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Recent progress and advanced technology in carbohydrate-based drug development

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Carbohydrates, one of the most abundant and widespread biomolecules in nature, play indispensable roles in diverse biological functions, and represent a treasure trove of untapped potential for pharmaceutical applications. Here, we provide a brief overview of carbohydrate-based drug development (CBDD) over the past two decades. More importantly, advanced techniques and methodologies related to CBDD are emerging, including enzymatic synthesis, metabolic engineering, site-specific glycoconjugation, carbohydrate libraries and microarrays as well as carbohydrate-gut microbiome evaluation. These technologies have dramatically accelerated the speed of CBDD. The recently approved drugs and emerging techniques summarized herein will inspire new sights into potential opportunities to discover novel carbohydrate drugs.

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Introduction

Carbohydrates are abundant resources in nature and play crucial roles in many biological processes such as cell–cell recognition, microorganism infection, immune-response and cell proliferation [1•,2]. The essential roles of carbohydrates in various physiological processes suggest that carbohydrate-based drugs can demonstrate high efficacy and specificity as novel therapeutic approaches [3,4]. To date, more than 170 commercial carbohydrate-based drugs have been successfully approved for the market by the United States Food and Drug Administration (FDA), European Medicines Agency (EMA), the Japan Pharmaceuticals and Medical Devices Agency (PMDA), and the Chinese National Medical Products Administration (NMPA). The versatile carbohydrates of these approved drugs include polysaccharides/oligosaccharides, small molecule glycosides and glycomimetics, glycopeptides and glycoproteins as well as carbohydrate-based vaccines (Figure 1).

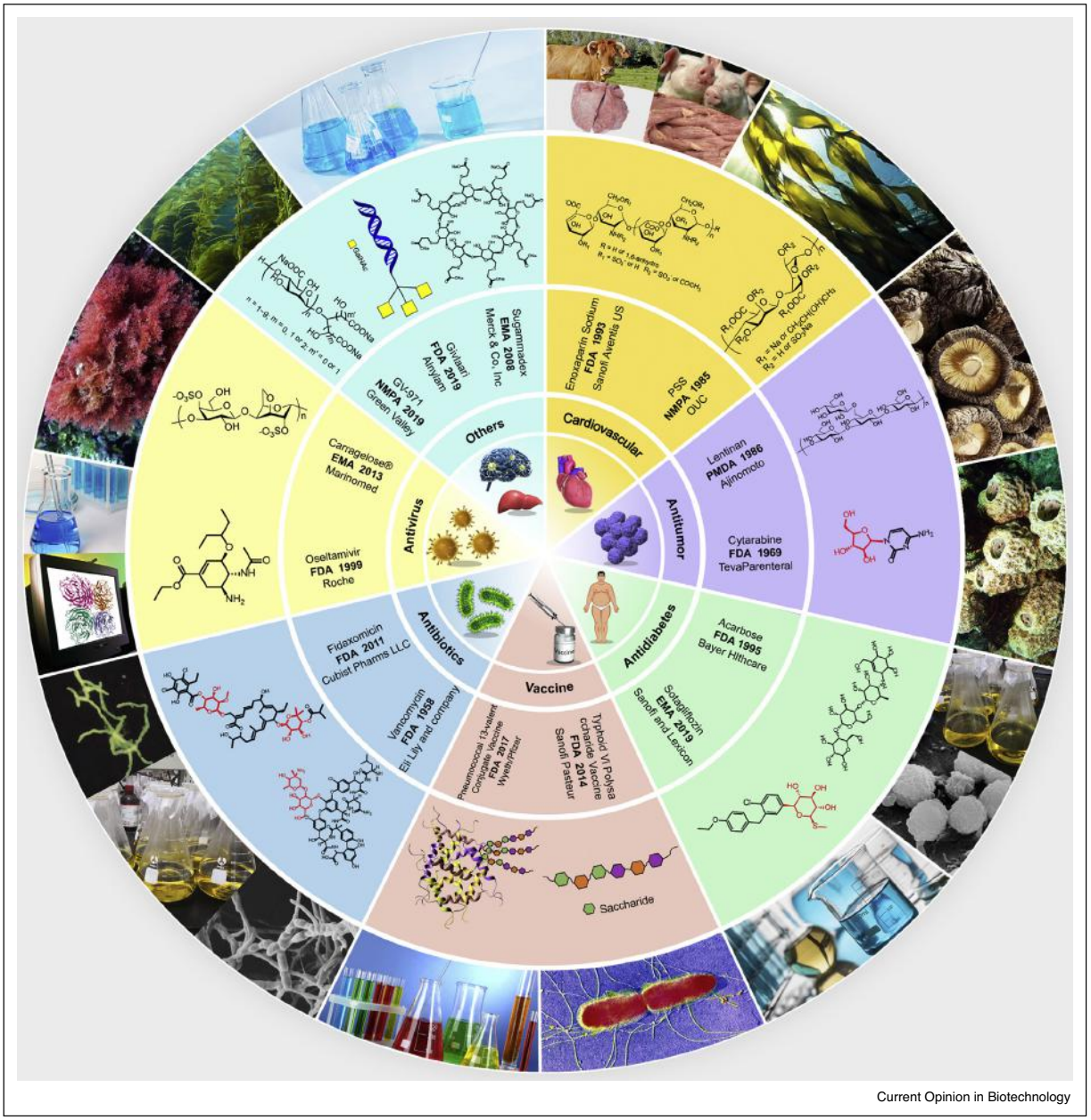
Because of the complex structure and diverse function of carbohydrates, it is worth noting that modern CBDD still lags far behind nucleic acid and protein drug development. Nonetheless, tremendous progress has been achieved in new technologies and methodologies in the past two decades [5••], dramatically boosting the discovery of novel carbohydrate-based drugs. Chemoenzymatic approaches, metabolic engineering, and site-specific glycoconjugation have significantly improved the efficiency of accessing more complex carbohydrates. The combination of computer-assisted virtual screening with carbohydrate databases and microarrays has sped up the discovery of bioactive substrates. Additionally, the explosive growth in gut microbiology provides useful tool to study the orally administered carbohydrates.

In this review, the progresses of the representative carbohydrate-based drugs approved by FDA, EMA, PMDA or NMPA in the last two decades are briefly reviewed. The emerging technologies for CBDD are commented on in terms of innovations and potential opportunities. In short, recent advances and achievements bring more opportunities in CBDD and provide reliable references for the development of novel carbohydrate drugs in the future.

Marketed carbohydrate-based drugs developed over the past two decades

Marketed polysaccharide/oligosaccharide drugs are conventionally developed from the isolated or synthetic glycans, such as heparin, hyaluronic acid, hydroxyethyl starch and fondaparinux, and so on. Small molecule glycosides and glycomimetics have also been well explored as antibiotics and glycosidase inhibitors for treating different diseases. Over the past two decades, the sources and indications of carbohydrate-based drugs are rapidly

Figure 1



Representative carbohydrate-based drugs approved by the FDA, EMA, Japan PMDA, and Chinese NMPA for the treatment of different types of diseases. The representative carbohydrate-based drugs listed herein include anticoagulants and cardiovascular drugs (e.g. Enoxaparin sodium, Propylene glycol alginate sodium sulfate), antitumor drugs (e.g. Lentinan, Cytarabine), antidiabetic drugs (e.g. Acarbose, Sotagliflozin), vaccines (e.g. Typhoid Vi Polysaccharide vaccine, Pneumococcal 13-valent conjugate vaccine), antibiotics (e.g. Vancomycin, Fidaxomicin), antiviral drugs (e.g. Oseltamivir, Carragelose®) and other related drugs (e.g. GV-971, Givlaari, Sugammadex). The approved time and corresponding institutions of these drugs are also indicated in Figure 1.

growing along with the well-understood action modes. In the first section of this review, we concentrate on the marketed carbohydrate-based drugs developed since the beginning of this century.

Polysaccharide/oligosaccharide derived drugs

Heparin and heparinoids

Heparin, one of the most successful anticoagulant drugs, has drawn global attention due to the heparin

contamination crisis in 2007. The FDA, the United States Pharmacopeial Convention (USP), and international stakeholders collaborated to redefine the quality expectations for heparin, thus bringing a better controlled and less susceptible medicine to the market [6]. Fondaparinux, a fully synthetic pentasaccharide inspired from heparin active site, was explored for the treatment of deep vein thrombosis with reduced risk of the side effects in clinic. In addition, bioengineered heparin and enzymatically synthesized oligosaccharides have been well established by the Linhardt and Liu groups in recent years, offering alternative solutions to access synthetic heparin and its derivatives [7].

Since the epidemic outbreak of novel coronavirus (SARS-CoV-2) in 2019, antiviral drugs are still considered the most effective therapeutic approach. Many studies have indicated that heparin and derivatives can effectively inhibit the interaction of SARS-CoV-2 spike glycoprotein (SGP) with the cell surface angiotensin-converting enzyme 2 (ACE2) receptor [8,9]. Recently, Chimerix (CMRX) initiated a phase II/III clinical study to evaluate Dociparstat sodium (DSTAT), a heparin derivative for the treatment of SARS-CoV-2 infection. Moreover, Carragelose® (Figure 1) [10], an innovative viral nasal spray, is being explored as adjuvant therapy against SARS-CoV-2. Substantial research has also indicated that heparinoids can potentially serve as broad-spectrum inhibitors of different viruses and provide the basis for the development of novel antiviral drugs [11•,12,13•].

Propylene glycol alginate sodium sulfate (PSS, Figure 1), an alginate derivative obtained by chemical modification, is developed as an effective cardio-cerebrovascular drug [14••]. Another marine sulfate polysaccharide Haikun Shenxi Capsule (HSP) was approved by the NMPA in 2003 for the treatment of chronic renal failure, and its degraded oligosaccharides are under investigation for the treatment of diabetes [15]. The carbohydrates derived from marine organisms commonly contain abundant uronic acid and sulfate groups and are promising resources for the development of heparinoid drugs.

Immunomodulatory drugs

The innate immune system can be activated by pathogen-related molecular patterns (PAMP), such as β-glucan and mannan, through specific pattern recognition receptors (PRR), including toll-like (TLR) and C-type lectin-like receptors, and so on [16]. Coriolusversicolor polysaccharide (polysaccharide K) and Lentinan (Figure 1), first approved by PMDA, have been used as antitumor adjuvants for more than 40 years in Asian countries. Recently, Merck developed β-glucan as an innate immune activator to treat advanced melanoma and colorectal cancer (NCT02981303). β-1,3/1,6-glucan (BG136) is currently under preclinical study for the development of new antitumor immunotherapeutic drug [17]. More recently, it was reported that the β-glucan-trained innate immunity (TII) promotes anti-tumor activity involving appropriate rewiring of granulopoiesis [18]. In brief, natural polysaccharides possess a great potential in the future development of immunomodulatory antitumor drugs.

Small molecule glycosides and glycomimetics

Antibiotics including macrolides, aminoglycosides, everninomycins and glycopeptides contain versatile glycan ligands. Their glycan portions primarily modulate the solubility, stability and molecular recognition of antibiotics [19]. Fidaxomicin, a representative antimicrobial agent approved by FDA for the treatment of *Clostridium difficile* infection (CDI) in adults, includes two different glycan components (Figure 1). Carbohydrate modification, has been proved to be a powerful strategy to combat bacterial resistance [20]. For example, modification of cladinose in Erythromycin A has decreased the bacterial resistance of Erythromycin, which provided new versions of macrolide antibiotics. Additionally, the sugar moieties also play crucial roles in blocking virus infection. Zanamivir, Oseltamivir, and Peramivir, developed as specific inhibitors of neuraminidase (NA), are the most commonly prescribed drugs for the treatment of influenza A virus (IAV) [21•,22].

Carbohydrates play crucial roles in biological processes, it is therefore not surprising that glycomimetics could have a powerful potential for drug development. Biotechnology companies are dedicated to the discovery of novel glycomimetic drugs to address urgent medical needs. Glycomimetics are primarily designed as small-molecule inhibitors of glycosidases. Three α-glucosidase inhibitors, Voglibose, Miglitol and Acarbose, have been developed for controlling type-2 diabetes. In recent years, the sodium-dependent glucose transporter 1/2 (SGLT1/2) inhibitor 'gliflozins' have shown a promising future as antidiabetic agents. Sotagliflozin (Figure 1), an SGLT-1/SGLT-2 dual-effect inhibitor, is clinically used as an adjuvant drug with insulin for adult patients with type-1 diabetes (T1D) [23,24]. Moreover, the iminosugar Miglustat, a glucocerebrosidase inhibitor, has been developed to manage type I Gaucher disease. Rivipansel and Uproleselan are under clinical evaluation for their ability to treat a variety of solid tumor and fibrotic diseases.

Advanced technologies and toolkits for carbohydrate-based drug development

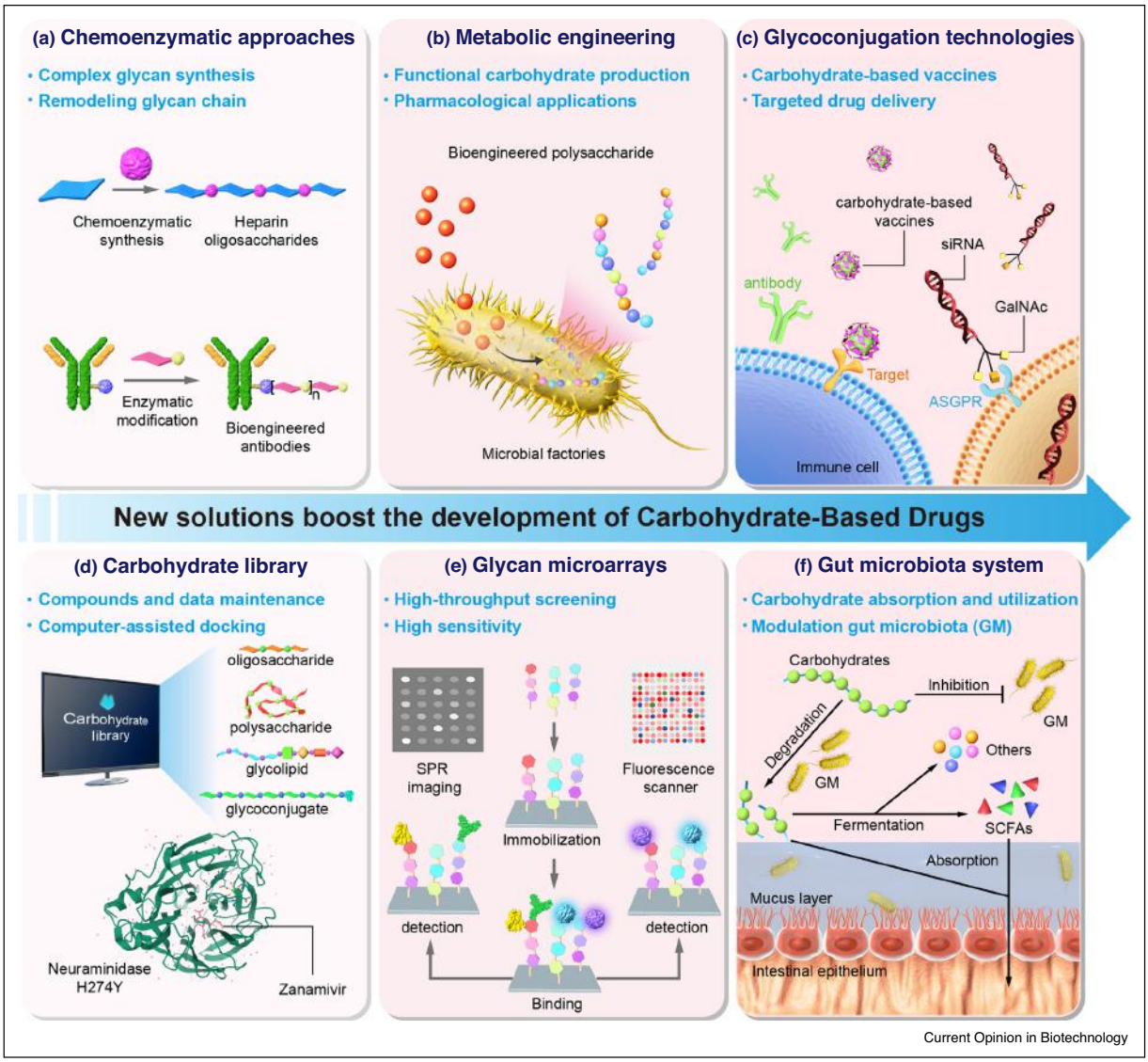
Advanced technologies and toolkits are gradually being established for accelerating CBDD mainly in two aspects (Figure 2). One category of technologies was adopted to provide better and easier access to complex carbohydrates with well-defined structures which relies on enzymatic approaches, synthetic biology and metabolic engineering as well as site-specific glycoconjugation and targeted drug design (Figure 2a–c). In addition to above tools, another group of technologies were employed to facilitate accurate quantifying of carbohydrate structures and further

determining their bioactivities, which include: construction of carbohydrate databases with computer-assisted virtual screening, carbohydrate microarrays and carbohydrate-gut microbial interaction (Figure 2d–f). In this part, we will summarize and discuss these tools.

Chemoenzymatic synthesis

The chemoenzymatic synthesis has proven to be a powerful approach to obtain complex polysaccharides/oligosaccharides, glycolipids, glycoengineered antibodies, and glycosylated natural products [25] (Figure 2a).

Figure 2



Advanced technologies and toolkits for carbohydrate-based drugs development. One category of technologies was adopted to provide better and easier access to complex carbohydrates with well-defined structures **(a)–(c)** and another group of technologies were employed to facilitate accurate simulation of carbohydrate structures and further exploring their bioactivities **(d)–(f)**. (a) Chemoenzymatic synthesis provides a powerful approach to acquire structurally well-defined polysaccharides and oligosaccharides, complex *N*-glycans as well as glycoengineered antibodies and glycosite-specific antibody-drug conjugates. (b) Metabolic engineering supplies abundant carbohydrate materials such as functional polysaccharides, glycoproteins, and glycosylated natural products. (c) With improved understanding of the interaction between carbohydrates and other functional biomolecules, glycoconjugation technologies are developed for multivalent glycoconjugate vaccines and targeting therapeutics. (d) Carbohydrate libraries and databases containing sufficient substrates for bioactive screening and computer-assisted simulation and docking. (e) Glycan microarrays are powerful tools for high-throughput screening of bioactive substrates with trace amounts of samples. (f) The gut microbiota has proven to be a useful system for the pharmacological evaluation of carbohydrates in recent years.

Tremendous progress has been made in recent years in the synthesis of heparin polysaccharides and oligosaccharides [26] as well as complex mammalian *N*-glycans [27]. Additionally, the chemoenzymatic synthesis of glycoengineered-Fc antibodies and glycosite-specific antibody-drug conjugates could dramatically improve the half-life and effector response of therapeutic monoclonal antibodies [28]. A promiscuous *C*-glycosyltransferase identified from *Aloe barbadensis* has been recently employed to synthesize *C*-glycosides with potent inhibition on SGLT2 [29]. The advantage of using a chemoenzymatic approach is the well-controlled assembly of biomacromolecules with stereo-selective and regio-selective carbohydrates. Substrate specificity and scale-up limitation of enzymes still need to be addressed for widespread applications.

Synthetic biology and metabolic engineering

Metabolic engineering using microbes is a promising approach for the large-scale production of complex polysaccharides and glycoproteins (Figure 2b) [30,31••]. Some functional polysaccharides (e.g. levan, heparosan) have been well explored by metabolic engineering, but the industrial applications of pharmacological polysaccharides (e.g. heparin) are still currently limited by the challenging post-modifications and downstream tailoring process. One successful application is the bioengineered expression of glycoprotein drugs including monoclonal antibodies, recombinant proteins, fusion proteins, growth factors, and cellular factors, and so on. The majority of FDA-approved biological therapeutics are recombinant glycoproteins. Glycans play significant roles in pharmacokinetics, tissue targeting, and the biological activities of glycoprotein drugs [28]. One example is Erythropoietin (EPO) with an identical protein sequence but variations in the structure of the pendant glycans, and the optimization of these glycan structures can help prolong its serum half-life [32]. Many studies have focused on glycolytic chain editing to improve the biocompatibility and bioactivity of glycoprotein drugs. The Danishefsky group reported the first total synthesis of homogeneous erythropoietin with consensus carbohydrate domains [33]. Therefore, the combination of biosynthesis and a chemical reaction could enable concise production of homogeneous glycoproteins for drug development.

Glycoconjugations for vaccine development and targeted drug delivery

Over the past few decades, the glycoconjugation of biomacromolecules has attracted great interest (Figure 2c) [34], especially in the field of glycoconjugate vaccines. Conventional conjugate vaccines mainly utilize natural bacterial capsular polysaccharides. Typhoid Vi Polysaccharide vaccine (Figure 1) is one of the most successful carbohydrate vaccines. Chemical and enzymatic approaches as well as the liposomal encapsulation of polysaccharides have been applied in vaccine development [35]. The licensed Pneumococcal 13-valent conjugate vaccine (Figure 1) was successfully developed using a chemical approach. Tumor-associated carbohydrate antigens are gradually understood in terms of their effects on immune response. The Wong group has developed a chemical approach to synthesize the carrier-based glycoconjugates with the unique epitope markers such as Globo-H, Lewis Y, Tn, and so on, [36]. Multiple carbohydrate-based conjugate cancer vaccines are under clinical studies in different countries [37,38]. Other glycoconjugate vaccine candidates have also been designed to combat tropical diseases, bacterial and viral infections.

Carbohydrates can effectively bind to cell surface receptors resulting in various physiological processes offering advantages in targeted drug delivery (Figure 2c). Givlaari (Figure 1) unveils a new era of RNAi therapy. The concept of givlaari is the conjugation of small interfering RNA (siRNA) to the multivalent *N*-acetylgalactosamine, an asialoglycoprotein receptor ligand, facilitating targeted delivery to hepatocytes [39•,40]. Carbohydrate-based transport is an effective therapeutic approach and has been comprehensively discussed in many reviews [41,42].

Carbohydrate library and computer-assisted techniques

Over the past two decades, several carbohydrate databases and websites have been well established, including the Consortium for Functional Glycomics (CFG, http://www.functionalglycomics.org/), Glycosciences.de (http://www.glycosciences.de), CSDB (http://csdb.glycoscience.ru) and Glyco3D (http://glyco3d.cermav.cnrs.fr), which provide detailed information on carbohydrate structures, taxonomy, bibliographies and NMR data, and so on. These carbohydrate databases and websites are useful tools for the structure analysis of saccharides and offer excellent opportunities for research in bioactivity profiling [43]. Moreover, the carbohydrate entity library has also been critical for drug screening and drug development. For example, a marine polysaccharide/oligosaccharide library has been established at Ocean University of China, which provided material basis for the development of marine drugs. Novel drugs are now being explored from this marine carbohydrate library [17].

The modern drug discovery research has made great progress during the big data era. The computer-assisted techniques have shown to be useful tools for the rapid discovery of bioactive molecules from the massive carbohydrate databases [44] (Figure 2d), during which, the artificial intelligence approaches such as deep learning and relevant modeling studies provide promising solutions in elucidating carbohydrate structures with high complexities and molecular flexibilities [45].

Carbohydrate microarray

Carbohydrate microarray, with remarkably high-throughput analysis of carbohydrate-mediated biological interactions, is another powerful tool for the efficient discovery

of carbohydrate lead compounds (Figure 2e) [46••]. As early as 2002, the Feizi group fabricated microarrays with lipid-conjugated oligosaccharides (termed neoglycolipids) for the first time. The noncovalent neoglycolipid-based microarrays provided a revolutionizing tool for studying the molecular basis of protein–carbohydrate interactions both in endogenous recognition systems and host–pathogen interactions. Over the past two decades, a variety of carbohydrate microarrays were constructed using noncovalent and covalent methods, including the site-specific and site-nonspecific properties [47]. The Cummings group developed a glycochip containing about 350 microbial carbohydrates from different species. Additional studies have focused on the well-controlled presentation of substrates, including density, spacing arms, and ligand orientation to improve carbohydrate microarray performance and capability. A novel glycan microarray has been recently fabricated by the Wu group on the cell surface to better probe glycan–protein interactions in real biological systems [48].

Carbohydrate–gut microbial interactions

In this decade, the gut microbiota represents a novel target for the orally administrated carbohydrates [49]. An increasing number of studies have shown that different types of carbohydrates, such as polysaccharide inulin, have prebiotic effects on the gut microbiota [50]. For example, GV-971 (Figure 1) has been approved by NMPA for its outstanding action of suppressing gut dysbiosis and modulating phenylalanine/isoleucine accumulation and attenuating neuroinflammation [51]. Recently, the mechanism of natural polysaccharide isolated from *Hirsutella sinensis* has been reported to target obesity-related gut microbes, providing useful information for the development of oral therapeutics against disorder of carbohydrate metabolism [52•]. Many cases have demonstrated that diseases associated with intestinal microbiota dysbiosis can be improved by therapeutic modalities involving microbiota. However, there is still a substantial gap in our understanding of how carbohydrates modulate the microbiota and how microbiota modulates the metabolism of the host. New tools and methods are always needed for the treatment of diseases associated with gastrointestinal microbiota dysbiosis. In the process of carbohydrate-based drug development, close attention should be paid to the carbohydrate-gut microbial interaction.

Conclusion and outlook

Carbohydrate-based bioactive entities including polysaccharides/oligosaccharides, small molecule glycosides and glycomimetics as well as more complex carbohydrates such as glycoproteins and derivatives, have shown great potential in drug development. Especially in terms of polysaccharides and oligosaccharides, marine organisms have proven to be a promising resource for searching and developing novel pharmaceutical agents. Carbohydrate digital databases and entity libraries, combined with carbohydrate microarrays and carbohydrate-gut microbial interaction studies have greatly promoted and accelerated the efficiency of identifying bioactive carbohydrates and their derivatives for drug development.

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Lin Pan: Investigation, Formal analysis, Data curation, Writing - original draft, Visualization. **Chao Cai:** Conceptualization, Project administration, Writing - review & editing. **Chanjuan Liu:** Investigation, Visualization, Writing - review & editing. **Di Liu:** Investigation, Visualization. **Guoyun Li:** Investigation, Visualization, Writing - review & editing. **Robert J Linhardt:** Writing - review & editing. **Guangli Yu:** Conceptualization, Supervision, Project administration, Writing - review & editing.

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- •• of outstanding interest

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