Natural Products as a Robust Source of New Drugs and Drug Leads: Past Successes and Present Day Issues

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The history of drug development has its foundation firmly set in the study of natural remedies used to treat human disease over centuries. Analysis of medicinal plants, bioactive cultures, and increased understanding of micronutrients in the food chain opened the door to the development of purified and defined chemical compounds as dose-controlled medicines. Thus, with the early discovery of cardiotonics in foxglove, salicylic acid in willow bark, morphine in poppies, and penicillin in mold, the pharmaceutical industry was launched. Such natural small molecules served as treatments for disease and ultimately, as pharmacologic tools to enable the understanding of the biochemical pathways and mechanisms of disease. In contrast, modern drug discovery technologies coupled with the powerful tools of biotechnology have prompted drug discovery organizations to focus on target-driven drug discovery at the molecular level by launching high-throughput screening programs using artificial biochemical assays. At a time when the pharmaceutical industry has come under scrutiny for high rates of drug development failure, it is interesting to see that natural products drug discovery has been marginalized in favor of this high-throughput biochemical screening paradigm. If modern drug development is once again to benefit from natural products as a source, then the limitations of artificial biochemical assays as applied to the screening of natural extracts must be realized in order to capitalize on the vast natural molecular diversity and rich ethnobotanic data that has emerged worldwide. Natural compounds can again become central players in the treatment of disease and in the understanding of disease mechanisms. **(Am J Cardiol 2008;101[suppl]:43D–49D)**

The pharmaceutical industry widely implemented biochemical assays and high-throughput screening in the 1990s, and as a result, natural product screening programs have been de-emphasized. The reasons for this are analyzed and debated in the literature, but the various analyses seem skewed toward uncertain philosophical generalizations about natural compound complexity, rational drug design versus "random" screening, and issues with the "soft science" of ethnobotany.[1–4] Given the historical successes[5] in finding new drugs and drug leads from natural materials, it would seem to be an immense oversight that drug discovery organizations would choose not to exploit the uniquely rich chemical diversity found in nature. Furthermore, given our dramatically improved analytic technologies[6,7] and purification methods,[8] the industry of drug development seems enabled to launch a well-resourced renaissance in the development of drugs derived from natural sources. Although the reasoning behind the de-emphasis of natural product screening is debatable, it is certain that neither the quality nor the structural diversity of compounds derived from natural sources has diminished over time. It would seem instead that the decision to move away from natural extract screening was made because of an increasing dependence on high-throughput biochemical screening technologies that are inappropriate for the screening of natural extracts. Natural product screening has been deemed by many to be impractical and obsolete in our modern biochemical screening arena.

Historical Perspective: Natural Compounds as Drugs and Pharmacologic Tools

The seminal discoveries leading to the use of pure drug substances occurred in the 18th and 19th centuries. These involved the study of plant preparations known historically to have medicinal properties. The dates of the identification of the pure drug substances belie the centuries of ethnobotanic use preceding them. Compounds that emerged from the study of ethnobotanic extracts became important as medicines and were enabling as pharmacologic tools in the elucidation of disease mechanisms. Those new natural drug substances were pivotal in forming entire therapeutic areas and in stimulating the formation of the modern pharmaceutical industry.

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Digoxin from foxglove (digitalis), 1785: William Withering published his treatment of heart patients with cardiotonic foxglove extract, also known as digitalis, in 1785.[9] This treatment led to the discovery of digoxin, marketed as the brand Lanoxin (GlaxoSmithKline, Research Triangle Park, North Carolina)[10] (Figure 1), and has been used to treat arrhythmia and congestive heart failure. Foxglove was previously known as gypsyweed because patients were literally going to bands of gypsies to get the extract.[11] This underscores the central importance of the ethnobotanic component of this early drug discovery and its role in informing and establishing safety and efficacy.

Thus, the discovery of the small molecule drug digoxin allowed development of a defined and dose-controlled medicine. Often overlooked, however, is the very important fact that such biologically active small molecules enabled pharmacology to elucidate the biochemical pathways of a disease. Over decades, the study of the pharmacology of digoxin led to an understanding of the biochemistry and the biology of the sodium-potassium adenosine triphosphotase pump,[12] providing an example of ethnobotanic information ultimately shaping an entire therapeutic area.

Morphine from poppies, 1806: Freidrich Serturner[13] analyzed opium poppy and isolated morphine in 1806. This investigation led to the discovery of morphine and the development of a defined and dose-controlled medicine for pain. Again, importantly, the pharmacology of the small molecule morphine enabled an understanding of the opiate receptors subtypes, and ultimately, an understanding of the endorphin and enkephalin pathways.[14]

An organic chemist considering the structure of morphine would be quick to point out that such a molecule would never have been conceived of by medicinal chemists engaged in a rational drug design program for pain. Without morphine as a small molecule tool for pharmacology and without its unique chemical structure for inspiration, drug discovery scientists might never have developed an analogously effective therapy for pain. A cursory glance at the evolution of opiates over the years speaks volumes to our dependence on the structure of the natural parent (Figure 2).

Aspirin from salicylic acid in willow bark, 1897: Felix Hoffmann, working with the Bayer Company at the end of the 18th century, synthesized aspirin from salicylic acid in willow bark.[15] This is an early example of a synthetic drug inspired by a natural product that had been isolated from a plant preparation with a rich ethnobotanic history. People had been using the extract from willow bark prepared as a tea for centuries to treat rheumatism and headache. Hoffmann observed that many patients had nausea on ingestion of the pure salicylate and hypothesized that this side effect was because of the particularly acidic salicylate functionality (Figure 3). An acetylation reaction to mask the phenol of salicylic acid as an acetate solved the acidity problem. Aspirin, the prodrug of salicylic acid, could be administered orally without nausea. Then, in the gut and circulation, it is short-lived and rapidly deacylated to deliver the natural parent drug, salicylic acid.

Once again, the monumental influence of ethnobotany and natural drug discovery on drug development and medicine in general is apparent here. Using salicylic acid as a pharmacologic tool, it was at once possible to delineate the mechanisms of inflammation[16] and subsequently to design and test a battery of new nonsteroidal anti-inflammatory agents, including acetaminophen, ibuprofen, and naproxen. Given this knowledge and the modern assay tools to test cyclooxygenase (COX) enzyme subtypes, medicinal chemists were able to design and synthesize the next-generation COX-2-selective inhibitors, Vioxx (Merck & Company, Inc., Whitehouse Station, New Jersey) and Celebrex (G.D. Searle LLC, division of Pfizer Inc., New York, New York).[17] The recent safety concerns[18] over the COX-2 inhibitors make the point that synthetic drugs, in contrast to those derived from ethnobotanic sources, have relatively undefined safety profiles and present an inherent safety risk despite rigorous clinical testing.[19]

Penicillin from mold, 1928: It is a well-known story that in 1928, Alexander Fleming discovered penicillin in mold.[20] The Fleming group had speculated that there were particular microorganisms responsible for disease, and they learned how to grow colonies of these microorganisms on agar in Petri dishes. Depending on which version of the story you wish to believe, astute scientific observation or sloppy serendipity or likely a fortunate blend of both led to the discovery of antibiotic action and the isolation of an antibiotic chemical component of penicillium mold. Whether it was science or serendipity, the discovery of penicillin and its impact on the treatment and understanding of infectious disease did more for human health than any other single discovery. The small molecule penicillin enabled the study of antibiotic action and infection to the point that countless penicillin and cephalosporin antibiotics (Figure 4) could be synthesized and studied for the treatment of various strains of bacteria and in response to antibiotic resistance.[21] An understanding of infectious disease was achieved, and an entirely new therapeutic approach was born.

The Pharmaceutical Industry Coming of Age

In the mid-1900s, the pharmaceutical industry expanded its drug discovery effort to include industrial sources of unnatural chemical entities, including the petrochemicals, the dyes and their synthetic intermediates that ultimately inspired the sulfa drugs.[22] Natural products continued to play a major role, and endogenous chemicals, such as the steroids, prostaglandins, and peptide hor-

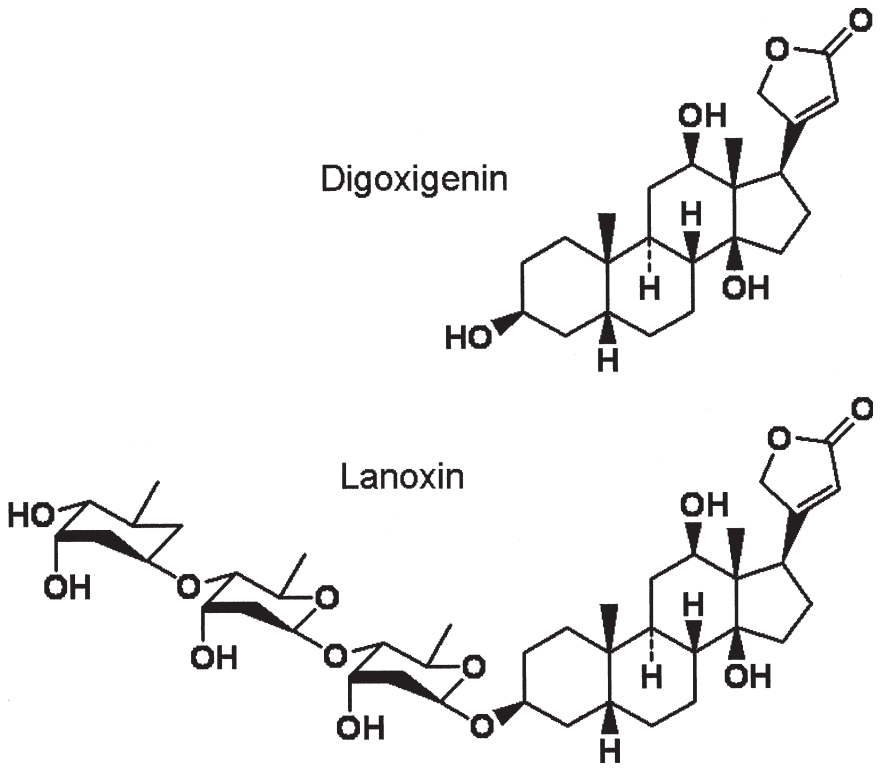

Figure 1. Cardiotonic agent digoxin (Lanoxin, GlaxoSmithKline, Research Triangle Park, North Carolina) and its parent aglycone digoxigenin.

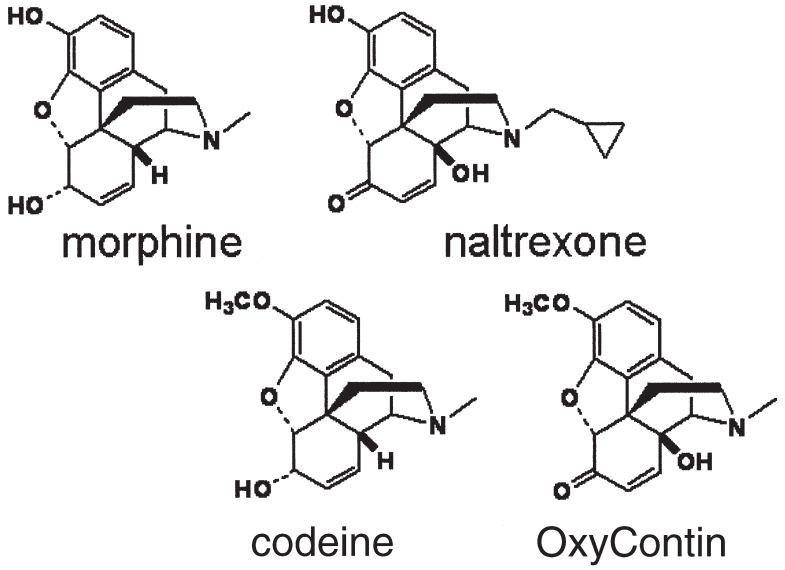

Figure 2. Morphine, codeine, and their synthetic offspring, naltrexone and OxyContin (Purdue Pharma LP, Stamford, Connecticut).

mones, provided the pharmaceutical industry with additional natural inspiration for drug discovery as the 20th century unfolded.[23]

In the last quarter of the 20th century, a refined understanding of enzymology and receptor pharmacology developed. The role of functional proteins in biology and in the biochemical pathways of disease became clear. Medicinal chemists, with the help of functional cellular assay tools and animal models of disease, could synthesize and screen small molecules in order to develop structure–activity relations to optimize potency and selectivity. Natural molecules often provided inspiration to design receptor binders, for instance, the nicotinic and muscarinic receptors. Antibiotics and antineoplastic agents could also be developed as selective toxins by using selective cytotoxicity assays and tumor models. These functional biologic assay tools were also useful for the screening and testing of natural product extracts and ethnobotanics. Selective cytotoxicity assays were

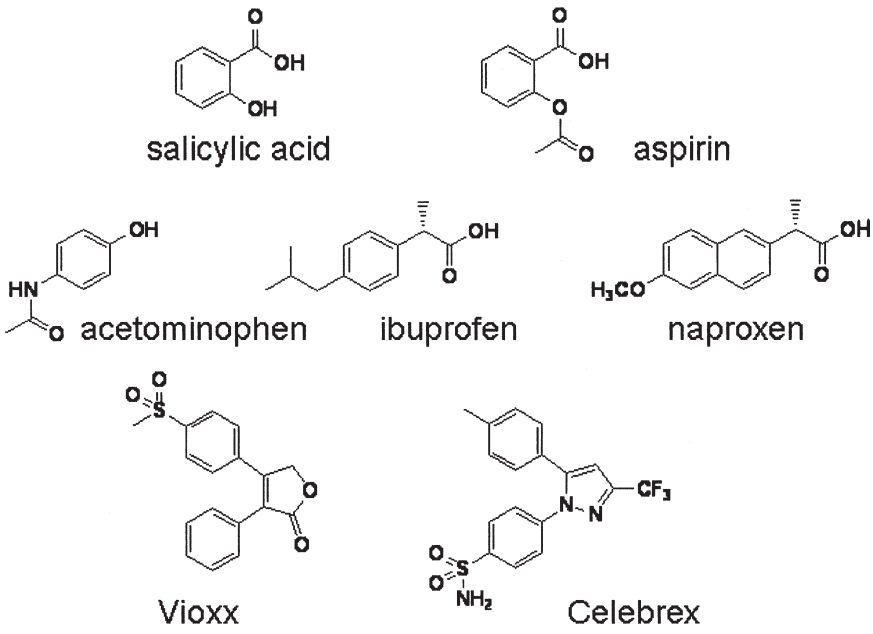

Figure 3. Evolution from natural salicylic acid to synthetic aspirin and the subsequent development of the various nonsteroidal anti-inflammatory drugs and the cyclooxygenase–2 inhibitors.

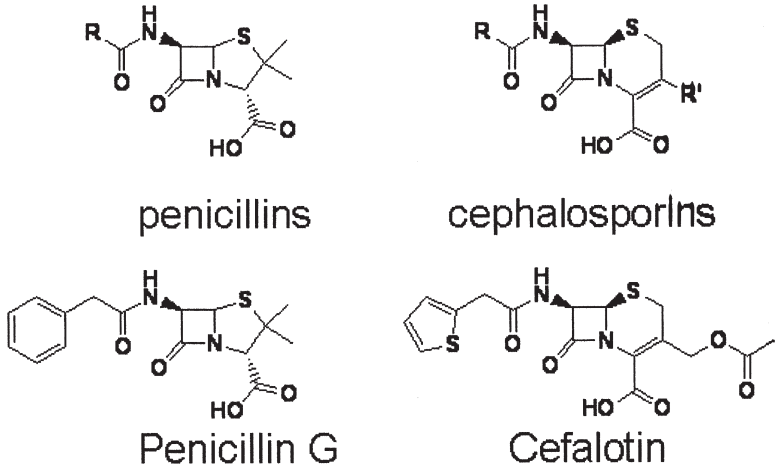

Figure 4. Penicillin and the family of β-lactam antibiotics.

a particularly effective way to identify new antibiotics from cultures and extracts by simply screening and fractionating to isolate selective activity.

Biotechnology Provides Access to Isolated Human Enzymes and Receptors

As of 1990, the pharmaceutical industry had embraced the tools of the emerging biotechnology industry. Cloning and expression technology now allowed the production of purified human enzymes and receptors. Enzyme inhibitors and receptor binders could be studied on the molecular level while interacting with their respective enzyme and targets. The biochemical assay arena would provide powerful tools for precisely measuring potency and selectivity of drug leads. Furthermore, many thought it would also provide a platform for high-throughput screening programs that might increase the rate of new drug discovery dramatically.[24] This was a vital tipping point for the pharmaceutical industry in that there seemed to be an overarching agreement that first-tier screening for drug discovery should be done using nonfunctional, nonbiologic enzyme inhibition assays and receptor-binding assays. Our drug screening emphasis had shifted suddenly from functional assays that measured biologic activity to artificial assays that measured molecular interaction. In hindsight, some would make the point that we had taken a step backward in the drug development process. The overall lull in the launch of new drug products annually over the past 2 decades would seem to support that point.

Biochemical Assays Prompt Marginalization of Natural Extract Screening

Recently, there has been much attention paid to the high rate of pharmaceutical industry failure in drug development and the low rate of production of new chemical entities approved as medicines,[25] despite dramatically increased research and development expenditures. Self-analysis in the pharmaceutical industry has raised questions of quality and tractability. Accordingly, new guidelines for "druggability,"[26] "druglikeness,"[27] "leadlikeness,"[28] and preclinical compound profiling[29] have emerged. Properties including chemical stability,[30] molecular weight, polarity, and solubility have now become metrics in determining the appropriateness of a compound for testing in a biochemical assay. Druglikeness filters have further marginalized natural products but for all the wrong reasons. Many compounds, natural or unnatural, that are problematic in biochemical screens currently were perfectly well-studied in functional biologic assays and selective cytotoxicity assays before 1990. The major problems with modern biochemical assay programs are not because of the compounds being screened but because, in large part, of the shortcomings of the biochemical assays themselves.

The effectiveness and productivity of biochemical assays and high-throughput screening technologies for drug discovery since 1990 is debatable. It is beyond debate, however, that biochemical assays are simply too sensitive and too prone to artifact because of issues of solubility, aggregation,[31] chemical reactivity,[30] and quenching effects (depending on the detection technology used in the assay) to be used effectively to screen natural extract mixtures. The wide adoption of biochemical assays and high-throughput screening has simply made natural product drug discovery impractical. The qualities and structural diversity of natural product extracts have not diminished over the past 20 years. It is the pharmaceutical industry's screening methods that have changed. To effectively screen for new drug leads in natural extracts, it would be preferable to use functional biologic assays and whole cell cytotoxicity assays, *not biochemical assays*. Fortunately, there has been great progress made recently in the development of relatively high-throughput functional whole-cell screening platforms.

Contingency Approaches for Exploiting Natural Extracts Effectively in the Biochemical Screening Arena

Ideally, and consistent with the above discussion, natural extracts would be screened using functional biologic assays. In today's screening arena, however, we are often limited to biochemical tools. A biochemical assay can be a perfectly effective tool when implemented with care. There are simple guidelines for the biochemical screening of extracts that will enhance the effectiveness of our biochemical assay tools.

Prefractionation: It is apparent that biochemical assays are too sensitive to screen complex extract mixtures. A prefractionation step using normal phase or reverse phase chromatography is certainly required. The resulting fractions need not be pure or fully characterized, although modern liquid chromatography/mass spectrometry analyses are applicable.[6,7] Fractionation would result in much more simplified fraction mixtures for assay, and it would provide the opportunity to discard very lipophilic compounds or very hydrophilic compounds, depending on the requirements of the program.

Chemical conditioning: Natural extracts contain chemically reactive compounds that are inappropriate for biochemical screens because they tend to modify target proteins covalently, inducing false-positive results in the assay.[30] These chemically reactive compounds in the extract can be exploited by chemical conditioning treatments[32] that destroy reactive agents and create novel and chemically stable natural product–derived material. The chemical conditioning should be followed by prefractionation and then screening. Chemical conditioning will afford the opportunity for the creation of novel drug-like chemical diversity and will open the door for a drug design component as well.

The Future of Natural Products and Ethnobotany in Drug Discovery

We have reached an important crossroads in the selection of a drug discovery paradigm moving into the future. The recent adoption of biochemical assays and high-throughput screening has created the impression that natural extract screening is somehow less effective or less practical than the high-throughput screening of large corporate compound collections. In fact, it is the high-throughput biochemical screening paradigm that has exceeded its own practicality. Massive investment in high-throughput screening campaigns result in thousands of biochemical "hits" that require confirmation, evaluation, and optimization to determine if they are meaningful leads or simply artifact. Medicinal chemistry follow-up is exceedingly resource intensive, and in the first stages, it is often focused on simply establishing functional activity in a cellular assay. Given the vast resources of the pharmaceutical industry and the incredible advancements that have been made in spectroscopic analysis and purification technologies, it is important to move forward with a new balance of perspective and screening paradigm that includes the rigorous and intelligent screening of natural extracts.

Ethnobotany has appeared often in the popular press of late. Articles lauding red wine, green tea, and curry have introduced the public to polyphenols, antioxidants, and the concept of micronutrients that appear to maintain and pro-

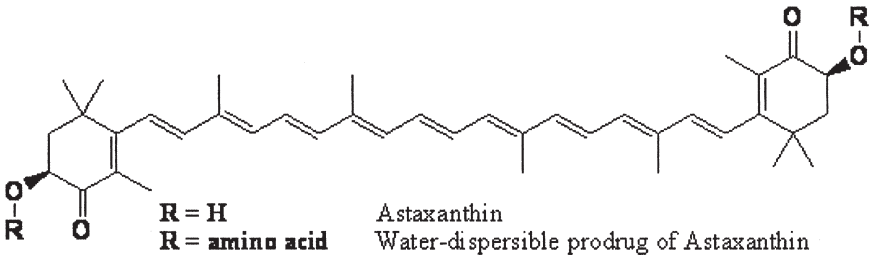

Figure 5. Astaxanthin and its water-dispersible and bioavailable synthetic analogues.

mote health and longevity. Laboratory investigations and clinical studies around the world are performed to confirm the efficacy of many plant extracts and micronutrients. It is the rare case indeed that rigorous data demonstrate bioavailability, exposure, delivery, and dose-dependent efficacy. However, it is likely that in the near future, these hopeful studies will begin to bear fruit in the clinic.

The Carotenoid Astaxanthin and the Improved Novel Derivatives

Ethnobotany takes many forms ranging from ancient Asian medicine to shaman-directed preparations used in South American jungles. We have become fascinated as well with the micronutrients that are present, or maybe lacking, in our own diets and how those might affect overall health. A particularly compelling example of such a micronutrient is the potent antioxidant astaxanthin. A structural relative of carotene, astaxanthin occurs naturally in marine algae. The algae are consumed by krill, and the krill are consumed by wild salmon and passed through the food chain accordingly. As years of aquaculture led to successful salmon farming, it was learned that nutrient-rich but unnatural feed had the effect of causing the salmon's flesh to lose its characteristic color. It was realized that if the feed was replaced by the natural krill, or supplemented with an astaxanthin-containing additive, the salmon's flesh color was restored. The restoration of the flesh color was also accompanied by an observed increase in body weight and improvement in reproductive health.[33–35] Subsequent studies in other animals have further demonstrated the beneficial effects of astaxanthin.[36]

The chemical structure of astaxanthin offers a rationale as to why it is the most potent antioxidant known. Astaxanthin possesses the conjugated polyene backbone characteristic of the carotenoids, but it is unique in having the α-hydroxy cyclohexenone headgroups. It is apparent from the structure that the intramolecular hydrogen bonds between the hydroxyl groups and the ketone carbonyl oxygen groups serve to polarize the conjugated polyene and to activate the molecule toward reaction with reactive oxygen species. The polar headgroups also serve to align astaxanthin in cell and mitochondrial membranes, essentially localizing astaxanthin to the vital organ tissues and vasculature. This combination of features suggests it would be an efficacious antioxidant, and cell studies and animal models have supported this thesis.[37,38]

Typical of micronutrients, orally ingested astaxanthin is absorbed in minute amounts and would seem to be maintained at suitable levels only with a steady diet of foods that contain it.[39] Pure astaxanthin is extremely hydrophobic and difficult to study in assays and animal models. In response to these challenges, novel and water-dispersible derivatives have been prepared as bioavailable and dose-controlled prodrugs of astaxanthin to be evaluated clinically as an antioxidant drug for the treatment of cardiovascular disease (Figure 5).[40,41]

Conclusion

This approach of improving natural compounds for use as drugs is not new. We will recall the Felix Hoffmann[15] conversion of salicylic acid to aspirin and the resulting dramatic effect on the development of a new drug and an entire therapeutic area. Similarly, the improved properties of these astaxanthin derivatives relative to astaxanthin may enable rigorous clinical evaluation leading to an entirely new understanding of the role of oxidative stress in human disease. This is perhaps, another example of a natural product leading to the development of a new drug candidate and inspiring an entirely new therapeutic approach to human disease.

Author Disclosures

The author who contributed to this article has disclosed the following industry relationships.

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