Recent Progress in the Design of Selectin Inhibitors

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Abstract: The selectins are a family of cell-adhesion proteins that mediate the early stages of leukocyte recruitment from the blood stream to sites of tissue damage through recognition of the carbohydrate epitope sialyl Lewis^x (sLe^x). Current development of small molecule based inhibitors of this process and their clinical potential to address numerous acute and chronic diseases are explored.

Keywords: Selectins; sialyl Lewis^x; adhesion molecules; antagonist; mimetics; inflammation.

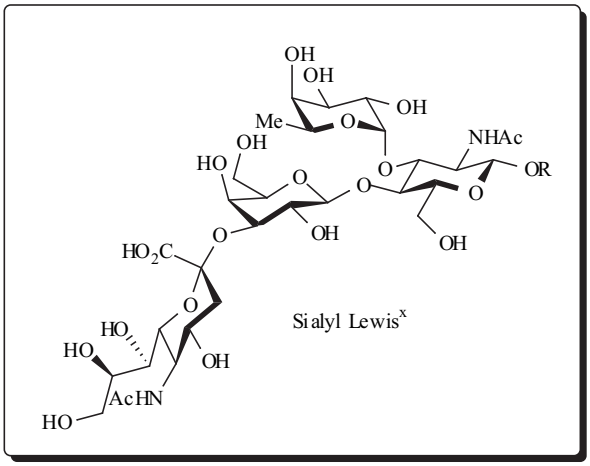
1. INTRODUCTION

Carbohydrates were once thought to be the components of animal and plant tissues which served primarily as energy sources/storage. However in the latter quarter of the 20th century, the roles of carbohydrates took a fresh perspective particularly in the area of interactions between outer cell surface carbohydrates with carbohydrate-binding proteins (lectins)[1]. Lectins are believed to mediate cell-cell communication, recognition and signal transduction. Among a myriad of important biological processes, lectins act as receptors in adhesion of pathogens to host cells in infection, fertilization, initial tethering of leukocytes to inflamed tissues and metastasis[1-4].

Selectins, a sub-class of lectins, represents a family of carbohydrate-binding proteins which are distributed mainly in the leukocyte-vascular system and participate in the first key recognition step in leukocyte recruitment [4-8]. This mobilisation ensures that disposal of any offending pathogens from the host cell is dealt with efficiently. Sometimes however the higly regulated leukocyte conscription may result in excessive recruitment of leukocytes to the site of trauma. This eventually may give rise to tissue damage and manifest itself in inflammatory disease states, such as rheumatoid arthritis, psoriasis, asthma and atherosclerosis [3, 4, 9, 10]. Accordingly, as early as in 1980's it was envisaged that disruption of these selectin-carbohydrate interactions might serve as a novel target for palliative treatment of acute and chronic inflammatory diseases [10].

1.1 Why Carbohydrate-Based Molecules as A New Class of Therapeutic Agents?

The implication of selectin - carbohydrate interactions in a number of disease states has stimulated fervent research across a wide range of scientific disciplines. Apart from the inflammatory diseases suggested earlier, a growing number of studies have identified the central role of this interaction in the pathological processes of cancer metastasis [10, 11]. These promising avenues for the discovery of novel therapeutic agents has provided a compelling impetus in the search for potent selectin antagonists. One such endogenously expressed ligand, sialyl Lewis^x tetrasaccharide (sLe^x), has attracted interest for its potential as an anti-inflammatory agent. This is evident from a number of excellent recent reviews which report an array of molecules designed to mimic key functionalities of sLe^x [10, 12-15].



This mini-review aims to describe the most recent sLe^x mimetics that have emerged over the past three years, with an emphasis mainly on small molecule inhibitors of the selectins. The review begins with a brief introduction to the biology of inflammatory events, followed by a brief discussion on sLe^x as a lead and the postulated binding motifs on sLe^x that have assumed great importance in the development of the numerous potent novel sLe^x mimetics generated to date. The future directions advanced by several groups are also discussed alongside some pertinent issues on biological evaluations of selectin inhibitors.

1.2 The Role of Selectins in Inflammatory Cascade

Leukocyte recruitment into inflammed tissues is a sequential adhesive multistep process governed by molecular interactions between the leukocytes and the endothelial cells [10, 16-18] Fig (**1**). Inducible selectins, E- and P-selectins are not present on the surface of normal endothelium. However, upon activation by pro-inflammatory stimuli released from damaged tissues, the endothelial cells express E- and P-selectins. P-selectin, stored in Weibel-Palade bodies in endothelial cells and α-granules in platelets, is

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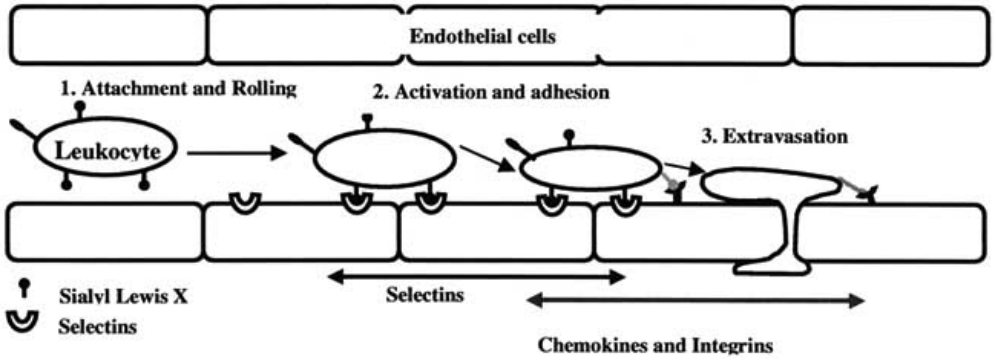


Fig. (1). Role of $sLe^x$and selectins in the inflammatory events.
The inflammatory cascade begins when cytokines are released by damaged tissues that lead to dilation of the capillary and eventually slowing down of blood flow. This allows circulating leukocytes to travel closer to the inflamed endothelial cells. The carbohydrate-bearing ligands expressed contitutively on endothelium and leukocytes are recognised by E-, P- and L-selectins. The selectins mediate the first step i.e. attachment and rolling of leukocytes to the endothelial cells. Rolling of leukocytes activates the second step which involves stronger adhesion interaction. The firmer attachment to the endothelium allows the leukocytes to transmigrate into the underlying tissue to the site of trauma.

immediately expressed on the surface of the endothelium and platelets. The expression of E-selectin, unlike P-selectin, is only evident 2 hours after initial stimulation since it is under transcriptional control. These selectins bind to selectin ligands on leukocytes bearing sLe^x such as P-selectin glycoprotein ligand-1 (PSGL-1) and E-selectin ligand-1 (ESL-1). Constitutively expressed and located on microvilli of circulating leukocytes, L-selectin also recognises and binds to similar carbohydrate-bearing ligands on the endothelial surface and leukocytes. Thus, on activated endothelial cells leukocytes are tethered to and rolled across the surface by means of the three selectins. Rolling then activates and induces a firmer adhesion interaction between integrins on leukocytes and an endothelial protein, intercellular adhesion molecule-1 (ICAM-1). This stronger adhesion leads to transmigration of leukocytes into the underlying tissues, with shedding of L-selectin from the leukocyte surface. One way to effectively combat offending inpathogens is by forming a cluster of leukocytes. This is believed to be achieved by leukocyte adherence to one another *via* L-selectin and its ligands. Platelets also may become attached to leukocytes and this leads to the formation of leukocyte-platelet aggregates.

1.3 Selectin Structure and its Ligands

Selectins are made up of five domains (Figure **2**): a cytosolic tail and a transmembrane domain, complement-like repeats, an epidermal growth factor (EGF) domain and an N-terminal carbohydrate-regconition domain (CRD). The EGF domain is believed to hold the CRD, containing the Ca^{2+} and sLe^x, in the right conformation. Four important crystal structures have been made available from two studies. The first by Graves [19a] shed light on the lectin domain of E-selectin and the second by Somers [19b] revealed the crystal structure of sLe^x bound to E- and P-selectin alongside PSGL-1 bound to P-selectin. Though the majority of selectin inhibitors designed are based on Graves' study, the recent revelation of the bound E- and P-selectin certainly offers further invaluable insight into the binding nature of

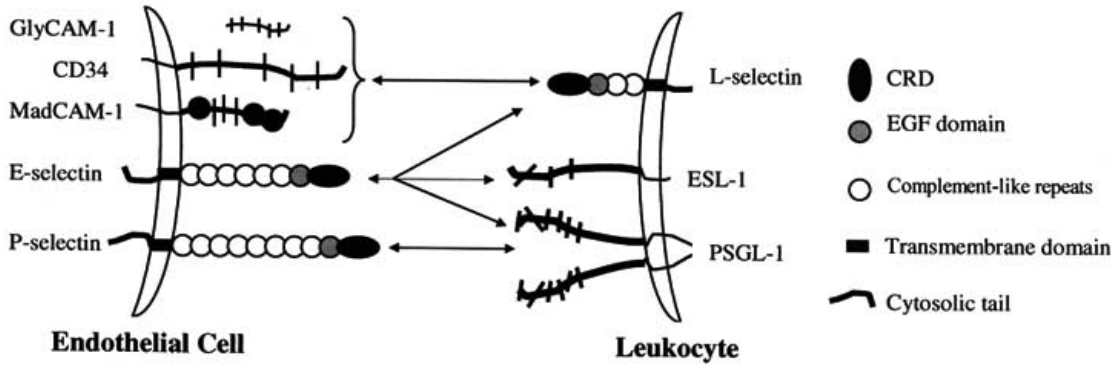


Fig. (2). The interactions between the selectins and its ligands (*adapted from Vestweber and Blanks* [8]). L-Selectin interacts with three glycoprotein ligands on the endothelial cell surface: GlyCAM-1, CD34 and MadCAM-1. P-Selectin interacts with only PSGL-1, which is the best-characterised physiological ligand to-date, found on leukocytes; whereas E-selectin performs multiple interactions with ESL-1, PSGL-1 and L-selectin, which forms the major carbohydrate presenting ligand on leukocyte for E-selectin.

the selectins and their ligands, and should facilitate the design of future selectin inhibitors.

1.4 Sialyl Lewis^x

The minimal terminal carbohydrate motif of a number of selectin ligands is identified as sialyl Lewis x (sLe^x) (Figure **3**). Attempts to turn sLe^x into a drug molecule has been marred due to the often protracted syntheses entailing complex protection-deprotection strategies. Furthermore the presence of *O*-glycosidic bonds makes sLe^x prone to degradation by gut glycosidases. In addition, quoted IC_{50} values of sLe^x in various binding studies are often in millimolar concentrations suggesting a weak interaction with the selectins [13]. Naturally these factors render sLe^x unsuitable as a drug. Nevertheless, sLe^x finds its use as a reference material in binding assays and the SAR study of minimal binding domains.

Though there are on-going debates on the definite binding motifs of sLe^x to the selectins, the key interactions recently established are: the 3- and 4-hydroxyls of fucose; the 4- and 6-hydroxyls of galactose and the carboxylic acid group on sialic acid [14d, 19b] (Figure **3**). Even though the *N*-acetylglucosamine does not seem to participate in the binding to the selectins, it is believed to be important in pre-organisation of the remaining trisaccharide into the bio-active conformation. Thus, by retaining the essential key motifs, systematic removal of saccharides from sLe^x has resulted in a plethora of selectin antagonists, both weak and potent, that afford insight into the SAR and further allow exploration into additional binding pockets of the selectins.

2. RECENT DEVELOPMENT OF SELECTIN INHIBITORS

The identification of the essential binding motifs on the sLe^x structure has paved the way for the development of smaller molecules with affinities comparable to or better than sLe^x. Whilst employment of multivalent mimetics is one of the strategies to improve avidity to the selectins, the majority of sLe^x mimetics are small molecules that display the functional groups deemed critical for binding to the selectins. Removal of inessential carbohydrate residues allows the design of inhibitors to be tailor-made for high affinity binding and stable to enzymatic degradation, enhancing both the bioavailability and pharmacokinetic profiles of such molecules. This approach may generate lead structures for drug development, and the reader is encouraged to turn to extensive discussion and reviews of these glycomimetics [10, 12-15].

The present mini-review covers the recent literature on glycomimetics of sLe^x and in-line with the past reviewers classifies the mimetics based on trisaccharides, disaccharides, monosaccharides and finally the non-carbohydrate antagonists that have shown improved affinity towards the selectins.

2.1 Glycomimetics

2.1.1 Trisaccharide and Disaccharide-Based Mimetics

Studies on trisaccharide-based mimetics secure the essential hydroxyl functionalities of galactose and fucose residues but substitute a number of anionic moieties in place of the expensive, but dispensible, sialic acid residue. Binding affinity suffers when the links are too rigid or flexible. Among the best inhibitors are those that replace the *N*-acetylglucosamine moiety with a cyclohexandiol or glucal residue, which may encourage optimal presentation of fucose and galactose into a bio-active conformation [12, 14d, 20].

The majority of work on trisaccharide and disaccharide-based mimetics was performed by researchers at Novartis and GlycoTech. Their work was based on original *in vitro* studies undertaken by Ramphal and co-workers, who showed that aromatic *N*-acylglucosamine derivatives of sLe^x inhibited E-selectin mediated cellular adhesion at

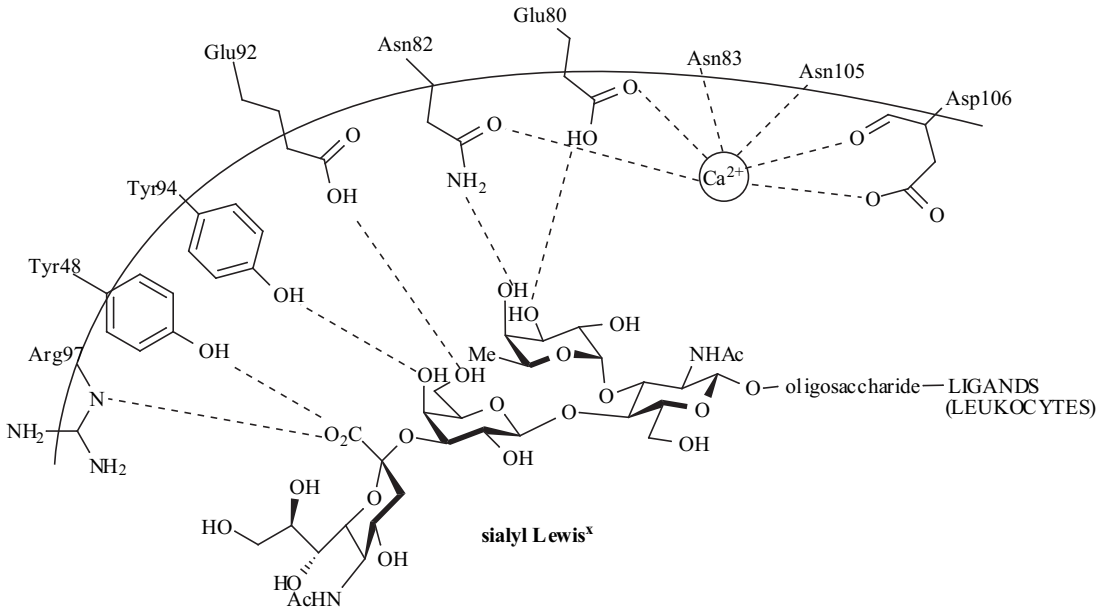
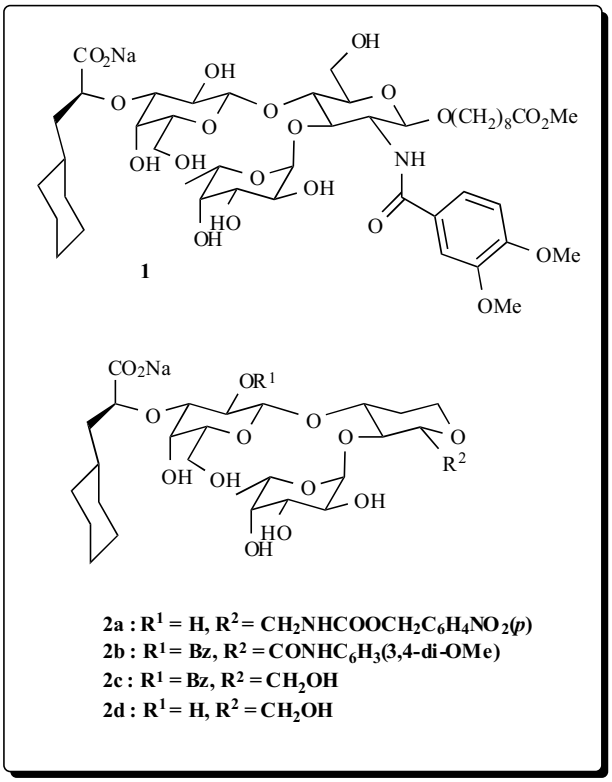


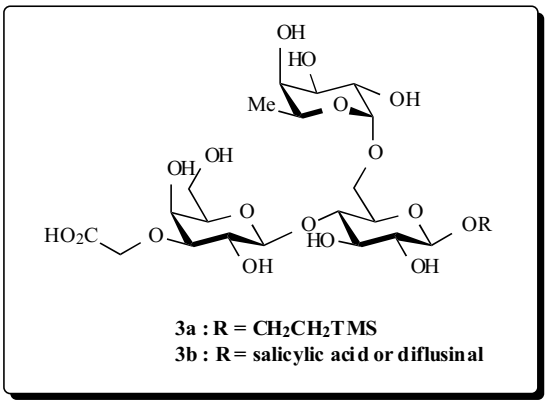
Fig. (3). Sialyl Lewis^x and postulated minimal binding motifs to E-selectin and bound calcium.

micromolar concentrations [21]. By combining this effective glucosamine modification with the previously successful replacement of the sialic acid moiety with (*S*)-cyclohexyllactic acid, Thoma and co-workers synthesised compound **1** and demonstrated it to be 30-fold more potent than sLe^x [22].



Earlier work, by the same group, had produced a variety of mimetics whereby the glucosamine residue had been replaced by various glucal derivatives [23]. Of these, **2a** showed a slight improvement in binding affinity compared to the native precursor **2d**. From this point, a mixture of accident, serendipity and rational design lead to compound **2b** [24]. Despite being unable to remove the Gal 2-*O*-benzoate during the synthesis, Thoma and co-workers nevertheless decided to test **2b** and revealed that it exhibited the highest potency to date (>100 fold when compared to sLe^x) in a dynamic flow assay. Surprisingly **2c**, the Gal 2-*O*-benzoate derivative of **2d** [25], also displayed a similar potency to **2b** against E-selectin. Though further study is needed , this does raise the question of where and how this benzoyl group binds in the E-selectin pocket.

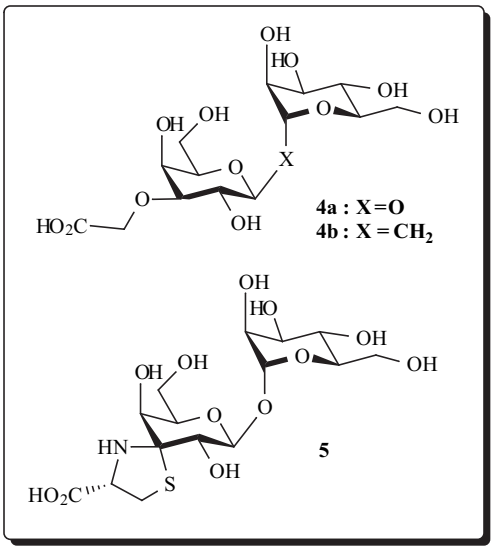
The most recent trisaccharide-based mimetic comprised a β-D-lactose core which was α-L-fucosylated on the glucose-*O*-6 position and carboxymethylated on the galactose-*O*-3 [26]. The authors suggested that the mimetic **3a** could be effective as a stand-alone selectin inhibitor or, if linked to an established antiinflammatory agent, could function as a targeting moiety for drug delivery (*e.g.* **3b**). Unfortunately, upon evaluation **3a** showed no inhibitory activity at concentrations up to 4 μM in a human polymorphonuclear leukocyte (hPMNL) adhesion assay to immobilised recombinant human E-selectin. In comparison, sLe^x displayed 68% inhibition at 1 μM.



Whilst the *N*-acetylglucosamine residue is retained in many trisaccharide-based mimetics, the studies of disaccharide-based mimetics entails several substitutions within this region [12, 13]. Hiruma and colleagues reported an *O*-linked disaccharide mimetic, β-D-galactopyranosyl-(1$\rightarrow$1)-α-D-mannopyranoside with a flexible carboxymethyl group **4a** that bound 5-fold more tightly to E-, P- and L-selectins than sLe^x [27]. This subsequently led to the development of two new disaccharide families based upon **4b** and **5**. The *C*-linked analogue **4b** is expected to offer more flexibility about the intersaccharide linkage than *O*-linked **4a**, and it should be more stable towards enzyme degradation. The modification may also allow conformational studies to be performed. Unfortunately, this compound has not yet been subjected to any binding studies [28]. In another study which retained the two sugar residues from the parent compound, a spiro ring was installed to position the carboxylate in a well defined orientation, *e.g.* **5** [29]. In the case of the latter, this was achieved by condensing D-cysteine with the unprotected 3-ulo precursor. The excellent potency of **5** compared to **4a** (IC_{50} values of 19 and 193 μM respectively) was however attributed to its increased hydrophobicity rather than incorporation of the locked carboxylate.

2.1.2 Monosaccharide-Based Inhibitors

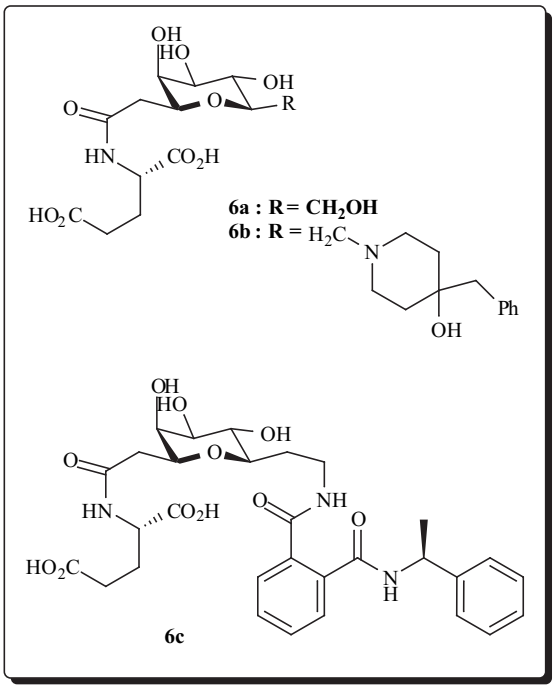
This class of inhibitors normally involves replacement of the dispensable lactosamine moiety with a flexible alkyl chain, or alternative rigid scaffolds, whilst retaining most of



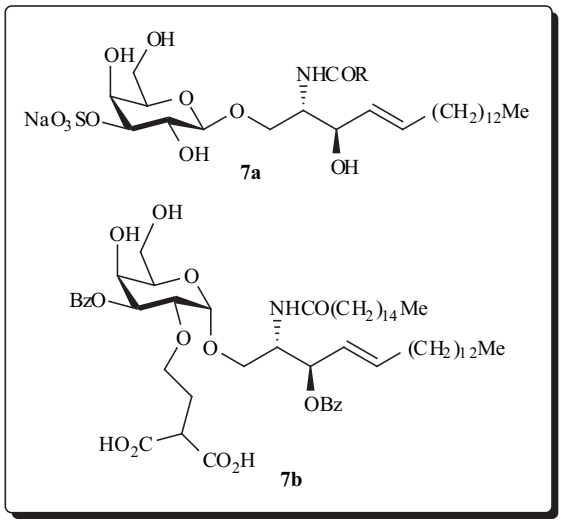
the key binding motifs. In most mimetics, fucose is preserved or replaced with the inexpensive D-galactose or D-mannose monosaccharides. These provide three of the six key hydroxyl motifs, assumed to be critical for interactions within the selectin binding pocket. The majority of single-carbohydrate based inhibitors are either glycoamino acid/glycopeptide mimetics or biphenyl-mannoside mimetics and were developed by numerous research groups but especially those of Wong and Kogan respectively [12]. They have laid the groundwork for most of the potent inhibitors to-date.

2.2 Glycopeptidomimetics

Many of these mimetics were designed and synthesized by Wong and co-workers, who published an extensive number of papers describing glycopeptide mimetics utilizing fucose, mannose and galactose as scaffolds to which amino acids are α- or β-linked. The researchers successfully employed solid-phase combinatorial chemistry to synthesise a library of fucopeptides [30]. Using cell-free binding assay, many mimetics in this class were shown to exhibit excellent binding activities against E-, P– and L-selectins. Encouraged by these results, Kaila and co-workers synthesized and evaluated both the α- and β-mannosyl glutamates and surprisingly found that the β-anomer **6a** also displayed excellent inhibition against E-selectin [31]. This spurred the synthesis of a range of β-*C*-mannosides where the researchers installed a number of substitutions at either C-1 or C-6 in the mannose residue. The discovery of potent inhibitor (**6b**) (IC_{50} = 1.6 μM) in turn provided the drive to construct two β-C-mannoside libraries employing solution-phase parallel chemistry and molecular modeling in tandem [32]. This generation of structurally-diverse molecules however did not result in many potent selectin inhibitors. The most potent member of the libraries **6c** showed activities against E-selectin (IC_{50} = 5 mM compared to sLe^x = 250 μM) and P-selectin (IC_{50} = 2.5 mM compared to sLe^x = 8 mM). The authors concluded the low inhibitory activities of their library members exemplified the difficulty in designing compounds that bind to the relatively flat surface of the P-selectin - sLe^x binding site; previously demonstrated by X-ray crystallography and modelling [19b].



Initiated by a publication that described sulfatides of general structure **7a** displaying an avidity to P-selectin that is equivalent to that of the myeloid HL-60 cell line, Marinier and colleagues recently published a study of sulfatide-based selectin antagonists [33]. On mapping key structural features of sLe^x to the general sulfatide scaffold, it was reasoned that the fucose residue could be replaced with the cheaper galactose mimetic, itself able to contribute the 4- and 6-hydroxy groups as calcium chelators. An additional acidic functionality had to be incorporated onto the 3-position of the galactose ring to mimic the sialic acid carboxylate of the sulfatide sulfate group. The inclusion of a malonate group at C-2 or C-3 was postulated to enhance interaction with the two lysine residues (Lys111 and Lys113) located within 4-5Å of each other in the selectin binding pocket. The C-2 malonate substituted galactoconjugates in general displayed better inhibitory activities against P-selectin *in vitro* and *in vivo* as compared to the C-3 malonate derivatives. For example, one of the C-2 malonate substituted compounds **7b** inhibited P-selectin in the range of IC_{50} = 8.5 - 12 μM. Thus, the study seemed to suggest this new family of sulfatide-based derivatives, in particular the 3-benzoylated C-2-malonate series hold the potential as interesting lead P-selectin antagonists.

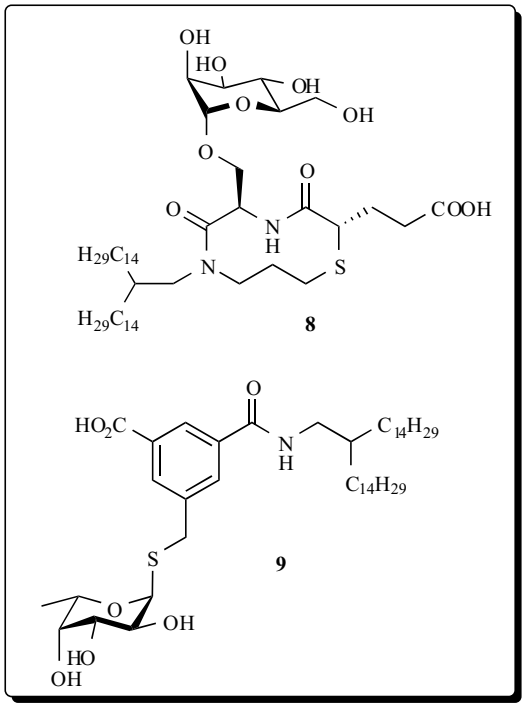


2.3 Scaffold-Based Mimetics

An increasing number of studies have implied that the use of a rigid core scaffold might improve the binding affinity of a molecule to the various selectins. Utilisation of the appropriate scaffolds may mimic the orientation of the fucose to galactose residue of the parent sLe^x. Recent trends have been in the employment of rigid structures, for example napthalene, biphenyl and heterocycles as scaffolds, since these may present the monosaccharides in optimal orientation to the selectins [12-15]. More importantly, generation of diverse libraries of potential selectin

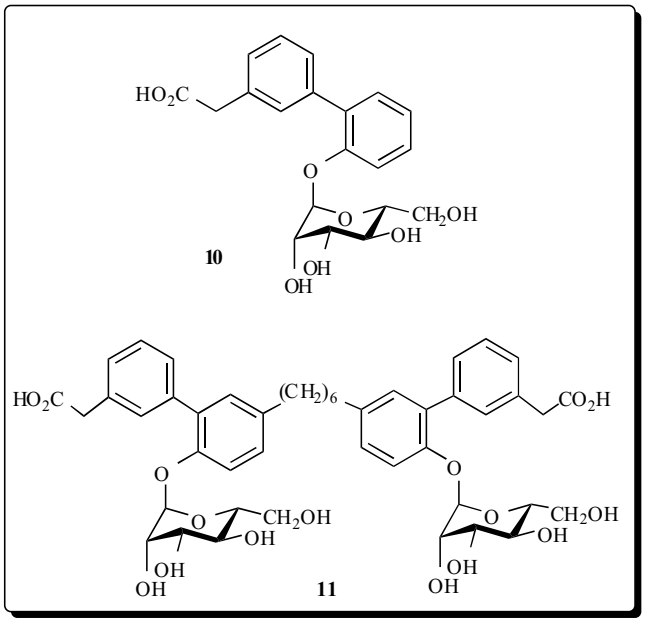
antagonists based upon these templates can be achieved *via* a combinatorial strategy.

The group of Kondo and co-workers synthesised several Ser-Glu dipeptide mimetics that exhibited type II and/or type II' β-turn conformations. These were found to inhibit E-, P- and L-selectins at micromolar concentrations *in vitro*. One of the inhibitors, a fucofuranosyl dipeptide was shown to improve pulmonary function and alveolar damage due to ischemia-reperfusion injuries in an *ex vivo* rat model. This success strongly suggests its potential as a useful template [34], and has subsequently led to the development of two relatively potent selectin inhibitors. Kurokawa, Kumihara and Kondo published the synthesis of **8**, a heterocyclic β-turn mimetic, using a solid-phase methodology. Though the 6-step synthesis afforded a poor yield before deprotection (11% overall starting from the original resin supported disulfide), the target compound was obtained in a highly stereoselective manner and in sufficient purity. In an ELISA based assay, mannoside **8** exhibited potent P- and L-selectin inhibitory activities (IC_{50} = 2.57 μM, P-selectin; 2.34 μM, L-selectin compared with sLe^x >1000 μM for both P- and L-selectin) but not towards E-selectin [35].

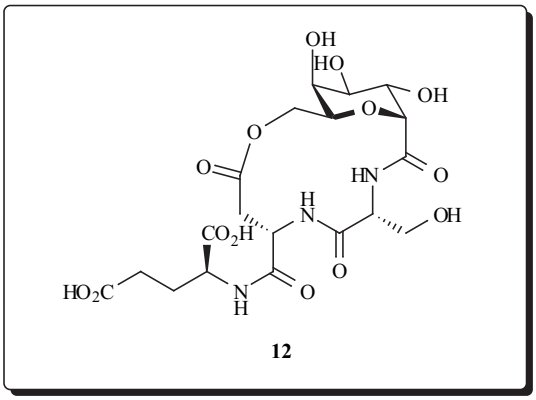


An alternative example described the synthesis of **9**, where a rigid tri-functionalised aromatic ring acted as the core scaffold. In this instance the fucose residue was retained but *S*-linked to the aromatic core. The carboxylic acid group served to mimic the negatively-charged sialic acid, and the long alkyl chain maintained to enhance hydrophobic interactions. The compound **9** was demonstrated to possess potent P-selectin *in vitro* antagonist activity (IC_{50} = 10.6 μM) in a P-selectin mediated cell adhesion assay [36]. Condensation of the benzoic acid **9** with either β-alanine or glutamic acid C^{α}-*N*-methyl amide afforded compounds with reduced potency.

Kogan and colleagues in their earlier studies demonstrated the potential of a rigid biphenyl core that strategically presented the three hydroxyl groups and the carboxylic acid for selectin binding. Instead of using a fucose residue, they incorporated the less costly mannose as the key monosaccharide. In their first study the biphenyl derivative **10** displayed only comparable activity to sLe^x [37a], however in their recent report the dimer **11** (TBC1269) was found to be six and fifty times more potent than sLe^x in its ability to inhibit the binding of sLe^x expressing HL-60 cells to E- and P-selectin IgG fusion proteins respectively [37b]. TBC1269 is currently in Phase II clinical trials for the potential treatment of asthma. In animal models, the dimer has been shown to afford beneficial effects in haemorrhagic shock and ischemic acute renal failure; whereas not in coronary bypass-associated myocardial injury [37c-f].



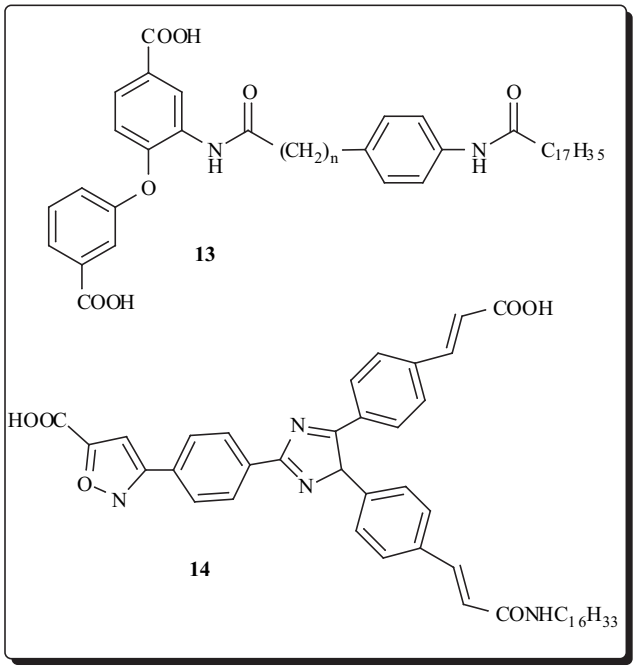
In another approach Wong and co-workers attempted the synthesis of a macrolide-centered inhibitor **12** to show the feasibility of scaffold-based mimetics. This study was based on one of their most potent glycopeptide inhibitors as the acyclic lead molecule [38]. The ring structure presumably offers an improved rigidity and therefore may bring about reduction in the energy need for the pre-organisation of the functional groups upon binding to the selectins. In agreement with the findings of Kondo's group, compound **12** showed more than 100-fold improvement (IC_{50} = 1 μM) when compared with its non-cyclised parent [38].



2.4 Non-Carbohydrate Mimetics

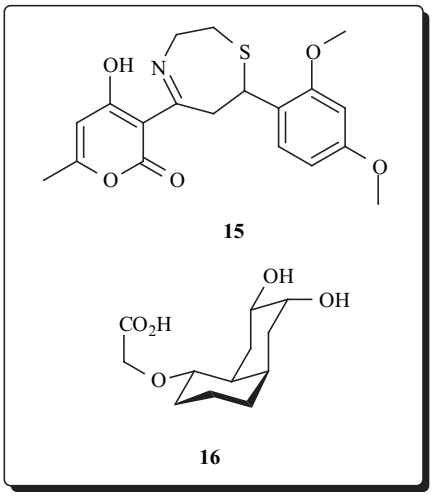
Though the aforementioned carbohydrate-based inhibitors retain most of the essential binding motifs of sLe^x, they are prone to suffer from enzyme degradation, possess poor pharmacokinetic and bioavailability profiles and rely upon often complex syntheses. Alternatively, small non-carbohydrate inhibitors are attractive as pharmaceutical leads since they tend to exhibit better pharmacokinetic profiles, are generally less cumbersome to synthesise and are amenable to combinatorial chemistry based strategies for drug discovery.

The conception of compound **13** (n=0) by Kondo and co-workers was derived from their earlier studies of a model of E-selectin bound to a potent selectin antagonist, 3'-sulfated Lewis^x, alongside their recent successes with the glycopeptidomimetic Ser-Glu dipeptides [34, 35, 39]. In this recent study, they screened 3D pharmacores based on a pharmacore model of the sulfated trisaccharide/E-selectin complex. Optimization of a potent lead compound obtained from 3D databases eventually led to the synthesis and evaluation of **13** (IC_{50} = 0.086 mM) [40]. Extensive modifications of **13** reveal the importance of the length and orientation of the alkyl chain, the position of carboxylic acid units and the length of methylene-spacer (n=0, 1 and 2) in relation to activity towards the selectins [41]. Another conclusion apparent from these studies was that replacement of the fucose residue with a carboxylic acid moiety, without loss in binding activity, may imply that the three fucose hydroxyl groups, assumed critical for binding to the selectins, are not necessary.



The discovery of the highly potent E- and P-selectin antagonist **14** was a culmination of optimisation and combinatorial syntheses of imidazole-based lead compounds obtained by means of high-throughput screening of libraries against P-selectin. Using molecular modelling, Slee and colleagues proposed a model that may elucidate the potency of **14** in its interactions with P-selectin binding pocket. In a similar manner, it is interesting to note that the carboxylic acid group in **14** also effectively acts as a calcium chelator replacing the fucose residue. Another similarity is their emphasis on the incorporation of long hydrophobic chains, which are found to be crucial in inhibitor interactions with the E- and P-selectin binding pockets. Compound **14** was found to exhibit excellent activities in cell-cell assays (IC_{50} P-selectin: 14 ± 19 μM; E-selectin: 30 μM). Following the *in vitro* studies, owing to its excellent aqueous solubility, it was administered as the corresponding bis-sodium salt to afford a dose-dependent, 30-50% reduction in cell infiltration in a mouse thioglycollate-induced peritonitis model [42].

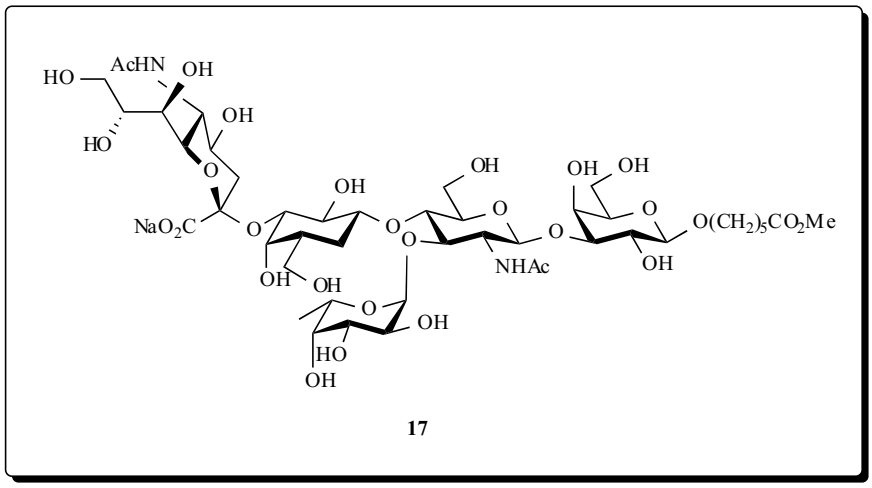
Ohta and colleagues have recently reported a novel P-selectin inhibitor based on a 7-phenyl-1,4-thiazepine core. Modifications at the phenyl ring comprised of mono-, di- and tri-substitutions with methoxy groups and it was demonstrated that the di-substituted **15** (KF38789) displayed an IC_{50} of 1.97 μM for P-selectin, with negligible activity against E- and L-selectins at concentrations up to 100 μM. *In vivo*, administration of 1mg/kg of **15** in a mouse thioglycollate-induced peritonitis model brought about a 34 ± 5% reduction in leukocyte infiltration. Compound **15** exhibited the highest potency so far in an *in vivo* animal model [43].



Another non-carbohydrate inhibitor **16**, comprising a semi-rigid *cis*-decalinic core has also been reported by Gravel an co-workers [44]. The novel scaffold serves as a mimic of the fucose and galactose rings. The ionic interaction with Ca^{2+} is mediated *via* the *cis*-diol, and the sialic acid residue is replaced with a carboxylic acid group. This inhibitor exhibited inhibitory activity comparable to sLe^x, showing activity at millimolar concentrations against E– and P-selectin. Facile synthesis, coupled with the potential of appending pharmacophoric substituents to this scaffold will allow exploration of additional binding sites thus potentially improving selectivity against the selectins. To-date, the simplest yet non-carbohydrate mimetic reported is 3-(4-methoxybenzoyl)propionic acid (MBPA) which was shown to markedly reduce arterial thrombosis in an animal model [45].

3. BINDING ASSAYS – SOME PERTINENT ISSUES

In the field of selectin medicinal chemistry, it is unfortunate that there is still no universal assay that one can



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rely upon, which accurately and reproducibly facilitates determination of the binding activity of potential selectin inhibitors. Various groups have employed numerous different assays, among them are competitive assays, glycolipid assays and rolling assays to evaluate the potential inhibitors. Most of these have inherent drawbacks such as reproducibility and quality of stocks. Multimeric interactions in ELISA-based assays may lead to complex formation and variability, therefore false positive results. As discussed by Kretzschmar, there is a huge disparity in terms of binding affinity when cell-based and cell-free assays were used to evaluate inhibitors and when acidic ion-exchange resins are used in synthesis of inhibitors [46]. Thus, *in vitro* assays are normally used to indicate and predict the performance of inhibitors *in vivo*, even though many a time a weak inhibitor may ultimately display excellent activity *in vivo*, as evidenced with Kogan's biphenyl dimer. These issues must be taken into consideration and addressed when evaluating potential new inhibitors *in vitro* [10, 12, 14].

4. CONCLUSIONS AND FUTURE DIRECTIONS

To many investigators in the field of carbohydrate-based therapeutics, the unsuccessful outcome of Cylexin **17** in clinical trials is probably synonymous with the bleak outlook of this field. On the contrary, this view is unjustified as recently several groups have successfully synthesised stable small molecule selectin antagonists, which showed excellent potency both in *in vitro* and *in vivo* assays. They hopefully display good pharmacokinetic profiles in clinical trials, unlike the enzymatically-labile *O*-linked pentasaccharide Cylexin.

The fact that the Kogan dimer is currently in Phase II clinical trials perhaps may serve as a reassurance and an indication to the bright outlook in this field. This exciting area promises a novel target for many established disease states and may also be supplemented by other approaches. They include reports of a very potent glycopeptide inhibitor based on PSGL-1 ligand; appendage of inflammatory drugs to a potential selectin inhibitor (drug delivery); combination selectin-integrin inhibitor using glycopeptides fused with sLe^x and the employment of fucoidan and heparin [29, 47-49].

ABBREVIATIONS

CD34	=	Cellular domain 34
CRD	=	Carbohydrate recognition domain
EGF	=	Extracellular growth factor
ELISA	=	Enzyme-linked immunosorbent assay
E-selectin	=	Endothelium selectin
ESL-1	=	E-selectin ligand-1
GlyCAM-1	=	Glycosylation cellular adhesion molecule – 1
L-selectin	=	Leukocyte selectin
MadCAM-1	=	Mucosal addresin cellular adhesion molecule
P-selectin	=	Platelet selectin
PSGL-1	=	P-selectin glycoprotein ligand-1
SAR	=	Structure-activity relationship

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