Pharmaceutical oligosaccharides

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Oligosaccharides, complex carbohydrates that comprise 2–30 monosaccharides linked by glycosidic bonds, are being developed as therapeutic agents for a variety of indications. The large-scale production of oligosaccharides achieved by enzymatic synthesis has encouraged the preclinical and clinical evaluation of these carbohydrates for efficacy in infectious and inflammatory diseases, transplantation, and other conditions. Many of the promising oligosaccharide drugs in development interfere with adhesion events between different cell types, and between antibodies and cells. In addition, complex carbohydrate-based agents are being developed for use in metabolic and cardiovascular diseases, as cancer vaccines, and in drug delivery. Also, diverse efforts to construct combinatorial libraries of complex carbohydrates are opening the door to broader screening approaches.

The emphasis of this review will be on oligosaccharides and their derivatives as potential pharmacological agents. For reviews on monosaccharides, large polysaccharides, and their derivatives, the reader is referred to other sources[1,2]. This article will focus on oligosaccharides that interfere with cell–cell and antibody–cell adhesion. For illustrative purposes, selected examples of other pharmacological applications of oligosaccharides, extracted from recent reports and communications, will be provided. These include mimetic molecules that imitate oligosaccharide structure and function, glycosidase inhibitors and carrier glycosidic amphiphiles for enhancement of pharmacophore bioavailability.

Competitive inhibition of adhesion

Glycoproteins and glycolipids, which are widely expressed on cell surfaces, participate in many cell–cell and other direct molecular recognition and binding processes, in health and in disease, and present a multitude of opportunities for therapeutic intervention. The recognition and binding processes generally proceed through the carbohydrate portion of these so-called glycoconjugates. The simplest approach to the disruption of recognition and binding processes is direct competitive inhibition of adhesion of pathogens or their toxins to host cells, antibodies to their carbohydrate antigens, lectins to their respective saccharide ligands and of adhesion between autologous cells (e.g. in inflammation and metastasis). Carbohydrates and their biomimics, which may both act as ligands in these adhesive interactions, can be administered in monomeric or multivalent form in solution, or presented immobilized on accessible surfaces, to block or arrest the targeted adhesion event. Three examples of competitive inhibition of recognition and adhesion events of relevance to disease states will be examined: adhesion of bacteria to epithelia, adhesion of leukocytes to endothelial cells and adhesion of antibodies to endothelial cells.

Inhibition of microbial adhesins

Most pathogenic microorganisms require host-tissue colonization for their survival and propagation. Adhesion to suitable host-tissue surfaces is the first event in colonization. This attachment is generally governed by the specific molecular recognition of host ligands, such as glycoconjugates on the surface of cells, extracellular substances, or basement membrane constituents, by microbial adhesins[3,4]. Disruption of microbial adhesion, either before or after attachment to host tissues, interferes with pathogenic colonization, as long as the pathogen has not been internalized by host cells. At a minimum, such disruption assists host defense mechanisms,

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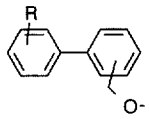
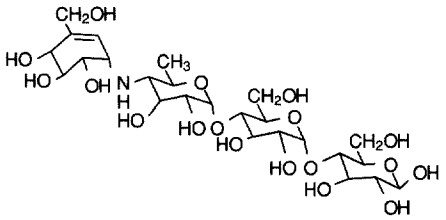
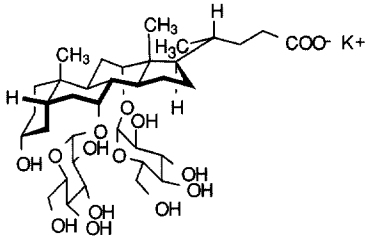
Box 1. Compounds mentioned in the text

Compound number	Name	Structure
1	Le^b (Lewis b)	Gal(β1-3)GlcNAc··· | | Fuc(α1-2) Fuc(α1-4)
2	3'SL (3'-sialyllactose)	Neu5Ac(α2-3)Gal(β1-4)Glc
3	'Ganglio' disaccharide	···GalNAc(β1-4)Gal···
4	LNnT (lacto-*N*-*neo*-tetraose)	Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc
5	'Globo' disaccharide	···GalNAc(β1-3)Gal···
6	[B] and O (or H) blood group antigen	[Gal(α1-3)]Gal(β1-4)GlcNAc··· | Fuc(α1-2)
7	sLe^x (sialyl Lewis X)	R3Gal(β1-4)GlcNAc··· | Fuc(α1-3) R = Neu5Ac(α2-)
8	Sulfo-Le^x	R = SO_3
9	Sulfo-Le^x-dialkyl[33]	SO_3-3Gal(β1-4)GlcNAcOCH$_2$CH($C_{14}H_{29}$)$_2$ | Fuc(α1-3)
10	(Mannopyranosyloxy) methylbiphenyl[39]	 R = carboxylate
11	Linear B trisaccharide (αGal-LacNAc)	Gal(α1-3)Gal(β1-4)GlcNAc
12	Linear B pentasaccharide (αGal-LNnT)	Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc
13	Acarbose[2]	
14	Sialyl Tn (STn)	Neu5Ac(α2-6)GalNAc(α1-Ser/Thr)
15	Thomsen–Friedenreich antigen	Gal(β1-3)GalNAc(α1-Ser/Thr)
16	Maltosyl trehalose[57]	Glc(α1-4)Glc(β1-4)Glc(α1-α1)Glc
17	Facial amphiphile[58]	
18	Cyclodextrin	Glc(α1-4)–Glc(α1-4) | Glc(α1-4) [Glc(α1-4)]$_{1-3}$ | Glc(α1-4)–Glc(α1-4)

whether immunological or physical, to expel the pathogen. A fully effective adhesion disruptor can, by itself, interrupt the pathogenic process[5,6]. The identification of microbial adhesins found on host tissues provides leads for the production of preventive and therapeutic inhibitors of microbial adhesion. A large number of oligosaccharidic ligands that are recognized by pathogenic microorganisms of the skin and gastrointestinal, respiratory and genito-urinary tracts have now been identified[7].

An example of great current interest is the bacterium *Helicobacter pylori*, which can cause gastritis and gastroduodenal ulcers, and has also been implicated in the etiology of gastric cancer[8]. Several carbohydrate adhesion ligands have been reported for this organism: sialyl (α2-3)galactoside[9] (3′S-GalR), the glycolipid sulfatide[10,11], and the Lewis^b blood group antigen[12,13] (Box 1, **1**). Sialyllactose [Neu5Ac(α2-3)Gal(β1-4)Glc, or 3′SL; **2**] is the only oligosaccharide that, as a monovalent sugar, potently inhibits initial bacterial adhesion to human gastrointestinal monolayers *in vitro*, is capable of detaching cell-bound bacteria (P.M. Simon and coworkers, unpublished), and reduces the gastric bacterial load in infected monkeys and gnotobiotic piglets (P.M. Simon and coworkers, unpublished). NE 0080, an (α2-3)sialylgalactoside developed by Neose Technologies as an orally administered *H. pylori* colonization antagonist, is presently undergoing clinical trials. Antiadhesive agents such as NE 0080 are not, by themselves, bactericidal, but they may enhance the antibacterial activity of antibiotics. Indeed, Mégraud and coworkers[14] report *in vitro* experiments showing that unattached *H. pylori* is more sensitive to antibiotics than cell-bound bacteria are.

Likewise, adhesion antagonists for several major pathogenic bacteria of the respiratory tract have been identified. *Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae* (NTHi) and *Moraxella catarrhalis* are the prevalent pathogenic bacteria causing community-acquired pneumonia, chronic bronchitis, sinusitis and otitis media. Early studies using isolated glycolipids identified a range of glycosides that were recognized by adhesins of the main pneumonia-causing bacteria[15]. It was found that oligosaccharides containing the GalNAc(β1-4)Gal group (**3**), found on asialogangliosides, serve as adhesion ligands for these bacteria. It now appears, however, that this group mediates the non-pathogenic colonization of the nasopharynx[16]. More recent studies using human airway epithelial monolayers have led to the identification of glycosides involved in the pathogenic colonization of respiratory epithelium. These ligands include lacto-*N*-*neo*-tetraose (LNnT; **4**), several sialyllactosamine derivatives and the globoside-associated oligosaccharide GalNAc(β1-3)GalR (**5**) (R. Barthelson and coworkers, unpublished). Various oligosaccharides with sialylated lactosamine groups at their non-reducing ends inhibit bacterial adhesion to airway epithelial monolayers at concentrations of 1–5 mM. In rabbits infected with virulent strains of *S. pneumoniae* or NTHi, 20 nmol of inhibitory oligosaccharide administered at the time of, or 24 h after, bacterial instillation in the lungs, greatly reduces or clears the infection (I. Idänpään-Heikkilä and coworkers, unpublished).

Several of the oligosaccharides that inhibit adhesion by *H. pylori* and *S. pneumoniae* are, in fact, found in human milk[17] and colostrum[18,19], which suggests that these complex carbohydrates provide a natural antimicrobial mechanism for newborn infants, whose immune system is still relatively immature[20].

Antagonists of microbial toxins

Bacterial toxins often bind carbohydrate structures on the membranes of exposed epithelial cells, as the first step in their cytopathic activity sequence. Common examples are cholera toxin, which binds the ganglioside G_{M1}, Shiga-like (or vero) toxin, which binds globo series Gal(α1-4)Gal residues[21], and the toxin from *Clostridium difficile*, which recognizes a complex carbohydrate containing the group Gal(α1-3)GalR (Ref. 22) – although interestingly, humans can only synthesize Gal(α1-3)[Fuc(α1-2)]Gal (**6**), the antigen for blood group B. Interference with the ability of *Clostridium* toxin to attach to host epithelial cells prevents penetration and the subsequent toxic effects, which are responsible for severe diarrhea especially in aged patients and in patients undergoing antibiotic therapy[23].

Selectin inhibitors

Selectins are oligosaccharide-binding proteins that mediate initial leukocyte arrest and recruitment to sites of inflammation[24]. Some selectins recognize glycosides terminating in the immunologically defined sialyl Lewis X group (sLe^x; **7**) and other related sialylated and sulfated structures (for example **8**)[25], which are displayed as receptors on leukocytes, platelets, endothelial, and other cells. Several reports indicate that the selectin ligands are repeating units of sLe^x [e.g. (sLe^x)$_2$] (Refs 26,27). Interestingly, and contrary to the generally accepted view[28], experiments reported in a recent patent application describe selectin ligands isolated from leukocytes that are not fucosylated[29].

Soluble sLe^x has been an effective anti-inflammatory agent in animal models[30–32]. Selectin-blocking oligosaccharides with alkyl chains at the reducing terminus have been synthesized (e.g. compound **9**); the attached alkyl chains cause micelle formation[33], which leads to ligand display in a multivalent array. In a separate attempt to augment circulatory half-life of selectin blockers, sLe^x pentasaccharide was conjugated to a phospholipid and incorporated into liposomes[34]. These liposomes also included lipid derivatives of poly(ethylene glycol), which retards circulatory clearance by macrophages[35,36]. Administered in this formulation, a dose of 400 μg/kg of sLe^x afforded protection against reperfusion injury following myocardial ischemia in a cat model[34]. In this model, 10 mg/kg of the free sLe^x was required for a similar effect[32]. The protection arose from inhibition of initial neutrophil–endothelial cell interaction in the ischemic tissue. Investigators at Kanebo (Osaka, Japan) have reported that sulfated analogs of Le^x with long-chain branched alkyl tails appended (e.g. **9**) also exhibit augmented anti-inflammatory activity compared with sLe^x tetrasaccharide in a mouse ear, IgE-induced inflammation model[33]. Increased activity by these sulfo-Le^x analogs may arise from micellar or multivalent presentation of these oligosaccharide derivatives following spontaneous association to albumin or other hydrophobic carriers, or from extended serum retention of the modified oligosaccharide.

Cytel Corporation has been conducting clinical trials with sLe^x pentasaccharide (known as Cylexin™). Intravenous administration of Cylexin before initiation of surgery was reported to reduce the risk of reperfusion injury following pulmonary thrombo-endarterectomy by 50%, when compared with the placebo group[37]. But a Phase II clinical trial with Cylexin in reperfusion injury following angioplasty treatment for myocardial infarction was recently terminated because, '...based upon the primary endpoint, there was no benefit in patients treated with Cylexin over those patients in the placebo control group'[38].

Non-oligosaccharide, glycomimetic selectin antagonists are being developed using rational design analysis to guide organic synthesis efforts, with the expectation of obtaining activity and bioavailability profiles comparable with or superior to that of the natural ligands. Kogan and coworkers[39] have identified three key molecular sites from the sLe^x and sLe^a tetrasaccharides (the carboxylate of sialic acid and the 2- and 3-hydroxyls of fucose; see Box 1, **7**) as being required for selectin active-site binding, and have undertaken a synthesis program to generate biomimetics using mannopyranosyl biphenyls (**10**) as a core scaffolding for these groups. Substituting the mannose 3- and 4-hydroxyls for the fucose 2- and 3-hydroxyls of sLe^x, and maintaining this mannosyl group intact, a series of substitutions have been introduced into the biphenyl structure[40]. *In vitro* studies measuring inhibition of binding of selectin–immunoglobulin fusion protein to HL-60 human myelogenous leukemia cells have identified a structure, named TBC-1269, with an IC_{50} value of 70 μM for P-selectin, compared with 2.6 mM for sLe^x tetrasaccharide. This compound is effective in sheep asthma (at 0.3 mg/kg) and canine cardiac reperfusion injury models (at 5–25 mg/kg)[41].

Natural antibodies: xenotransplantation

A further opportunity for adhesion-disrupting oligosaccharides is found in xenotransplantation. The shortage of human organ donors has generated interest in animal organs. Large primates are not likely donors for routine organ transplantation into humans because some species are endangered, animals are difficult to obtain, there are ethical objections to their use, and there are concerns about the dangers of transmission of xenozoonotic pathogens into phylogenetically closely related, immunosuppressed recipients[42]. The use of non-primate mammals as organ donors overcomes these objections.

Tissues from vertebrates other than old-world monkeys, apes and humans express oligosaccharide chains terminating in the unfucosylated Gal(α1-3)GalR (αGal or linear B) group (**11**). Apes and humans express this sequence, but only as part of the fucosylated B blood group oligosaccharide (see Box 1, **6**). In the absence of the αGal antigen, humans and higher primates produce very large quantities of anti-αGal antibody, as much as 1–3% of all circulating immunoglobulin[43]. Approximately 1% of all B cells are committed to producing anti-αGal antibody[44]. Transplantation of organs from pigs, a donor species of interest, into baboons or cynomolgus monkeys, used as models for humans, is met by the swift deposition of naturally occurring anti-αGal antibodies, leading to the very rapid, complement-mediated destruction of the organ in a process called hyperacute rejection (HAR)[45]. Prevention of HAR for a brief period (approximately 2 weeks) following transplantation is expected to permit the organ to function and survive, even in the continuing presence of anti-αGal antibodies, a process referred to as 'accommodation'[46,47]. Permanent engraftment may then be possible with the use of more conventional immunosuppressive regimens, similar to those currently used in the transplantation of human organs.

Of the various attempts to overcome HAR, the simplest in concept is the competitive blockade of anti-αGal antibodies *in vivo*, or their extracorporeal removal from the circulation. Neose Technologies, Dextra Labs (UK) and Syntesome (Munich) are currently testing αGal oligosaccharides for just these purposes. The pentasaccharide Gal(α1-3)Gal(β1-4)-GlcNAc(β1-3)Gal(β1-4)Glc (or αGal-LNnT; **12**), part of porcine renal endothelium glycolipids, has been identified as a natural ligand for anti-αGal antibodies[47]. The non-reducing terminal trisaccharide is a better inhibitor of anti-αGal antibody-mediated activities *in vitro* than the Gal(α1-3)Gal disaccharide[48,49]. Neose Technologies has synthesized αGal oligosaccharides, including the αGal-LNnT pentasaccharide, and is evaluating these sugars *in vitro* and in baboons undergoing porcine organ xenotransplantation.

Other approaches to bridging the species barrier between pigs and humans are the production of transgenic animals that express various human proteins, and rendering patients immunologically chimeric through porcine donor bone marrow transplantation. For a discussion of these strategies, the interested reader is referred to other sources[50]. One transgenic scheme, however, deserves noting. In this strategy[51], animals are transfected with the (α1-2)fucosyl- (or H) transferase gene, which leads to the biosynthesis of the H blood group oligosaccharide, from which the B blood group antigen (**6**) is built. The fucosylated structure (H substance) is not a substrate for the porcine linear B α-galactosyltransferase. But, unless the linear B α-galactosyltransferase gene can be simultaneously knocked out in such animals, the fucosyl- and the galactosyltransferases will likely compete intracellularly for the terminal Gal, and some αGal groups may yet be generated. If so, xenograft rejection may still occur.

Prevention of HAR with αGal oligosaccharides will be required for any successful scheme being considered for the xenotransplantation of porcine organs into humans.

Natural antibodies: blood group incompatibility

Earlier, with a similar rationale, A and B blood group trisaccharides were synthesized by Chembiomed (Alberta, Canada) to block blood group incompatibility reactions. The trisaccharides were tested clinically in hemolytic neonatal disease induced by fetal–maternal blood group antigen disparity. In premature infants exposed to maternal antibodies, the resulting hemolysis leads to the accumulation of bilirubin in the circulation, which is neurotoxic. Romano and coworkers infused A and B trisaccharides into bilirubinemic infants to block the hemolytic activity of the respective maternal antibodies. The treatment resulted in lower bilirubin levels, and a marked reduction in the number of transfusions required[52]. Blockade of anti-blood group antibodies may also be useful in transplantation, because ABO-mismatched donor tissue is generally disqualified for use.

Other applications

Oligosaccharide biosynthesis modulation

A more indirect approach to the pharmacological manipulation of oligosaccharide-mediated events is intervention in glycosylation and deglycosylation. It is possible to target metabolic steps in the intracellular phases of complex carbohydrate biosynthesis, as well as aspects of extracellular carbohydrate tailoring. The obvious targets in this strategy are the enzymes involved in oligosaccharide assembly (glycosyltransferases) and degradation (glycosidases). Most of the compounds tested as inhibitors of these enzymes are derivatives of monosaccharides, frequently imino sugars, and these will not be addressed in this paper. (For information on the subject the reader is referred to other reports[2,3].) One exception mentioned here for the purpose of illustration is Acarbose (**13**; developed by Bayer)[2,53]. It is a tetrasaccharide obtained by fermentation, an α-glucosidase inhibitor, which is used as an antidiabetes agent. This agent, and others like it, inhibit intestinal disaccharidases, thereby reducing the final breakdown of dietary complex carbohydrates, which leads to a lower uptake of glucose into the blood.

Vaccines

Cancer cells occasionally display immunologically detectable surface antigens that are suitable candidates for vaccine development. An example is the mucin MUC-1, which contains short carbohydrate chains such as Tn [GalNAc(α1-Ser/Thr)], sialyl Tn [Neu5Ac(α2-6)Tn, or STn; **14**] and the Thomsen–Friedenreich antigen [Gal(β1-3)-GalNAc(α1-Ser/Thr); **15**]. Theratope®, a conjugate of STn and the immune-adjuvant keyhole limpet hemocyanin, is synthesized by Biomira, Inc. (Edmonton, Canada). In ongoing Phase II clinical trials with breast cancer patients, when dosed in conjunction with a single intravenous administration of the immunomodulator cyclophosphamide, patients acquire higher anti-STn antibody titers and experience prolongation of survival[54]. Many other tumor-associated carbohydrate antigens have been identified and could become candidates for vaccine development[55].

Heparinoids

Heparin and other glycosaminoglycans have diverse effects on cells with heparin-binding proteins. Inhibition of smooth muscle proliferation is one activity of interest in the prevention of arteriosclerotic lesion formation and forestalling of restenosis[56]. With these objectives in view, investigators at Hoffmann-La Roche, Inc. (Basel, Switzerland) have prepared small-molecular-weight heparinoid-like oligosaccharides. Highly sulfated maltosyl(β1-4)trehalose (**16**) is an example of a synthetic non-uronic-acid-containing oligosaccharide with antiproliferative activity similar to that of heparin, but without any anticoagulant effects[57].

Drug delivery

Pharmacophores with limited bioavailability may become transmucosally permeable when combined with permeation-enhancing compounds. Glycoderivatives of bile acids (**17**) synthesized by Transcell Technologies (Monmouth Junction, NJ) are facially amphiphilic, i.e. the polar and non-polar surfaces of the molecule are apposed along the long axis, in contrast to head-to-tail amphiphiles. Facial amphiphiles self-assemble to form complexes[58] with a hydrophilic inner surface that can cradle a polar drug (e.g. gentamicin) and a lipophilic outer surface that interacts with biological membranes, enhancing drug penetration and oral bioavailability[59]. Compounds of this type also improve transfection of cells with DNA by enhancing transmembrane permeabilization[60], and may find a use in gene therapy efforts.

In a related, but inverse strategy, lipophilic drugs were complexed with the hydrophobic interior of cyclodextrins (**18**). These annular hexa-, hepta- and octaglucosides, variously derivatized, have been used to enhance oral bioavailability and to extend the half-life in serum of a number of water-insoluble drugs[61].

Combinatorial libraries

Combinatorial libraries of nucleotides, peptides and other chemical families, make enormous numbers of molecular species available via the coupling of subunits to give polymeric products[62–64]. Unlike amino acids and nucleotides, which form linear polymers, the glycosylation of monosaccharides and oligosaccharides may lead to a great number of regio- and anomeric isomers, which presents special challenges for those attempting to generate libraries, whether by organic synthesis[65,66] or with the use of enzymes[67], either on solid supports or in solution. The analytical deconvolution of oligosaccharide and glycoconjugate mixtures creates additional difficulties. A discussion of the various strategies under investigation appeared in a recent issue of this journal[68].

Manufacturing issues

Pharmaceutical development requires manufacturing technology that can produce large quantities of a drug. Preclinical toxicokinetic evaluation alone can require multi-kilogram quantities of a drug candidate.

In the synthesis of complex carbohydrates, the synthetic organic chemist faces considerable challenges. There are the obvious difficulties arising from the need to synthesize highly complex molecules with multiple stereo- and regiospecific variations in the presence of many hydroxyl groups of similar reactivities[69]. Careful and elaborate blocking and unblocking of functional groups is necessary, which requires multi-step synthesis strategies and consequently makes obtaining adequate final yields difficult. Large-scale chemical synthesis of complex oligosaccharides has not been achieved.

Enzymatic synthesis overcomes many of the difficulties encountered in organic synthesis, owing to the specificity and efficiency of glycosyltransferases. These enzymes, when expressed in recombinant microorganisms, provide a practical and scalable manufacturing process. Sugar nucleotides are required for the enzymatic synthesis of complex carbohydrates. Ingenious schemes for the use of multi-enzyme, cyclical reactions at the laboratory scale permit the addition of saccharide units with minimal input of sugar nucleotides[70]. But the large-scale practicality of this strategy has not yet been demonstrated. Fortunately, the demand for activated sugars arising from the advent of industrial-scale oligosaccharide synthesis is being met by commercial suppliers. Some activated forms of sugars that are not found in nature have been synthesized, and were shown to be substrates for glycosyltransferases[71–74]. Conversely, acceptor specificity can be altered by manipulating reaction conditions (e.g. pH), causing addition of sugars to acceptors not otherwise recognized by the glycosyltransferase[75]. Thus enzymatic technology can also be useful in the synthesis of non-natural oligosaccharides.

Oligosaccharides are currently being produced in multi-kilogram amounts by methodology developed at Neose Technologies, with one tetrasaccharide now being scaled to a manufacturing rate of approximately 1,000 kg per month.

Concluding remarks

The principal reason for the until now largely unexploited pharmacology of oligosaccharide drugs has been the difficulty in achieving practical and cost-effective syntheses of complex carbohydrates. The advent of scalable enzymatic synthesis has made clinical trials with potentially therapeutic oligosaccharides a reality. From an abundance of therapeutic opportunities for complex carbohydrates, their derivatives and non-saccharidic glycomimetics, the earliest diseases being targeted involve cell-surface adhesion events in inflammation, infectious diseases and xenotransplantation, in which oligosaccharides compete with natural carbohydrate ligands and inhibit the adhesion events involving leukocytes, endothelial cells, microorganisms and antibodies. This beginning marks the emergence of a period that will see ever greater integration of complex carbohydrates into the repertoire of therapeutic, diagnostic and nutritional agents.

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