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Review

Targeting Heparanase in Cancer: Inhibition by Synthetic, Chemically Modified, and Natural Compounds

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Heparanase is an endoglycosidase involved in remodeling the extracellular matrix and thereby in regulating multiple cellular processes and biological activities. It cleaves heparan sulfate (HS) side chains of HS proteoglycans into smaller fragments and hence regulates tissue morphogenesis, differentiation, and homeostasis. Heparanase is overexpressed in various carcinomas, sarcomas, and hematological malignancies, and its upregulation correlates with increased tumor size, tumor angiogenesis, enhanced metastasis, and poor prognosis. In contrast, knockdown or inhibition of heparanase markedly attenuates tumor progression, further underscoring the potential of anti-heparanase therapy. Heparanase inhibitors were employed to interfere with tumor progression in preclinical studies, and selected heparin mimetics are being examined in clinical trials. However, despite tremendous efforts, the discovery of heparanase inhibitors with high clinical benefit and minimal adverse effects remains a therapeutic challenge. This review discusses the key roles of heparanase in cancer progression focusing on the status of natural, chemically modified, and synthetic heparanase inhibitors in various types of malignancies.

INTRODUCTION

Cancer is a major health problem throughout the globe and the second leading cause of death in the United States with an increasing number of new cancer cases annually (Siegel et al., 2017). The frequency of cancer-related mortality is increasing at a frightening rate in developing countries (Uzma et al., 2018). Somatic mutations, inheritance of mutated genes, lifestyle, and environmental factors are the major contributing factors for cancer development (Siveen et al., 2014). Cancer cells possess the characteristics of uncontrolled proliferation, anti-apoptosis, angiogenesis, and metastasis. Compelling evidence ties heparanase with all steps of tumor formation, including tumor initiation, growth, metastasis, and chemoresistance (Vlodavsky et al., 2016, 2018; Rivara et al., 2016). The enzyme is highly implicated in the cross talk between tumor cells and their microenvironment, a consequence of cleavage of heparan sulfate (HS), remodeling of the extracellular matrix (ECM) underlying epithelial and endothelial cells, and regulating the bioavailability of HS-bound growth- and angiogenesis-promoting factors (Figure 1). Tumor cell cross talk with the immune system is well documented. With regard to heparanase, cytokines and enzymes secreted by macrophages, for example, promote heparanase expression and processing (Lerner et al., 2011). On the other hand, heparanase and HS degradation fragments activate macrophages (Goodall et al., 2014), which may promote or suppress tumor growth (Gutter-Kapon et al., 2016). Importantly, it was recently reported that heparanase, secreted by tumor- and/or immune-cells, may play a critical role in the ability of chimeric antigen receptor (CAR)-T lymphocytes (Caruana et al., 2015) and natural killer (NK) cells (Putz et al., 2017) to infiltrate and reach their target tumor cells. Collectively, heparanase secreted by tumor cells, stromal cells, and immune cells appears to play a dual, possibly double sword, role in modulating tumor growth.

The ECM is the framework of all the tissues and organs that provide physical scaffolding and assists the biochemical processes that are essential for tissue morphogenesis, differentiation, and homeostasis (Frantz et al., 2010; Kinoshita et al., n.d.). The biochemical composition of the ECM is primarily contributed by supramolecular proteins (collagen, elastin, laminin, fibronectin), proteoglycans (HS, chondroitin sulfate, keratan sulfate, dermatan sulfate (DS), all representing glycosaminoglycan (GAG) chains covalently attached to the proteoglycan core protein), and polysaccharides (hyaluronic acid). HS is one of the major types of GAG and the chief constituent of basement membranes. HS is a highly acidic, strongly anionic

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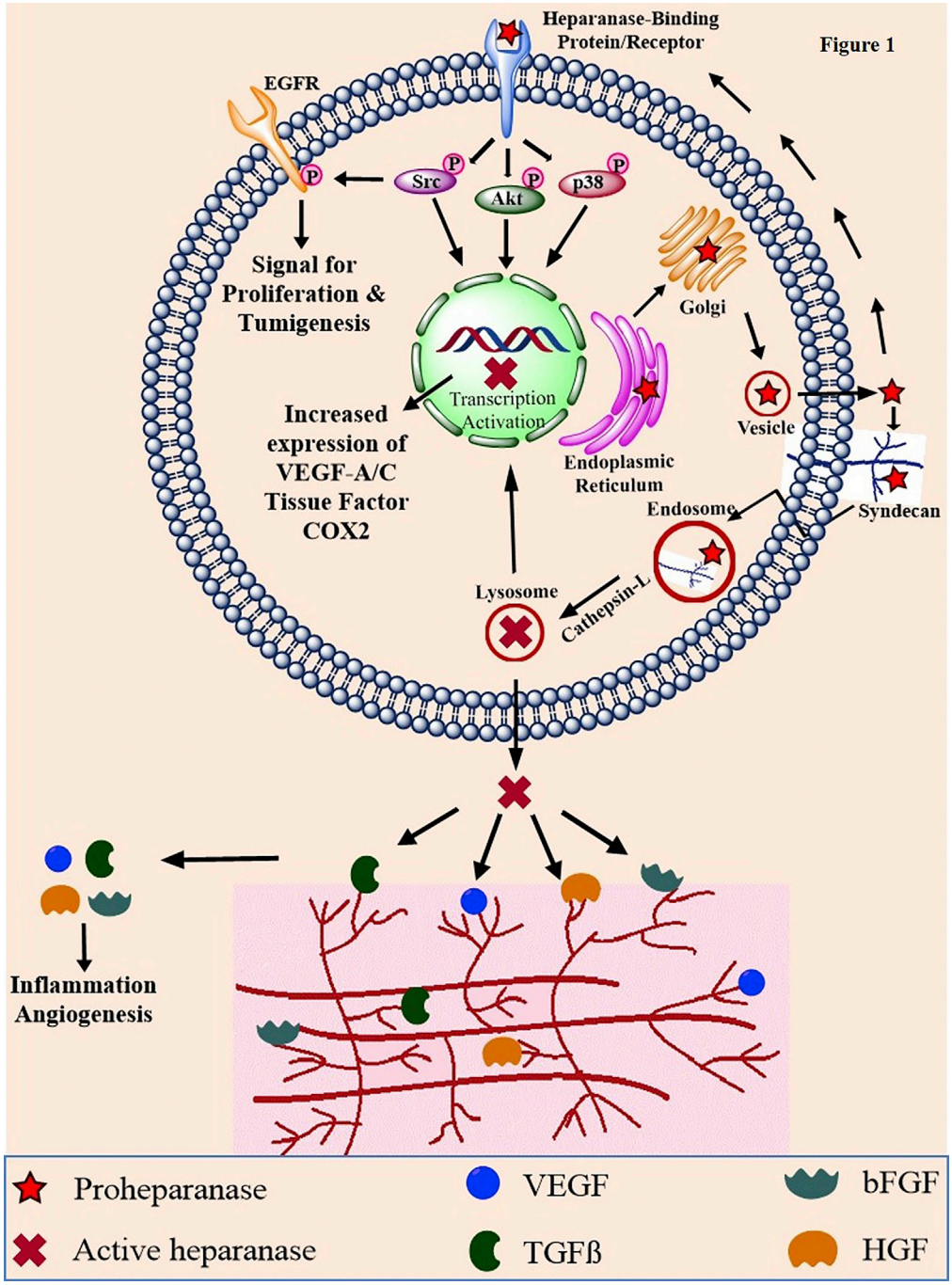

Figure 1. Enzyme-Activity-Dependent and Enzyme-Activity-Independent Functions of Heparanase

Translation of heparanase mRNA on the endoplasmic reticulum (ER) leads to the synthesis of proheparanase followed by its transportation to the Golgi apparatus and secretion into the extracellular space. Proheparanase has a dual role: (1) interacting with membrane-bound signaling receptor and (2) cellular uptake to form active heparanase. Briefly, proheparanase may interact with heparanase-binding protein or receptor on the cell membrane, resulting in activation of Src, EGFR, Akt, p38, and thereby contributing to the increased expression of VEGF-A/C, tissue factor, and COX2 leading to enhanced cell proliferation, adhesion, motility, and tumorigenesis. Proheparanase is also internalized by interacting with cell surface syndecans, low-density lipoprotein receptor-related protein (LRP), and mannose-6-phosphate receptor. Internalization leads to proteolytic cleavage by cathepsin-L to form the active

Figure 1. *Continued*
heparanase heterodimer. Active heparanase may translocate into the nucleus where it entangles in the transcription of target genes. Secretion of active heparanase into the ECM results in the release of HS-bound growth factors and cytokines to promote angiogenesis, inflammation, and epithelial-mesenchymal transition.

GAG made up of repeating disaccharides of glucuronic acid (GlcA) or iduronic acid and D-glucosamine that are covalently linked to proteins to form HS proteoglycans (HSPGs) (Simon Davis and Parish, 2013). Despite the seemingly simple single repeating structural motif, these sugar polymers show a great deal of structural diversity generated by a complex pattern of deacetylation, sulfation, and epimerization (Lindahl et al., 2015). Each saccharide unit can be sulfated at different hydroxyl groups, and the sulfation is unevenly distributed along HS. Moreover, HS contains regions with repeated disaccharide units of -GlcA-GlcNAc-, forming low-sulfated domains and regions with repeating disaccharide units forming highly sulfated domains (Peterson and Liu, 2013). The sulfated saccharides create binding sites for ECM and cell surface proteins, as well as numerous bioactive molecules (i.e., growth factors, cytokines, chemokines, and enzymes) (Figure 1) that together orchestrate cellular responses to both normal and pathological situations. By interacting with other macromolecules such as laminin, fibronectin, and collagens I and IV, HSPGs contribute to the structural integrity, self-assembly, and insolubility of the ECM and basement membranes, thus intimately modulating cell-ECM interactions (Bernfield et al., 1999).

HEPARANASE IN HOMEOSTASIS

Heparanase is the sole endoglycosidase that cleaves HS polysaccharide chains in HSPGs into smaller fragments and thereby regulates the functions of HS (Li and Vlodavsky, 2009; Peterson and Liu, 2013). Heparanase, initially purified from human placenta (Klein and Figura, 1976) and mastocytoma (Ogren and Lindahl, 1975), is produced as an inactive precursor pro-heparanase (65 kDa), which is then processed by cathepsin L proteolytic removal of a 6-kDa linker peptide to form the active noncovalent heterodimer of 50- and 8-kDa subunits (Abboud-Jarrous et al., 2008). In its latent form, pro-heparanase interacts with heparanase-binding proteins or receptors at the cell surface, leading to the activation of Akt, p38, and Src, which in turn enhance the transcription of genes involved in angiogenesis, cell proliferation, and tumorigenesis (Fux et al., 2009b). Heparanase may also be translocated into the cell nucleus where it affects gene transcription (He et al., 2012). A number of studies have shown that secreted or exogenously added latent heparanase rapidly interacts with HS (i.e., syndecan) on the cell surface, followed by internalization and processing into a highly active enzyme (Gingis-Velitski et al., 2004b) (Figure 1), a process that may be used by cancer cells to increase their own levels of heparanase. Heparanase uptake is regarded as a pre-requisite for the delivery of latent heparanase to late endosomes and lysosomes where it undergoes proteolytic processing and activation (Vlodavsky et al., 2016, 2018). Thus heparanase resides primarily within endocytic vesicles, assuming a polar, perinuclear localization and co-localizing with lysosomal markers. Residence and accumulation of heparanase in lysosomes indicate that the enzyme may function in the normal physiology of this organelle (i.e., HS turnover, autophagy) (Shteingauz et al., 2015; Vlodavsky et al., 2018). This implies that heparanase function is not limited to the extracellular milieu but can operate inside the cell as well (Ilan et al., 2015).

Notably, HSPGs provide a reservoir for several growth factors including vascular endothelial growth factor (VEGF), transforming growth factor β (TGF-β), basic fibroblast growth factor (bFGF), keratinocyte growth factor, and hepatocyte growth factor (HGF) (Figure 1), all involved in maintaining homeostatic conditions and disease processes, including morphogenesis, tissue repair, inflammation, vascularization, and cancer metastasis (Bernfield et al., 1999; Vlodavsky et al., 2002; Kreuger et al., 2006). These growth factors serve as ligands for respective receptors to relay signals for various cellular activities. Heparanase may thereby regulate tissue remodeling and the bioavailability and activity of growth factors, chemokines, and cytokines (Folkman et al., 1988; Mundhenke et al., 2002; Sanderson et al., 2004). Immunohistochemical analysis revealed the expression of heparanase in the capillary endothelium, keratinocytes, cytotrophoblasts, platelets, neutrophils, and macrophages (Vlodavsky and Friedmann, 2001). Heparanase expression is increased during early pregnancy in mice, and administration of heparanase inhibitor suppresses implantation (D'Souza et al., 2007).

In addition, elevated activity of heparanase is observed in activated immune cells, fibroblasts, and endothelial cells (Edovitsky et al., 2006; Arvatz et al., 2011b; Barash et al., 2014; Gutter-Kapon et al., 2016; Digre et al., 2017). Mechanistically, heparanase was identified as a key mediator of macrophage activation

(Goodall et al., 2014). In particular, a signaling cascade was described in which heparanase activates Erk, p38, and JNK signaling in macrophages, leading to increased levels of c-Fos and induction of cytokine expression (Gutter-Kapon et al., 2016).

HEPARANASE IN TUMORIGENESIS

Heparanase is up-regulated in essentially all human tumors examined (Ilan et al., 2006; Parish et al., 2001; Vreys and David, 2007; Vlodavsky et al., 2012; McKenzie, 2007). A direct role of heparanase in tumor metastasis was demonstrated by the increased lung, liver, and bone colonization of cancer cells following overexpression of the heparanase gene, and by a marked decrease in the metastatic potential of cells subjected to heparanase gene silencing (Edovitsky et al., 2004). A significant role of heparanase in tumor angiogenesis and lymphangiogenesis was demonstrated applying a similar approach (Cohen-Kaplan et al., 2008). Notably, patients with cancer exhibiting high levels of heparanase had a significantly shorter postoperative survival time than patients whose tumors contained low levels of heparanase (Ilan et al., 2006; Vlodavsky et al., 2012). Collectively, these results indicate that heparanase is causally involved in cancer progression and hence is a valid target for anticancer drug development. This statement was reinforced by *in vivo* studies indicating a marked inhibition of human tumor growth (e.g., myeloma, mesothelioma, lymphoma, glioma, sarcoma, and carcinomas of the lung, stomach, and pancreas) in mice treated with heparanase-inhibiting drugs (e.g., roneparstat, pixatimod) (Bashenko et al., 2007; Dredge et al., 2011; Ritchie et al., 2011), now in clinical trials for various malignancies (Dredge et al., 2018; Galli et al., 2018). Of increasing significance are observations that enzymatically inactive heparanase promotes VEGF, HGF, and tissue factor expression, as well as Akt, Src, and epidermal growth factor (EGF) receptor phosphorylation (Gingis-Velitski et al., 2004a; Fux et al., 2009b), emphasizing the notion that non-enzymatic activities of heparanase play a significant role in heparanase-driven tumor progression (Ilan et al., 2006; Fux et al., 2009a). Various studies indicate that heparanase, expressed by the tumor or host cells, mediates a vicious proinflammatory and protumorigenic cross talk in the tumor microenvironment by regulating gene expression, ECM remodeling, shedding of syndecan-1, and facilitating growth factor bioavailability, and signaling cascades (Vlodavsky et al., 2012; Ramani et al., 2013). Notably, the crystal structure of heparanase was solved (Wu et al., 2015) and specific domains critical for substrate recognition, processing, secretion, and non-enzymatic functions of the heparanase protein were identified (Vlodavsky et al., 2016), paving the way for rational design of site-specific heparanase inhibitors (Rivara et al., 2016; Wu et al., 2017) and neutralizing antibodies (Weissmann et al., 2016). Altogether, basic and clinical research led to a much better understanding of the mode of action and involvement of heparanase in cancer progression and the associated prognostic and therapeutic applications.

SUBSTRATE SPECIFICITY

Heparanase hydrolyzes the internal glycosidic bonds of GlcA residues at a limited number of specific locations within HS to release smaller HS fragments. The hydrolysis occurs via a double displacement mechanism with net retention of anomeric configuration, and so heparanase is classified as a retaining glycosidase (Wilson et al., 2014). Several substrate specificity studies indicate that heparanase cleaves the linkage between a GlcA and an N-sulfoglucosamine residue carrying either a 3-O-sulfo or 6-O-sulfo group. 2-O-sulfated hexuronic acid appeared required for substrate recognition by heparanase (Pikas et al., 1998). It was later found that the minimum recognition sequence is a trisaccharide, consistent with a recent X-ray crystallographic study (Wu et al., 2015). Structurally, −2 N-sulfate and +1 6-O-sulfate appear to be the main determinants for recognition, because these directly contact the enzyme through hydrogen-bonding networks (Wu et al., 2015). These studies suggest that heparanase recognizes certain sulfation patterns rather than specific monosaccharide sequences and that cleavage occurs at the non-reducing side of highly sulfated domains (Mao et al., 2014). Heparanase has also been shown to display plasticity in its substrate specificity depending on the structural features around the cleavage site (Peterson and Liu, 2013).

ASSAY SYSTEMS

Heparanase is well established as a cancer drug target, and several inhibitors have progressed to clinical trials (Rivara et al., 2016; Vlodavsky et al., 2016; Galli et al., 2018; Dredge et al., 2018). Heparanase has also been implicated in a range of other diseases, such as diabetes and its complications, kidney disease, atherosclerosis, and viral infections, to name a few (Rivara et al., 2016), which continues to fuel research into this protein. The somewhat limited progress in the development of heparanase inhibitors could be

attributed in part to the lack of a robust, accurate and rapid assay for enzyme activity. A plethora of assays have been developed over the years, to advance heparanase research and identify new inhibitors; however, no single method has emerged as the "gold standard." Indeed, the continued publication of new heparanase assays highlights the limitations of currently used methods and the need for improved assays (Chhabra and Ferro, 2018). Chhabra and Ferro have arbitrarily grouped the assays into those that use either heterogeneous heparin or HS-based substrates or homogeneous HS oligosaccharide-based substrates (Chhabra and Ferro, 2018).

The first group of assays yielded semiquantitative results, usually as an IC_{50}, making comparisons of the potency of various inhibitors from different sources, difficult. A homogeneous heparanase substrate that is commercially available is fondaparinux, the fully synthetic methyl glycoside of the antithrombin (AT)-activating pentasaccharide sequence of heparin, marketed as the anticoagulant drug Arixtra. An improved assay was developed using fondaparinux as the substrate based on the detection of the newly formed reducing disaccharide (Hammond et al., 2010) by reaction with the water-soluble tetrazolium salt 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (or WST-1). WST-1 reacts with the reducing end of disaccharide **3** to form a water-soluble formazan detected by UV absorbance at 584 nm. The assay enabled the rapid kinetic characterization of inhibitors, which were readily discriminated by their Ki values, with the data being directly comparable across studies. The simplicity and robustness of this method have been used successfully in drug discovery programs, e.g., leading to the discovery of the clinical candidate PG545 (Dredge et al., 2010). Although useful for screening and characterizing inhibitors, a limitation of the assay is its unsuitability for assaying biological samples because of interference by other reducing compounds that also react with WST-1.

A set of β-glucuronidase-specific activity-based probes (ABPs) based upon the cyclophellitol aziridine scaffold was found to allow the rapid and quantitative visualization of exo-acting β-glucuronidase and endo-acting heparanase in biological samples, providing valuable tools to rapidly determine enzyme activities within their native physiological contexts (Jiang et al., 2016). The discovery that aziridine ABPs label heparanase (Wu et al., 2017) paves the way for more rapid and practical methods to assess the activity of this enzyme, and may inspire the development of probes to assay other endo-glycosidases. Optimization efforts will be aided by the use of competitive ABP techniques, which were demonstrated to be viable methods for assessing selective inhibitors of individual β-glucuronidases (Wu et al., 2017).

SELECTED INHIBITORS OF HUMAN HEPARANASE

Heparin and Its Derivatives

Heparin is a polysaccharide that belongs to the family of GAGs, an endogenous anticoagulant, and a potent inhibitor of heparanase, which structurally resembles its natural substrate (Naggi et al., 2005; Casu et al., 2008). The use of heparin as a therapeutic agent against cancer is restrained due to its potent anticoagulant activity and induction of adverse bleeding complications. Low-molecular-weight heparin (LMWH: dalteparin, tinzaparin, enoxaparin, etc.), chemically modified heparins, and heparin-related polysulfated compounds with reduced anticoagulant activity are possible alternatives (Heyman and Yang, 2016; Jia and Ma, 2016). A systemic review and meta-analysis report suggested that LMWH treatment for preventing pulmonary embolism in patients with advanced stage cancer prolonged overall survival (Lazo-Langner et al., 2007), indicating that the antitumor effect of unfractionated heparin and LMWH is possibly mediated via anti-heparanase activity (Casu et al., 2008). Another study also presented the significant improvement in overall survival in patients with cancer treated with LMWH (Kuderer et al., 2007). In contrast, Sanford and coworkers reported that LMWH did not show a survival benefit in patients with cancer (Sanford et al., 2014). In another report, LMWH did not improve the overall survival in patients with lung cancer (Macbeth et al., 2016), indicating that the use of LMWH as a possible therapeutic agent against cancer remains controversial. The inconsistent results may be due to the use of different species of heparins with different anti-metastatic activity profiles (Oduah et al., 2016). In another report (Cassinelli et al., 2018), supersulfated LMWH exerted potent anti-heparanase activity (IC_{50}: 25 ng/mL), inhibited anchorage-independent growth and Matrigel invasion of sarcoma cells, and downregulated activation of receptor tyrosine kinases (insulin-like growth factor receptor 1 [IGF1R] and insulin receptor [IR]). It yielded an additive growth inhibitory effect on synovial sarcoma cells exposed to standard chemotherapeutic agents. Moreover, the combination of supersulfated LMWH with BMS754807 (IGF1R/IR inhibitor) suppressed synovial sarcoma tumor growth and metastases (Cassinelli et al., 2018).

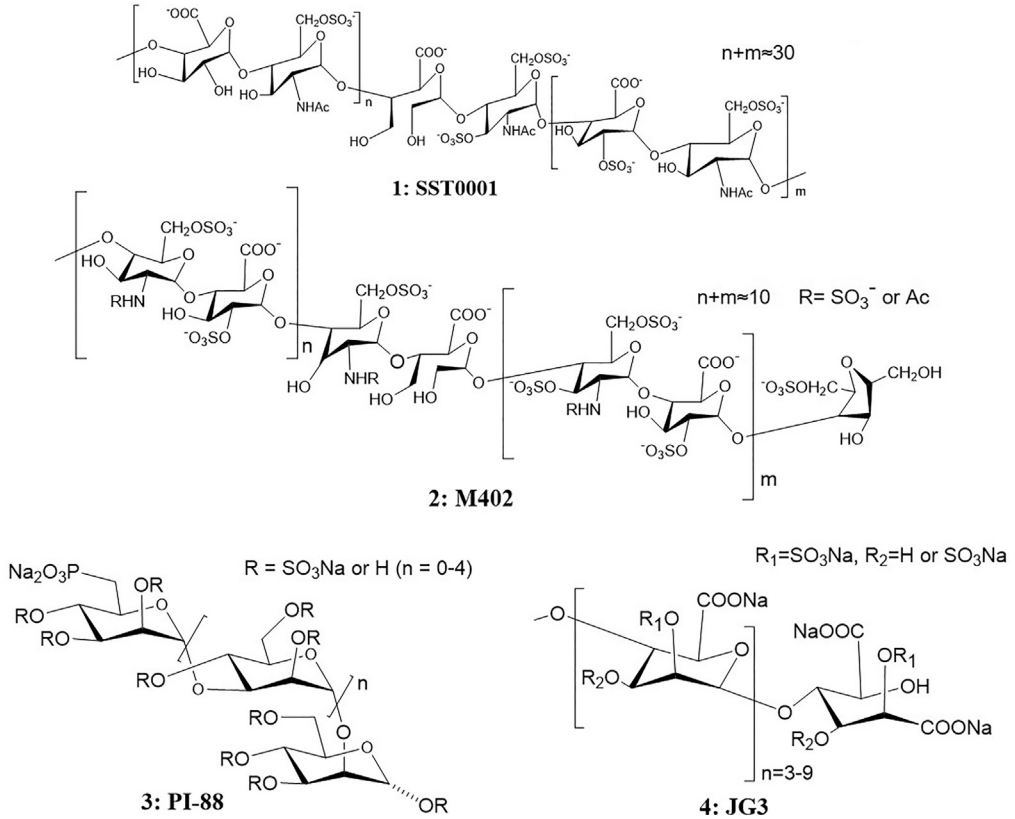

Figure 2. Chemical Structures of SST0001 (1), M402 (2), PI-88 (3), and JG3 (4)

Gomes et al. reported that a unique non-anticoagulant analog of heparin isolated from the bivalve mollusk *Nodipecten nodosus* inhibits P-selectin, heparanase activity, and experimental metastasis (Gomes et al., 2015). This mollusk species of heparin or HS was suggested as a potential alternative to heparin for inhibiting P-selectin- or heparanase-mediated events involved in metastasis and inflammation. A number of preclinical and clinical studies have suggested that heparin and LMWH may improve survival of patients with cancer with mechanisms that are different from its antithrombotic or anticoagulation effects but are linked to the ability of influencing various aspects of tumor biology such as angiogenesis, cell proliferation, metastasis, complement activation, and the above-mentioned interaction with heparanase and selectins (Franchini and Mannucci, 2015).

SST0001 (Roneparstat)

In an attempt to produce potent non-anticoagulant heparin devoid of bleeding complications, Naggi and colleagues applied desulfation strategies and controlled glycol splitting to produce chemically modified heparins. Among the generated modified heparins, a fully N-acetylated and 25% glycol-split derivative named ^{100}NA,RO-H (currently known as roneparstat = SST0001, **1**) (Figure 2) showed potent heparanase inhibitory activity, significantly reduced the release of ECM-bound fibroblast growth factor (FGF)-2, and failed to stimulate its mitogenic activity (Naggi et al., 2005). The glycol splitting of heparin involves a nearly complete abolishment of its anticoagulant activity due to cleavage of the C2-C3 bond of the GlcA of the pentasaccharide sequence, which leads to loss of affinity of the glycol-split heparin toward AT (Alekseeva et al., 2014). This approach led to the generation of heparin-based heparanase inhibitors with little or no adverse effects (Ritchie et al., 2011; Galli et al., 2018). Roneparstat (=SST0001) is one of the most potent inhibitors of heparanase with an IC_{50} value in the subnanomolar range. Syndecans are single transmembrane domain proteins that act as co-receptors, especially for G protein-coupled receptors. These core proteins carry three to five HS and chondroitin sulfate chains, which allow the interaction with a large variety of ligands, including FGF, VEGF, fibronectin, and AT-1. In some cases, a ternary complex composed of a ligand (i.e., bFGF), its high-affinity receptor, and HS is required for most efficient signaling (Park, 2018). Syndecan-1

is a potent growth-promoting factor for myeloma (Sanderson and Yang, 2008). In preclinical evaluation, SST0001 inhibited heparanase-mediated cleavage of syndecan-1 HS chains; downregulated HGF, VEGF, and MMP-9 expression; and inhibited myeloma tumor growth and angiogenesis *in vivo*. It was also demonstrated that SST0001 in combination with dexamethasone effectively inhibited myeloma tumor growth via attenuation of the heparanase/syndecan-1 axis (Ritchie et al., 2011). In sarcoma cells, SST0001 caused a significant reduction in the levels of HS-bound proangiogenic (VEGF, VEGF-C, platelet-derived growth factor [PDGF]-AA, angiopoietin) and protumorigenic (Endothelin-1 [ET-1] and endocrine gland derived-VEGF) factors, as well as ECM remodeling proteins (Matrix metalloproteinase 9 [MMP9], pentraxin, and urokinase-type plasminogen activator [uPA]) (Cassinelli et al., 2013). Subsequent experiments revealed that the combination of SST0001 with bevacizumab (anti-VEGF antibody) or sunitinib (multi-kinase inhibitor) markedly regressed the growth of Ewing sarcoma TC71 tumors (Cassinelli et al., 2013). In sarcoma subtypes, SST0001 induced inhibition of multiple receptor tyrosine kinases (FGF, IGF, ERBB, and PDGF receptors). SST0001 in combination with irinotecan (topoisomerase I inhibitor) displayed a significant enhancement in antitumor activity against A204 rhabdoid xenografts (Cassinelli et al., 2016). Roneparstat was also highly effective in lymphoma and pediatric sarcoma mouse models (Cassinelli et al., 2013).

Roneparstat showed a significant anti-myeloma effect in murine models, either alone or in combination with dexamethasone, bortezomib, or melphalan (Ritchie et al., 2011; Ramani et al., 2015). Based on this preclinical evidence an open-label, multicenter, phase I, first-in-human study (NCT01764880) was designed to assess the safety and tolerability profile of roneparstat in patients with relapsed or refractory multiple myeloma (MM) (Pala et al., 2016). Roneparstat was well tolerated and safe at all doses tested, and patients could be exposed to the drug at dose levels of 200 and 400 mg/day without any clinically relevant toxicities (Galli et al., 2018). The involvement of heparanase in regulating the cross talk between the tumor and the host myeloma microenvironment, and the preclinical activity of roneparstat in combination regimens, has been widely described (Ritchie et al., 2011; Ramani et al., 2016). Of particular interest, Ramani et al. (Ramani et al., 2015) reported a very significant effect on tumor burden when roneparstat was combined with bortezomib or melphalan in the treatment of mice bearing aggressive myeloma in an *in vivo* model applying CAG human myeloma cells expressing high levels of heparanase. Therefore exploration of roneparstat in combination regimens for the treatment of myeloma is well justified and should be examined in a follow-up clinical trial (Galli et al., 2018). Together, these studies comprehensively demonstrate the potency of glycol-split heparins in exerting antitumor activity.

M402 (Necuparanib)

M402 (**2**) is an HS mimetic glycol-split LMWH prepared by controlled depolymerization of unfractionated heparin followed by sequential periodate oxidation and borohydride reduction to produce modified heparin of 5,500–6,500 Da (Figure 2). The compound was rationally tailored to overcome the risk of anticoagulation associated with unfractionated heparin and to withhold the growth factors' binding property and hence exert more effective antitumor, anti-metastatic, and antiangiogenic properties. The engineered M402 showed potent heparanase inhibitory activity (IC_{50}: 5 μg/mL); possessed lower anticoagulant activity than LMWH (dalteparin); showed an equivalent affinity for FGF2, VEGF, SDF-1α, and HGF; and suppressed P-selectin interaction when compared with dalteparin. It exhibited a potent anti-metastatic activity as a single agent and in combination with cisplatin or docetaxel and prolonged survival in a murine breast carcinoma model (Zhou et al., 2011). In another study, pancreatic tumor cells and stellate cells were co-cultured to mimic pancreatic cancer system and study the effect of M402 on epithelial-stromal interactions. Co-culturing was necessary to make the cancer cells invasive under *in vitro* conditions and M402 suppressed the invasive behavior in a dose-dependent manner (Krause et al., 2015). The results of phase 1 clinical trial (NCT01621243) demonstrated acceptable safety and encouraging signals of activity (i.e., reduction in CA19.9 levels; overall disease-control rate of 63%) in patients with metastatic pancreatic cancer receiving necuparanib, nab-paclitaxel, and gemcitabine (O'Reilly et al., 2017).

PI-88 (Mupafostat)

Carbohydrate-based HS-mimicking compounds show high selectivity toward heparanase inhibition and reduced anticoagulatory property allowing for a higher therapeutic window (Heyman and Yang, 2016). PI-88 (**3**) is a hydrolytic product of the extracellular phosphomannan of the yeast *Pichia holstii* NRRL Y-2448. PI-88 (Figure 2) potently inhibits heparanase, prevents the cleavage of HS, and competes with growth factors for binding to HS and hence impacts antiangiogenic and anti-metastatic activity (Parish et al., 1999; Ferro et al., 2001). Chemically, PI-88 is a mixture of highly sulfated monophosphorylated

mannose oligosaccharides (phosphosulfomannan) that ranges between di- and hexasaccharides. The tetra- and pentasaccharides are the major components of the mixture accounting for ~30% and ~60%, respectively (Karoli et al., 2005). The sulfated oligosaccharides that are closely related to PI-88 produced via semisynthetic route display inhibitory activity against human platelet heparanase. It is important to note that tetra- and pentasaccharides were presented as potent competitive inhibitors of isolated heparanase whereas the di- and trisaccharides did not completely inhibit the enzyme at saturating concentrations (Fairweather et al., 2008). Besides heparanase inhibition, PI-88 inhibits angiogenesis by preventing the release of angiogenic growth factors (FGF, VEGF) and their interaction with respective receptors (Parish et al., 1999; Cochran et al., 2003; Ferro et al., 2007; Kudchadkar et al., 2008). Tissue factor pathway inhibitor (TFPI) is the anticoagulation protein that modulates the severity of a wide variety of bleeding and clotting disorders (Wood et al., 2014). The administration of PI-88 to monkeys (1 mg/kg) significantly increased the levels of TFPI. A similar increase in TFPI was observed in cultured human umbilical vein endothelial cells (HUVECs) exposed to PI-88, suggesting that PI-88 is capable of releasing endogenous TFPI, similar to other GAGs (Demir et al., 2001). PI-88 was found to selectively reduce the malignant cell load in rodent models of human myeloid leukemias (Iversen et al., 2002), associated with reduced cell proliferation and angiogenesis, enhanced apoptosis, and a substantial reduction in the number of invasive carcinomas (Joyce et al., 2005). Two human extracellular endoglucosamine 6-sulfatases (HSulf-1 and HSulf-2) are upregulated in several types of cancer. The activities of both HSulf were significantly inhibited by PI-88 in a concentration-dependent manner (Hossain et al., 2010). A recent phase III trial (hepatocellular carcinoma; PATRON/NCT01402908) revealed that whereas the disease-free survival was not improved in the overall treatment group, PI-88 could significantly prolong the disease-free survival in the microvascular invasion subgroup (40% of the trial population) supporting PI-88 as single therapy or in combination with other anticancer agents for future hepatocellular carcinoma adjuvant therapy trials (Chen et al., 2017). These and other studies encourage the development of PI-88 as an anticancer drug against human malignancies that exhibit elevated levels of heparanase expression. Notably, heparin mimetics, including PI-88, exhibit anti-inflammatory properties, attributed primarily to their ability to interact with complement system proteins, selectins, chemokines, and heparanase, each of which function differently to facilitate inflammation (Mohamed and Coombe, 2017).

Sulodexide

Previous studies have shown that sulodexide (a highly purified GAG isolated from porcine intestinal mucosa) can control proteinuria and podocyte damage by inhibiting heparanase (IC_{50} = 5 μg/mL) (Abaterusso and Gambaro, 2006; Masola et al., 2014). Sulodexide consists of 80% LMWH and 20% of DS. The heparin fraction has a molecular weight of 7,000 D and a low degree of sulfation (Masola et al., 2012). DS is a polydispersed polysaccharide with anticoagulant and antithrombotic activities. The findings highlight the capacity of sulodexide to inhibit heparanase due exclusively to its heparin component (Masola et al., 2012). It appears that sulodexide could protect against renal fibrosis sustained by FGF2-induced epithelial-mesenchymal transition, thereby preventing the progression of chronic kidney disease (diabetic nephropathy in particular) to end-stage renal disease (Masola et al., 2012).

Oligomannurarate Sulfate (JG3)

In an effort to identify heparanase inhibitors, Zhao and coworkers screened a library of marine-derived carbohydrates. JG3 (**4**) (Figure 2), a semisynthetic, highly sulfated oligosaccharide ranging in size from tetra-to decasaccharides derived from marine oligomannurarate, was identified as lead-substrate-based heparanase inhibitor (Zhao et al., 2006). JG3 inhibits heparanase by interacting with its KKDC and QPLK substrate-binding domains and suppresses the release of HS-bound bFGF from the ECM and subsequent association with its signaling receptors (Zhao et al., 2006). Evidently, it also suppresses heparanase-driven formation of focal adhesions and spreading of heparanase-overexpressing CHO-K1 cells (Li et al., 2009). JG3 collectively inhibits tumor angiogenesis and metastasis (Zhao et al., 2006). In addition, JG3 blocks tumor growth by inhibiting constitutive activation of proteins upstream to the nuclear factor (NF)-κB signaling pathway, independent of heparanase expression. Interestingly, JG3 has no growth-inhibitory effect against NF-κB-negative cell lines (Zhang et al., 2010a). Furthermore, JG3 selectively inhibits doxorubicin-induced activation of NF-κB, ataxia telangiectasia mutated, and subsequent MEK/ERK/P90Rsk signaling pathway, thereby sensitizing tumor cells to doxorubicin treatment (Zhang et al., 2012). Miao and coworkers presented JG3 as an antimitotic agent that exerts anticancer activity *in vitro* and *in vivo* by binding to tubulin at a unique site other than those of colchicine and vinblastine. Affinity chromatography, liquid chromatography-tandem mass spectrometry, and surface plasmon resonance indicate tubulin as a target protein of

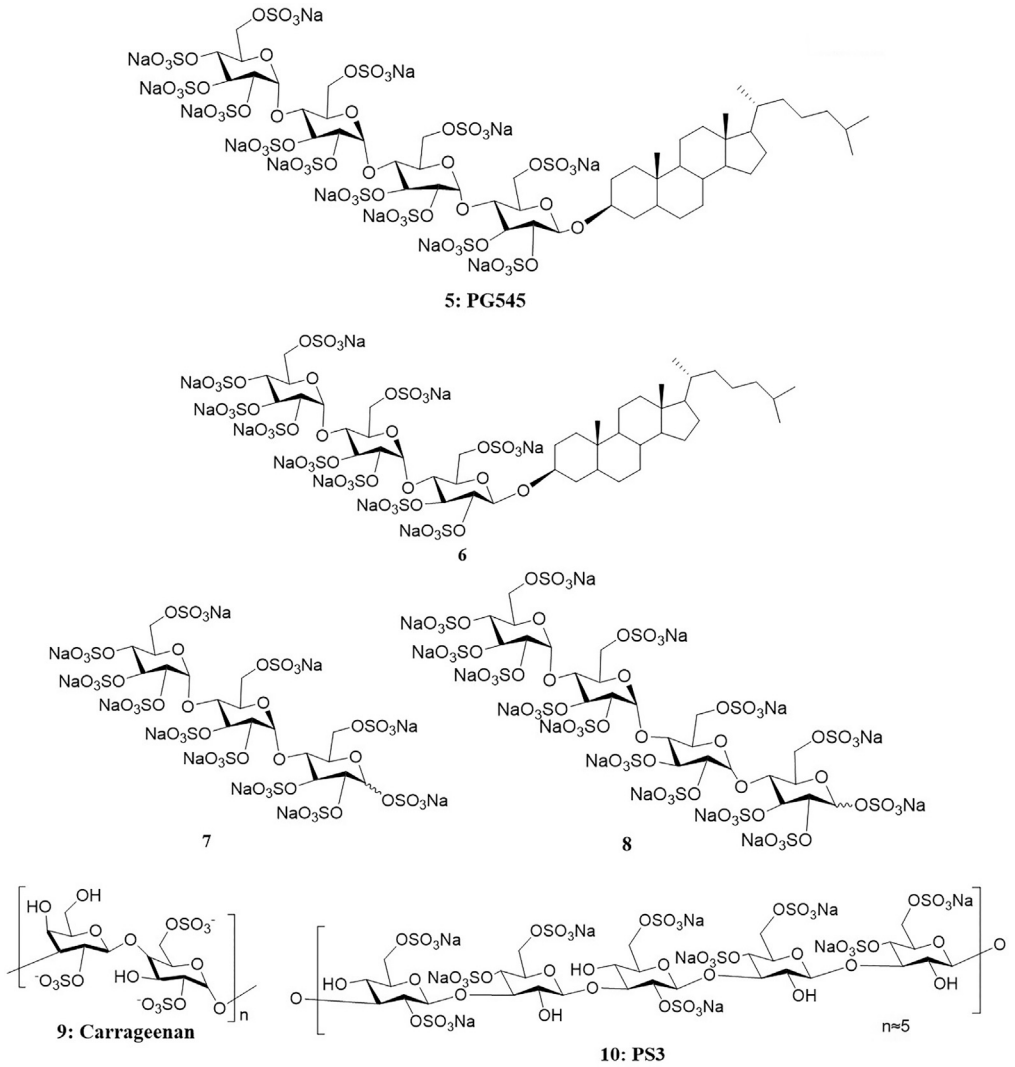

Figure 3. Chemical Structures of PG545 (5) and Other Three Analogs (6–8), Carrageenan (9), and PS3 (10)

JG3 (Miao et al., 2010). Importantly, JG3 displayed relatively low toxicity than other polyanionic compounds, likely attributed to its poor anticoagulant activity (Ding, 2016).

PG545 (Pixatimod)

The PG500 series is a library of chemically designed HS mimetics, fully sulfated oligosaccharide glycosides modified at the reducing end by the incorporation of an aglycone (Dredge et al., 2010). In preclinical models, PG500 series compounds displayed good heparanase inhibition; high affinity for FGF-1, FGF-2, and VEGF; and relatively reduced anticoagulant activity than PI-88 (Dredge et al., 2010). The compounds also possess good antiangiogenic and antitumor activities in *ex vivo* and *in vivo* models (Dredge et al., 2010). Considering the efficacy and consistency in different experimental models, PG545 (**5**) (Figure 3), a fully sulfated tetrasaccharide functionalized with cholestanyl aglycone, was chosen as a lead clinical candidate and dual inhibitor of heparanase and angiogenesis (Dredge et al., 2010). Encouragingly, several oligosaccharides conjugated with steroid or long-chain alkyl group inhibited heparanase and angiogenic growth factors with enhanced antitumor and anti-metastatic activity (Ferro et al., 2012). In another study, Hammond and coworkers investigated the mode of interaction of PG545 and other three analogs (tetrasaccharide without cholestanyl aglycone, trisaccharide with cholestanyl aglycone, and trisaccharide without cholestanyl aglycone) with the active site of heparanase. The tri- and tetrasaccharides functionalized with cholestenol (**PG545 and 6**) possess a higher affinity for heparanase than their corresponding

unconjugated tri- (**7**) and tetra- (**8**) saccharides (Figure 3) (Hammond et al., 2013). This study indicates that the hydrophobic modification in the oligosaccharide via the addition of steroid permits the binding to a hydrophobic pocket near or within the active site of heparanase, in addition to the substrate binding site(s) (Hammond et al., 2013). PG545 revealed anti-metastatic activity in breast, prostate, liver, lung, colon, and head and neck human cancers, as well as in melanoma murine models, possibly due to the inhibition of heparanase enzymatic activity. PG545 in combination with sorafenib increased the antitumor effect in a liver cancer model (Dredge et al., 2011). It also suppressed the proliferation, migration, and colony formation of pancreatic cancer cells and significantly increased animal survival in intraperitoneal [i.p.] and genetic models of pancreatic ductal adenocarcinoma (Ostapoff et al., 2013). In a study by Hammond et al., PG545 was found to inhibit primary tumor growth and spontaneous metastasis in the orthotopic 4T1 breast carcinoma model and significantly enhanced the overall survival of mice (Hammond et al., 2012). Importantly, administration of sorafenib did not inhibit metastasis, indicating that the heparanase inhibitory activity of PG545 underlies its anti-metastatic activity (Hammond et al., 2012). It also promoted apoptosis and exerted synergistic effects with gemcitabine in pancreatic tumor cells and an orthotopic mouse model (Jung et al., 2015). Moreover, PG545 suppressed Wnt/β-catenin signaling by interacting with Wnt3a and Wnt7a in pancreatic tumor xenografts (Jung et al., 2015). In another advancement, PG545 in combination with paclitaxel significantly reduced tumor burden in human ovarian carcinoma model (A2780 model), supporting clinical testing of PG545 alone or in combination with other chemotherapeutic agents in patients with ovarian cancer (Winterhoff et al., 2015). Vlodavsky and Pass have recently reported (Barash et al., 2018) that PG545 markedly attenuated mesothelioma tumor growth. Briefly, luciferase-labeled human mesothelioma cells were inoculated (i.p.) into severe combined immunodeficiency (SCID) mice and tumor growth was visualized by IVIS along treatment with PG545. Inhibition of tumor growth was associated with a profound increase in the survival time of mesothelioma-bearing mice (Barash et al., 2018) (Figure 4). Notably, PG545 was more potent than conventional chemotherapy (i.e., cisplatin) in extending the survival of mesothelioma-bearing mice (Figure 4), lending optimism that heparanase inhibition would prove efficacious in the clinic. In a related study, Katz et al. reported that PG545 treatment was highly effective in patient-derived xenograft (PDX) models of lung cancer, exerting inhibition of tumor growth in more than 85% of the PDX cases. Importantly, PG545 was highly effective in PDX that did not respond to conventional chemotherapy (cisplatin), whereas other PDX tumors responded well to cisplatin and to a lesser extent to PG545, emphasizing the concept and need for personalized medicine (Katz et al., 2018). Spyrou et al. demonstrated that PG545 efficiently inhibited heparanase activity in pediatric brain tumor cells, attenuating their proliferation, invasive capacity, and *in vivo* tumor growth (Spyrou et al., 2017). Likewise, PG545 (= pixatimod) was highly effective as a single agent in myeloma and lymphoma models (Weissmann et al., 2016), thus making this compound attractive for the treatment of hematological malignancies, as well. It appears, however, that in lymphoma cells PG545 exerts both heparanase-dependent and heparanase-independent (i.e., apoptosis, endoplasmic reticulum [ER] stress, NK cell activation) (Brennan et al., 2016; Weissmann et al., 2018) functions that together underlie its high potency in preclinical cancer models.

Collectively, these reports support the potent *in vitro* and *in vivo* antitumor, anti-metastatic, antiangiogenic, and heparanase inhibitory activity of PG545. Intravenously (i.v.)-infused PG545 completed a phase 1 clinical trial (NCT02042781) to evaluate its safety and tolerability in subjects with advanced solid tumors (Supramaniam et al., 2018). Ongoing is a phase Ib study of the safety and tolerability of IV-infused PG545 in combination with nivolumab in patients with advanced solid tumors with an expansion cohort in patients with metastatic pancreatic cancer (ACTRN12617001573347).

Carrageenan

Carrageenans (**9**) are a family of polysulfated polygalactans found in red algae (Figure 3). Several forms of carrageenans have been identified, and the major forms are κ-, ι-, and λ-types. The variants of carrageenans vary from one another in the content of 3,6 anhydro-D-galactose and D-galactose and the position and number of ester sulfation (Hoffman et al., 1995). The studies of Parish et al. showed that λ- and κ-carrageenan displayed significant inhibition of ECM breakdown *in vitro*, λ being superior over its counterparts. Overall, it was suggested that these sulfated polysaccharides exhibit anti-metastatic activity by inhibiting tumor-derived heparanase (Parish et al., 1987). Carrageenans inhibit heparin-binding growth factors including bFGF, PDGF, and TGF-β. ι-Carrageenan is a potent inhibitor of endothelial and prostate cancer cell proliferation with relatively lower anticoagulant activity and toxicity than λ-carrageenan (Hoffman et al., 1995). λ-Carrageenan displayed complete inhibition of heparanase at 2.5 μg/mL and suppressed the release of ECM-bound bFGF to less than 5% (Ishai-Michaeli et al., 1990). In another report, λ-carrageenans

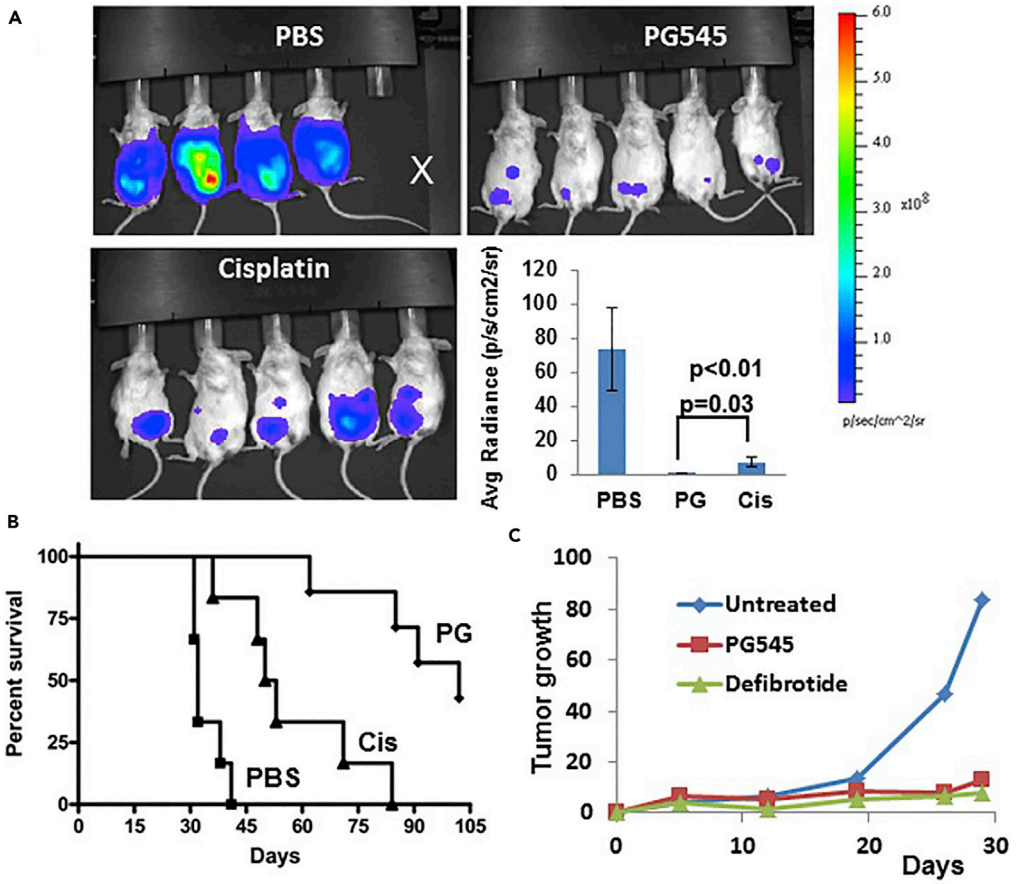

Figure 4. Heparanase: A Valid Target in the Pathogenesis of Mesothelioma

(A) Tumor growth. Luciferase-labeled MSTO-211H human mesothelioma cells (2×10^6) were inoculated i.p. into SCID mice. Mice were treated with PG545 (400 μg/mouse; once a week), cisplatin (once/2 weeks; 3 mg/kg), or control vehicle (PBS), and tumor development was inspected by IVIS. Quantification of the luciferase intensities is shown graphically in the lower right panel.

(B) Survival. The effect of PG545 and cisplatin on the survival of mice is plotted as Kaplan-Meier curves. Note that PG545 is significantly more effective than cisplatin in inhibiting orthotopic mesothelioma tumor growth and prolonged survival of the mice.

(C) Defibrotide. Luciferase-labeled MSTO-211H human mesothelioma cells (0.5×10^6) were inoculated i.p. into SCID mice. Mice were treated with defibrotide (8 mg/mouse; twice a day), PG545 (400 μg/mouse; once a week), or control vehicle (PBS), and tumor development was inspected by IVIS over time (figure taken and modified from: Barash et al., JNCI, 110:1102–1114, 2018).

with a degree of polymerization ranging from two to eight were produced, and among the new oligosaccharides, λ-carraheptaose showed potent heparanase inhibition and inhibited bFGF-induced proliferation of endothelial cells (Niu et al., 2015). To minimize the proinflammatory properties of high-molecular-weight carrageenans, several attempts have been made to produce depolymerized low-molecular-weight variants. A recent study suggested that low-molecular-weight glycol-split λ-carrageenan is a potent anti-heparanase agent (IC_{50}: 7.32 ng/mL), not cytotoxic to endothelial cells up to 500 μg/mL, and the anticoagulant activity is close to zero (Poupard et al., 2017a, 2017b). Moreover, low-molecular-weight carrageenans are potent antiangiogenic agents (Chen et al., 2007).

PS3

PS3 (**10**) is a semisynthetic, sulfated derivative of β-1,3-glucan phycarin isolated from sporophytes of *Laminaria digitata* with a molecular weight of 10,000, average polymerization of 25 units and one or two C6 ramifications with glucose (Figure 3) (Alban et al., 2009). PS3 possesses relatively potent anti-inflammatory activity and weaker anticoagulant activity than heparin (Alban et al., 2009). PS3 exhibits

potent anti-heparanase activity (IC_{50}: 0.08 μg/mL), which may underlie its anti-metastatic activity (Schoenfeld et al., 2014). Among the two sulfated polysaccharides obtained from the red algae *Delesseria sanguinea*, PS3 displayed superior anti-inflammatory and anti-metastatic activities (Groth et al., 2009). It also exhibits inhibitory activity against P- and L-selectins suggesting that it may be developed as an effective be a therapeutic agent for both cancer and inflammatory diseases (Alban et al., 2009).

Sulfated Tri Mannose C-C-Linked Dimers

Considering the role of heparanase and selectins in cancer metastasis and the significance of compounds that target both heparanase and selectins, Vismara and coworkers showed the preparation of semisynthetic sulfated tri mannose C-C-linked dimers (STMCs) (**11**) (Figure 5) that are endowed with stability and dual inhibition (Coletti et al., 2014). STMCs effectively inhibited heparanase and attenuated metastasis primarily by inhibiting P-selectin in murine models (Borsig et al., 2011). The therapeutic potential of this dual inhibitor can be extrapolated to inflammatory diseases and renal dysfunction (Borsig et al., 2011).

Trachyspic Acid

Shiozawa and colleagues isolated a metabolite from the culture broth of fungus *Talaromyces trachyspermus* SANK12191 and called it *trachyspic acid* (**12**) (Figure 5). Trachyspic acid inhibited heparanase in a concentration-dependent manner with an IC_{50} value of 36 μM, and it had no inhibitory activity against bovine liver β-glucuronidase, indicating its specificity toward heparanase (Shiozawa et al., 1995). The synthetic stereoisomers of trachyspic acid (**13, 14, 15, 16**) (Figure 5) showed heparanase inhibitory activity with IC_{50} values ranging from 24.5 to 33.5 μM. Polo-like kinase 1 (Plk1) is a serine-threonine protein kinase involved in the regulation of mitotic cell division and often overexpressed in various malignancies. The C terminus of Plk1 has polo-box domain (PBD), which is essential for interaction with other proteins and subcellular localization (Nogawa et al., 2017). A metabolite named trachyspic acid 19-butyl ester (**17**) (Figure 5) along with trachyspic acid was isolated from uncharacterized fungus RKGS-F2684 (Nogawa et al., 2017). Trachyspic acid 19-butyl ester and trachyspic acid showed inhibition toward PBD-dependent binding with an IC_{50} value of 102 and 128 μM, which was comparable with purpurogallin (IC_{50}: 114 μM), which served as positive control (Nogawa et al., 2017). This result shed light on other probable cellular targets of trachyspic acid derivatives.

RK-682

RK-682 (**18**) (3-hexadecanoyl-5-hydroxymethyltetronic acid) (Figure 5) was isolated from the mycelia of *Streptomyces* sp. 88-682 and originally identified as protein tyrosine phosphatase (Hamaguchi et al., 1995). The culture broth of actinomycete strain of RK99-A234 showed heparanase inhibition (IC_{50}: 17 μM) among ~10,000 microbial samples, and purification of the active metabolite yielded RK-682 (Ishida et al., 2004b). RK-682 was chemically modified to produce new derivatives and to increase the heparanase inhibitory efficacy (Ishida et al., 2004a). Among the rationally designed derivatives, benzylation of the 4-position of RK-682 resulted in a selective inhibition toward heparanase (17 μM) (ibid). Moreover, 4-benzyl-RK-682 remarkably inhibits migration and invasion of HT1080 cells, whereas the parent compound has no inhibitory effect at 100 μmol/L (Ishida et al., 2004a).

NUCLEIC ACID-BASED INHIBITORS

Defibrotide

Defibrotide (**19**) is an orally bioavailable nucleic acid-based (oligonucleotide) heparanase inhibitor (Figure 5), isolated from porcine intestinal mucosa prepared by controlled depolymerization of DNA. It is predominantly single-stranded with a mean molecular weight of 17 ± 4 kDa approved for the treatment of severe hepatic veno-occlusive disease (Guglielmelli et al., 2012). Defibrotide reduces expression of the heparanase gene and inhibits enzyme activity and tumor growth in MM (Mitsiades et al., 2009) and mesothelioma (Barash et al., 2018). Unexpectedly, it did not significantly affect the viability of MM, mesothelioma, breast carcinoma, and colon cancer cells. However, defibrotide increases chemosensitization of MM and mammary carcinoma xenografts *in vivo*. Furthermore, defibrotide decreases adhesion of MM and bone marrow stromal cells. It was suggested that defibrotide modulates interactions between stromal cells and endothelia in the tumor microenvironment (Mitsiades et al., 2009). In a multicenter phase 1 or 2 trial, defibrotide in combination with melphalan, prednisone, and thalidomide exhibited well tolerability and antitumor activity in patients with relapsed or refractory MM (Palumbo et al., 2010). Barash and coworkers

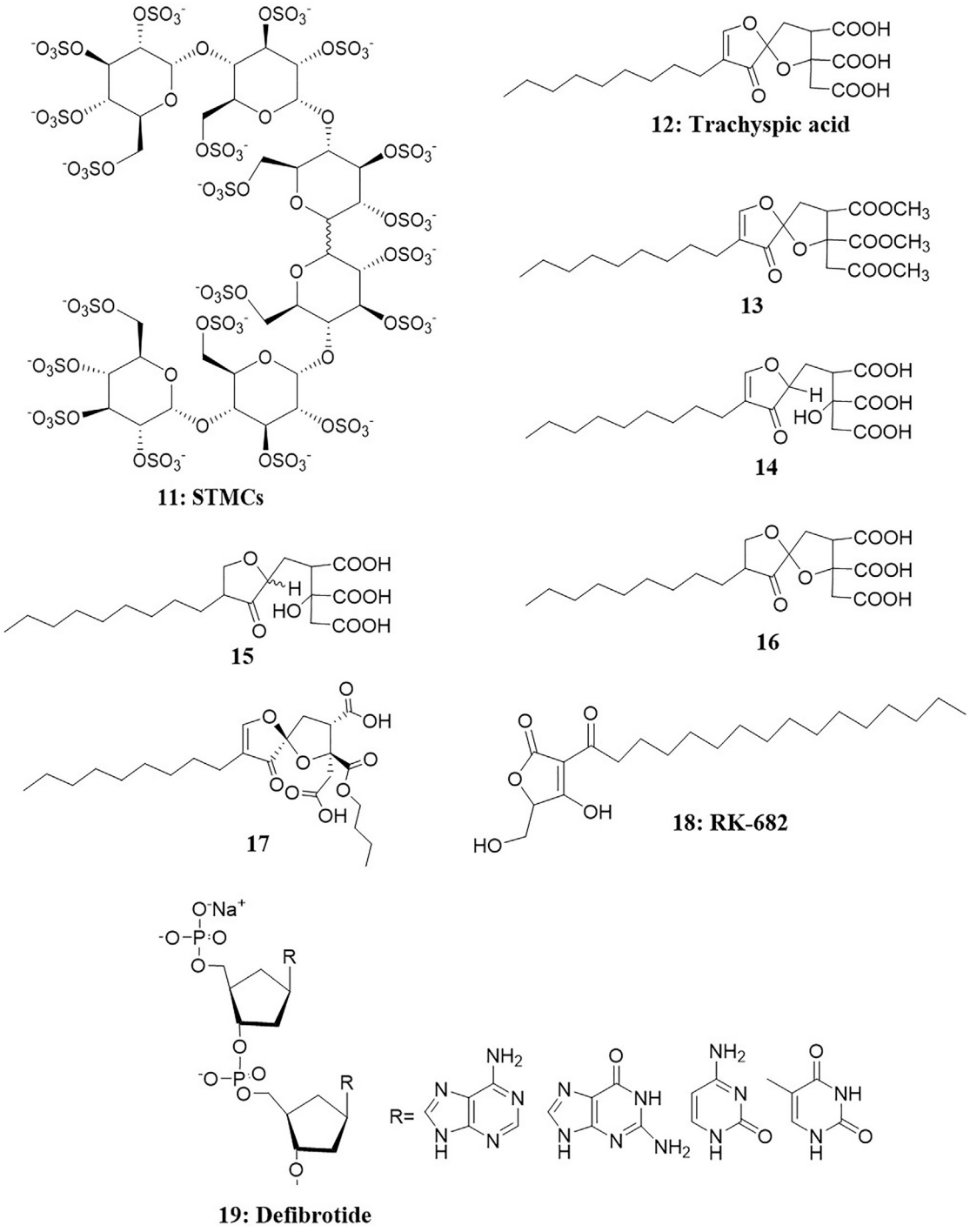

Figure 5. Chemical Structures of STMCs (11), Trachyspic Acid (12) and Its Stereoisomers (13–16), Trachyspic Acid 19-Butyl Ester (17), RK-682 (18), and Defibrotide (19)

demonstrated the role of heparanase in the pathogenesis of mesothelioma and presented heparanase as a likely major mediator of mesothelioma tumor progression. Furthermore, defibrotide exerted a potent tumor growth inhibition in mesothelioma xenografts, suggesting clinical evaluation of defibrotide as a therapeutic modality for mesothelioma (Barash et al., 2018).

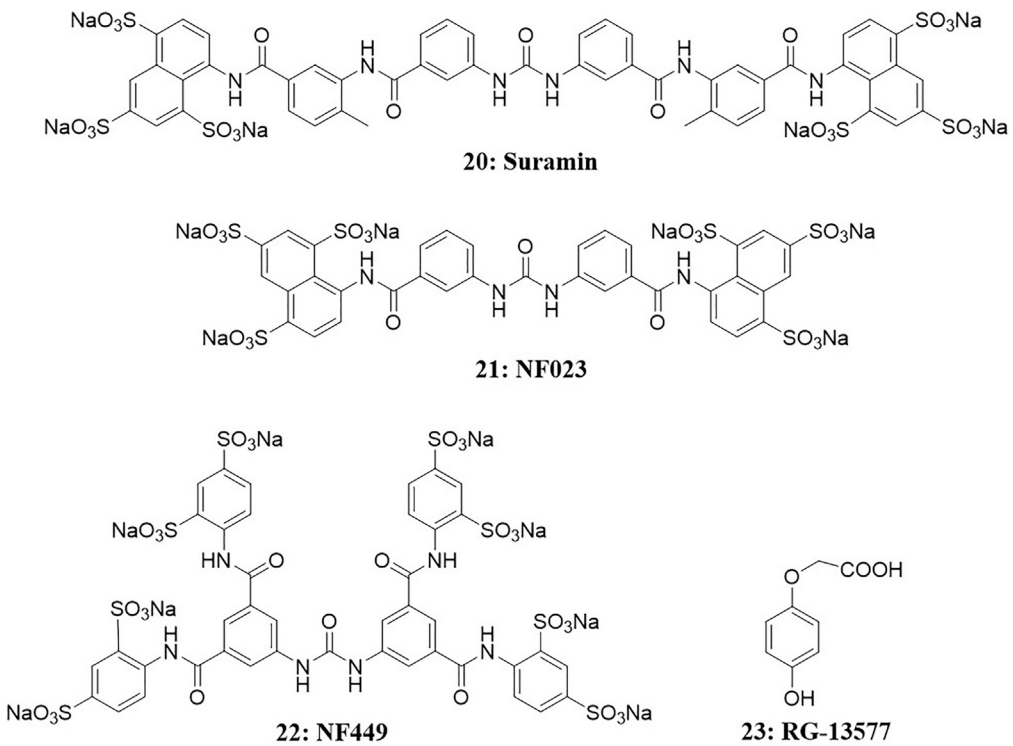

Figure 6. Chemical Structures of Suramin (20), NF023 (21), NF449 (22), and RG-13577 (23)

Anti-Heparanase Aptamers

Aptamers are small single DNA or RNA polynucleotide sequences typically ranging in length from 22 to 100 bases. They are used for diagnostic and therapeutic purposes because of their small size (8–25 kDa), high affinity, and target specificity without triggering an immune response (Gold et al., 1995; Osborne et al., 1997; Famulok and Mayer, 1999; Hicke et al., 2001). AS1411 is representative of a DNA aptamer that was advanced to clinical trials in renal carcinoma (NCT00740441) and acute myeloid leukemia (NCT00512083), indicating that AS-1411 is a promising candidate for cancer treatment in these pathologies (Mongelard and Bouvet, 2010). Simmons et al. produced single-stranded DNA aptamers targeted toward the active form of heparanase using a modified systematic evolution of ligands by exponential enrichment protocol. The aptamer binds to heparanase with high affinity and inhibits its enzymatic activity. The enzyme recognition by the aptamer is in some cases superior over polyclonal anti-heparanase antibody (Simmons et al., 2012). Furthermore, a short anti-heparanase aptamer (30 bases) inhibits tissue invasion, does not exhibit cytotoxicity on oral carcinoma cells, and is stable in human serum without significant binding to serum proteins suggesting, the potential application of aptamers as therapeutic agents (Simmons et al., 2014; Vayrynen et al., 2018).

SYNTHETIC HEPARANASE INHIBITORS

Apart from the development of sugar mimetics and natural compounds as heparanase inhibitors, the discovery of synthetic small molecules that target heparanase is highly essential. Small molecules remain as a choice of interest because of their more conducive pharmacokinetic profiles and ability to optimize for oral administration (Madia et al., 2018). The following section describes some of the reported heparanase inhibitors of synthetic origin and biological effects.

Suramin

Suramin (**20**) is a polysulfonated naphthylurea-based small molecule originally used to treat onchocerciasis and trypanosomiasis (Figure 6) (De Clercq, 1979). Suramin inhibits melanoma heparanase (ID_{50}: 46 μM), and B16 melanoma cell invasion and metastasis, but failed to induce antiproliferative activity toward B16-BL6 melanoma cells (Nakajima et al., 1991). In preclinical studies, suramin attenuated the activity of hepatic

heparanase, reduced the expression of FGF-2 and caspase-3, displayed good antitumor activity in hepatocellular carcinoma model, and showed hepatoprotective effects (Tayel et al., 2014). In another study, it significantly inhibited the growth of ovarian and cervical cancer cells in a dose- and time-dependent manner and its growth inhibitory activity was mediated through reduced expression of heparanase mRNA and protein (Li et al., 2015). Besides heparanase inhibition, it exhibits a broad range of *in vitro* inhibitory activity against enzymes including DNA topoisomerase II, DNA polymerase α, DNA primase, RNA polymerase, and reverse transcriptase, which may be held responsible for its anticancer property (Ono et al., 1988). Suramin failed to advance into clinical trials owing to its toxicity including adrenal insufficiency, renal toxicity, neurotoxicity, and anticoagulant-mediated blood dyscrasias (Ding, 2016). To overcome the toxicity issues and indiscriminate inhibition of many enzymes, several suramin analogs have been developed (Firsching et al., 1995; Gagliardi et al., 1998; Marchetti et al., 2003). Some of these analogs displayed enhanced antiproliferative and angiostatic activity when compared with the parent compound (Gagliardi et al., 1998). STAT5a is an inducible transcription factor and a major cancer therapeutic target (Liao et al., 2010). Recently, suramin and its analogs (NF023 [**21**] and NF449 [**22**]) (Figure 6) were identified as inhibitors of the SH2 domains of STAT5a/b with IC_{50} values ranging between 1.4 and 12.7 μM (Berg and Berg, 2017).

RG-13577

To study the interaction of polyanionic compounds with proteins, Regan and colleagues prepared a library of negatively charged, non-sulfated aromatic compounds with low anticoagulant activity and heparin-mimicking cellular effects (Regan et al., 1993). Among the nine polyanionic polymers, a compound named RG-13577 (**23**) (Figure 6) exerted complete inhibition of heparanase at 2.5 μg/mL, reduced the release of ECM-bound bFGF, and suppressed thrombin-, bFGF- or serum-induced smooth muscle cell proliferation (Benezra et al., 2002). RG-13577 is a synthetic, linear, noncarbohydrate polymer of 4-hydroxyphenoxy acetic acid, heparin-mimicking polyanionic compound and an antagonist of FGF-2 (Miao et al., 1997). In addition, RG-13577 effectively reduces central nervous system inflammation accounting for its high efficacy in ameliorating experimental autoimmune encephalomyelitis. Its activity is mediated primarily by inhibiting heparanase activity in lymphocytes and astrocytes (Irony-Tur-Sinai et al., 2003).

Benzazoles (Benzimidazole and Benzoxazole)

2,3-dihydro-1,3-dioxo-1H-isoindole-5-carboxylic acid (**24**) (Figure 7) was identified as a heparanase inhibitor using high-throughput screening (Courtney et al., 2004). To identify benzoxazole derivative with increased potency and selective inhibitory efficacy toward heparanase, **24** was used as template structure for derivatization. Among the eight derivatives, compound with 2-methoxy substitution in the central phenyl ring (**25**) showed betterment in heparanase inhibition (IC_{50}: 3 μM) with potent angiogenesis inhibitory activity (IC_{50}: 2 μM) over the template structure (**24;** IC_{50}: 8 and 40 μM, respectively) (Courtney et al., 2004). In addition, coupling of boronic acid with 5-bromobenzoxazole resulted in 18 new derivatives and some of the fluoro-substituted compounds displayed significantly improved heparanase inhibitory activity (IC_{50}: 0.4 μM) (Courtney et al., 2004). Among the coupled products, methylenedioxy derivative (**26**) (Figure 7) was the most potent inhibitor of heparanase (IC_{50}: 0.2 μM) and angiogenesis (IC_{50}: 3 μM) (Courtney et al., 2004). To overcome the limitation in solubility of coupled products, potentially solubilizing substitutions ($O(CH_2)_2OMe$, $O(CH_2)_2NMe_2$, $NH(CH_2)_2OMe$) were introduced on the central phenyl ring and the enzyme inhibitory efficacy ranged between IC_{50} values of 0.25 to >10 μM. $NHPr^n$ (**27**) was the most potent heparanase inhibitor (IC_{50}: 0.25 μM) with a marked antiangiogenic activity (IC_{50}: 15 μM), which led to further optimization via introduction of substitutions in the pendant phenyl ring. 4′-F (**28**) and 4′-Cl (**29**) substitutions (Figure 7) resulted in selective heparanase inhibition (IC_{50}: 0.5 and 0.2 μM, respectively) over human β-glucuronidase with appreciable antiangiogenic activity (0.25 and 1 μM, respectively) (Courtney et al., 2004).

A class of N-(4-{[4-(1H-benzoimidazol-2-yl)-arylamino]-methyl}-phenyl)-benzamides were reported as inhibitors of the catalytic activity of heparanase (Xu et al., 2006). Initially, high-throughput screening presented compound **30** (benzimidazole derivative) (Figure 7) as a hit, and it was chosen as a template structure based on its synthetic convenience (Xu et al., 2006). Notably, a compound with 4-ethoxy substitution (**31**) on ring A showed better heparanase inhibition (IC_{50}: 1.2 μM). 3-fluoro-4-methoxy and 3-chloro-4-methoxy substitutions on ring A dramatically decreased the inhibitory activity (IC_{50}: >15 μM) (Xu et al., 2006). Further efforts were made to improve the efficacy of the title compounds using **31** as a template (Figure 7). The structural modifications were marked on ring B, and substitution with methyl (**32**, IC_{50}: 1.6 μM) or

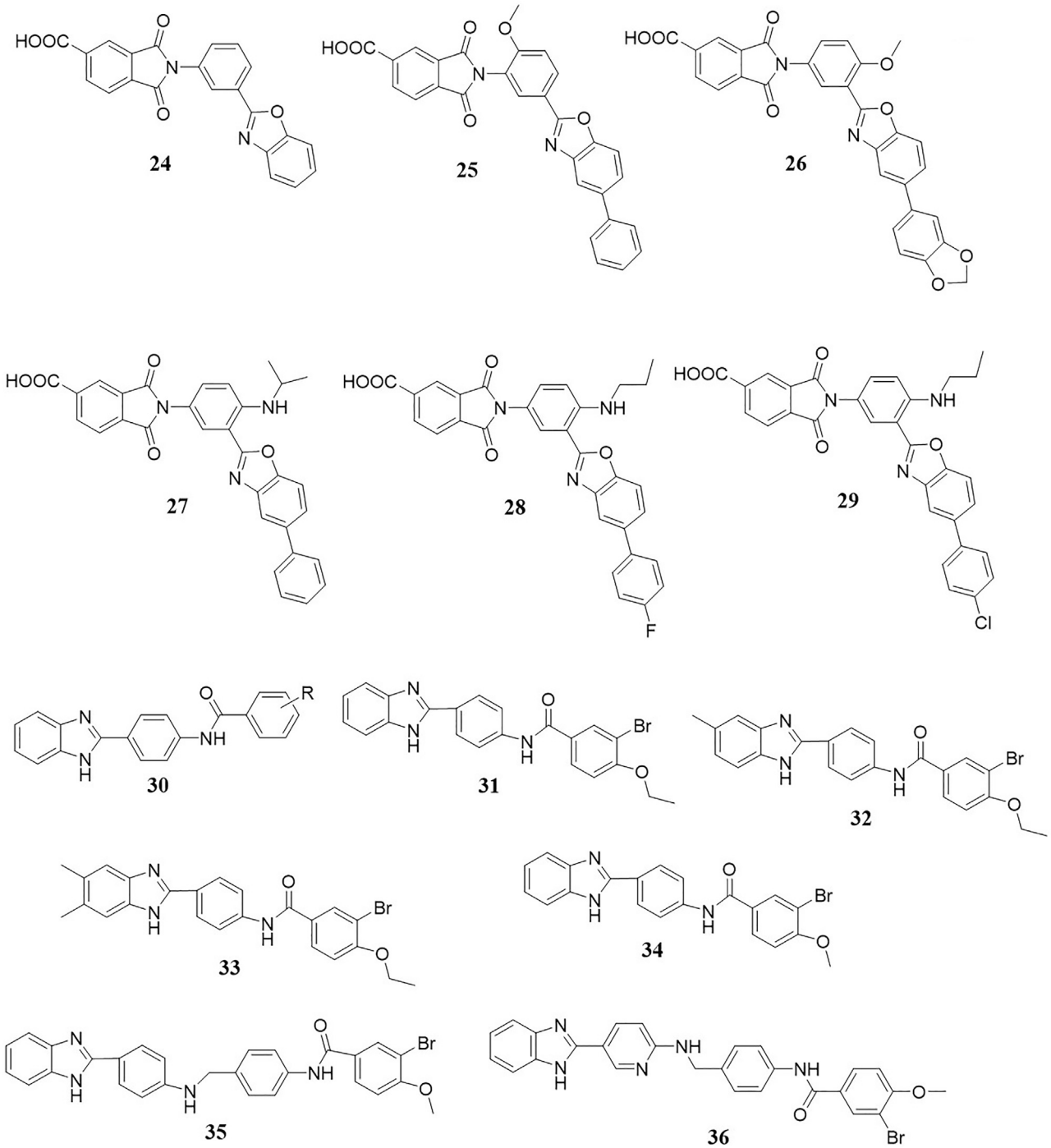

Figure 7. Chemical Structures of 2,3-Dihydro-1,3-dioxo-1H-isoindole-5-carboxylic Acid Derivatives (24–29) and N-(4-{[4-(1H-benzoimidazol-2-yl)-arylamino]-methyl}-phenyl)-benzamides Derivatives (30–36)

dimethyl (**33**, IC_{50}: 1.1 μM) resulted in retained heparanase inhibitory activity (Xu et al., 2006). No significant improvements were found in the biological activity of these compounds over the template. Pharmacokinetics study revealed that the plasma concentration of **34** was 0.67 μM by 4 h after i.p. dosing (50 mg/kg), which is superior over lead compound (**31**) (Xu et al., 2006). To improve the heparanase inhibitory activity and pharmacokinetic properties, the amide linkage in **34** (Figure 7) was replaced with a

reversed amide, sulfonamide, an aminomethyl linkage, and urea (Xu et al., 2006). Notably, the flexible compound **35** showed good heparanase inhibition (IC_{50}: 0.23 μM), whereas most of the other analogs showed diminished activity. Furthermore, **35** was chosen as a template for structural modifications. The structure-activity relationship and optimization experiments suggest that overall linear, yet slightly "bent," disposition of the molecule is vital for heparanase inhibition (Xu et al., 2006). Compound **36** (Figure 7) generated by this approach where the central phenyl group was replaced with a pyridine moiety exhibited improved heparanase inhibition (IC_{50}: 0.29 μM). Oral administration of **36** in ethanol/Tween 80/PEG 400 to mice (30 mg/kg) resulted in plasma concentration of 3.8 μM at 1 h and up to 5.5 μM by 4 h (Xu et al., 2006).

In another study, 1-[4-(1H-benzoimidazol-2-yl)-phenyl]-3-[4-(1H-benzoimidazol-2-yl)-phenyl]-urea derivatives were presented as heparanase inhibitors (Pan et al., 2006). Among the small library of compounds, **37** displayed good heparanase inhibition, whereas **38** (Figure 8), a tetra-methyl-substituted derivative of **37** had a potent anti-heparanase activity (IC_{50}: 0.075 μM) (Pan et al., 2006). Notably, substitutions on the phenyl ring had dramatic effects on the catalytic activity of heparanase. Based on these results, a series of unsymmetrical urea derivatives were generated (Pan et al., 2006). Among them, methyl-substituted compound (**39**) (Figure 8) exhibited improved anti-heparanase activity, whereas methoxy substitutions exerted relatively diminished inhibition (Pan et al., 2006). The inhibitory activity profile of asymmetric urea derivatives was in agreement with the results of symmetric urea derivatives (Pan et al., 2006). The SAR of the urea derivatives also showed a preference for an overall linear, yet "bent" disposition of the molecule. The more "bent" molecules resulted in improved heparanase inhibitory activity. Compound **37** was chosen as a representative candidate for preclinical studies (Pan et al., 2006). The plasma concentration of **37** was 31 μM at 1 h, and was 23 μM by 4 h after its i.p. administration into mice, and these levels were much higher than the IC_{50} value (0.27 μM) toward heparanase (Pan et al., 2006). Compound **37** also showed around 50% inhibition of B16 melanoma lung metastasis compared with the vehicle-treated group (Pan et al., 2006). These results encourage further development of benzimidazole derivatives as potent inhibitors of heparanase.

The synthesis of a series of benzimidazole and benzoxazole derivatives was described, and their anti-heparanase activity was reported (Madia et al., 2018). Among the newly generated compounds, **40** (Figure 8), a benzimidazole derivative, was presented as a lead inhibitor with an IC_{50} of 0.16 μM and its non-fluorinated form showed an IC_{50} of 0.37 μM (2-fold increase). Similarly, benzimidazolyl derivative (**41**) and benzoxazolyl counterpart (**42**) inhibited heparanase with IC_{50} values of 2.86 and 2.56 μM, respectively, whereas the replacement of the fluorine atom by hydrogen (**43** and **44**) (Figure 8) resulted in decreased inhibitory activity with IC_{50} more than 10 μM for both compounds, indicating the significance of fluorine atom in improving the anti-heparanase activity (Madia et al., 2018). In addition, functionalization of the acetic acid portion with glycine in the benzimidazole (**45,** IC_{50}: 0.64 μM) and glutamate in the benzoxazole (**46,** IC_{50}: 0.82 μM) resulted in improved heparanase inhibitory activity compared with their parent compounds (**41 and 42**), respectively (Figure 8) (Madia et al., 2018). The most active heparanase inhibitors (**40, 45, 46, and 47**) except **40**, did not show antiproliferative activity against heparanase-expressing HT1080 (fibrosarcoma), U2OS (osteosarcoma), and U87MG (glioma) cells up to 2.5 μM (Madia et al., 2018). Notably, **40** exerted potent anti-metastatic potential and quantitative real-time PCR analysis revealed that **40** moderately downregulated the expression of genes that encode for proangiogenic factors (FGF1/2, VEGF, MMP-9, and heparanase-1) (Madia et al., 2018). These reports indicate that benzazole heterocycles are therapeutically important pharmacophores that may serve as templates for the development of heparanase inhibitors.

Thiazoles

A series of furanyl-1,3-thiazol-2-yl acetic acid derivatives were described as inhibitors of heparanase and angiogenesis (Courtney et al., 2005). Initially, furanylthiazole acetic acid (**48**) (Figure 9) was identified to weakly inhibit the enzyme with an IC_{50} value of 25 μM. To optimize the structure, analogs of title compounds were prepared by diazotization reaction of 2-chloro-4-nitroaniline on 2-acetyl furan, with subsequent α-bromination and a thiazole ring. This was followed by reduction of the nitro group to introduce desired amides. Among the newly synthesized compounds, **49** showed relatively good heparanase (IC_{50}: 1.5 μM) and angiogenesis (IC_{50}: 0.5 μM) inhibitory activity (Courtney et al., 2005). Further extension of the amide moiety to a cinnamoyl group resulted in the synthesis of five compounds and 4-bromo derivative (**50**) that exhibited a much better anti-heparanase (IC_{50}: 0.4 μM) and antiangiogenesis (IC_{50}: 1 μM) activity compared with other structural analogs. However, **50** displayed poor bioavailability with a short half-life of 0.5 h in mice (Courtney et al., 2005). To improve the drug metabolism and pharmacokinetic profile,

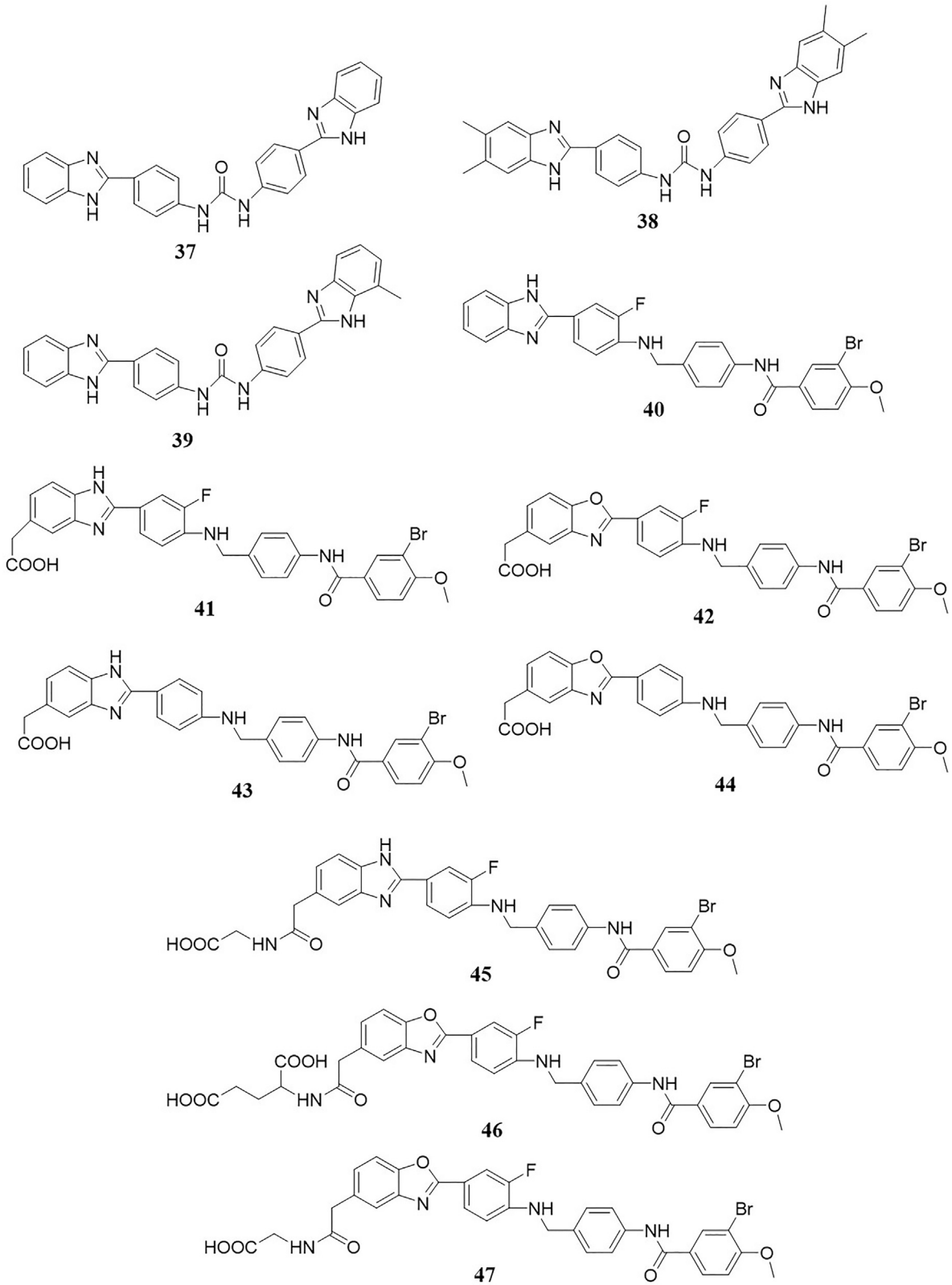

Figure 8. Chemical Structures of 1-[4-(1H-benzoimidazol-2-yl)-phenyl]-3-[4-(1H-benzoimidazol-2-yl)-phenyl]-urea Derivatives (37–39) and Benzazoles (40–47)

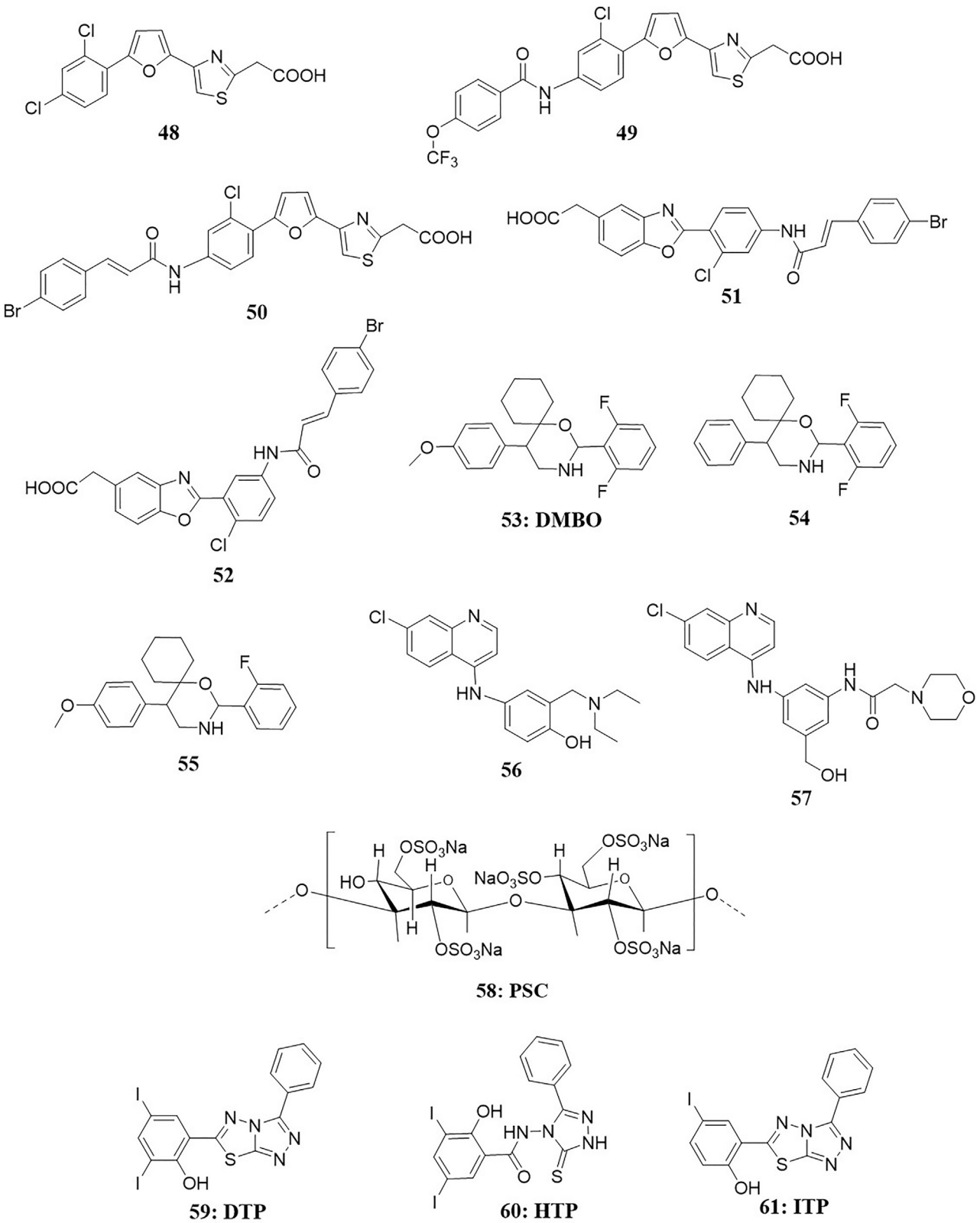

Figure 9. Chemical Structures of Furanyl-1,3-thiazol-2-yl (48–50) and benzoxazol-5-yls (51–52) Acetic Acid Derivatives, Oxazines (53–55), Quinolines (56–57), PSC (58), and Triazolo-thiadiazoles (DTP, HTP, and ITP, 59, 60, and 61, Respectively)

attempts were made, using molecular overlays, to substitute furanylthiazole acetic acids with bicyclic heteroaromatic (benzoxazole) acetic acids. Two positional isomers (**51** and **52**) (Figure 9) were synthesized using this approach, and interestingly, **51** showed comparable heparanase inhibitory activity (IC_{50}: 0.4 μM) with **50**. The structural isomer **52** was less effective (IC_{50}: 8.5 μM) toward the target enzyme. Further modifications resulted in the formation of cinnamic acid amide and reversed cinnamic acid amides. Overall, the study provided the basis for the design and optimization of heparanase inhibitors as therapeutic agents (Courtney et al., 2005).

Oxazines

Several preclinical studies have demonstrated oxazines as good anticancer agents in various malignancies and their mode of action has not been fully understood (Mohan et al., 2014, 2018; Bharathkumar et al., 2015; Srinivas et al., 2015; Nirvanappa et al., 2016; Sulaiman et al., 2016; Uzma et al., 2018). In continuation of our efforts to prepare chemical inhibitors toward heparanase, we designed oxazine-based small molecule (DMBO, **53**) (Figure 9) that structurally mimics the pyranoside ring of HS and reported their heparanase inhibitory activity. DMBO was synthesized by cyclization of 1-[2-amino-1-(4-methoxy-phenyl)-ethyl]-cyclohexanol monoacetate with 2,6-difluoro benzaldehyde (Basappa et al., 2010). Given that DMBO resembles pyranoside, we speculated that these oxazines may interact with heparin-binding growth factors. DMBO displayed significant affinity toward VEGF, tumor necrosis factor-α, and heparin-binding EGF-like growth factor (HB-EGF) as detected by surface plasmon resonance. Further analysis revealed that DMBO inhibits the catalytic activity of heparanase (IC_{50}: 65 μM) and exerts *in vivo* anti-metastatic and antiangiogenic effects (Basappa et al., 2010).

In another study, Song and colleagues structurally optimized DMBO to better suppress heparanase catalytic activity. The removal of methoxy group from the benzene ring (**54,** IC_{50}: 4.47 μM) or removal of a fluorine substitution (**55,** IC_{50}: 8.36 μM) of DMBO resulted in relatively better catalytic inhibition among the new structural analogs (Figure 9). The growth inhibition studies against HeLa and Siha cells presented **55** as a lead reagent. It also inhibited cell migration, induced cell apoptosis, and sensitized the cells to the cytotoxic effect of nedaplatin. Importantly, **55** strongly reduced the mRNA and protein levels of heparanase. This study suggested that targeted inhibition of heparanase may prove to be an effective therapy against cervical cancer (Song et al., 2016).

Quinolines

Before the resolution of the heparanase crystal structure (Wu et al., 2015), a structural model using sequence homology was developed and applied for virtual screening of known heparanase inhibitors, commercially available drugs, and drug-like compounds (Gozalbes et al., 2009, 2013; Mosulen et al., 2011). Besides, biophysical methods (nuclear magnetic resonance [NMR] and surface plasmon resonance [SPR]) revealed higher affinity (K_D = 52.6 μM) of amodiaquine (**56**) (Figure 9) toward the target. Furthermore, the subset of amodiaquine was obtained by a combination of chemical modifications. The compounds under investigation retained 4-aminophenyl-chloroquinoline scaffold of amodiaquine. However, subsequent NMR and SPR analyses revealed that none of these analogs improved the affinity observed for amodiaquine. It was concluded that the presence of nitrogen or oxygen as a heteroatom or bromine atom in the *para*-position of the non-fused aromatic ring is essential for interaction with heparanase, and no interaction was seen in the absence of these heteroatoms. The morpholine derivative **57** (Figure 9) displayed the relatively favorable interaction (K_D = 75 μM) among the structural analogs. Replacement of the morpholine hydrophilic and polar groups such as 4-hydroxypiperidine resulted in a dramatic reduction in the inhibitory activity. Apart from these modifications, substitution with 2-piperidin-1-yl-acetamido chain at the *meta*-position resulted in favorable interaction with heparanase when the benzyl alcohol is esterified with polar piperidine acetic acid. Overall, two polar unbulky substitutions on the aromatic ring are essential for activity (Gozalbes et al., 2013).

Glucans

Four glucan derivatives were prepared from β-(1→3)-D-glucan and carboxymethyl glucan using modified Wolfrom's method (Wang et al., 2012). The β-(1-3)-D-glucan and carboxymethyl glucan were independently subjected to degradation with sulfuric acid to get fractions of approximately 8,000 Da followed by their sulfonation. Among them, PSC **(58)** (Figure 9) showed 96% inhibition of heparanase activity at a concentration of 1.6 μg/mL with an IC_{50} value of 0.4 μg/mL. Furthermore, PSC inhibited bFGF-induced migration of MDA-MB-435 breast cancer cells (IC_{50}: 0.98 μg/mL) and HUVECs (IC_{50}: 18.3 ng/mL). Also, PSC reduced bFGF-induced phosphorylation of ERK1/2 and JNK in HUVECs (Wang et al., 2012).

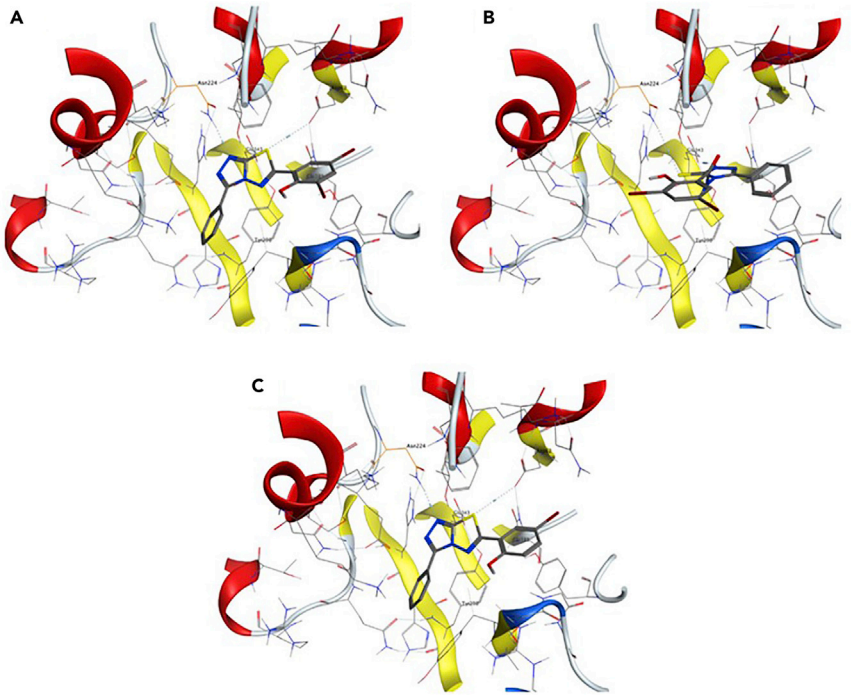

Figure 10. *In Silico* Docking into Heparanase of Triazolo-thiadiazoles

Selected docked poses for active compounds DTP, HTP, and ITP (**A**, **B** and **C**, respectively), showing similar active site interaction modes. DTP and ITP are shown to interact with both Asn-224 and Asp-62 via the triazolo-thiadiazole backbone, and HTP is shown to interact with Asn-224, and the active site acid-base Glu-343 (Figure 4 is taken from Baburajeev et al., 2017).

Triazolo-Thiadiazoles

In a recent study, we screened 150 chemically diverse scaffolds bearing quinolones, oxazines, benzoxazines, isoxazoli(di)nes, pyrimidinones, quinolines, benzoxazines, and 4-thiazolidinones, thiadiazolo[3,2-a] pyrimidin-5-one, 1,2,4-triazolo-1,3,4-thiadiazoles, and azaspiranes, toward the catalytic activity of recombinant human heparanase and identified 1,2,4-triazolo-1,3,4-thiadiazole as the lead scaffold (Baburajeev et al., 2017). Furthermore, the lead scaffold was derivatized and evaluated for anti-heparanase activity. Among the new derivatives, 2,4-diiodo-6-(3-phenyl-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-6yl)phenol (DTP, **59**), 2-hydroxy-3,5-diiodo-N-(3-phenyl-5-thioxo-1H-1,2,4-triazol-4(5H)-yl)benzamide (**60**), and 4-iodo-2-(3-phenyl-[1, 2, 4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)phenol (**61**) (Figure 9) showed maximum inhibition up to 20 μg/mL. DTP completely inhibited the release of radioactive HS degradation fragments from [^{35}S]-labeled ECM at 10 μg/mL. HepG2 (hepatocellular carcinoma) and LLC (mouse Lewis lung carcinoma) cells showed low and moderate heparanase activity, respectively, and DTP exhibited good anti-proliferative and anti-invasive effects toward moderately heparanase-expressing LLC cells. Molecular docking analysis revealed that triazolo-thiadiazole backbone interacts with Asn-224 and Asp-62 of the enzyme (Figure 10) (Baburajeev et al., 2017).

FCE27266

FCE27266 is a sulfonated distamycin, a derivative that possesses inhibitory activity toward bFGF, PDGF-β, and VEGF. FCE27266 (**62**) (Figure 11) is generated by replacing benzene moieties of suramin with *N*-methylpyrrole rings characteristic of distamycins (Rondanin et al., 2017). Hence, it has been regarded as a hybrid of suramin and distamycin. Rondanin and colleagues identified FCE27266 and other structurally related compounds as heparanase inhibitors. Initially, asymmetric compounds (carbamates and ureas) and derivatives in which a pyrrole ring has been replaced with benzene were prepared and examined for their anti-heparanase activity (Rondanin et al., 2017). FCE27266 exerted heparanase inhibition at low micromolar concentrations (IC_{50}: 2.0 μM) (Rondanin et al., 2017). Among the investigated structurally related compounds, asymmetric carbamates (**63**) and ureas (**64 and 65**) (Figure 11) were completely devoid of inhibitory

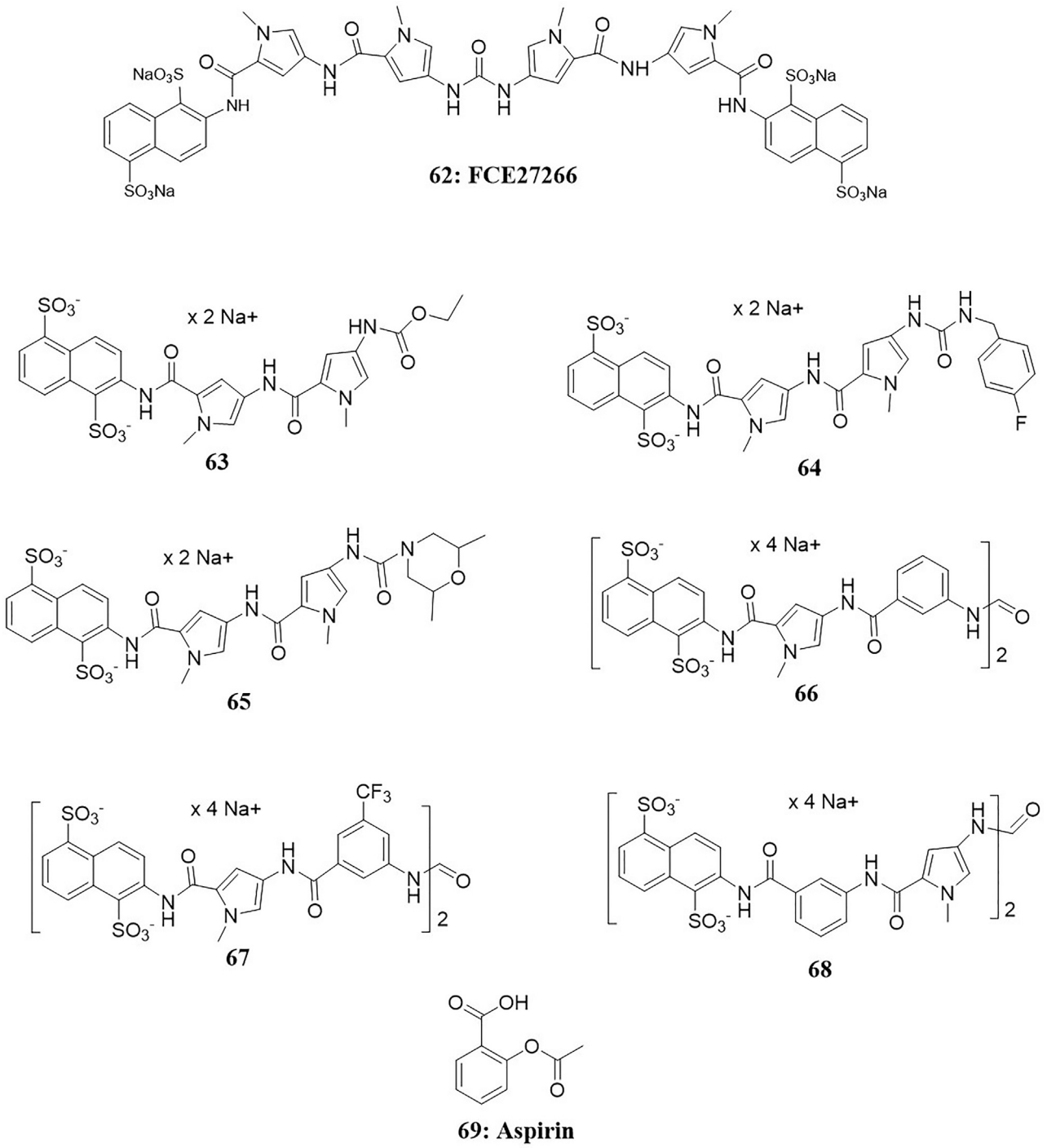

Figure 11. Chemical Structures of Distamycin- and Suramin-Related Compounds (62–68) and Aspirin (69)

activity (Rondanin et al., 2017). However, the replacement of the pyrrole ring with a benzene unit resulted in compound (**66**) with improved inhibitory efficacy. The modified structure resembles suramin more than distamycin. Moreover, the introduction of the trifluoromethyl group on the newly introduced benzene significantly suppressed the activity (**67**; IC_{50}: 0.66 μM) (Rondanin et al., 2017). The dipyrroleurea compound (**68**) (Figure 11) showed anti-invasive activity with more than 90% inhibition. In addition, **68** and FCE27266 potently modulated the expression of heparanase (Rondanin et al., 2017).

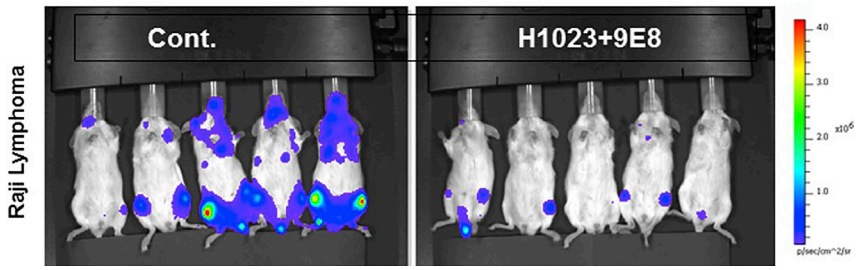

Figure 12. Heparanase-Neutralizing mAbs Attenuate Tumor Growth

Non-obese diabetic (NOD)/SCID mice were inoculated (i.v.) with luciferase-labeled Raji lymphoma cells. Mice were treated with mAb 9E8 plus mAb H1023 (500 μg/mouse every other day) directed against heparanase, and tumor growth was evaluated by IVIS imaging (figure taken and modified from: Weissmann et al., 2016).

Aspirin

Aspirin (**69**) is a non-steroidal anti-inflammatory drug that has been extensively used as an analgesic and for the treatment of inflammatory diseases (Figure 11) (Kumar et al., 2018). Several preclinical studies have demonstrated that long-term administration of aspirin has significant clinical advantage such as decreased cancer incidence, weakening the risk of metastasis, and reducing cancer-related mortality, although its mode of action in cancer has not been elucidated (Burn et al., 2011; Rothwell et al., 2012; Patrignani and Patrono, 2016; Dai et al., 2017). In a recent study, aspirin was found to inhibit heparanase enzymatic activity possibly by directly interacting with Glu225 in the active site. Aspirin was found to inhibit heparanase-driven cancer cell migration, VEGF release, angiogenesis, and tumor metastasis, both *in vitro* and *in vivo* (Dai et al., 2017).

HEPARANASE-NEUTRALIZING ANTIBODIES

Monoclonal antibodies (mAbs) against cancer-related targets have met with success owing to their specificity and long half-life in humans, yet none have been advanced toward clinical development (Weissmann et al., 2016). Three potential heparin- or HS-binding domains of heparanase were identified (Levy-Adam et al., 2005). Particular attention was given to the Lys_{158}-Asp_{171} heparin-binding domain (designated HBD1) because a peptide (termed KKDC) corresponding to this sequence physically interacts with heparin and HS, with high affinity, and inhibits heparanase enzymatic activity (Levy-Adam et al., 2005). A panel of mAbs was next generated in an attempt to target the interaction of heparanase with its HS substrate. Two mAbs (9E8, H1023) that neutralize heparanase enzymatic activity were selected (Weissmann et al., 2016) and found to substantially decrease the cellular uptake of latent heparanase, an HS-dependent mechanism that limits extracellular retention of the enzyme and enables intracellular processing of the latent enzyme into its active form (Gingis-Velitski et al., 2004b; Ilan et al., 2006). It, therefore, appears that the antibodies not only neutralize the enzyme extracellularly but also diminish uptake and accumulation of heparanase inside the cell (Weissmann et al., 2016). Both the 9E8 (IgM) and H1023 (IgG) mAbs markedly inhibit cell invasion and tumor metastasis, the hallmarks of heparanase function. Moreover, both mAbs inhibit the spontaneous metastasis of ESb mouse lymphoma cells from the subcutaneous primary lesion to the liver (Weissmann et al., 2016). Importantly, treatment with mAb 9E8 or mAb H1023, as a single agent, attenuated the growth of human CAG myeloma and Raji lymphoma tumors, and even greater inhibition was observed when combining the two mAbs together (Weissmann et al., 2016) (Figure 12), in agreement with the notion that combining two different mAbs increases the inhibitory outcome. Not surprising, mAb 9E8 and H1023 are not cytotoxic to lymphoma or myeloma cells, implying that the mAbs do not exert a direct effect on the tumor cells but rather affect the tumor microenvironment. This was best demonstrated with Raji Burkitt's lymphoma cells that lack intrinsic heparanase activity due to gene methylation (Shteper et al., 2003), yet tumor xenografts produced by the same cells exhibit typical heparanase activity (Weissmann et al., 2016). Thus the ability of mAbs 9E8 and H1023 to attenuate the growth of these tumors is due primarily to neutralization of heparanase contributed by cells in the tumor microenvironment (Weissmann et al., 2016). This implies that the host contributes a significant amount of heparanase and that inhibition of this fraction is sufficient to attenuate tumor growth. This notion was further supported by showing that mouse El-4 lymphoma cells developed larger (3.5-fold) tumor xenografts when transplanted in heparanase-overexpressing transgenic (*Hpa-tg*) mice versus wild-type

C57BL mice (I.V., unpublished data). In contrast, smaller (8.5-fold) tumors developed when the same cells were inoculated into heparanase knockout (*Hpa-KO*) mice versus wild-type mice, further implying that *host* heparanase plays a decisive role in tumor progression (Gutter-Kapon et al.; our unpublished data). The finding that EL4 lymphoma cells grew aggressively in *Hpa-tg* but not *wt* animals is particularly instructive because EL4 lymphoma cells lack heparanase expression, leaving only host cells to contribute heparanase (Gutter-Kapon et al., 2016).

CONCLUSIONS AND PERSPECTIVES

Compelling evidence ties heparanase with all steps of tumor formation including tumor initiation, growth, metastasis, and chemoresistance (Arvatz et al., 2011a; Barash et al., 2014; Boyango et al., 2014; Hammond et al., 2014; Ramani et al., 2015, 2016; Shteingauz et al., 2015). Mechanistic studies on heparanase action have focused primarily on its expression by tumor cells and revealed that heparanase promotes an aggressive tumor behavior via multiple mechanisms. However, non-tumor (host) cells including T lymphocytes, B lymphocytes, neutrophils, monocyte/macrophages, endothelial cells, osteoclasts, and fibroblasts can also upregulate heparanase expression upon activation and thereby contribute not only to cancer progression (Edovitsky et al., 2004; Arvatz et al., 2011b; Lerner et al., 2011; Vlodavsky et al., 2012; Barash et al., 2014; Ramani et al., 2016), but also to acute and chronic inflammation (Li et al., 2008; Vlodavsky et al., 2012; Goldberg et al., 2013), autoimmunity (de Mestre et al., 2007; Li et al., 2008), atherosclerosis (Vlodavsky et al., 2013; Aldi et al., 2019), tissue fibrosis (Secchi et al., 2015), kidney dysfunction (van den Hoven et al., 2007; Garsen et al., 2016a; Garsen et al., 2016b), ocular surface dysfunction (McKown et al., 2009; Zhang et al., 2010b), diabetes (Parish et al., 2013), and diabetic complications (Gil et al., 2012; Wang et al., 2013).

The importance of heparanase in health and disease (Shu and Santulli, 2019) has led to numerous efforts over the years to develop assays to monitor its activity and to screen for new inhibitors as potential drug candidates. Despite these efforts and the commercialization of a few kits, most heparanase assays are still complex, labor-intensive, costly, or have limited application (Chhabra and Ferro, 2018). The somewhat limited progress in the development of heparanase inhibitors could be attributed in part to the lack of a robust, accurate, and rapid assay for enzyme activity. Advances in the synthesis of simple synthetic oligosaccharide substrates with a single point of cleavage are expected to ultimately lead to a "gold standard" assay for detailed kinetic analyses. Progress continues to be made with both HS and oligosaccharide-based assays, and it is anticipated that in the future more optimized assays will be commercially available. It remains to be seen whether a single, universal assay will emerge, or whether there will be different assays for different applications (Chhabra and Ferro, 2018).

The current review summarizes the results of intense effort to develop heparanase-inhibiting compounds (heparin or HS mimics, small molecules, neutralizing antibodies) starting soon after revealing the enzyme in the late 1970s. Now that the crystal structure of heparanase has been resolved, an important challenge in the field rests on structure-based rational development of clinically effective inhibitors of heparanase that will be applied to treat cancer, inflammation, and other diseases. This, together with the development of quantitative, high-throughput screening systems is expected to accelerate progress in the design and synthesis of clinically applicable heparanase inhibitors.

Heparin or HS-mimicking compounds are being tested in clinical trials for various cancers (Galli et al., 2018; Hammond et al., 2018) and the development of heparanase-inhibiting small molecules and neutralizing antibodies is ongoing. Notably, heparin or HS mimetics and other sulfated saccharides are multifunctional and hence may elicit off-target effects, dependent or independent of their heparanase-inhibiting activity. Some of these compounds were shown to inhibit angiogenesis (via interaction with heparin-binding pro-angiogenic factors) or promote anticoagulation, complement activation, TLR activation, and signal transduction, raising the issue of the pros and cons of target specificity in clinical settings. For example, pixatimod (=PG545) was shown to inhibit lymphoma tumorigenesis via TLR9-dependent NK cell activation (Brennan et al., 2016). Moreover, it was reported that the potential adverse effects of certain heparanase inhibitors might limit the antitumor activity of the inhibitors and should be considered when systemically treating patients with cancer with heparanase inhibitors (Putz et al., 2017). Noteworthy, heparanase is up-regulated in patients with various hematological malignancies following high-dose chemotherapy. It, therefore, appears that heparanase facilitates chemoresistance and that heparanase inhibitors used in tandem with chemotherapeutic drugs will overcome initial chemoresistance and enhance tumor killing,

providing a strong rationale for testing anti-heparanase therapy in combination with existing anticancer drugs (Ramani et al., 2016). Thus, the efficacy of heparanase inhibitors can best be exploited in rational combination therapy with other antitumor agents. Yet, only a few studies address this aspect of the therapeutic applications of heparanase inhibitors.

Remarkably, heparanase inhibitors were effective even when the xenografted tumor cells were devoid of heparanase, emphasizing the significance of heparanase contributed by cells residing in the tumor microenvironment (Weissmann et al., 2016). It appears that targeting both the tumor and the tumor microenvironment by heparanase inhibitors enhances the antitumor activity of approved therapies, further providing a strong rationale for applying anti-heparanase therapy in combination with conventional anticancer drugs (Ramani et al., 2015, 2016). Collectively, the emerging premise is that heparanase expressed by tumor cells and cells of the tumor microenvironment is a dominant regulator of the aggressive phenotype of cancer, an important contributor to the poor outcome of patients with cancer and a prime target for therapy.

Limitation

Studies on the pleiotropic modes of heparanase action in normal cellular functions and in promoting cancer, inflammation, and other diseases are beyond the scope of this review.

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AUTHOR CONTRIBUTIONS

Conceptualization, K.S.R, C.D.M., B., and I.V.; Writing – Original Draft, C.D.M., S.H., H.D.P., S.R., U.B., and S.C.N.; Writing – Review and Editing, C.D.M., B., N.I., S.C.N., V.K.G., and I.V.; Supervision, K.S.R, C.D.M., B., and I.V.

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