3S6S → IdoA2S → GlcNS6S (where Glc is glucosamine, IdoA is iduronic acid and GlcA is glucoronic acid, which are either sulphated or acetylated), comprises the AT-binding domain and is responsible for the anticoagulant activity of heparin. The 3-*O*-sulphate group at position F is responsible for strong and specific interactions with AT [26]. The pentasaccharide unit only inhibits the activity of FXa mediated by AT; however, a much larger oligosaccharide is required for the AT-mediated inhibition of thrombin [27]. The structural requirements for the binding of heparin (Fig. 1a) to AT, as shown in Fig. 1, were determined by analysing the crystal structure and by determining the structure–activity relationships of a series of pentasaccharides using various combinations of sulphate and carboxylate groups [28,29]. This approach helped to establish that charged groups, as depicted in Fig. 1, are absolutely essential for the activation of AT (highlighted in the blue boxes) and required to increase the biological activity (in the red boxes). Moreover, hydrophobic interactions between the heparin pentasaccharide and AT also contribute to increasing the binding affinity [30]. Several review

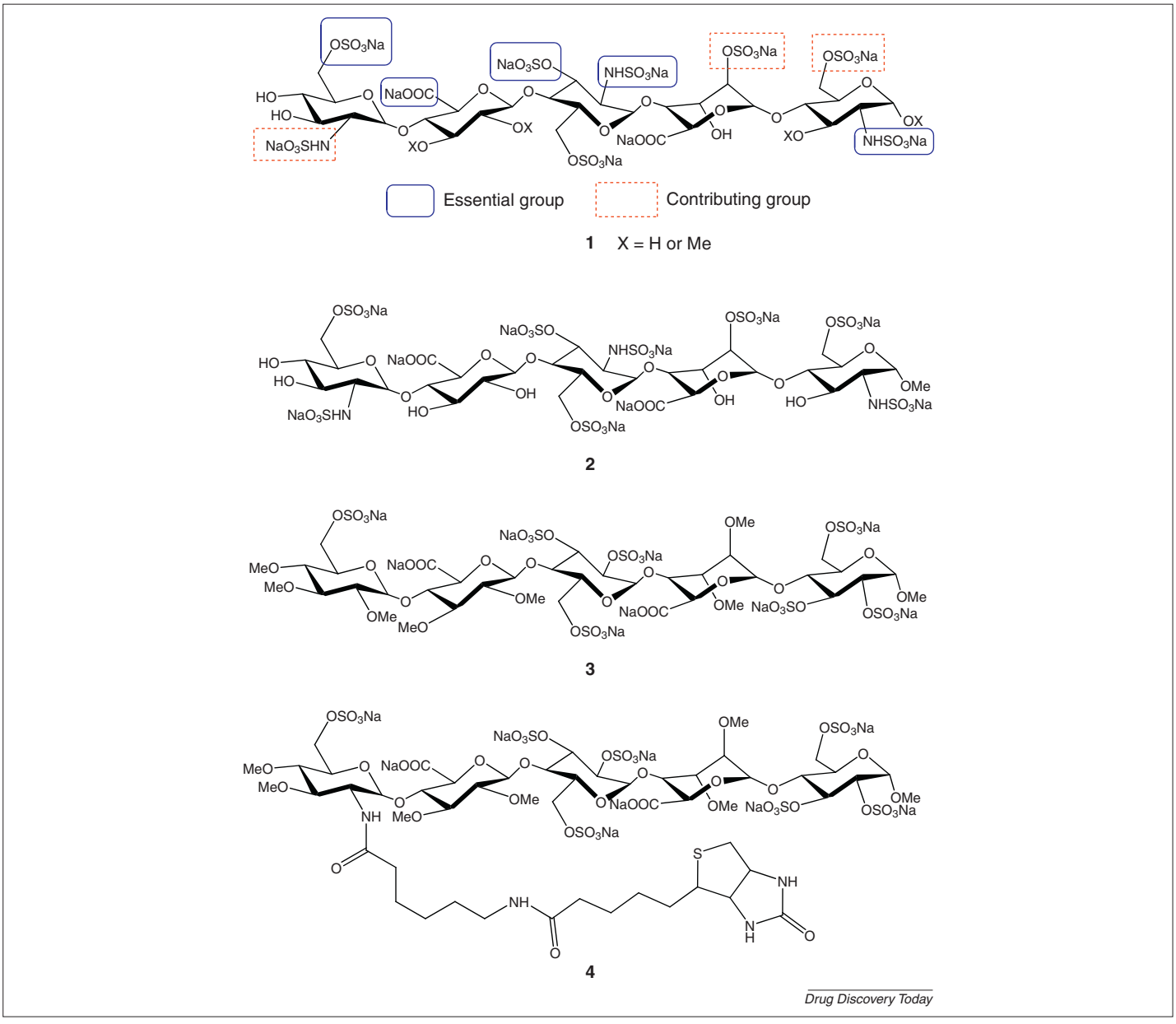

FIGURE 1

Chemical structures of heparin pentasaccharide derivatives. (**a**) The AT-binding pentasaccharide motif. Highlighted functional groups are essential for AT activation. (**b**) Structure of Fondaparinux. (**c**) Structure of Idraparinux. (**d**) Structure of Idrabiotaparinux. Natural heparin and synthetic pentasaccharides differ in their substitution pattern (synthetic pentasaccharides contains –OMe substitutions).

articles have been published describing the structure–activity relationship and mechanism of action of heparin mimetic anticoagulants [24,28,29,31,32].

Research on heparin mimetic anticoagulants has gained momentum since the successful clinical development programs of the 1990s. GlaxoSmithKline registered Fondaparinux (Fig. 1b) (SR90107, Org31540) as a new anti-thrombotic drug under the name Arixtra® after being granted approval from the US FDA and the European Committee for Proprietary Medical Products [33]. SR123781 is a short-acting hexadecasaccharide analogue of heparin with *N*-sulphate groups replaced by *O*-sulphates and alkylated hydroxyl groups in the AT-binding domain. It has tailor-made FXa- and thrombin-inhibitory activities combined with more selectivity in its mode of action. Sanofi-Aventis discontinued the development of SR123781, however, after the success of heparin mimetic AVE 5026 [34].

Idraparinux (SanOrg34006, SR34006)

Idraparinux (Fig. 1c) is a synthetic pentasaccharide analogue of Fondaparinux, in which the hydroxyl groups are methylated and the *N*-sulphate groups are replaced by *O*-sulphates [35]. Idraparinux (K_d of 1 nM) interacts more strongly with AT than Fondaparinux (K_d of 50 nM) through non-ionic interactions [36] and also exhibits superior anti-Xa activity (1600 versus 700 U μg^{-1}) [35,37,38]. Idraparinux can be synthesized more easily than Fondaparinux because of the presence of a 'pseudo'-alternating sequence that can be easily prepared from glucose [39]. The crystal structures of the complexes of a pentasaccharide analogue of Idraparinux (modified by the addition of a sulphate at the H unit) [40] with a dimer consisting of activated AT and latent AT [41], and with an intermediate state [42], have been reported. Analysis of these structures explains the lower affinity of heparin for AT on the basis of induced conformational changes in AT, such as the expulsion of the hinge region and the closure of β-sheet A to the normal five-stranded form. This then leads to the activation of AT and the allosteric inhibition of coagulation factors IXa, Xa and thrombin.

Idraparinux has been evaluated in clinical trials for the treatment and secondary prevention of venous thromboembolism (VTE) and the prevention of thromboembolic events associated with atrial fibrillation (AF) [43]. The pharmacokinetics, pharmacodynamics and tolerability of Idraparinux were evaluated in several phase I studies [44,45]. Idraparinux has a long half-life (80–120 h) in the bloodstream, thus enabling once-weekly administration [46]. The phase II dose-ranging PERSIST study established that subcutaneous once-weekly administration of 2.5 mg of Idraparinux, with an increased elimination half-life of approximately 600 h, was as effective as warfarin and demonstrated dose-dependent increases in major bleeding for the secondary prevention of deep vein thrombosis (DVT) [47–49]. Idraparinux was also evaluated for the long-term treatment of patients with DVT and pulmonary embolism (PE) using a subcutaneous once-weekly dose of 2.5 mg in the three van Gogh trials [50,51]. Unfortunately, the results of these trials indicated that the rate of recurrent VTE was considerably higher with Idraparinux than with conventional therapy. In addition, the elimination half-life of 60 days led to a prolonged anticoagulant effect after completion of the therapy [52]. These findings also explained the complication of increased bleeding during a 12-month treatment period compared to a six-month treatment period in patients randomly treated with Idraparinux in the DVT and PE study, as carried out in the van Gogh Extension trial [53,54]. However, repeated doses of reversal agents, such as rFVIIa, are required in bleeding patients during surgery to neutralize the anticoagulant effect of Idraparinux [55].

The van Gogh and Amadeus phase III clinical trials of Idraparinux established that its pharmacokinetics were best described by a three-compartment model in patients with DVT, PE or AF at risk of thromboembolic events [56]. The terminal half-life was measured to be 66.3 days, and the half-life during steady state was determined to be 35 weeks. Idraparinux clearance was notably related to subject weight, creatinine clearance, sex and age. The phase III AMADEUS non-inferiority trial enrolled patients with AF at risk for thromboembolism to compare the efficacy and safety of Idraparinux to therapy with vitamin K antagonists [57]. Idraparinux proved as effective as vitamin K antagonists; however, the trial was cut short because of an excess of bleeding complications in Idraparinux-treated patients and a few documented ischemic events. Idraparinux remains in the late clinical development pipeline of Sanofi-Aventis because of its advantageous (compared to oral anticoagulants and Fondaparinux) once-a-week dosing regimen.

Idrabiotaparinux (SSR126517) (Fig. 1d) is a novel synthetic anticoagulant linked to biotin at position 2 of the non-reducing end of glucose in Idraparinux [58]. Linkage of biotin at this position in the pentasaccharide prevents interaction of the pentasaccharide with AT or FXa *in vitro* [59]. The optimal length of the spacer was found to be a 6C-length arm. Administration of Idrabiotaparinux to rats by either the intravenous or the subcutaneous route resulted in a similar pharmacokinetic profile to that of Idraparinux. Further animal studies into Idrabiotaparinux showed that the injection of avidin triggered the immediate elimination of the molecule from the bloodstream, resulting in the complete neutralization of the anti-thrombotic activity of Idrabiotaparinux. Sanofi-Aventis has halted the development of Idrabiotaparinux in AF in phase III trials, because of its lack of potential benefit over oral anticoagulants, such as vitamin K antagonists, which are currently being evaluated in clinical trials [60].

AVE5026

AVE5026 (Sanofi-Aventis) is in clinical development for the prevention of VTE [61]. This molecule is a complex mixture of oligomeric ultra-low-molecular-weight heparin (LMWH) fragments (molecular weights 2000–3000 Da) with a polydispersity index of approximately 1.0. It is prepared by partial and controlled chemioselective depolymerization of porcine unfractionated heparin (UFH). AVE5026 primarily targets FXa and has only a minimal effect on thrombin. It exhibits a higher ratio of FXa to anti-Factor IIa activity (>30:1). In addition, it shows dose-dependent anti-thrombotic activity in a rat microvascular thrombosis disease model, suggesting that this agent might provide the optimal treatment for cancer-associated thrombosis [62]. When given subcutaneously, the half-life of AVE5026 is 16–20 h, enabling once-daily administration. AVE5026 is excreted renally and, like Fondaparinux, its anticoagulant effects are not neutralized by protamine sulphate. An elective total knee replacement surgery study demonstrated a highly statistically significant dose-dependent response with AVE5026 for the prevention of VTE in patients

undergoing knee arthroplasty [63]. A 20 mg dose of AVE5026 was selected for further evaluation. An extensive phase III trial is currently comparing AVE5026 with the LMWH Enoxaparin for the prevention of VTE in patients undergoing hip, knee or abdominal surgery and in cancer patients receiving chemotherapy.

M118

Momenta Pharmaceuticals developed M118 (Fig. 2), a novel anticoagulant for the treatment of patients with acute coronary syndrome. It is currently being evaluated in a phase II clinical trial with patients undergoing percutaneous coronary intervention [64]. M118 is an optimized polysaccharide compound engineered from UFH using a specific enzymatic depolymerization process. It is designed to act at multiple points in the coagulation cascade by selectively binding to both AT and thrombin, two crucial factors involved in the formation of clots [65]. Preclinical and phase I studies have shown that M118 has the positive attributes of both UFH (reversibility, monitorability and broad inhibition of the coagulation cascade) and LMWH (adequate bioavailability and predictable pharmacokinetics that enable subcutaneous administration) and can thus be administered both intravenously and subcutaneously [66]. M118 exhibits clear dose-dependent inhibition of FXa and Factor IIa, with an anti-Xa:anti-IIa ratio that is constant over time [67,68]. M118 was found to be effective at preventing thrombosis in diseased arteries in a photochemical carotid artery injury model in $ApoE^{-/-}$ mice [69]. The reduced polydispersity (the ratio of weight averaged to number-averaged molecular weight) of M118 contributes to a more predictable pharmacokinetic profile. M118 lacks drug–drug interactions when co-administered with aspirin and clopidogrel or with glycoprotein IIb/IIIa inhibitors such as Eptifibatide [70].

EP42675 and EP217609

EP42675 is the first representative of a new class of synthetic parenteral anticoagulants with a dual mechanism of action combining the properties of an indirect FXa inhibitor and a direct thrombin inhibitor [71]. EP42675 is being trialled in patients with acute coronary syndrome undergoing percutaneous coronary intervention. The structure of EP42675 contains an AT-binding pentasaccharide (an indirect FXa inhibitor) coupled to a peptidomimetic α-NAPAP analogue (a direct inhibitor of the active site of both free and clot-bound thrombin). This dual mechanism imparts a unique pharmacological profile to EP42675: (i) inhibition of both fibrin-bound and fluid-phase thrombin owing to the presence of a direct thrombin-inhibiting moiety, (ii) inhibition of FXa in the presence of AT, (iii) a favourable pharmacokinetic profile that ensures prolonged anticoagulant coverage with improved control over its therapeutic window owing to the presence of the Fondaparinux pentasaccharide, and (iv) no cross-reaction with platelet factor 4 antibodies.

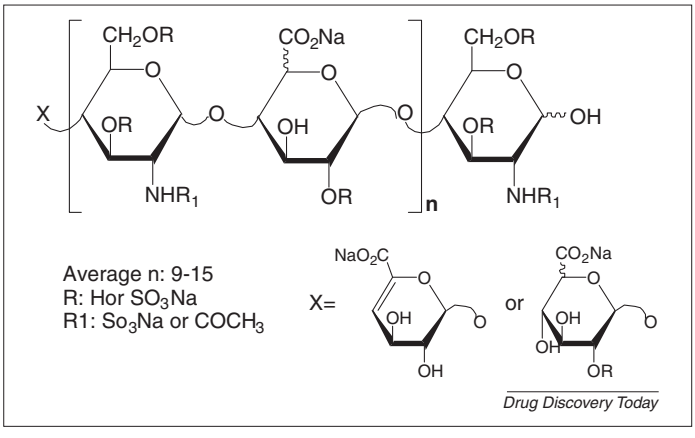

FIGURE 2

Chemical structure of M118, a LMWH characterized by a weight-averaged molecular mass between 5500 and 9000 Da and a polydispersity of approximately 1.0. M118 has the structural formula $C_{12m}H_{14m+1}O_{10m}N_mNa_mR_{3m-1}R1_m$, $C_{12m}H_{14m+2}O_{10m+1}N_mNa_mR_{3m}R1_m$, where n is equal to the average number of disaccharide repeats, $m = 1 + n$, R is H or SO_3Na and R_1 is SO_3Na or $COCH_3$.

The use of anticoagulants can result in haemorrhagic adverse events and hence the availability of an antidote is highly desirable. An antidote can also be useful in case an anticoagulated patient needs to be operated on urgently. In the development of EP42675, therefore, a biotin entity was covalently linked to the spacer between the pentasaccharide portion and the direct thrombin inhibitor portion of the molecule to give EP217609 (Fig. 3), which enables it to be neutralized upon administration of avidin [72]. EP42675 has successfully completed phase I trials with 100 healthy subjects, where it was found to be well tolerated and showed predictable pharmacokinetic and pharmacodynamic profiles, with low intra- and inter-subject variabilities [73]. The half-lives of EP42675 and EP217609 in rats was determined to be approximately three hours [31]. In animals, the pharmacokinetic/pharmacodynamic profiles of EP217609 and EP42675 were found to be similar.

Non-anticoagulant heparin/HS mimetics

Heparin is known to inhibit the synthesis, expression and/or function of adhesion molecules, cytokines, chemokines, proteases and viral proteins [74]. Consequently, attention has been focused recently on the non-anticoagulant properties of heparin, which are known to inhibit inflammation [63,75] and the metastatic spread of tumour cells [7,76].

PI-88 (Muparfostat)

Heparanase is an endoglycosidase enzyme that has vital roles in inflammation, tumour cell invasion, metastasis and angiogenesis [77,78]. Heparanase is the enzyme responsible for processing HS. Several sulphated sugar molecules such as cyclitols and glycol-split derivatives have been identified as selective inhibitors of heparanase–heparin interactions [79]. PI-88 (Progen Pharmaceuticals) (Fig. 4) is one such inhibitor. PI-88 inhibits heparanase and the cleavage of HS by binding competitively with HS, thereby preventing the release of growth factors, such as FGF-1, FGF-2 and VEGF, involved in angiogenesis [80]. PI-88 has progressed to clinical trials to treat inflammatory diseases, thrombosis, viral infections and cancer [81].

PI-88 is a phosphomannopentose sulphate (6-*O*-PO_3H_2-α-D-Man-(1 → 3)-α-D-Man-(1 → 3)-α-D-Man-(1 → 3)-α-D-Man-(1 → 2)-D-Man) (Fig. 4), wherein the chain length, sugar composition and glycosidic linkages α(1 → 3) and α(1 → 2) play important parts in its anticoagulation activity, compared with the anticoagulant activity of sulphated glucose-containing oligosaccharides with β(1 → 4) and β(1 → 3) linkages [82]. PI-88 is known to consistently prolong the activated partial thromboplastin time through the

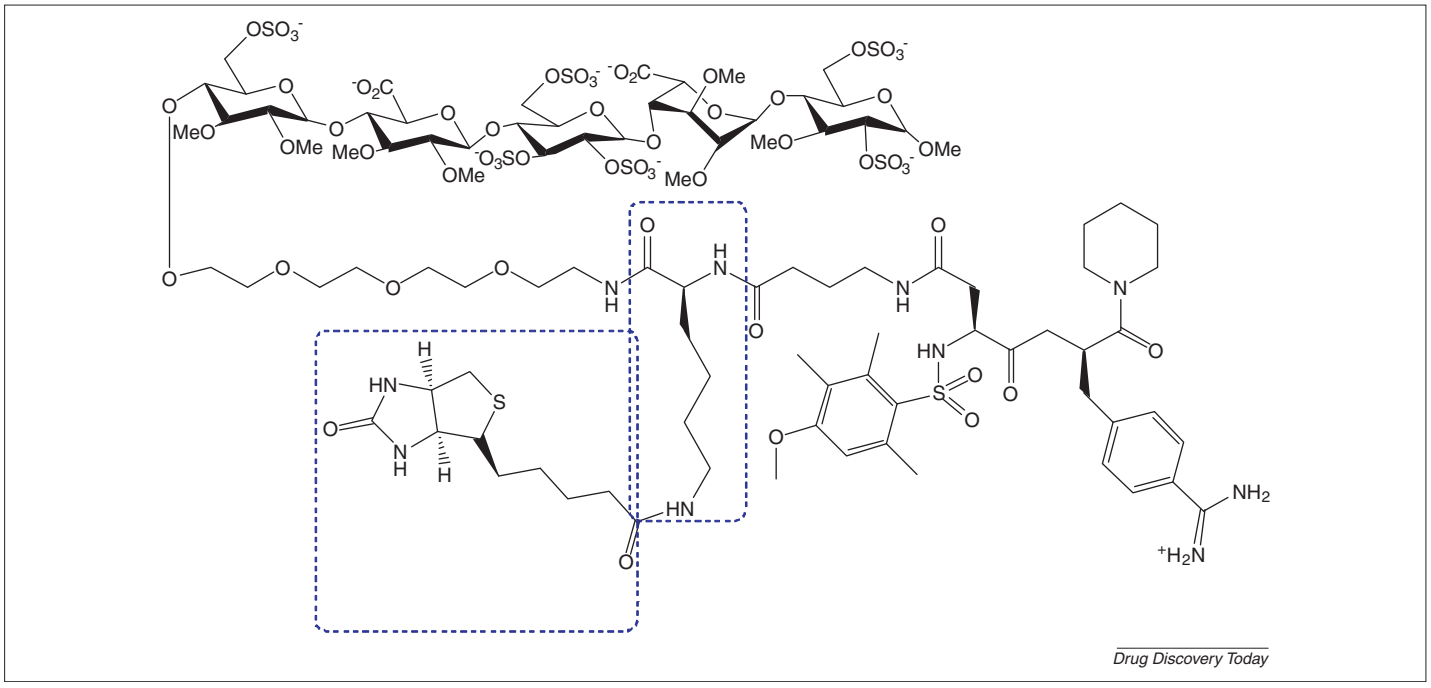

FIGURE 3

Chemical structures of EP217609 and EP42675. EP42675 and EP217609 are the first representatives of a new class of synthetic, parenteral anticoagulants with a dual mechanism of action combining the properties of an indirect FXa inhibitor and a direct thrombin inhibitor. The structure of EP42675 can be inferred by deleting the biotin and lysine moieties (shown in dotted rectangles) from the structure of EP217609.

activation of the endogenous heparin cofactor II. Apart from its anticipated anticoagulant effects, PI-88 was well tolerated in animal studies.

In the first phase I trial with patients with malignant disease, PI-88 was administered subcutaneously for four consecutive days either bimonthly or weekly [83]. Prolongation of the activated partial thromboplastin time was seen in only 2 of 14 patients. The recommended dose of PI-88 administered daily for four days every week was established to be 250 mg. Dose-limiting toxicity occasionally resulted in thrombocytopenia (at a dose of 2.28 mg/kg/day for 14 days) in patients with advanced malignancies, which seemed to be immunologically mediated through the development of anti-heparin platelet factor 4 complex antibodies [84]. The second phase I trial evaluated the safety, toxicity, pharmacological properties and biological activity of PI-88 with fixed weekly docetaxel (chemotherapy) in patients with advanced solid malignancies [85]. Sixteen patients received docetaxel at a 30 mg/m^2 dose on days 1, 8 and 15 of a 28 day cycle, with PI-88 injected subcutaneously for four days per week. Minor toxicity responses during the course of the therapy included fatigue, dysgeusia, thrombocytopenia, diarrhoea, nausea and emesis. Docetaxel and PI-88 did not alter the pharmacokinetics of each other. In another phase I trial, the recommended dose of PI-88 was reported to be 190 mg/m^2 alone and 1000 mg/m^2 in combination with dacarbazine every three weeks [86]. A phase I/II trial of daily PI-88 alone or with dacarbazine in patients with malignant melanoma was subsequently undertaken; however, the trial was stopped owing to cases of major febrile neutropenia [87]. A phase II trial of PI-88 in patients with advanced melanoma evaluated a fixed dose of 250 mg/day given by injection for four consecutive days followed by three drug-free days in a 28 day cycle [88]. Some patients developed serious bleeding events, with hemorrhagic cerebral metastases and arterial thrombosis. Nonetheless, in patients with advanced melanoma, PI-88 demonstrated noteworthy activity, but further investigations are needed of its use in combination with chemotherapy. A phase III trial investigating PI-88 as a post-resection treatment for hepatocellular carcinoma (liver cancer) was designed to establish the efficacy and safety of PI-88, but no results have been reported.

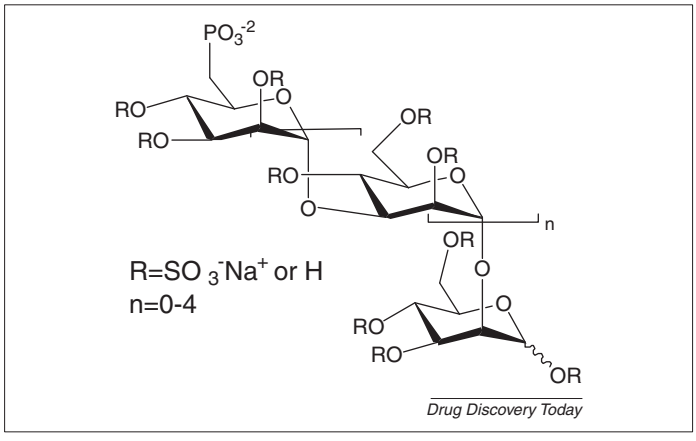

FIGURE 4

Chemical structure of PI-88. PI-88 is primarily composed of sulphated phosphomannopentaose and phosphomannotetraose oligosaccharide units. PI-88 is a potent anti-angiogenic, anti-tumour and anti-metastatic agent because of its inhibition of the heparan sulphate-degrading enzyme heparanase.

The PG500 series is a collection of newly designed compounds that are anomerically pure and fully sulphated and have single entity oligosaccharides attached to a lipophilic moiety, such as aglycone, at the reducing end of the molecule [89]. Compared with PI-88, some of these compounds are more potent inhibitors of

angiogenesis and metastasis and show strong anti-tumour activity in some aggressive tumour models [90]. These compounds are believed to interfere in processes such as tumour development, namely angiogenesis via inhibition of VEGF, FGF-1 and FGF-2, and metastasis via inhibition of heparanase [91]. PG545 was selected as the lead molecule based on its efficacy, pharmacokinetics, toxicology and ease of manufacture [92,93]. This compound has been in preclinical trials and administered subcutaneously once a week in mice for the treatment of cancer.

Tramiprosate (Alzhemed™)

HS/heparin have been widely reported to be associated with neuritic plaques in Alzheimer's disease (AD) [94]. HS has also been shown to promote the aggregation of amyloid β-peptide (Aβ) and have a pivotal role in plaque formation [95]. Several molecules have been proposed to be used to prevent HS-induced aggregation of Aβ: derivatives or fractions of heparin and other GAGs, sulphated compounds that act as HS mimetics (e.g. pentosan polysulphate and dextran sulphate) [96], small-molecule anionic sulphonates or sulphates [97], and amyloidophilic, sulphonated dyes, such as Congo Red and Thioflavin S.

Tramiprosate (also referred to as 3-amino-1-propanesulfonic acid, 3-aminopropylsulfonic acid, 3-APS, homotaurine or NC-531) is a GAG mimetic designed to interfere with the actions of Aβ early in the cascade of amyloidogenic events [98–100]. Structurally, Tramiprosate is a modification of the amino acid taurine (Fig. 5). It binds preferentially to soluble Aβ peptide and maintains Aβ in a random-coil/α-helical rich conformation and in non-fibrillar form, thereby inhibiting aggregation and hence plaque formation and deposition [101]. It can cross the blood–brain barrier effectively [102]. Recently, it has been reported that Tramiprosate also alters tau aggregation [103].

A phase II trial demonstrated that Tramiprosate reduces $A\beta_{42}$ in the cerebrospinal fluid of patients with mild to moderate AD [104]. The US phase III trial involved patients with mild to moderate AD, who were randomly assigned to receive placebo or 100 mg or 150 mg twice-daily doses of Tramiprosate. Although treatment was well tolerated, the study failed to demonstrate efficacy upon long-term clinical testing of cognitive improvement [105]. The European phase III trial has been discontinued. No further reports on the drug are available except columetric magnetic resonance imaging findings, which suggested less hippocampal shrinkage upon treatment with Tramiprosate [106]. Bellus Health (formerly Neurochem Inc.) has been promoting this medication as a nutraceutical, Vivimind™, which is being put forward as protecting against memory loss [107].

Bellus Health has recently initiated a phase I clinical trial of NRM8499, a prodrug of Tramiprosate for the treatment of AD. NRM8499 increases brain exposure to Tramiprosate, which might help improve the therapeutic effect on cognitive function and other clinical results in AD. This randomized, double-blind, placebo-controlled study is expected to investigate the safety, tolerability and pharmacokinetic profile of NRM8499 in a group of up to 84 young and elderly healthy subjects. Preclinical studies conducted in rodents showed that NRM8499 increased plasma and brain exposure to Tramiprosate by 1.5–3-fold.

Eprodisate sodium (NC-503, Kiacta® and Fibrillex™)

Eprodisate (1,3-propanedisulfonic acid disodium salt) is a low-molecular-weight, negatively charged sulphonated molecule (Fig. 6) that shares certain structural similarities with HS and is known to bind to serum amyloid protein A (SAA) [108]. Eprodisate binds to the GAG-binding site of SAA and competes with naturally occurring sulphated GAGs, thus targeting amyloid fibril polymerization and inhibiting amyloid deposition in tissues [97,109]. Eprodisate inhibits the development of amyloid deposits in *in vivo* mouse models of amyloid protein A (AA) amyloidosis [110]. In preclinical pharmacokinetic studies, Eprodisate has good bioavailability if administered orally; it is not metabolized, it does not bind to plasma proteins, and it is excreted primarily by the kidney, although pharmacokinetics analyses in its phase I trial revealed high inter-individual variability in its plasma concentrations [111]. Although Eprodisate is eliminated by the kidney, plasma concentrations were seen to increase as renal function decreased, resulting in a considerable increase in drug systemic exposure. An approximate terminal half-life of 10–20 h was derived from a multiple rising oral dose study. The efficacy and safety of Eprodisate was tested in a single phase II/III trial in AA amyloidosis patients [112]. Eprosidate was well tolerated, and its adverse events profile was comparable to placebo. Eprodisate can also be used with other types of amyloidosis. The results of a recent trial showed that it might slow the progression of AA amyloidosis-related renal disease [113], but no effect was seen on SAA levels, progression to end-stage renal disease or death, proteinuria and amyloid content of abdominal fat [114]. Despite having previously been granted orphan and fast-track status, the FDA and the EMEA both requested an additional confirmatory phase III trial before approval [114].

Studies using a preclinical rat model of diabetes and metabolic syndrome have confirmed that Eprodisate decreases glucose, cholesterol and triglycerides in the blood of obese diabetic Zucker rats compared with the control group, while preserving 40% more

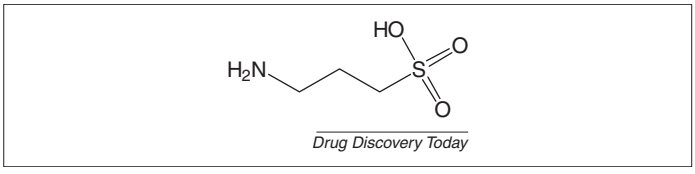

FIGURE 5

Chemical structure of Tramiprosate, an anti-amyloidogenic agent. In the context of Alzheimer's disease, this molecule acts by preventing and slowing the formation and the deposition of heparin/HS-induced amyloid fibrils in the brain and by binding to soluble beta-amyloid protein to reduce the amyloid-induced toxicity on neuronal and brain inflammatory cells.

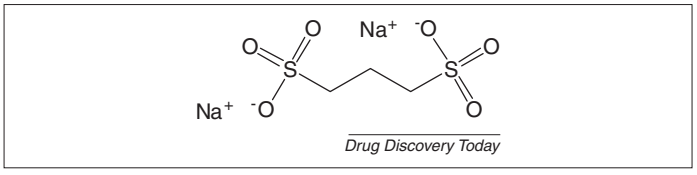

FIGURE 6

Chemical structure of Eprodisate. Eprodisate is a promising agent designed to prevent the worsening of renal function in patients with AA amyloidosis. It inhibits the polymerization of amyloid fibrils and the deposition of the fibrils in tissues by interfering with interactions between amyloidogenic proteins and heparin/HS.

pancreatic islet cells compared with the control group and showing some protective effect on renal function. However, Bellus Health has discontinued the development of Eprodisate in relation to diabetes after a phase IIa clinical proof-of-concept trial because the study did not meet its primary efficacy endpoint [115]. Instead, the company has pushed preclinical development of a prodrug of Eprodisate for the treatment of Type II diabetes and related metabolic syndromes.

Other novel GAG mimetics

Substrate-optimized glycans

Zacharon Pharmaceuticals has focused its research on small-molecule inhibitors of GAG biosynthesis for lysosomal storage disease. Mucopolysaccharidosis (MPS) is a form of lysosomal storage disease caused by a deficiency in the enzymes responsible for the degradation of GAGs, which makes lysosomes fill with partially degraded GAGs and resulting in serious systemic disease.

Zacharon uses substrate optimization therapy, which is a novel therapeutic approach for selectively modifying glycan structure without reducing the overall amount produced or altering normal glycan function [116]. This selective modification renders the glycan molecule more readily degradable, despite the presence of specific enzyme deficiencies. In MPS, this is accomplished by selectively and favourably modifying the glycan sulphation pattern. In MPS II, an inhibitor of the biosynthetic step involving the addition of 2-*O* sulphates to GAGs would produce GAGs with less 2-*O* sulphation and increased 6-*O* sulphation (Fig. 7). These GAGs would be easier to degrade for an MPS II (2-sulphatase-deficient) patient. To identify potential drug candidates, a library of 74,000 drug-like small molecules for inhibitors of HS biosynthesis were screened using cell-based assays. Of the 264 hit compounds identified in the primary screen, 30 were found to inhibit HS biosynthesis in cultured cells. Four of the compounds were found to reduce GAG accumulation in primary human fibroblasts obtained from MPS patients. ZP2345 was then chosen as the starting point for the development of a GAG mimetic based on substrate optimization therapy. ZP2345 is an HS inhibitor that reduces 2-*O* sulphation in a dose-dependent manner in a cultured human cell model of MPS-II [116]. Ongoing studies are focusing on analogue design, synthesis and testing to improve the potency and efficacy of these inhibitors.

Heparin mimetics in cancer

Exogenous heparin, LMWH and their mimetics have been shown to exert anti-metastatic and anti-angiogenic properties affecting cancer progression, such as inhibition of heparanase, blocking of P- and L-selectin-mediated cell adhesion, and inhibition of angiogenesis [117]. Heparanase has been targeted with several heparin-related inhibitors such as aza sugar derivatives, glycol-split derivatives and cyclitols that block the active site of the enzyme or the heparin/HS binding sites, or both [118]. Several LMWH derivatives have been described, among which are a deoxycholic acid conjugate and the fragmentation of a periodate-oxidized heparin, which have anti-angiogenic and anti-metastatic activities in different types of cancer models [119,120].

Endotis Pharma has created a platform for the development of anti-cancer 'small-glyco' drugs. These molecules are short, chemically synthesized oligosaccharides with potent affinity and selective inhibition of several growth factors and proteins (VEGF-A, FGF-2, PDGF-B, SDF-1α and heparanase) involved in tumour growth and dissemination [121]. A library of more than 100 synthetic oligosaccharides of different sizes containing various substitutions has been evaluated for their affinity for specific targets and their efficacy on cell proliferation and migration and *in vitro* endothelial tubule formation. The structure–activity relationship indicates that affinity and selectivity of these molecules for different targets can be fine-tuned through chemical substitutions. EP80061 is the lead compound in the series, and it induced a very potent anti-metastatic effect on a disseminated tumour model in C57Bl/6 mice [122]. However, the structure of these series has not yet been disclosed.

Momenta Pharmaceuticals presented preclinical data for M402, a HS mimetic containing a mixture of linear sugar chains and engineered to have potent anti-metastatic properties [123,124].

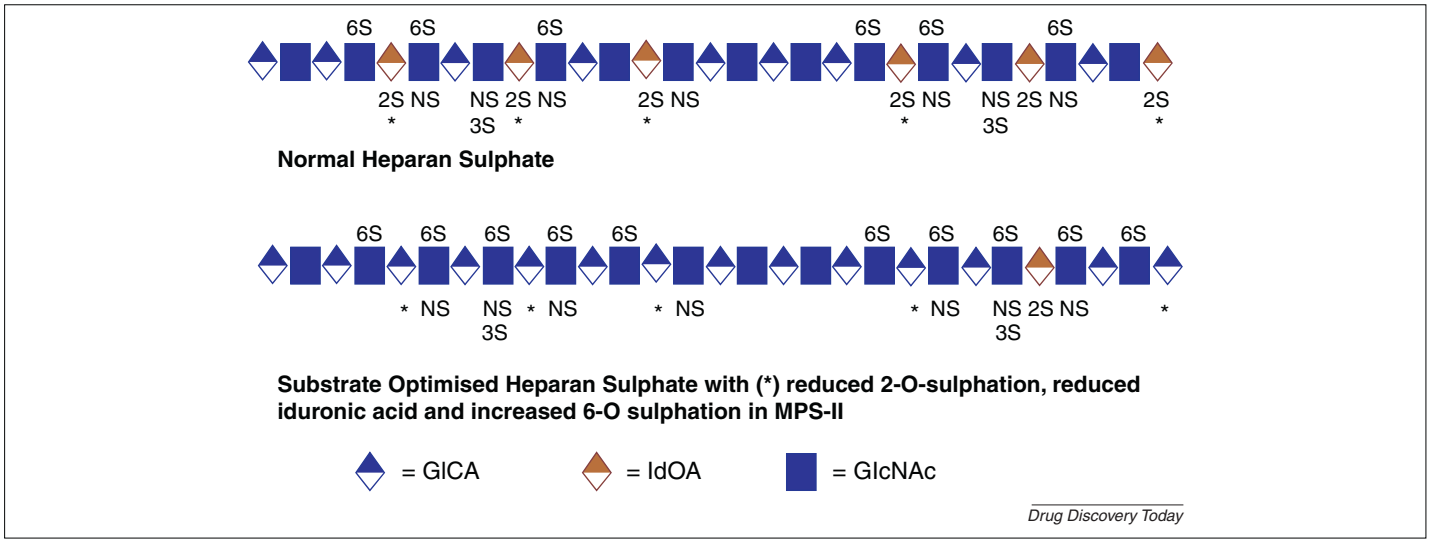

FIGURE 7

Substrate-optimized glycans (e.g. HS in MPS).Figure modified, with permission, from Ref. [159], Zacharon Pharmaceuticals.

Both *in vitro* and *in vivo*, M402 showed reduced anticoagulant activity and inhibited tumour metastasis through modulation of multiple factors, such as P-selectin, VEGF, FGF-2 and SDF-1α [124,125]. M402 as a monotherapy or in combination with chemotherapeutics showed statistically significant survival benefits in animal models with aggressive tumours [123,125]. In combination with gemcitabine, M402 produced much prolonged survival and reduced metastasis compared to groups treated with saline solution alone or gemcitabine alone in a murine pancreatic model [126]. Mice treated with M402 showed reduced epithelial-to-mesenchymal transition, a key step in the progression of tumour cells towards a more invasive phenotype.

Regenerating agents

Regenerating agents (RGTAs) are large biopolymers engineered to replace HS specifically bound to matrix proteins and growth factors destroyed after chronic tissue injury [127]. These polymers protect proteins bound to the extracellular matrix from proteolysis. RGTAs can interact with many heparin-binding growth factors, such as FGF-2 [128], transforming growth factor-β [129] and VEGF [130]. In addition to their heparin-binding-growth-factor-protecting and stabilizing properties, RGTAs have been found to inhibit human leukocyte elastase [131], plasmin [132,133] and heparanase [134]. RGTA-induced matrix therapy is a possible alternative to cell or gene therapy in regenerative medicine [135]. RGTA derivatives are potent activators of tissue repair in various *in vivo* wound-healing models: wound [136], bone defect [137,138], infarcted myocardium [139], colic ulceration [140] and periodontitis [141]. These RGTAs have also been shown to stimulate satellite cell growth and differentiation in primary cultures [142].

RGTAs are dextran derivatives with defined amounts of substituted carboxymethyl, benzylamide and sulfonate groups (Fig. 8). By varying the relative proportion of these substitutions, a library of heparin-mimetic biopolymers was produced. RGTAs with an increased level of sulphation and benzylamidation have shown anti-prion activity by blocking the conversion of prion protein PrP^C into the abnormal forms in scrapie-infected GT1 cells [143] and scrapie-infected and bovine-spongiform-encephalopathy-infected mice [144]. RGTA polymers are easier and less costly to produce, store and handle than growth factors. One such molecule is OTR4120 (alternatively called RGD120 or RG1192), derived from a glycosidic polymer of dextran T40 and functionalized with a 1.13 level of substitution of sulphate residues and a 0.46 level of substitution of carboxymethyl residues, with a maximal level of substitution of 3.0 [145], rendering this molecule structurally similar to heparin but having at least ten times less anticoagulant activity than heparin [146]. Nuclear magnetic resonance (NMR) analysis has shown that this polymeric compound is composed of a 15 sugar unit sequence statistically repeated along the molecule [147,148]. OTR4120 is known to enhance tissue repair in several animal models, including peripheral nerve injury in rats [149], burned skin in rats [150], chronic skin ulcers in mice [135] and cutaneous wound repair in rats [151]. Pharmacokinetics studies performed in a muscle crush model indicated that OTR4120 could replace degraded HS-GAG after tissue injury and bind to the heparin-binding sites present on many extracellular matrix proteins that have been freed from occupation by their endogenous GAGs [152]. In a recent clinical pilot study, an OTR4120 ophthalmic solution was found to improve the healing of severe corneal ulcers and dystrophy [153]. OTR3 is currently marketing CACIPLIQ20®, an active device based on RGTA for the treatment of chronic ulcers, diabetic foot ulcers, pressure ulcers, venous ulcers and burns.

Heptagonists

Use of heparin, LMWH, Fondaparinux and Idraparinux in cardiovascular surgeries often leads to a high incidence of bleeding complications. Protamine and LMW protamine are antidotes employed in heparin reversal; however, they can cause severe adverse reactions. PolyMedix has developed novel small synthetic salicylamide derivatives called heptagonists, which act as universal anticoagulant-reversing agents and are active against heparin, LMWH, Idraparinux and Fondaparinux [154–157]. One of the company's so-called 'heptagonists', PMX-60056 [157], can effectively neutralize the AT and anti-Xa activities of LMWH. PMX-50056 has been shown to completely reverse the anticoagulant effects of heparin and normalize blood clotting time in six human subjects in less than 10 min in a phase Ib clinical trial.

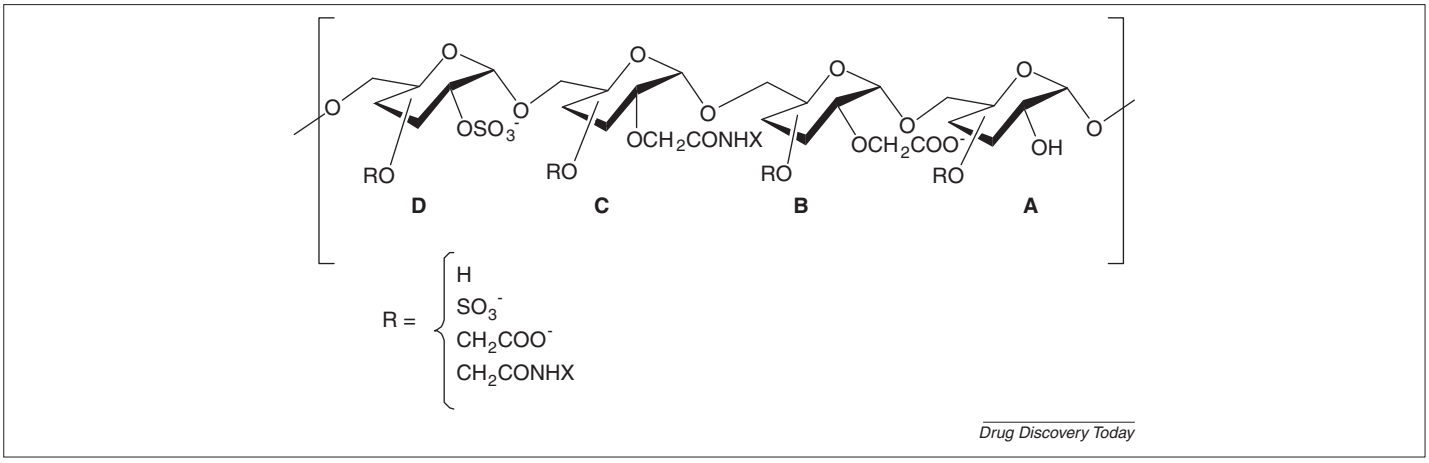

FIGURE 8

Schematic chemical structure of RGTA. Four differently substituted units, A (<1%), B (=32%), C (=0%) and D (=67%), can be present in OTR4120, as reported by titrimetry and ^{1}H NMR [147]. R represents the proportion of substituted group in the global C3 and C4 positions arranged to define the global dextran sulphate of each group.

Concluding remarks

Past research has highlighted the drawbacks of using native heparin oligosaccharides as drugs. Their anionic nature can result in large interactions with multiple, physiologically important proteins, leading to many side-effects. In addition to their lack of affinity, heparin oligosaccharides suffer from low tissue permeability, short serum half-life and poor stability. Consequently, the pharmacodynamic and pharmacokinetic properties of heparin make it inadequate for its direct therapeutic application. In addition, the multi-step synthesis of heparin/HS oligosaccharides poses challenges for medicinal chemists, both at the drug development and the production scale [158]. Furthermore, new therapeutic applications of sulphated GAGs now include the treatment of infectious diseases and inflammation and the control of cell growth in wound healing and cancer. These new applications require the elimination of the anticoagulant activity of heparin oligosaccharides and the engineering of appropriate pharmacokinetic properties and optimal oral bioavailability.

GAG mimetics are designed to overcome these shortcomings. Detailed insight into GAG–protein interactions has predominantly been provided by recent progress in NMR spectroscopy, X-ray crystallography and molecular modelling techniques. Identification of the bound conformation of heparin/HS to a protein enables the design of GAG mimetics and the identification of negligible and replaceable functional groups. As a consequence, the development of GAG mimetics that have improved absorption, distribution, metabolism and excretion properties can be accomplished.

The development of HS/heparin-based drugs is a fertile field of research that is providing enormous opportunities for the discovery of improved treatments for many diseases. This is evidenced by the existence of many newly established companies, such as Intellihep, Zacharon Pharmaceuticals, GlycoMimetics, Endotis Pharma, Polymedix, Progen, OTR3 and Momenta, to name a few, which are beginning to exploit the untapped potential of the structural diversity of heparin/HS in various therapeutic and biomedical applications.

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