The Development of Inhibitors of Heparanase, a Key Enzyme Involved in Tumour Metastasis, Angiogenesis and Inflammation

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Abstract: Heparanase is an endo-β-glucuronidase that degrades the glycosaminoglycan heparan sulfate, a major component of the extracellular matrix and basement membranes, and has been implicated in such processes as inflammation, angiogenesis and metastasis. The identification of inhibitors of heparanase is an attractive approach towards developing new therapeutics for metastatic tumours and chronic inflammatory diseases. This review focuses on heparanase inhibitors that have been isolated or synthesised to date. More recent developments in the understanding of heparanase structure and function that may ultimately aid in the future design of inhibitors with improved potency and specificity, are also discussed.

Keywords: Heparanase, endo-β-glucuronidase, heparan sulfate, metastasis, angiogenesis, inflammation, inhibitors.

INTRODUCTION

Heparanase is an endo-β-glucuronidase that cleaves the heparan sulfate (HS) side chains of proteoglycans that are found on cell surfaces and as a major constituent of the extracellular matrix (ECM) and basement membranes surrounding cells (for recent reviews see references [1-6]). HS, a member of the glycosaminoglycan (GAG) family of polysaccharides, is a linear polysaccharide of up to 400 sugar residues in length, consisting of uronic acid-(1→4)-D-glucosamine-α-(1→4) repeating disaccharide subunits [7] (Fig. (**1a**)). Though ubiquitous, HS is difficult to isolate in large quantity and as such much of the work on heparanase has been performed using the closely related naturally occurring GAG, heparin (Fig. (**1b**)). The substrate specificity of heparanase is poorly understood, however, some progress has been made in determining the exact oligosaccharide sequence(s) recognised and cleaved by this enzyme [8,9]. In particular, the recent work by Okada *et al.* [9] suggests that recognition may be achieved with as little as a highly sulfated trisaccharide (Fig. (**1c**)).

Only one gene (HSPE) has been shown to encode for a protein with heparanase activity [10-13]. The product of this gene is a 61 kDa polypeptide which is post-translationally modified by glycosylation and proteolysis to give a mature heterodimer consisting of two subunits, 50 and 8 kDa in size [14]. While the three dimensional structure of heparanase has yet to be determined, it shows sequence homology to other glycosyl hydrolases, particularly in families 10, 39 and 51 [15]. Sequence analysis has also revealed that heparanase is ubiquitous in mammals, with similar sequences found in rat, mouse and cow. Apart from functions in tissue reorganisation during development and embryo implantation, heparanase plays a key role in the degradation of the ECM by metastatic tumour cells [11,16,17] and leucocytes [18], facilitating their spread to remote sites where they can form secondary tumours, or cause inflammation, respectively. Heparanase is also involved in angiogenesis, i.e., the growth of new blood vessels from pre-existing ones surrounding a tumour [19]. It is able to liberate active HS-bound angiogenic growth factors from the ECM [20] and it aids in the remodelling of the ECM and surrounding tissue associated with new blood vessel growth. In addition, heparanase has been linked to the proliferation of smooth muscle cells in blood vessel walls [21]. The medical significance of such processes and the discovery of a single heparanase gene makes this protein an attractive target for new drug development.

This review covers the heparanase inhibitors that have been isolated or synthesised, including heparin and modified heparin derivatives, various natural and synthetic polyanionic polymers used as heparin mimetics, and smaller molecules presumed to act as transition state analogues. It should be noted that, because of the diversity in heparanase assays, the IC_{50} data cited here are not directly comparable between different sources and are given only as an approximate indication of potency. Additionally, these IC_{50} data were obtained directly from the published sources in either μg/mL or μM and no attempt has been made to provide uniformity to these values. Many of the heparanase inhibitors described below, particularly those that are heparin or heparan sulfate mimetics, also impact on a wide range of other biological processes and these are beyond the scope of this review.

HEPARIN AND MODIFIED HEPARINS

The ability of heparin to slow the digestion of HS by heparanase is well documented [22,23]. As well as displaying inhibitory activity, heparin can also act as a substrate because most preparations of heparin contain sequences which can be cleaved by heparanase (see Fig. (**1a,b**)) [24-26]. While technically considered a sulfated polysaccharide (see next section), the wealth of information drawn from research surrounding heparin-heparanase interactions deserves special attention. Such studies have led to the discovery of potential therapeutics which have anti-heparanase activity but with diminished undesirable properties such as anticoagulation, and have also provided

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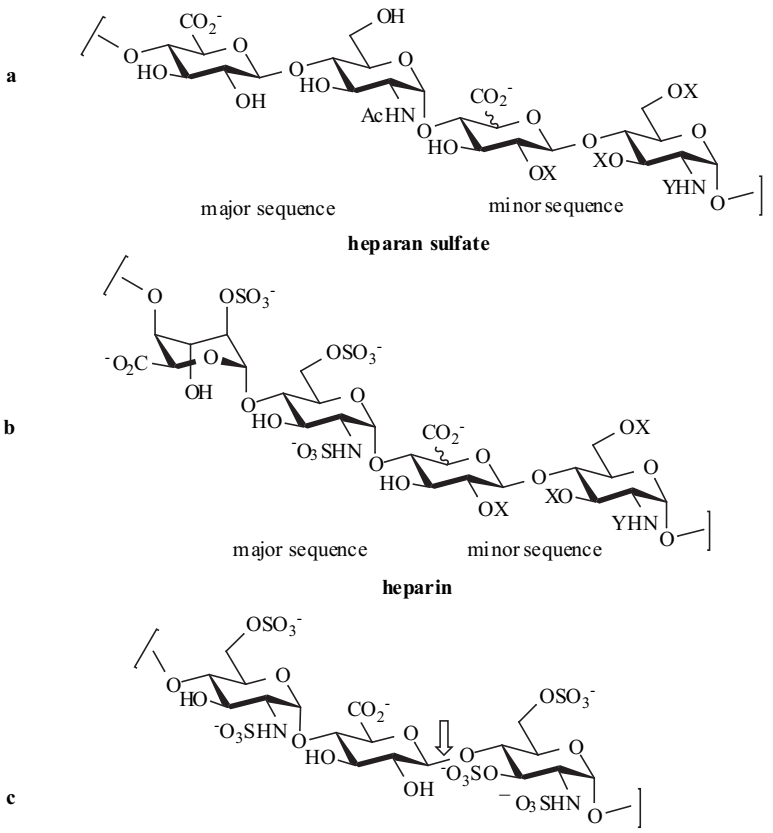


Fig. (1). Major and minor disaccharide repeating units in (**a**) heparan sulfate and (**b**) heparin (X = H or SO_3^-, Y = Ac, SO_3^-, or H). (**c**) Minimum heparan sulfate substrate recognition sequence for heparanase [9]. The scissile bond is indicated by an arrow.

valuable information about the structural requirements for the interaction of these polymers with heparanase.

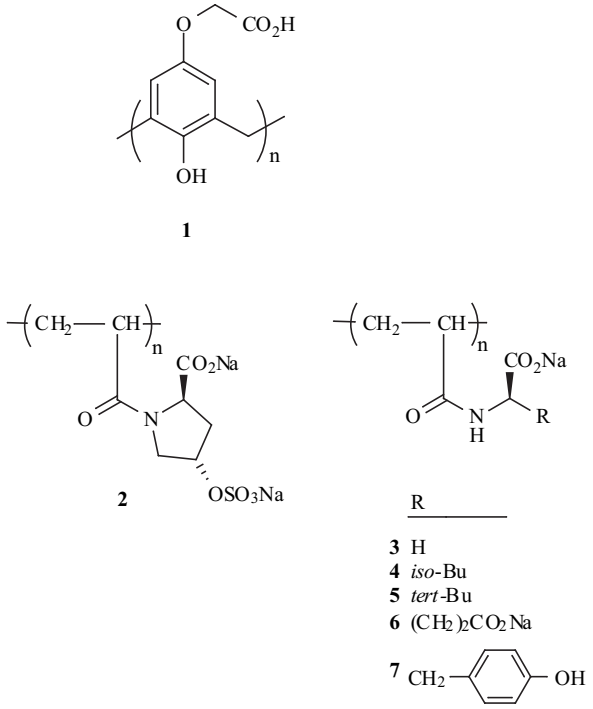


Fig. (2). Structures of non-carbohydrate heparin mimetic polymers.

Selective modifications of heparin GAGs have shown which functional groups are important for the inhibition of heparanase. A general trend in the results of these experiments is that some degree of sulfation is required for heparin to interfere with heparanase catalysis but that the location or linkage type of the sulfo group is not very important. When *O*-sulfo groups are removed, a retention (or even an increase) in heparanase inhibition by the modified heparin is most often observed [22,27,28]. An exception is the work of Vlodavksy *et al.* [29] who observed that *O*-desulfonation of heparin from porcine intestinal mucosa abolished its heparanase inhibitory activity. The substitution of *N*-sulfo groups on the D-glucosamine residues with *N*-acetyl groups generally had little or no effect on heparanase inhibition unless also accompanied by *O*-desulfonation of the uronic acid residues, in which case the inhibitory activity was reduced [22,27,29]. If the *N*-sulfo groups are removed and the amine remains unsubstituted the heparin loses much of its inhibitory activity [22,27,29]. This may be due to repulsion between the positively charged amino group and the heparanase active site which is itself likely to contain positively charged residues for coordinating sulfo and/or carboxylate groups of the substrate [15].

The uronic acid residue of heparin appears to require at least one formal negative charge for heparin to retain its inhibitory activity towards heparanase. The carboxylate group can be reduced to an alcohol or the 2-*O* and 3-*O* positions of the saccharide backbone can be desulfonated and heparanase inhibition remains high [28]. If both types of

modifications are present, however, the heparin's potency is significantly diminished [28].

Apart from substitution of functional groups on the polysaccharide backbone of heparin, modifications to some of the saccharide units also modulate heparanase inhibition. "Glycol split" heparin (produced by periodate oxidation of a non-sulfated glucuronic acid followed by borohydride reduction), in which between a quarter and half of the total number of uronic acid residues were modified, is an even more potent inhibitor of heparanase than the corresponding native heparin[1] [28,30,31]. Since glycol splitting also involves disruption of the highly specific pentasaccharide sequence required for activation of antithrombin, these derivatives have very low anticoagulant activity.

A typical heparin preparation contains a polydisperse mixture of polysaccharides with molecular weights ranging from ~5 to ~25 kDa (average ~12 kDa) [32]. Many studies have detailed the use of heparin oligomers as heparanase inhibitors but often without diligent quality controls. Some experimental results may be affected by the presence of heparin fragments considerably larger than the average. However, one very thorough, systematic examination of heparanase inhibition by fragments of heparin derived from a preparation from porcine intestinal mucosa showed that when fragment size declined below 14 saccharides in length (approximately 3.9 kDa) heparanase inhibition also declined to a point where four saccharide units displayed virtually no inhibition [29]. The sulfate content of heparin fragments smaller than 5 kDa shows a positive correlation with their ability to inhibit heparanase [29] and in a separate study a heparin-derived tetrasaccharide sulfonated at all available positions (11 sulfo groups) showed heparanase inhibition with a K_i of 290 μM in comparison to a typical heparin-derived tetrasaccharide (6 sulfo groups) which showed no heparanase inhibition [33].

HEPARIN MIMETIC POLYMERS

Sulfated Polysaccharides

Sulfated linear polysaccharides, readily available from natural sources or by chemical sulfonation of neutral polysaccharides, can be considered as crude heparin mimetics [34,35]. Parish and coworkers showed that some naturally occurring and semi-synthetic sulfated polysaccharides could block the breakdown of subendothelial ECM by rat mammary adenocarcinoma 13762 MAT cells and that this was probably due to the inhibition of heparanase produced by the MAT cells [36]. In addition to heparin, λ-carrageenan, fucoidan, pentosan polysulfate and dextran sulfate all showed significant heparanase inhibition. Their inhibitory potencies were similar and all were shown to inhibit tumour-cell metastasis. Hyaluronic acid, chondroitin-4-sulfate and chondroitin-6-sulfate, on the other hand, showed no significant activity, whilst κ-carrageenan showed weak inhibition of ECM breakdown but did not inhibit metastasis [36]. Subsequently, it was shown that heparin and sulfated 6-*O*-carboxymethyl chitin (SCM-chitin) III inhibited the invasion of B16-BL6 melanoma cells through basement membrane Matrigel™ [37]. SCM-chitin III and heparin also inhibited heparanase from B16-BL6 melanoma cells in a dose dependent manner, with complete inhibition of heparanase activity being achieved at a concentration between 10 and 100 μg/mL of SCM-chitin III.

More recently, laminarin sulfate was shown to inhibit heparanase with an IC_{50} of 1 μg/mL which compared favourably with heparin (IC_{50} = 2.5-3 μg/mL) [38,39], whilst calcium spirulan, an L-rhamnose-containing sulfated polysaccharide isolated from the blue green alga *Spirulina platensis* [40-42], inhibited heparanase from B16-BL6 melanoma cells with an IC_{50} = 56 ng/mL [43], more than 1000-fold lower than that of SCM-chitin III on a molar basis.

Phosphorothioate Oligodeoxynucleotides

Phosphorothioate oligodeoxynucleotides (PS ODNs) are wholly synthetic, polyanionic, heparin mimetic polymers. It was observed that the base composition in the oligonucleotide backbone had a significant effect on the potency of a PS ODN with those composed of guanine and thymine being stronger inhibitors than those composed from adenine or cytosine, indicating some specificity in the binding to heparanase. The length of PS ODNs also had a significant effect on their heparanase inhibition: longer chains bound more tightly than shorter chains [39,44]. From a variety of synthetic PS ODNs tested for heparanase inhibition, one consisting of 30 nucleotides and rich in guanine repeats was the most efficient inhibitor (IC_{50} = 24 μM) [44]. These inhibitors are, however, promiscuous, inhibiting a variety of enzymes, some of which have structurally related substrates (e.g., bacterial heparinases I, II and chondroitinases A, B and C) and others with unrelated substrates (e.g., phospholipases and protein kinases).

Non-Carbohydrate Heparin Mimetic Polymers

Synthetic, linear, non-carbohydrate polyanionic polymers have been studied for many years as heparin mimetics. A series of polymers of carboxylated phenols [45] was tested for inhibition of heparanase-mediated degradation of HS in the subendothelial ECM. Several compounds, including a polymer of (4-hydroxyphenoxy)acetic acid of MW ~5 800, designated RG-13577 (**1**), showed almost complete inhibition of heparanase activity at a concentration of 2.5 μg/mL [46]. RG-13577 also inhibited central nervous system inflammation in experimental autoimmune encephalomyelitis. Addition of RG-13577 at a concentration of 5 μg/mL to lymphocytes incubated with radiolabelled ECM completely inhibited their heparanase activity [47].

A series of poly(*N*-acryl amino acids) of MW range 3000 to 60 000 with lipophilic or polar side chains were synthesised by radical polymerisation of *N*-acryl amino acid monomers [48]. Poly(*N*-acryl amino acids) containing hydroxyl groups were also sulfonated to yield polymers such as (**2**). All polymers were assayed for heparanase inhibitory activity. Compounds (**2-7**) showed a similar inhibitory potency to heparin, resulting in 70-80% inhibition of

[1] See also (a) Naggi, A.; Perez, M.; Torri, G.; Giannini, G.; Penco, S.; Pisano, C.; Vlodavsky, I.; Casu, B. *XXIst International Carbohydrate Symposium*: Cairns, Australia, **2002**; p. OP100(PP096); (b) Vesci, L.; Aulicino, C.; Casu, B.; Naggi, A.; Giannini, G.; Poli, M.; Giavazzi, R.; Vlodavsky, I.; Carminati, P.; Pisano, C. *Eur. J. Cancer* **2002**, *38,* Suppl. 7, 76.

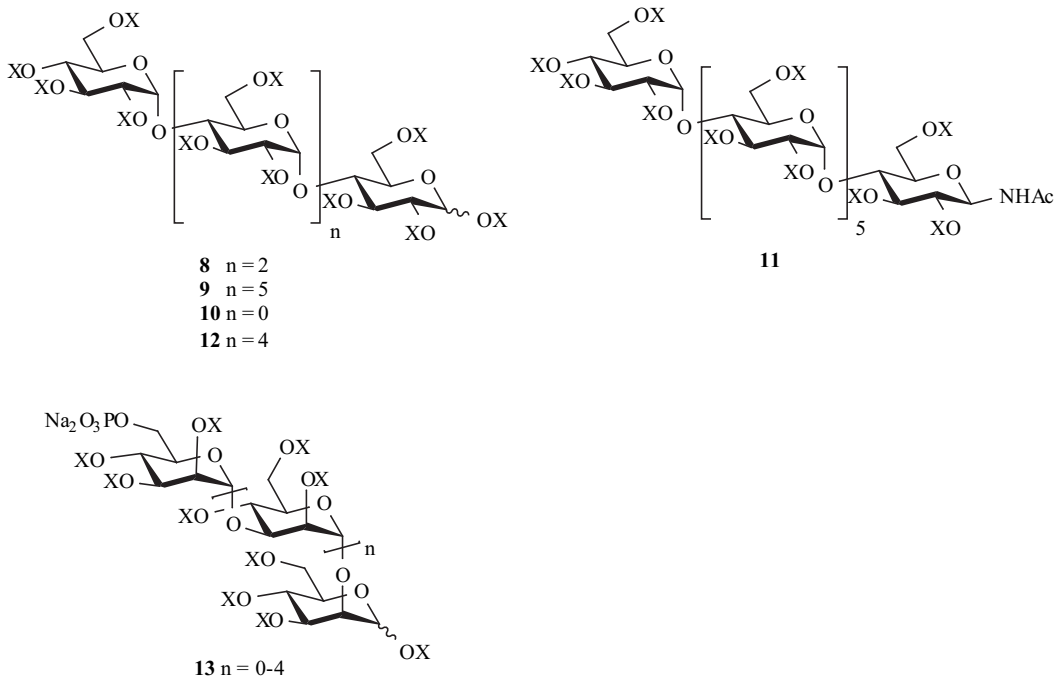


Fig. (3). Structures of sulfated maltooligosaccharides and of PI-88 (**13**). X = SO_3Na or H.

heparanase activity at a concentration of 1 μg/mL. The most effective inhibitor was poly(*N*-acrylleucine) (**4**).

SULFATED OLIGOSACCHARIDES AND OLIGOSACCHARIDE MIMETICS

The polyanionic polymers described above have a number of problems, in particular their heterogeneity and associated difficulty in characterisation, and their wide range of biological activity, including anticoagulant activity. This has lead to the investigation of smaller sulfated oligosaccharides and derivatives thereof as heparanase inhibitors. Once again, this class of compounds can be considered as heparin mimetics.

Sulfated Malto-Oligosaccharides

Sulfated malto-oligosaccharides, ranging from di- to heptasaccharide, were prepared and tested for inhibitory activity against rat hepatoma heparanase [49]. The inhibitory activity compared favourably with heparin and exhibited a dependence on chain length and degree of sulfation. Sulfated maltotetraose (**8**) (IC_{50} = 9.0 μg/mL) was slightly less effective than heparin (IC_{50} 4.1 μg/mL). Sulfated maltoheptaose (**9**) had the same activity as heparin, but sulfated maltose (**10**) showed no activity at greater than 200 μg/mL. A related sulfated maltoheptaoside, GM1474 (**11**), also inhibited rat hepatoma heparanase with the same potency as heparin (IC_{50} = 4.5 μg/mL) [50].

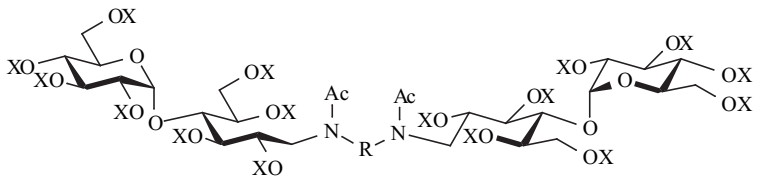
Phosphosulfomannans

Parish and coworkers [51] prepared a number of different sulfated oligosaccharides, including sulfated malto-oligosaccharides and cyclodextrins, and tested their inhibitory potency against heparanase [26] using a novel *in vitro* assay [52]. The observed inhibition trends were similar with regard to chain length and degree of sulfation observed in earlier work [49]. The two most potent inhibitors, which had activity comparable to heparin (IC_{50} = 1 μg/mL), were found to be sulfated maltohexaose (**12**) (IC_{50} = 1.5 μg/mL) and the phosphosulfomannan PI-88 (**13**) (IC_{50} = 2 μg/mL). PI-88 (**13**) (Fig. (**3**)) is a mixture of highly sulfated, monophosphorylated manno-oligosaccharides ranging from di- to hexasaccharide with the penta- (~60%) and tetrasaccharides (~30%) as major components [53,54]. PI-88 (**13**) strongly inhibited *in vitro* angiogenesis, whereas heparin had no activity in this assay, and showed potent inhibition of tumour growth and metastasis in animal models [51]. It was thus identified as a potential antitumour drug and has successfully undergone Phase I and Ib clinical trials in healthy volunteers and in cancer patients, with low toxicity being demonstrated [55]. One Phase II trial has recently been completed (for relapsed or refractory multiple myeloma) and several more are planned to establish the efficacy of PI-88 (**13**) in patients with advanced malignancies.

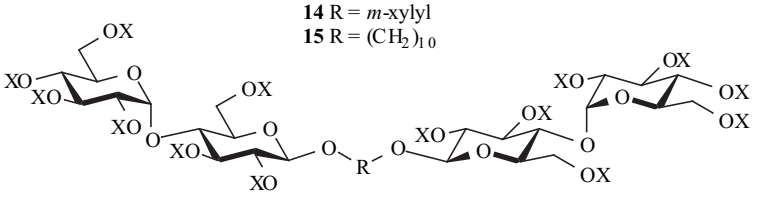
Sulfated Spaced Oligosaccharides

Banwell and coworkers[2] prepared several open and closed chain sulfated oligosaccharides linked by alkyl or aryl spacers as exemplified in Fig. (**4**). The two series of compounds were prepared by reductive amination with appropriate diamines, or by glycosylation with appropriate diols, respectively, followed by sulfonation. The compounds were tested for inhibition of heparanase and all of them had

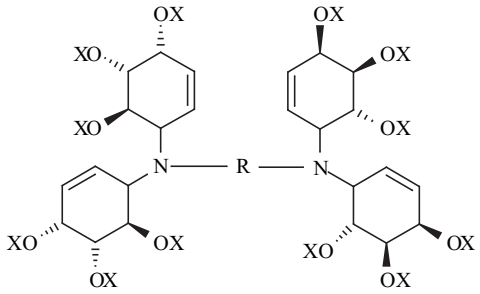
[2] (a) Armitt, D.J.; Banwell, M.G.; Ferro, V.; Freeman, C.; Parish, C.R. *World Chemistry Congress 2001*; The Royal Australian Chemical Institute: Brisbane, Australia, **2001**; p. 174; (b) Freeman, C.; Bezos, A.; Parish, C.R.; Armitt, D.; Liu, L.; Ferro, V.; Banwell, M.G. *XXIst International Carbohydrate Symposium*: Cairns, Australia, **2002**; p. PP238.

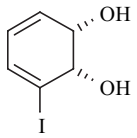


14 R = *m*-xylyl
15 R = $(CH_2)_{10}$



16 R = alkyl or aryl spacer





18

17 R = alkyl or aryl spacer

19 R =

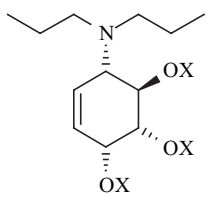


Fig. (4). Structures of sulfated linked oligosaccharides and linked cyclitols. X = SO_3Na or H.

some activity with IC_{50} values ranging from 8-30 μg/mL. In general, the open chain derivatives were more potent inhibitors than those with closed chains, with (**14**) and (**15**) being the most effective (IC_{50} values of 3 and 4 μg/mL, respectively).

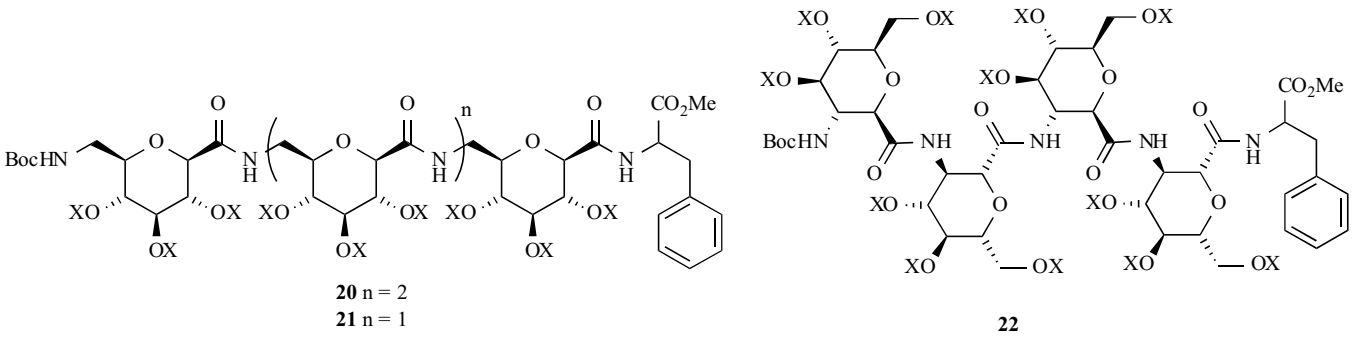
Sulfated Linked Cyclitols

Banwell and coworkers also prepared a series of sulfated alkyl- or aryl-linked cyclitols with the general structure (**17**), also shown in Fig. (**4**)[3] [56]. The compounds were prepared from enantiomerically pure *cis*-1,2-dihydrocatechol (**18**), readily obtained by the microbial dihydroxylation of iodobenzene [57]. All the compounds had some inhibitory activity against heparanase, although in general the activity was significantly less than that of PI-88 (**13**). IC_{50} values ranged from 9-42 μg/mL. However, a more highly sulfated compound (**19**) having an additional cyclitol attached to the linker was almost as active as PI-88 (**13**), with an IC_{50} of 3.5 μg/mL.

Sulfated Oligomers of Glycamino Acids

Ichikawa and coworkers [58-60] have self-condensed a series of glucose-derived glycamino acid building blocks (derivatives of *C*-glycosides that possess a carboxyl group at the C-1 position and an amino group in place of a hydroxyl group at either the C-2, 3, 4 or 6 position) *via* standard peptide coupling techniques to form β-linked homo-oligomers (di- to tetramers). Homo-oligomers with β(1→2) [58] and β(1→6) [59] linkages were sulfated and tested against a partially purified heparanase [60]. The most potent compounds were the β(1→6)-linked tetramer (**20**) (IC_{50} = 0.6 μM), the β(1→6)-linked trimer (**21**) (IC_{50} = 20 μM) and the β(1→2)-linked tetramer (**22**) (IC_{50} = 10 μM).

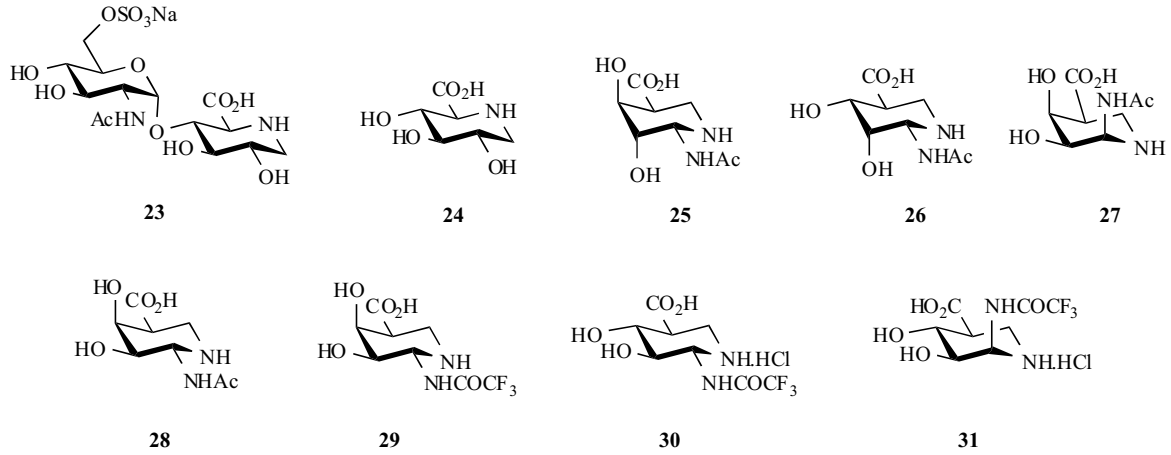
[3] See also (a) Banwell, M.G.; Ferro, V.; Freeman, C.; Liu, L.; Parish, C.R. *World Chemistry Congress 2001*; The Royal Australian Chemical Institute: Brisbane, Australia, **2001**; p. 392; (b) Freeman, C.; Bezos, A.; Parish, C.R.; Armitt, D.; Liu, L.; Ferro, V.; Banwell, M.G. *XXIst International Carbohydrate Symposium*: Cairns, Australia, **2002**; p. PP238; (c) Parish, C.; Armitt, D.; Liu, L.; Freeman, C.; Bezos, A.; Ferro, V.; Banwell, M. *XXIst International Carbohydrate Symposium*: Cairns, Australia, **2002**; p. IL03.



20 n = 2
21 n = 1

22

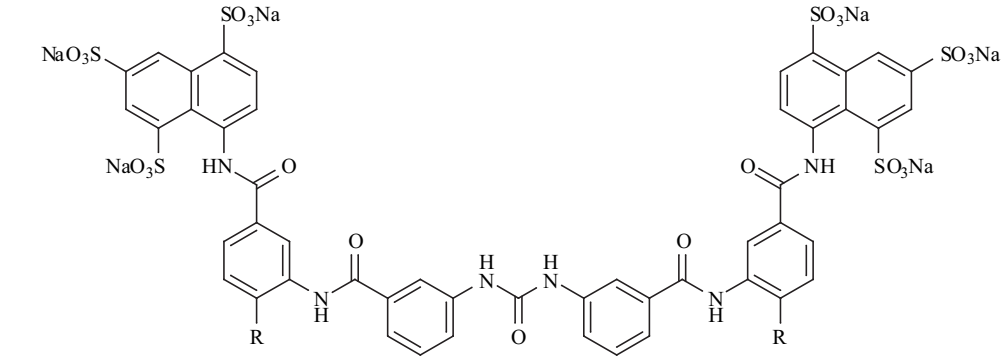
Fig. (5). Structures of sulfated oligomers of glycamino acids. X = SO_3Na or H.



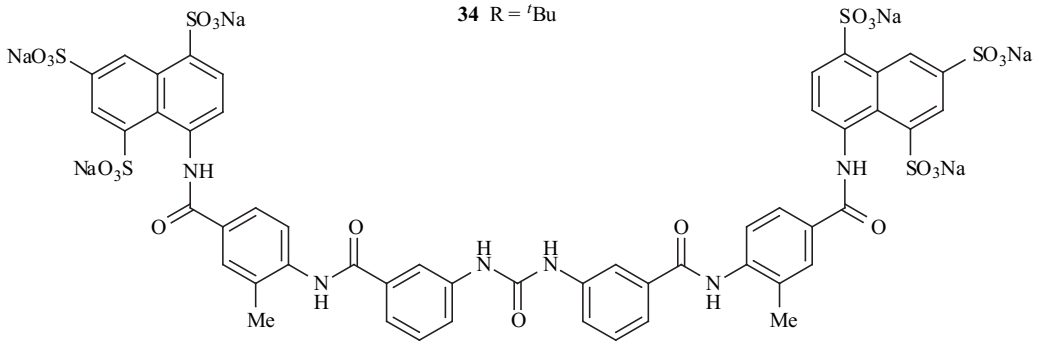
23 **24** **25** **26** **27**

28 **29** **30** **31**

Fig. (6). Structures of putative transition state analogue inhibitors of heparanase.



32 R = Me
33 R = Et
34 R = ^{t}Bu



35

Fig. (7). Structures of suramin and suramin analogues.

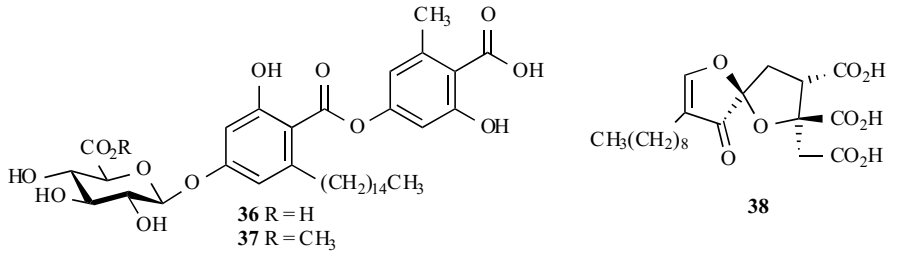


Fig. (8). Structures of fungal metabolites CRM646-A and -B, and trachyspic acid.

PUTATIVE TRANSITION STATE ANALOGUES

Glycosyl hydrolases can be classified as either retaining or inverting, depending upon whether they catalyse the hydrolysis of the glycosidic bond with net retention or inversion, respectively, of anomeric configuration [61,62]. Though not yet fully characterised, heparanase is believed to operate with a retaining mechanism [15]. Both inverting and retaining glycosidases catalyse hydrolysis *via* transition states with substantial oxocarbenium ion character and considerable flattening of the ring compared to the normal $^4C_1$ chair conformation. An attractive approach to designing potent inhibitors of glycosidases with high specificity is to use compounds that mimic such transition states [63]. This approach has proven successful with many glycosidases, using inhibitors isolated from natural sources or synthetic analogues [63]. Thus far only relatively few transition state analogue inhibitors have been found or synthesised for heparanase.

Pseudodisaccharides

Takahashi *et al.* synthesised the pseudodisaccharide (**23**), a mimic of the basic repeating disaccharide unit of HS, as a potential heparanase inhibitor [64,65]. Compound (**23**) is composed of a 2-acetamido-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl moiety and 2,6-dideoxy-2,6-imino-L-gulonic acid (**24**). The latter, a member of the 1-deoxynojirimycin family of transition state analogue inhibitors that mimics a positively charged endocyclic oxygen, is a powerful inhibitor of human liver β-glucuronidase. Compound (**23**) inhibited heparanase from B16-F10 melanoma cells and colon 26N-17 cells with IC_{50} values ranging from 58-140 μM [65]. Although compound (**24**) was not assayed, further investigation may well prove fruitful, as other β-glucuronidase transition state mimics show heparanase inhibition (see below).

Siastatin B Derivatives

During the course of screening for new heparanase inhibitors, Takatsu *et al.* isolated A72363 A-1, A-2 and C (**25-27**) from the culture filtrate of *Streptomyces nobilis* SANK 60192 [66]. Compounds (**25-27**) are diastereomers of siastatin B (**28**), a neuraminidase inhibitor [67], also produced by the organism. Compound (**27**) reversibly inhibited heparanase with an IC_{50} of 12 μM, whereas the other diastereomers did not inhibit the enzyme at all up to a concentration of 350 μM [68]. Compound (**27**) was also a potent β-glucuronidase inhibitor (IC_{50} = 1.6 μM), whilst siastatin B (**28**) and compound (**25**) also showed some activity against this enzyme (IC_{50} = 39-50 μM).

Uronic Acid-Type *Gem*-Diamine 1-*N*-Iminosugars

Nishimura *et al.* replaced the acetamido group of siastatin B with a trifluoroacetamido group to yield an inhibitor (**29**) [69,70] with enhanced inhibitory potency towards β-glucuronidase (IC_{50} = 0.065 μM) [71]. Nishimura *et al.* more recently tested compound (**29**) for inhibition of recombinant human heparanase [71]. Also tested were the newly synthesised analogues with the D-gluco- (**30**), D-manno (**31**), L-ido and L-gulo configurations. Compounds (**29-31**) all inhibited the enzyme (IC_{50} = 1, 11 and 29 μM, respectively), whilst the L-ido and L-gulo analogues showed no inhibition up to a concentration of 3.3 mM.

OTHER INHIBITORS

The remaining known heparanase inhibitors of quite diverse structure have been grouped together in this section. Other than suramin and its analogues (**32-35**) which can be regarded as crude heparin mimetics because of the sulfonate groups at both ends of the molecules, these remaining compounds have been identified as inhibitors by screening and it is not clear from their structures how they inhibit heparanase.

Suramin and Suramin Analogues

Suramin (**32**), a polysulfonated naphthylurea with antiangiogenic and antiproliferative activity, inhibited heparanase with a K_i = 48 μM [33]. The inhibition was shown to be primarily noncompetitive. Parish *et al.* [51] also demonstrated suramin inhibition of heparanase (IC_{50} = 8 μg/mL compared to heparin IC_{50} = 1 μg/mL). Suramin has undergone extensive clinical evaluation as an anticancer agent over the past decade but has limited clinical usefulness because of severe toxicity associated with indiscriminate inhibition of many other enzymes. The suramin analogues (**33-35**), chosen because they possess higher antiangiogenic activity than suramin itself, were also more potent heparanase inhibitors (IC_{50} = 20-30 μM) [72]. The structurally related polysulfonated dyes, trypan blue and Evans blue, were poorer heparanase inhibitors (IC_{50} values ranging from 310-320 μM) [33].

Fungal Metabolites: CRM646-A and -B, and Trachyspic Acid

CRM646-A (**36**) and -B (**37**) are novel fungal metabolites isolated from *Acremonium* sp. MT70646, which inhibited the invasion of B16-F10 melanoma cells through laminin-coated Matrigel™ [73]. Both compounds inhibit

heparanase with an IC_{50} value of 10 μM [74] and also show activity against bacterial heparinase [73] and telomerase [75].

Trachyspic acid (**38**) is a metabolite isolated from the culture broth of *Talaromyces trachyspermus* SANK 12191 [76]. Its relative configuration has recently been determined following the total synthesis of racemic trachyspic acid, however, its absolute configuration is not yet established [77]. Trachyspic acid inhibited heparanase with an IC_{50} of 36 μM (15 μg/mL) but showed no inhibitory activity towards bovine liver β-glucuronidase, suggesting some specificity towards heparanase. Reduction products of trachyspic acid lacking the spiroketal functionality or the C=C double bond of the α,β-unsaturated ketone also showed some inhibitory activity (IC_{50} = 36-145 μM), but citric acid, whose structure is contained within trachyspic acid, did not inhibit heparanase up to a concentration of 1 mM [76].

Diphenyl Ether, Carbazole, Indole and Benz-1,3-Azole Derivatives

A recent series of patent applications describes several aromatic compounds that inhibit recombinant human heparanase with micromolar IC_{50} values [78-81]. These compounds, presumably identified as inhibitors by screening of compound libraries, were separated into four classes: diphenyl ether derivatives (IC_{50} = 5-45 μM), carbazole derivatives (IC_{50} = 12-25 μM), indole derivatives (IC_{50} = 10-26 μM) and benz-1,3-azole derivatives (IC_{50} = 1.5-36 μM). The best inhibitors from each class are shown in Fig. (**9**). The diphenyl ether derivative (**39**) [IC_{50} = 5 μM] inhibited mouse melanoma primary tumour growth [78] whilst the carbazole derivative (**40**) [IC_{50} = 12 μM] [79] and the indole derivative (**41**) [IC_{50} = 11 μM] [80] inhibited metastasis as well as the primary tumour growth. The most potent inhibitor described, the benz-1,3-azole derivative (**42**) [IC_{50} = 1.5 μM], reportedly abolished the tumour [81].

MONOCLONAL ANTIBODIES

An alternative approach for the inhibition of heparanase is the development and use of monoclonal antibodies to the protein. Recently, a monoclonal antibody has been reported which effectively abolishes the activity of recombinant heparanase when used in a protein:antibody ratio of 1:10 [82]. Furthermore, when trialed on heparanase purified from human placenta, activity was abolished when used in a ratio of 1:20. Three companies recently entered into an alliance to develop monoclonal antibodies to heparanase and the lead antibody (OGS-MDX-001) has since entered pre-clinical safety assessment [83].

CONCLUDING REMARKS AND FUTURE PROSPECTS

Heparanase represents an excellent target for new drug development, with such drugs having potential as antimetastatic, antiangiogenic and anti-inflammatory agents. In fact, of the heparanase inhibitors reviewed here, one compound, PI-88 (**13**), shows promise as an anticancer drug and is currently in clinical trials. Many of the heparanase inhibitors described here suffer from a lack of enzyme specificity and a number of problems have hampered the development of inhibitors with enhanced specificity and potency. In particular, the lack of sufficient quantities of enzyme, and of an efficient assay to allow for rapid screening of potential inhibitors and more facile determination of useful (and comparable) kinetic parameters, needs to be

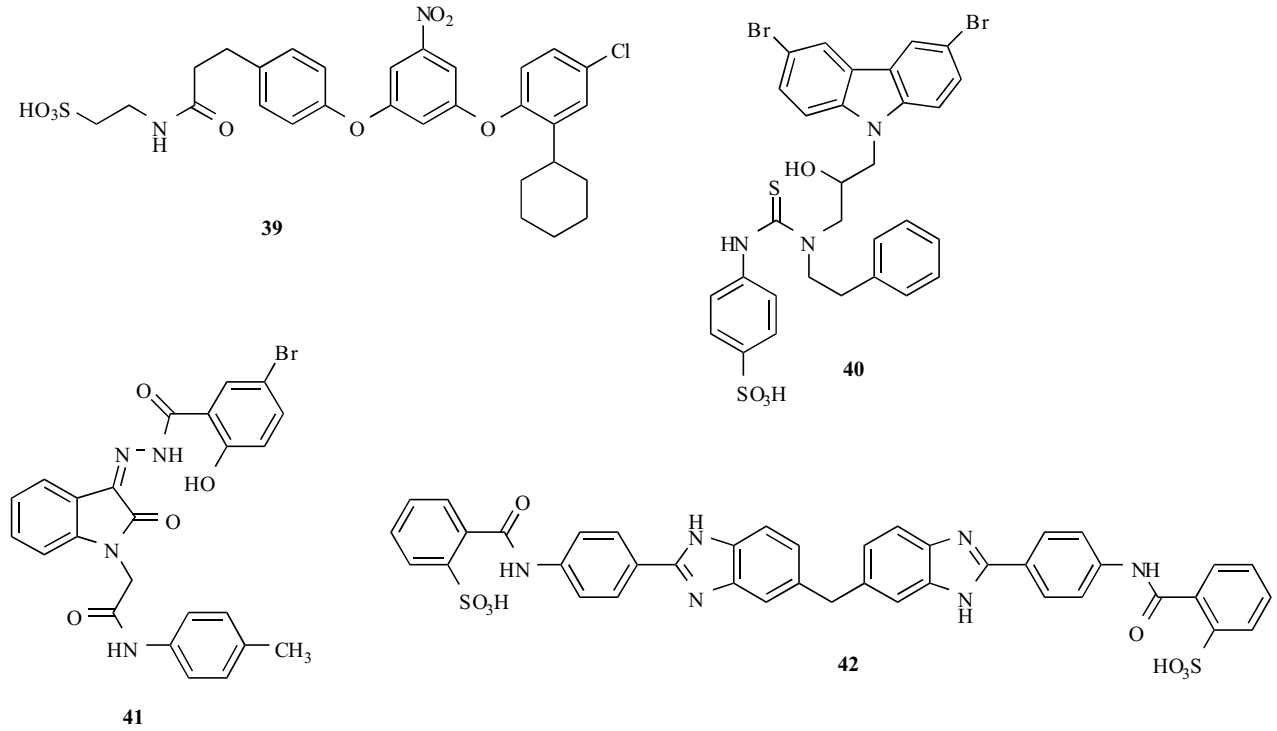


Fig. (9). Structures of diphenyl ether, carbazole, indole and benz-1,3-azole derivatives.

overcome. Such problems may be addressed in part by continued work on the development of improved expression systems for the recombinant enzyme, e.g., the very recently reported expression of the active heterodimer form of heparanase in insect cells [84]. It would seem plausible that further defining the protein's evolved functions (catalytic mechanism and substrate specificity) and the determination of an X-ray crystal structure would be invaluable for pursuing a more 'rational' approach to developing inhibitors of heparanase.

NOTE ADDED IN PROOF

After submission of this manuscript another class of compounds, phthalimide carboxylic acid derivatives, were reported as heparanase inhibitors with IC_{50} values ranging from 0.2 to 5.0 μM: Courtney, S. M.; Hay, P.A. *PCT Int. Appl.* WO 03/074516.

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