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Iminosugars past, present and future: medicines for tomorrow

Graeme Horne, Francis X. Wilson, Jon Tinsley, David H. Williams and Richard Storer

Summit Plc[1], 91 Milton Park, Abingdon, Oxon, OX14 4RY, UK

Iminosugars comprise the most attractive class of carbohydrate mimetics reported to date and are ideally positioned to take advantage of our increasing understanding of glycobiology in the search for new medicines. First-generation iminosugar drugs suffered from lack of adequate selectivity, resulting in considerable side-effects in the clinic. Current efforts directed towards second-generation compounds, encompassing a much greater range of structures and addressing a wider selection of biochemical targets, are enabling the identification and development of suitable candidates that benefit from improved activity and selectivity. Furthermore, second-generation compounds can address a variety of established targets that have previously proved refractory to other compound classes. This review focuses on the breadth of opportunities provided by second-generation leads from iminosugars (Seglins™).

Introduction

The importance of nucleic acids and proteins in providing biochemical targets in the search for new drugs is well established and has been successfully exploited; however, these molecular classes alone do not account for the biology and function of pathways, cells, tissues and physiological systems. Carbohydrates and lipids comprise two other important major molecular classes that fulfil crucial functions. The diverse structures displayed by carbohydrate structures, together with their recognition and handling processes, provide a substantial opportunity for the identification of new chemotherapeutic targets and the development of new therapies. A class of molecule ideally suited to exploit the numerous elements of these opportunities is iminosugars.

Iminosugars, whether of natural or synthetic origin, are small organic compounds that mimic carbohydrates or their hydrolysis transition states but contain a nitrogen atom instead of oxygen in the ring system template [1,2]. Such substitution enables not only monocyclic but also bicyclic

Corresponding author:. Storer, R. (richard.storer@summitplc.com)

[1] Summit Corporation Plc is a public company based in Abingdon UK and listed on AIM (SUMM). Summit specializes in second generation iminosugars (Seglins™) in the search for new drug candidates across a broad range of therapeutic areas.

Graeme Horne
Graeme Horne is a senior scientist at Summit where he leads the development of the Seglin™ platform. He holds a PhD in biochemistry from the University of Leeds and has been involved in carbohydrate drug discovery for over 10 years, 8 of which have been in iminosugars. After completing his doctoral studies in glycopeptide synthesis he spent several years working in therapeutic applications of the inositol phosphates before joining MNLpharma as senior chemist overseeing the development of their iminosugar chemistry programme. In 2006 he joined Summit and has played an integral role in the development of the Seglin™ Technology. He is a member of the RSC Carbohydrate Group committee.

Richard Storer
Richard Storer received a D.Phil from the University of Sussex and is a Fellow of the Royal Society of Chemistry. During a 35 year career in the pharmaceutical industry he has held senior positions in Glaxo in the UK, Biochem Pharma in Canada and Idenix Pharmaceuticals in the USA and France prior to joining the Board of Summit as Chief Scientific Officer in 2006. He has over 150 published papers and patents many in areas having a significant carbohydrate involvement. He has played leading roles in the discovery and early development of a number of marketed drugs and clinical candidates including the antivirals Epivir (3TC), Relenza, Telbivudine and IDX 899 and also SMT C1100 for treatment of Duchenne muscular dystrophy. He was a co-recipient of the 1996 Canadian Prix Galien for the discovery of 3TC.

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GLOSSARY

Glycobiology the study of the entire complement of carbohydrates in an organism (the glycome) through the systematic analysis and profiling of all carbohydrate structures of a given cell type or organism evaluating composition, association, expression and organization using a diverse set of novel technologies (glycomics) to determine the structure, biosynthesis and physiological roles of carbohydrates in biological systems.
Glycospace the description of the structural diversity and space defined by the glycome including analysis of abundance, stereochemistry, chemical functionality, recognition and biological function.
Iminosugar a natural or synthetic carbohydrate mimic in which the endocyclic oxygen has been replaced by nitrogen. The iminosugar motif is present in several classes of monocyclic and bicyclic compounds, leading to a large and structurally diverse class of molecules.
Seglin™ second-generation leads from iminosugars building upon the clinically validated first-generation iminosugars such as Zavesca and Glyset. Seglins benefit from an extensive rational design process allowing for wider diversity, utility and selectivity. Seglin™ is a registered trademark of Summit plc.

templates in which the nitrogen might also be introduced at a position common to the two rings (Fig. 1a). Substitution of the template is typically by hydroxyls, but other functional groups such as carboxylic acids and amides are found in nature, and in synthetic analogues, many variations are possible.

Unsurprisingly, iminosugars share many chemical features with mono- and disaccharides. They occupy an area of chemical space very similar to carbohydrates but distinct from that typically occupied by the usual small heterocyclic molecule screening libraries (Fig. 1b). Fig. 1b defines chemical space using axes representing molecular weight, AlogP and topological polar surface area. Location within this space of Summit's iminosugar collection is shown in green, whereas the typical heterocyclic 10,000-member drug-like screening collection adhering to Lipinski's 'rule-of-five' oral drug physiochemical criteria is shown in red.

At one level, on the basis of this analysis, iminosugars provide access to a new area of chemical space, and such compounds are actively sought by many Pharma companies to complement their own in-house collections. The attraction of iminosugars, however, extends beyond simply being different. As small polar molecules, their resemblance to carbohydrates might be responsible for endowing them with several special attributes as potential drug candidates, for example efficient uptake [3]. At the same time, they remain sufficiently distinct from carbohydrates to avoid processing by other carbohydrate-modifying systems and have both chemical and biological stability. This unique combination of properties singles out iminosugars as a special class in the search for new drug molecules [4–6]. Developments in the depth of understanding of glycobiology increasingly identify new targets to which iminosugars can appropriately be applied in the search for drug candidates. In addition, they provide potential access to a range of established targets, including those where typical screening collections have had limited success. Heightened interest in the field of iminosugars is evidenced by consistent substantial

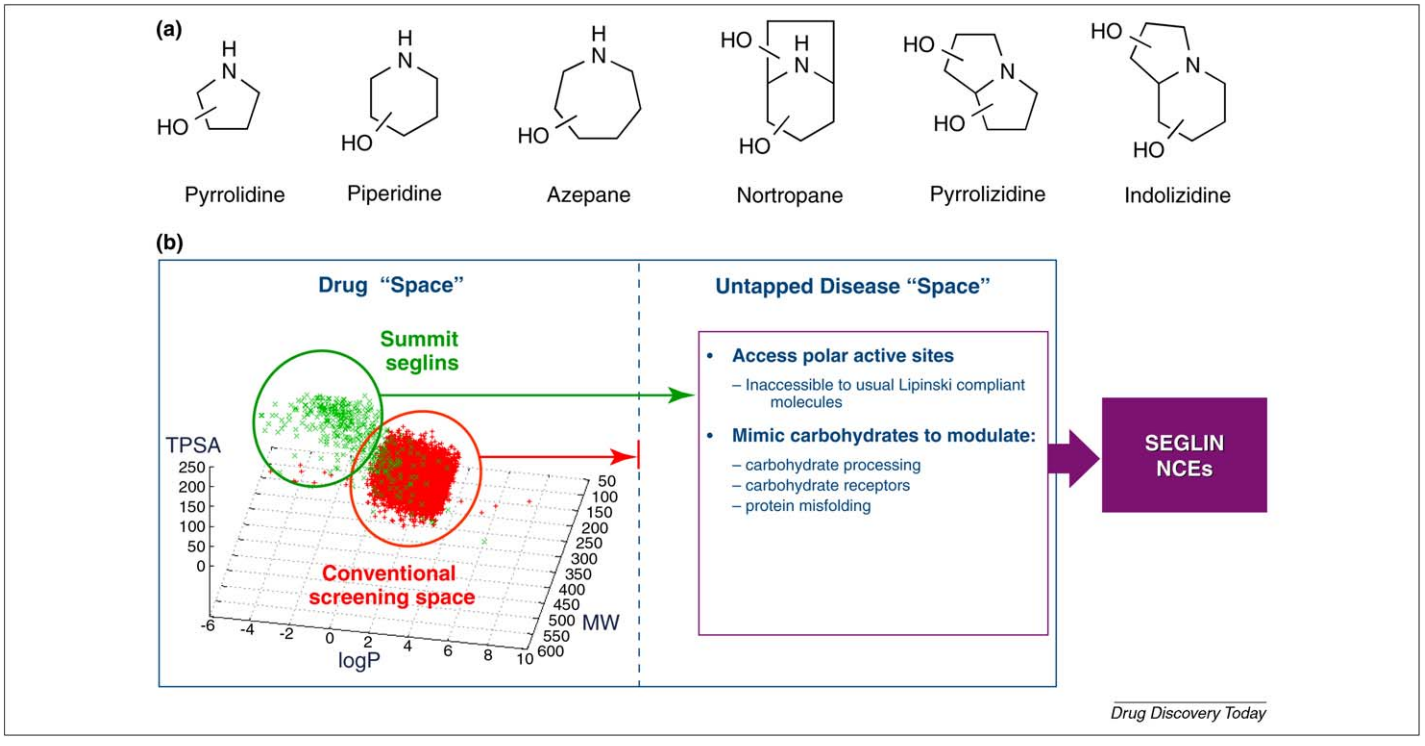

FIGURE 1

Common structural classes and physiochemical properties of iminosugars. **(a)** Examples of structural motifs commonly found in synthesis and nature. **(b)** Comparative analysis of the molecular landscape between Summit's Seglin Platform (shown in green) and a conventional screening library (shown in red). Advantageous physiochemical properties of Seglins enable access to untapped disease space with potential to develop new medicines in unmet areas.

increases in the number of publications appearing in the primary literature.

Origins and historical perspective of first-generation iminosugars

Early interest in iminosugars followed their discovery as natural products from many plant and bacteria species and has been extensively reviewed [7–13]. As highly water-soluble compounds, they would typically have been missed and discarded with the aqueous phase in the extensive efforts made in the 1980s by Pharma to isolate new leads for medicinal chemistry programmes by extracting plant materials using organic solvents. Indeed, there is increasing evidence that many of the historically interesting biological properties ascribed to plant extracts might have been due to the presence of iminosugars as active principles [14,15].

Biological activities of specific isolated iminosugars have typically been either for the parent natural compounds or for simple derivatives or prodrugs thereof. The diversity of such first-generation compounds studied, therefore, has been very limited. Although these compounds principally attracted initial attention from the pharmaceutical industry in the 1980s as anti-cancer or anti-HIV agents [16–18], they have subsequently demonstrated activity against a range of biochemical targets in diverse therapeutic areas [19–22]. The targets responsible for their activities have typically been carbohydrate-handling enzymes, and many of these early compounds act through inhibition of glycosidases. Other known mechanisms now include allosteric modulation of enzyme activity, active-site-specific chaperoning, inhibition of glycosyltransferases and acting as agonists for carbohydrate sensors [23–25].

First-generation clinical and marketed compounds

The development of iminosugars as therapeutics has its beginnings in the use of herbal and plant extracts in traditional medicines. As an example, the extracts of the white mulberry (*Morus alba*), which are rich in iminosugars, have had a pharmaceutical history for more than a thousand years [26]. These and other naturally occurring iminosugars have been the starting point for the development of this class of compound as therapeutic agents. The first-generation iminosugars have been founded on three natural products: swainsonine **1**; deoxynojirimycin (DNJ, **2**) and castanospermine **8** (Fig. 2). As will be seen below, however, issues with lack of adequate potency and selectivity have restricted the number of compounds developed from this generation that have progressed beyond the discovery phase. Despite these drawbacks, a selection of first-generation iminosugars has progressed to the clinic, of which two are now marketed drugs [5].

A first-generation iminosugar that suffered in the clinic because of inadequate potency and selectivity can be seen in the indolizidine D-swainsonine (GD0039, **1**). D-Swainsonine has been isolated from several plant species, including locoweed, in which it is thought to be its primary toxin and responsible for a variety of neurological disorders in livestock [27]. It is known to have several pharmacological effects in human, including inhibition of *N*-linked glycosylation [28]. It is a potent inhibitor of Golgi alpha-mannosidase II, an immunomodulator and a potential chemotherapeutic [29]. Despite its complex profile, it has been the subject of several trials as an anti-cancer drug. Some positive effects noted were offset by severe hepatotoxicity (at least in part associated with non-specific mannosidase inhibition), which prevented its further development. Nevertheless, the positive results observed in the initial stages of treatment for some patients (reductions in tumour size and tumour regression) highlight the potential for the utility of more selective and more potent second-generation leads from iminosugars (Seglins) in cancer therapy (see the section 'Current Seglin programmes').

The two marketed first-generation iminosugar drugs Glyset **3** and Zavesca **4** are closely related to the naturally occurring iminosugar DNJ **2**. Glyset has been licensed for use in the treatment of type II diabetes since 1996. Its proposed mode of action is through inhibition of intestinal α-1,4-glucosidases leading to a reduction in glucose absorption from the gut. Although this mechanism leads

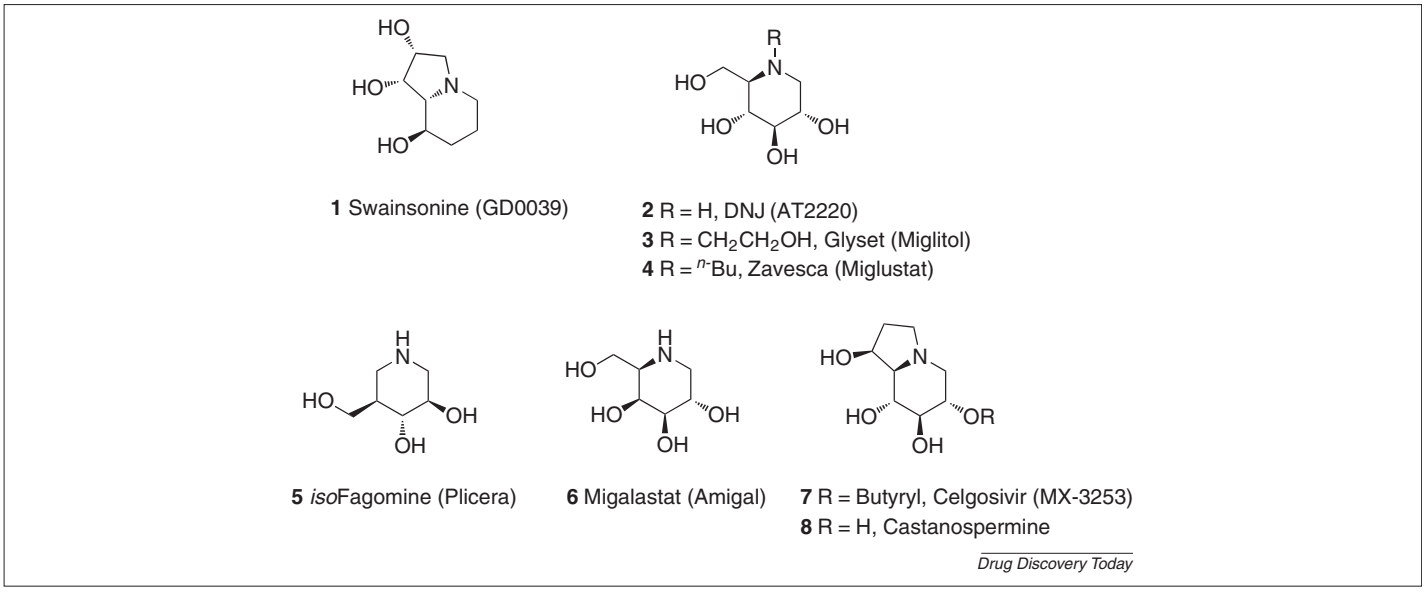

FIGURE 2

Selected first-generation iminosugar clinical candidates and marketed drugs.

to the lowering of plasma glucose without the risk of inducing hypoglycaemia, it has an inbuilt side-effect related to its mechanism of action. The prevention of carbohydrate breakdown in the upper gastrointestinal tract means that the carbohydrates are available as a microbial food source in the large intestine, which causes a buildup of gas that leads to abdominal pain, flatulence and the possibility of diarrhoea. With type II diabetes approaching near-epidemic proportions in many societies, the availability of antidiabetic drugs that are well tolerated and with minimal side-effects is increasingly important. Despite the side-effects associated with Glyset, the progression of this first-generation iminosugar to market highlights the potential opportunity for the development of Seglins as more selective and potent antidiabetic agents (see the section 'Current Seglin programmes').

The *N*-alkylated DNJ derivative Zavesca (Miglustat) is licensed for the treatment of type I Gaucher's disease and Niemann-Pick type C (NPC) disease. Its proposed mechanism of action is through inhibition of glucosylceramide synthase, a glycosyl transferase responsible for the synthesis of many glycosphingolipids [30]. Type I Gaucher's patients have a dysfunctional glucocerebrosidase enzyme leading to the accumulation of intracellular glucosylceramide [31]. Among other disease symptoms, this genetic defect causes liver and spleen enlargement. Reducing the activity of the glucosylceramide synthase lowers buildup of glucosylceramide (a process known as substrate reduction therapy), thus alleviating some of the disease symptoms. A similar principal effect is seen in NPC, another disease characterized by the accumulation of intracellular glycosphingolipids, albeit by an inherited defect in lipid trafficking [32]. Zavesca is currently indicated for the treatment of progressive neurological manifestations in adult and paediatric patients with NPC in several regions. However, because Zavesca contains the same DNJ scaffold present in Glyset, it is unsurprising that the side-effects exhibited by Glyset are also seen with Zavesca. These adverse effects might also be implicated in the suspension of a phase II trial for Pompe's disease using DNJ (AT2220, **2**). Pompe's disease is a glycogen storage disease associated with a deficiency in the lysosomal enzyme acid α-glucosidase [33]. The capacity of iminosugars to bind to lysosomal hydrolases and enhance the stability of the mutant forms, enabling the enzyme to attain a functional conformation, is well documented [34–36]. This process of using an active-site-specific chaperone (ASSC) is well suited to stabilizing lysosomal hydrolases and has been used in the clinical development of several iminosugars for the treatment of lysosomal storage disorders (LSDs). DNJ [37] was generally safe and well tolerated in phase I, but at the high dose administered at the start of phase II, adverse effects were seen in several participants and enrolment was ceased.

Two more recent first-generation iminosugars that have progressed to the clinic for the treatment of LSDs serve to reinforce the view that superior drug candidates will emerge from second-generation compounds. The first of these provides an alternative to substrate reduction therapy for the treatment of Type I Gaucher's using the iminosugar as an ASSC. *iso*Fagomine (AT2101, Plicera, **5**) is believed to bind to misfolded glucocerebrosidase enzyme, enabling correct processing and trafficking to the lysosome by the endoplasmic reticulum, thereby restoring its intended biological function of degrading glucocerebroside [38]. Although preliminary results from the phase II study indicated that treatment with Plicera was well tolerated, meaningful clinical improvements were observed in only one patient. On the basis of these results, Plicera is not expected to advance into phase III development at this time (http://www.ir.amicustherapeutics.com/ReleaseDetail.cfm?ReleaseID=413437). Migalastat **6** has recently reported successful results in phase II clinical trials for Fabry's disease (http://www.ir.amicustherapeutics.com/ReleaseDetail.cfm?ReleaseID=444486). Fabry's disease patients possess inherited mutations in α-galactosidase A, an enzyme responsible for degradation of the lipid globotriaosylceramide in the lysosomes [39]. These mutations lead to generation of an unstable protein, which is rapidly degraded, resulting in substrate buildup causing adverse effects. Migalastat seems to stabilize the mutant enzyme by binding to the catalytic site, enabling correct processing and trafficking to the lysosome by the endoplasmic reticulum [40].

In addition to applications in oncology and LSDs, the first-generation iminosugars have shown potential as antivirals. Most antiviral drugs target gene products unique to a specific pathogen. Despite good specificity and well-tolerated drugs, this strategy is vulnerable to the generation of drug-resistant strains, especially because RNA viruses possess high mutagenic rates. One approach to circumvent the problem of resistance is to target a cellular process in the host organism, and one pathway that has been targeted is *N*-glycan processing [41]. Celgosivir **7**, an ester prodrug of natural product castanospermine **8**, is the most advanced clinical-stage antiviral iminosugar targeting Hepatitis C virus (HCV) infection [42]. This first-generation compound inhibits several different glycosidases but is thought to exert its antiviral effect by preventing the maturation of *N*-glycans. This leads to incorrect folding and assembly of HCV envelope proteins E1 and E2, thus causing a reduction in virus production [43]. Celgosivir has shown some efficacy in phase II patient studies in combination with ribavirin and pegylated alpha interferon, most notably in patients that had previously not responded to existing therapy [44]. Despite encouraging data, the progression of Celgosivir to a marketed drug might be hampered by moderate potency and lack of glycosidase specificity. These characteristics are observed in all the first-generation iminosugar clinical candidates and marketed drugs and have, as discussed above, been partly responsible for the halted trials and observed side-effects of these first-generation compounds.

Perspective on first-generation iminosugars

The current status of the iminosugar field is very reminiscent of that of the nucleoside field in the mid-1970s. Then, early examples such as idoxuridine, trifluridine and vidarabine in the antiviral area and cytarabine in the anti-cancer area had demonstrated sufficient activity to generate some initial excitement. These early compounds themselves were insufficiently potent or selective to provide effective drug molecules of major commercial importance. Only with the advent of second-generation nucleoside analogues such as acyclovir and epivir (3TC) in the antiviral area and gemcitabine in the oncology area over the following years did the full potential of nucleosides begin to be realized [45]. Many other parallels with the nucleoside field can be drawn, and useful lessons we might benefit from in the development of the iminosugar field will be discussed later in this article.

The relative lack of success of the first-generation iminosugar compounds has deterred many people from working in this field rather than inspiring them to search out the full potential of iminosugars. In addition to the lack of specificity and undesirable side-effect profile of advanced candidates, iminosugars as a chemical class have evoked many of the same concerns and prejudices about their tractability and suitability as drug molecules as have been levied more generally at the field of carbohydrates, particularly with regard to synthesis, stability and the ability to conduct structure–activity optimizations. It is opportune to challenge these reservations in light of recent developments in the field and to make the case that this class is now ripe for exploitation through the identification of Seglins that can yield major new drugs in the future.

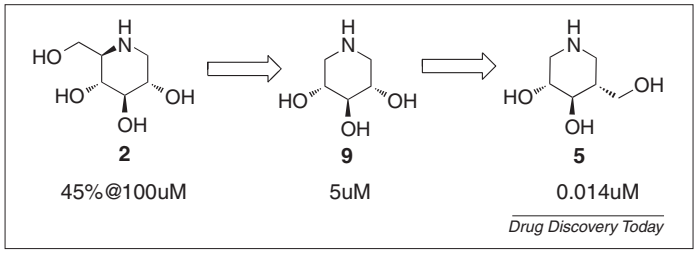

FIGURE 3

Iminosugar lead optimization. The scheme depicted shows selected data generated from screening the Seglin Platform against β-GluCerase. This sequence shows a hypothetical process of SAR optimization and does not necessarily reflect the process used in putting forward Plicera as a clinical candidate for Gaucher's disease.

Seglins: what can be done?

The key to opening up this area with second-generation compounds is the ability to access a full range of structures that reliably replicate glycospace and to optimize both potency and selectivity while retaining the favourable pharmaceutical attributes of iminosugars.

Synthesis and lead optimization

Chemical synthesis of iminosugars has often been cited as a primary reason for avoiding this area, owing to a combination of the polar nature of the polyhydroxylated structures and their stereochemical complexity. Although certain specialized technical expertise has to be acquired to work efficiently in this area, an appropriate choice of starting materials and synthetic approach can minimize synthetic complexity and in many cases, compounds of this class are as accessible as other small molecules about which there would be no such concerns. The wide variety of increasingly cheap molecules available from the chiral pool and rapid progress in the development of methods for facile stereochemical interconversions make the synthesis of target structures increasingly accessible [46–52]. Many issues around isomer separation can be avoided by judicious choice of approach, but even in those cases where isomer separation is necessary, a range of industrial-scale methodology is now available. In addition to synthesis from the chiral pool, the field is also now beginning to attract further interest in *de novo* and enzymatic synthesis, which might offer alternatives in certain cases [53–58]. The polar nature of the molecules, coupled with their lack of UV chromophores, has also required specialized analytical methods to be developed. With these capabilities now in place, it is no longer appropriate to single out this field as being one in which synthetic access and cost of goods provide a sufficient reason to avoid the area. For example, initial syntheses for the preparation of **1** suffered from low overall yields and effectively allowed only for the synthesis of milligram quantities [59–61]. Through a combination of synthetic ingenuity and judicious process development, a scalable route with an overall yield of 38% was realized [62]. Many such examples of facile routes for scale up of individual iminosugars have now been reported.

A second chemistry-related concern that can be addressed is that it will not be possible to effect lead optimization of iminosugars in the conventional manner. By way of exemplification, the hypothetical route towards the development of **5** for clinical use in Gaucher's disease shown in Fig. 3, using real data, demonstrates how fairly typical lead optimization steps could progress activity from >100 μM to 14 nM. The enzyme target β-glucocerebrosidase is responsible for the degradation of glucosylceramide to glucose and ceramide. Gaucher's patients possess a mutant form of the enzyme that can be corrected through the use of iminosugar ASSCs (see above). Taking glucose mimetic DNJ **2** as a start point for optimization, it can be seen that removal of the hydroxymethyl moiety to afford *xylo*-configured trihydroxy piperidine **9** leads to an improvement in potency from 45% inhibition at 100 μM to an IC_{50} of 5 μM. Further optimization of **9** to generate *iso*-structure **5** yields an additional major improvement in activity with an IC_{50} of 14 nM. What can be seen from this example is how the exact same principles of lead optimization that characterize traditional heterocyclic drug discovery can be applied to the iminosugar field with great success. As highlighted later, the application of these principles to the Seglins is expected to yield numerous clinical candidates across a wide range of therapy areas (see the section 'Current Seglin programmes').

One possibility would be to establish a collection of molecules in which all the important structural changes are exemplified in a systematic manner, such that the major features essential for activity can be delineated rapidly from first-round screening. A second stage would then be to address more subtle modifications around the optimal templates. We describe below a fuller account of the rationale for building such a compound collection.

Selectivity considerations

Regarding the biological profile of iminosugars, lack of specificity of action has been a primary limitation of first-generation compounds, and it is our contention that this is linked simply to the availability of analogues. The wealth of synthetic approaches now developed and available for the preparation of iminosugars has provided access to an expanding range of structures. Using the compounds already present in our own collection, we have clearly demonstrated that such a wider range of structures enables us to identify compounds with substantially improved selectivity compared with first-generation compounds, and we strongly believe that the identification of adequately selective drug candidates from this class will not be an issue. To build up a picture of the potential selectivity of action of all new compounds added to the collection, each is examined against a primary panel of enzymes (Table 1). For compounds of interest, subsequent fol-

TABLE 1

Relative inhibition of selected clinical candidates depicted in Fig. 2

Enzyme	Current clinical candidate				
	2	**3**	**5**	**6**	**7**
Non-human					
α-D-Glucosidase (rice)	Potent	Potent	Limited	Moderate	Potent
α-D-Glucosidase (*B. sterothermophilus*)	Potent	Potent	Moderate	Limited	Limited
α-D-Glucosidase (*S. cerevisiae*)	Moderate	Limited	Moderate	Limited	Limited
β-D-Glucosidase (almond)	Limited	Potent	Potent	Moderate	Moderate
Amyloglucosidase (*A. niger*)	Moderate	Potent	Potent	Limited	Limited
α-D-Mannosidase (jack bean)	Limited	Limited	Limited	Limited	Limited
β-D-Mannosidase (*C. fimi*)	Limited	Limited	Limited	Limited	Limited
α-D-Galactosidase (green coffee bean)	Limited	Limited	Limited	Potent	Limited
β-D-Galactosidase (bovine liver)	Limited	Potent	Potent	Limited	Limited
α-L-Fucosidase (bovine kidney)	Limited	Limited	Limited	Moderate	Limited
Neuraminidase (*C. perfingens*)	Limited	Limited	Limited	Limited	Limited
***N*-Acetyl-β-D-glucosaminidase (bovine kidney)**	Limited	Limited	Limited	Limited	Limited
***N*-Acetyl-β-D-hexosaminidase (*A. oryzae*)**	Limited	Limited	Limited	Limited	Limited
***N*-Acetyl-β-D-Glucosaminidase (jack bean)**	Limited	Limited	Limited	Limited	Limited
α-L-Rhamnosidase (*P. decumbens*)	Limited	Limited	Limited	Limited	Limited
Naringinase (*P. decumbens*)	Limited	Limited	Limited	Limited	Limited
β-D-Glucuronidase (bovine liver)	Limited	Limited	Limited	Limited	Limited
Human					
Acidic α-D-glucosidase	Potent	Potent	Moderate	Limited	Potent
Neutral α-D-glucosidase	Potent	Moderate	Limited	Moderate	Potent
β-GluCerase	Moderate	Moderate	Potent	Limited	Moderate
α-D-Galactosidase	Limited	Moderate	Limited	Potent	Moderate
Hexosaminidase A (human placenta)	Limited	Limited	Limited	Limited	Limited

Inhibition determined as % inhibition observed at inhibitor concentration of 0.8 mmol against non-human and 0.1 mmol against human enzymes.

low-up using mammalian enzymes is typically employed as the next step. The range of primary enzyme screening opportunities available is not restricted to those listed but can be expanded to cover additional relevant hydrolases and transferases that are themselves implicated in several disease states [63–67]. In addition to this, developments in analysing the glycome have afforded opportunities to explore a myriad of carbohydrate processes, many of which are involved in disease, through the use of lectin arrays and glycochips [68–70]. All of these methods enable full exploration and exploitation of the full potential of the glycome and are amenable to the throughput screening of iminosugars.

As can be seen from the table, problems with several compounds now known to have selectivity issues in later stages of development could have been anticipated from their profile against this panel. It is also apparent that there is clear potential for obtaining very selective compounds from this class of molecule. However, the combination of screening against the enzyme panel and screening in disease models not only is restricted to identifying selective inhibitors and the potential side-effects of hit compounds but also has utility in identifying hit compounds that exhibit no inhibitory activity against the panel. Such hits might mediate their activity through mechanisms other than hydrolase inhibition and provide leads into new areas. One example of a second-generation iminosugar that falls into this category is C2100 (see the section 'Current Seglin programmes').

Advantageous properties of iminosugars

Iminosugars have several intrinsic properties that are positive attributes as potential drug candidates.

The problem of water solubility of lead molecules has occupied a prominent place in discussions initially between chemist and biologist and subsequently between research and development departments. Many potent classical small molecules have had only marginal water solubility, and enormous resources have been deployed in trying to address this issue both in medicinal chemistry and in formulation. No such problem burdens the iminosugar field, where adequate water solubility is an important feature. Chemical stability of iminosugar molecules is another favourable attribute, particularly so where stockpiling of drug is likely to be a requirement.

Iminosugars are typically metabolically stable molecules – as might be anticipated from their highly oxygenated nature – and, therefore, are usually excreted unchanged in urine. Compared to most small polar molecules, iminosugars are well absorbed and in some cases can benefit from transport mechanisms designed to handle carbohydrates. In certain cases (e.g., Celgosivir **7**), compounds have been preferentially administered as prodrugs, and the structures of iminosugars lend themselves to that approach where necessary.

As would be expected for molecules primarily cleared by glomerular filtration, iminosugars are eliminated more rapidly in the mouse than in the rat – which, in turn, eliminates them more quickly than human [71]. Despite this, residence time in the mouse has proved adequate to enable demonstration of potency in a variety of *in vivo* models. Cellular transport mechanisms are also undoubtedly the reason why some iminosugars have been found to cross the blood–brain barrier [72]. Such properties distinguish iminosugars from other small polar molecules and further increase the attraction of using them as drug molecules.

Creating a Seglin collection

Many parallels exist between the current state of the iminosugar field and the early stages of research into nucleoside analogues in the mid-1970s. Over the past 35 years, the nucleoside field has advanced from early unimpressive lead compounds to the point where more than 20 drugs, mainly in the antiviral area, have been discovered and developed to the market. There was no concerted systematic evaluation of the field as a whole by any one or more group(s) to exploit the opportunity. It is interesting to reflect that had one possessed a set of the right nucleoside analogues (Fig. 4) at the outset and screened them against the important viral classes as they assumed prominence, one would have obtained a lead into each of the different series that yielded marketed products.

This set of approximately 200 members would have included both purine and pyrimidine natural bases and sugar modifications including D-ribo, 2′-deoxyribo-, 2′3′-dideoxyribo-, arabino-, lyxo-, xylo- and corresponding analogues in the L-series, in addition to acyclic analogues. With the benefit of hindsight, there is an opportunity to exploit the iminosugar field in a more systematic way and in a much shorter timeframe than was achieved with nucleoside analogues. The required number of compounds in a corresponding fundamental set will be higher, reflecting the more diverse templates and increased stereochemical complexity of the glycospace mimicked by iminosugars. This will be offset by an additional advantage because – unlike nucleosides, which have found utility principally as antiviral agents – we anticipate that iminosugars will have utility in a wide range of therapeutic areas across the glycobiology spectrum and beyond. A selection of therapy areas and biochemical targets are discussed in detail below (see the section 'Current Seglin programmes').

Early syntheses of iminosugars primarily focussed on the synthesis of natural compounds and close analogues rather than library creation [73–79]. Libraries, when generated, were primarily directed at modification of a defined stereochemical scaffold (e.g., by *N*-alkylation or *O*-esterification) rather than through the synthesis of a larger set of stereochemically diverse scaffolds [80–86]. The scaffolds used for these libraries were typically arrived at as naturally occurring iminosugars, close analogues or through specific design based on a known biological target. Although such methods have led to the generation of two marketed drugs (**3** and **4**) and several others in clinical development, they fail to capitalize on the true potential that a structurally and stereochemically diverse iminosugar collection mapping all glycospace would offer. As our knowledge and understanding of glycobiology and specifically its role in disease has increased over the past 10 years, the potential opportunities available for screening a diverse portfolio of glycomimetics have also grown. As highlighted earlier, if such a collection had existed within the nucleoside field, then it is highly likely that the time taken to identify and develop current marketed drugs would have been greatly reduced. An opportunity exists to apply these principles to the generation of a structurally diverse collection of iminosugars defined through the systematic evaluation and appropriate replication of glycospace. Advances in synthetic and analytical methods now allow for the compilation of such a rationally and systematically designed set without limitations imposed by accessibility.

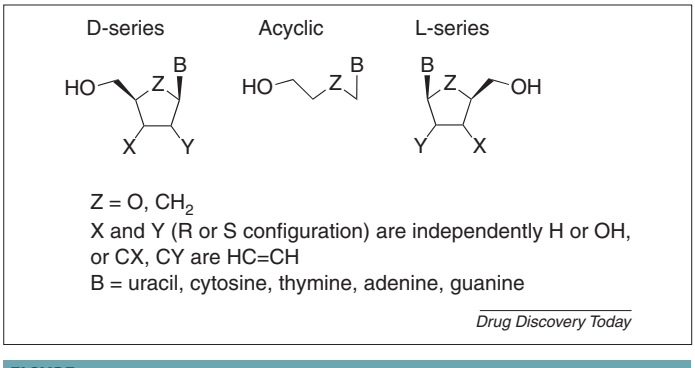

FIGURE 4

Ideal nucleoside screening set. Stereochemistry across the D- and L-series depicts that of the four pentose sugars (arabinose, lyxose, ribose and xylose) affording eight scaffolds. Mono-deoxygenation of either enantiomer at position 2 (Y as depicted in scheme) or 3 (X) yields an additional eight scaffolds. Di-deoxygenation allows for a further two scaffolds, with the unsaturated equivalent and acyclic form affording one scaffold each. The combination of 22 scaffolds and five nucleobases generates an initial screening library of 210 structures. In all relevant cases, the glycosidic bond is β-configured.

There are several ways in which one can conceive of a representative set of iminosugars for the construction of such a screening collection, an attractive option being on the basis of carbohydrate mimicry rather than on purely chemical considerations. Providing representation of the full range of carbohydrate molecules to be mimicked and complete coverage of the full range of attendant carbohydrate stereochemistries within each major template class is clearly the objective for this approach, which is described below. Subsequent objectives will include representation directed towards specific classes of biological targets and, in particular, making use of what is learnt from screening results, as well as from advances in our knowledge and understanding of glycobiology.

Turning first to the hexoses, we identified four basic templates with which to mimic each of the sixteen relative stereochemistries possible for the eight hexose sugars, affording an initial set of 64 structures (Fig. 5). The same methods have also been applied to the four pentose sugars, affording an additional set of structurally diverse compounds. These provide a comprehensive basic set covering all possible carbohydrate stereochemistries for initial screening. Results from screening at this level thus identify compounds of interest to serve as starting points for more detailed analogue synthesis and evaluation.

Establishing this initial phase of the collection required development of extensive expertise in iminosugar synthesis and analysis and facilitated a second phase of work focused on introducing additional motifs, ring systems and isomeric forms. Analysis of the structural diversity present in mammalian glycospace highlights the key part played by several functional motifs in many carbohydrate handling processes [87]. The occurrence of other functional groups (such as carboxylic acids), amides or modifications (such as deoxygenation) demonstrates that carbohydrate recognition is not restricted to the action of hydroxyls alone [88]. In the second phase of development, this functional diversity is being replicated across the four key templates to provide an extensive and unique screening portfolio. In addition to this, structural analogues such as the indolizidines and pyrrolizidines, as well as the *iso*-analogues, enable elaboration of the base set to encompass the full diversity of glycospace in a

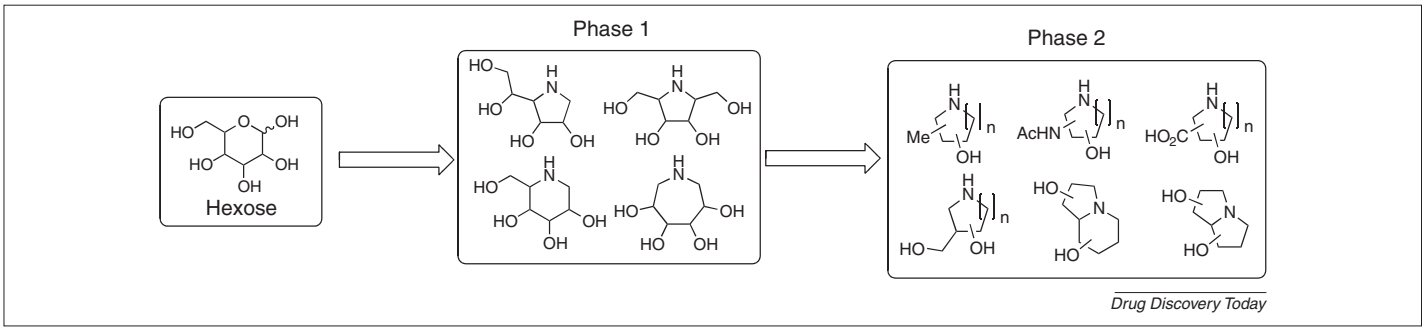

FIGURE 5

Development of the Seglin screening set. The process of replicating therapeutic glycospace has been divided into two phases. Phase 1 concerns replication of the eight hexose forms in four structurally defined Seglin templates across both enantiomeric series, affording a total of 64 structures (because of symmetry relationships in the 2,5-pyrrolidnes and azepanes, this first phase comprises 52 stereochemically distinct structures). Extension of this process to cover additional recognition motifs (e.g., acid, amide, amine, and deoxy) cyclic forms (e.g., pyrrolizidine, indolizidine) and *iso*-analogues is covered in phase 2. Completion of the initial screening set provides a comprehensive base covering all relevant glycospace for screening against a range of carbohydrate and non-carbohydrate targets the results of which feed back into the synthesis program after traditional SAR procedures.

comprehensive fashion. This offers many advantages over traditional small-molecule libraries and provides for strong intellectual property protection. Seglin technology enables access to biological targets that have not been fully exploited by conventional drug discovery and the platform has been validated in several therapy areas including anti-infectives, cancer and type II diabetes (see the section 'Current Seglin programmes').

Opportunities for Seglins

The full impact of the pioneering work of Dwek, Varki, and others in exploring the importance of glycobiology is becoming increasingly apparent [89–91]. The range of new targets across different therapeutic areas is vast and current efforts barely scratch the surface of what might be possible in the search for new drug molecules [92–96]. Figure 6 shows an overview of current areas

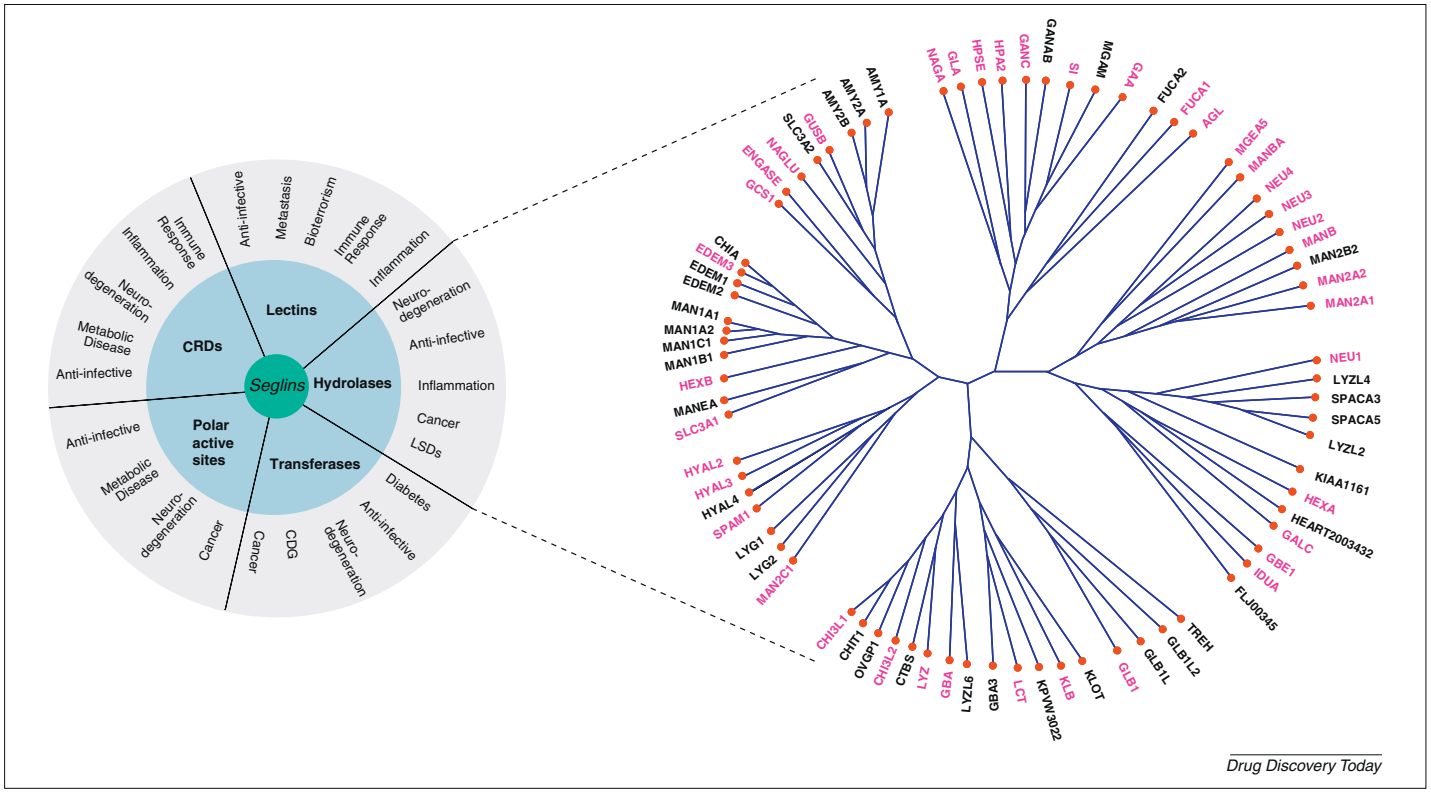

FIGURE 6

Seglin screening opportunities. The wheel on the left depicts the range of therapeutic targets amenable to screening with the Seglin platform. The inner section highlights the breadth and variety of target classes from glycoside hydrolases to carbohydrate recognition domains (CRDs) and polar active sites. The outer section identifies some of the therapeutic areas associated within each class. Looking at one target segment in greater detail, in this case the mammalian glycoside hydrolases, highlights the wealth of opportunity for direct drug discovery with the Seglin platform. In this example, those enzymes that have an association with disease are highlighted in red. This analysis was based on a combination of literature survey and review of the OMIM and Entrez Gene databases (http://www.ncbi.nlm.nih.gov/).

of interest on the basis of particular drug target classes, all of which can be addressed by Seglins. This is exemplified below by considering the area of the glycoside hydrolases.

Glycoside hydrolases hydrolyse the glycosydic bond between two or more carbohydrates or between a carbohydrate and a non-carbohydrate moiety and can be classified into families on the basis of substrate specificity and/or molecular mechanism. Currently, there are 115 families of glycoside hydrolases, of which 29 are human. Figure 6 shows the diversity across the 29-family-member human glycoside hydrolases demonstrated by phylogenetic analysis. More than 50% of the human glycoside hydrolases identified to date are believed to be implicated in disease and metabolic disorders (Fig. 6). This represents a substantial opportunity for direct drug discovery and provides an excellent opportunity for Seglins. When one extends this analysis to bacterial, viral and protozoal glycoside hydrolases, the number of potential targets greatly increases, offering extensive opportunities for the development of novel anti-infective agents. Further extension of this analysis across the glycobiology spectrum to include transferases, lectins and glycan-binding proteins reinforces the extent of the opportunities.

In most cases, these are targets related to carbohydrates and carbohydrate processing, but the opportunities for Seglins to interact with targets as small polar molecules with special properties rather than through carbohydrate mimicry open up a raft of additional targets. These opportunities are not just restricted to the glycobiology field but extend to many additional targets, across many protein families (Fig. 6).

Enzyme targets that have polar active sites and that, to date, have proved largely intractable were considered to present a suitable challenging opportunity to investigate this possibility with Seglins. Their low molecular weight (typically <200 Da) and polar functional nature makes them attractive not only as leads in their own right but also as fragments. Such small-molecule scaffolds provide excellent templates for the exploration of such chemotherapeutic targets. In line with our interests, in Summit we have successfully screened our Seglin Platform against HCV NS3 helicase (see the section 'Current Seglin programmes'). Structural studies with compounds of interest are in progress, and the extension of this approach to other suitable targets is being actively pursued.

Current Seglin programmes

By way of substantiating the claims made above about the potential of Seglins, the section below gives a brief summary of selected programmes in progress [97–104] (Table 2).

Oncology

C2100 is a lead anti-cancer candidate being developed by Summit as a treatment for melanoma and other advanced cancers. Melanoma is one of the most common forms of malignancy with a very poor five-year survival rate of less than 20% for advanced disease and no treatments that improve prognosis. The compound has shown proof of concept in *in vivo* models of metastatic melanoma, working against a novel therapeutic target and triggering a specific immunological effect. This effect is to enhance cell-mediated killing through the innate immune system, a property that could also be used to enhance the therapeutic effect of existing biological cancer treatments such as Herceptin™ and Rituxamab™ [105]. C2100 mediates its therapeutic effect through interaction with a novel C-type lectin receptor, leading to an enhanced Th1 cytokine profile and effect in an antigen-challenged environment.

Antivirals

Current standard of care for hepatitis C (HCV) consists of combination therapy with peginterferon alfa and ribavirin for 24 or 48 weeks, depending on the patient's HCV genotype. The limitations of this treatment leave major unmet needs in HCV requiring new drugs with improved tolerability and efficacy. Summit is using its Seglin technology against a set of HCV targets that includes the

TABLE 2

Active areas of Seglin research.

Therapeutic area		Target	Organization	Refs
Antivirals	HCV	NS3 Helicase	Summit plc	http://www.summitplc.com
		p7	Astbury Centre/MRC Virology Unit	[97]
	Broad-spectrum	Glucosidase I/II	United Therapeutics	http://www.unither.com
		Glucosidase I/II	Drexel Institute	[98]
	HIV	Glucosidase I/II	Oxford Glycobiology Institute	[99]
Genetic disorders	Cystic fibrosis	–	University of Oxford/Summit plc	[100]
		Glucosidase I/II	Actelion	http://www.actelion.com
Neurodegeneration	Parkinsons Disease	GCase	Amicus Therapeutics	http://www.amicustherapeutics.com
	Alzheimer's Disease	Protein misfolding		
Bioterrorism	–	C-type Lectin	Summit plc	http://www.summitplc.com
Oncology	Melanoma	C-type Lectin		
	Glioblastoma	GMII	EPFL/CHUV Lausanne	[101]
Angiogenesis	Diabetic retinopathy	MMP/TACE	Gifu Pharmaceutical University	[102]
Metabolic disease	Type II diabetes	–	Summit plc	http://www.summitplc.com
	Type II diabetes	GlcCer synthase	Department of Medical Biochemistry, University of Amsterdam	[103]
Cardiovascular	Atherosclerosis			[104]

Selection of therapy areas in which iminosugars have shown activity in the past two years. The lysosomal storage disorders have not been included for the sake of clarity because work in these areas has resulted in several clinical candidates discussed in detail within this article. Many of the *in vitro* screens have been performed directly against cell-based assays, demonstrating the ability of iminosugars to cross the membrane and confer activity within the cell. Another noteworthy observation is that all the positive *in vivo* animal studies performed have delivered the iminosugar through the oral route, again confirming the potential for these molecules to become drugs.

NS3 helicase protein, an enzyme that unwinds the double-stranded RNA complex enabling the virus to replicate. HCV helicase is a validated target that has proved intractable for more than a decade despite the major efforts made by the pharmaceutical industry. Preliminary studies have identified compounds having low micromolar activity against the enzyme, which are being further progressed. This serves to exemplify the wider potential of Seglins to access certain classes of previously intractable targets.

The HCV p7 protein plays a crucial part in the development and replication of the virus and has attracted the interest of several groups, including Drexel and the Oxford Antiviral Drug Discovery Unit, as a potential target for new therapies. Several compounds inhibit p7 function, including the first-generation iminosugar *N*-nonyl DNJ [97]; however, this compound exhibited a genotype-dependent pattern of sensitivity. Furthermore, *N*-nonyl DNJ had a global effect on glycoprotein processing, raising questions over whether its antiviral effect is a consequence of inhibition of the p7 channel or through the modulation of viral glycoproteins and highlighting the need for developing more specific and potent compounds against multiple p7 sequences.

Flaviviruses – in addition to HCV, potential bioterror threats and the need for improved or alternative compounds for areas where some treatments already exist – provide a wide range of opportunities for the Seglin platform. Summit has already identified compounds active against herpes viruses (HSV, VZV and CMV) and respiratory viruses such as RSV where there are continuing unmet needs. Compounds active against HIV, dengue, Vaccinia virus (a surrogate for smallpox) and both monkeypox and cowpox have also been obtained. The literature reports also describe compounds of this class as active against other viruses such as hepatitis B [106,107]. Work is continuing to progress these opportunities.

Recent pioneering advances in drug delivery systems have prompted the Oxford Antiviral Drug Discovery Unit to re-evaluate the usefulness of Zavesca as a potential addition to the anti-HIV arsenal [99]. By targeting the host pathway of calnexin-mediated glycoprotein folding, the researchers hope to have exposed a major weakness within HIV, minimizing the possibility for viral escape.

Diabetes

There are currently approximately 250 million people worldwide that suffer from diabetes, and this number is estimated to grow to 380 million by 2026. No available therapy for either type I or type II diabetes completely restores normal function, so even in the best responding patients there is still a risk of many complications including hypertension, retinal damage, renal failure and cardiovascular disease. New approaches to meet this high unmet medical need remain a top priority in metabolic disease research.

SMT 14224, which is being developed by Summit, shows excellent efficacy results in well-validated acute and chronic *in vivo* models, increasing insulin levels via a glucose-dependent mechanism, lowering HbA1c levels (a clinical measure of disease control) and lowering overall blood triglyceride levels. The compound's effects are observed without the characteristic weight gain observed with many other diabetes treatments and suggest that this second-generation iminosugar operates via a new mechanism of action. Unlike Glyset, SMT14224 does not function via glucosidase inhibition nor does it inhibit the activity of SGLT2 encouraging glucose excretion, which is the mechanism of action of the advanced clinical-stage compound Dapagliflozin. The chemical structure combined with the *in vivo* profile of SMT14224 suggests carbohydrate sensing as a potential mechanism. Additional studies are ongoing to reinforce data already generated to confirm the potentially unique clinical position of SMT14224 and to provide further insight into the compound's mechanism of action.

Cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive genetic disorder that leads to a multi-system organ dysfunction and is the most common fatal genetic disorder in the Caucasian population [108]. CF involves all epithelial cells, classically impacting the lungs, sinuses, pancreas, liver, bile ducts, intestines, reproductive tract, bones and sweat glands. The most serious consequence of CF is respiratory disease. CF is caused by functional defects of the CFTR protein, a chloride channel that controls ion and water content in epithelial cells. Many mutations can affect the CFTR protein, delF508 being the most frequent one. This mutation gives rise to a CFTR protein that retains some function but is not transported properly to the plasma membrane. As a result, ion and water movements through the epithelial cell membrane are abnormal, causing mucus hyperviscosity. Current therapy for CF involves mucolytics, antibiotics to prevent bacterial colonization and lung infection, and nutritional management. Recent studies have shown that the second-generation compound *iso*LAB is able to correct the transfer of mutated CFTR protein to the plasma membrane, restoring its function [100]. Unlike Zavesca, which has also shown efficacy in CF models [109], *iso*LAB is non-inhibitory towards glycoside hydrolases, suggesting a novel mechanism of action for this Seglin. These observations suggest that pharmacological agents such as *iso*LAB could improve not only the chloride channel activity of CFTR but also other CFTR-dependent cellular functions without the side-effects exhibited by the first-generation iminosugars.

Concluding remarks and future directions

Iminosugars are an extremely interesting and versatile class of compounds that are special in several ways and offer many advantages as potential drug candidates. At one level, they occupy a different area of chemical space to compounds typically found in pharmaceutical screening libraries and 'new chemical space' is a concept much prized and sought after by pharmaceutical companies seeking to expand their range of structures. Increasingly, the ability of these small polar compounds to access targets previously found to be intractable to the more conventional screening libraries is generating much interest. At another level, the structural characteristics they share with carbohydrates make them unique in providing an opportunity to access carbohydrate-related targets. In all cases, they benefit from privileged drug-like properties.

The first-generation iminosugars have already demonstrated their potential to provide drug molecules, with two marketed compounds and several more in clinical development. Recent advances in glycochemistry and glycobiology have served to dispel many of the reservations associated with the carbohydrate field generally and reveal the full extent of the opportunity that can be realized using Seglins and which is now wide open for exploitation of its full potential. Key to this is the assembly of a sufficiently extensive and diverse screening collection to enable identification of suitable starting points for optimization coupled

with the capabilities to enable efficient identification of the best candidate compounds having regard for potency, selectivity and drug-like properties. Biologically, the range of potential targets for Seglins is extremely rich in therapeutic utility, containing both soluble proteins and receptors and covering many protein families including glycoside hydrolases and transferases, inositol utilizing or metabolizing enzymes, carbohydrate receptors and polar active sites.

The Seglin platform, which has already been validated in several therapy areas, is extremely rich in therapeutic utility. Full exploitation of this unique compound class represents a major opportunity for drug discovery in the near and medium term.

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