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Revisão

TROPICAL BIODIVERSITY: HAS IT BEEN A POTENTIAL SOURCE OF SECONDARY METABOLITES USEFUL FOR MEDICINAL CHEMISTRY?[#]

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The use of natural products has definitely been the most successful strategy in the discovery of novel medicines. Secondary metabolites from terrestrial and marine organisms have found considerable use in the treatment of numerous diseases and have been considered lead molecules both in their natural form and as templates for medicinal chemistry. This paper seeks to show the great value of secondary metabolites and emphasize the rich chemical diversity of Brazilian biodiversity. This natural chemical library remains understudied, but can be a useful source of new secondary metabolites with potential application as templates for drug discovery.

Keywords: natural products; medicinal chemistry; tropical biodiversity.

INTRODUCTION

Human culture has been deeply influenced by biodiversity and plant species, particularly those identified as possessing medicinal uses. This has been established by a long selection process undertaken by ancient populations throughout the planet. Nowadays, the remaining knowledge regarding the medicinal properties of plants has been the focus of scientific research and of societies all over the world, due to the great advances that plant science has afforded modern phytochemistry and medicinal chemistry with the arrival of the so-called post-genomic era.

History presents a wealth of evidence as to how human relationships have been affected by plants in all aspects, including economic and social evolution of different populations from different regions. Examples of the importance of nature, particularly biodiversity, to humankind are the wooden ships of the Vikings inhabiting Scandinavia, the exquisitely sculptured houses built by the Maoris in New Zealand, the three-meter long blowguns of the ship-tribes from the Peruvian Amazon, and the adobe homes as well as the beautifully patterned carpets of the Navajos inhabiting deserted areas in North America.[1-3]

Plant species produce a wide variety of secondary metabolites that play an essential role in the survival of the species themselves as well as in the maintenance of environmental equilibrium. These natural products also perform very important functions in plant/plant and plant/insect interaction, resistance against pests and diseases, attraction of pollinators, and interaction with symbiotic microorganisms. Therefore, natural compounds are treasure troves for synthetic organic chemists and drug discovery researchers.[4] The oldest medical text comes from Mesopotamian ancestors, who described ca. 1000 plants, such as *Commiphora myrrha*, *Papaver somniferum*, and *Cedrus* sp, as being useful remedies. In fact, many of the ancient medicinal plants and formulations are still being used today.[5] So far, humankind has stored these fantastic experiences, which has contributed to the great development seen in the area of plant-derived drugs over the succeeding millennia, even after the combinatorial era.[6]

Over the last two decades, drug discovery has witnessed unprecedented advances owing to the utilization of natural products. The latter have been the most important source of secondary metabolites with countless applications in the fields of medicine, nutraceuticals, cosmetics, agrochemicals, and biology.[7] Some traditional examples include morphine (**1**), codeine (**2**), reserpine (**3**); the anticancer drugs vincristine (**4**), vinblastine (**5**), and paclitaxel (**6**);[8,9] the antilipidemic compound mevastatin (**7**, compactin) and the homologous lovastatin (**8**, mevinolin);[10] as well as the anticholinesterasic agent galanthamine (**9**), as shown in Figure 1.[11] The successful part played by natural products in the discovery of new medicines, especially with respect to cancer treatment,[9] demonstrates the great potential of screening natural products in the search for novel classes of bioactive compounds. Despite the recent advances in combinatorial chemistry, which enable the synthesis of libraries of compounds comprising a wide range of chemical properties,[12,13] the high structural diversity and complexity typical of natural products is unique, placing them in such a successful position. However, some of these compounds occur in very low

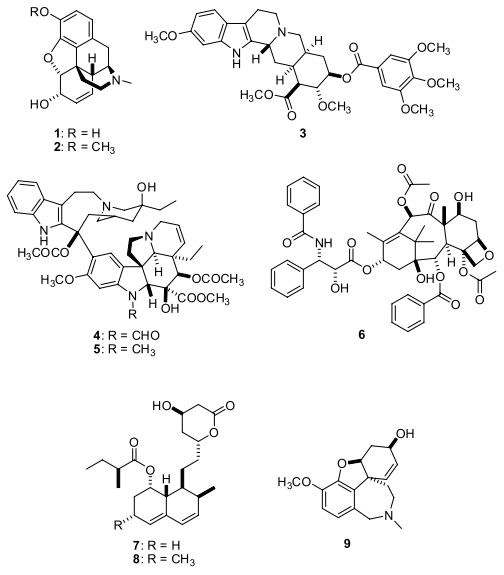

Figure 1. *Some important naturally-derived drugs and lead compounds*

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[#]Artigo em homenagem ao Prof. Otto R. Gottlieb (31/8/1920-19/6/2011)

amounts in the plant, which makes their commercialization unfeasible. The purpose of medicinal chemistry or biotechnology is to preserve biodiversity (chemical diversity libraries) and develop new drugs as an alternative to the production of the corresponding secondary metabolites. Even when bioactive natural products are not identified with the ideal potency or pharmacological properties for the development of new drugs, their chemical structures can be used as valuable models for the design of new analogues with optimized biological properties.

The latest review published by Kinghorn and collaborators[14] emphasized the importance of natural products in drug discovery. Of the 210 small-molecule therapeutic agents included in the recent World Health Organization Model List of Essential Medicines, 17 are plant-derived.[15] The Food and Drug Administration (FDA) approved ca. 16 new plant-derived drugs between 2001 and 2010. Among these compounds, several were obtained via modification of old plant-derived drugs (Figure 2). One example is tiotropium bromide (**10**),[16] an atropine derivative (Spiriva®) used in the treatment of chronic obstructive pulmonary disease (COPD) and COPD exacerbation that was introduced onto the market in 2002.[17] In the mid-1980s, several medicinal chemistry studies on Δ^9-tetrahydrocannabinoid derivatives were conducted.[18] However, only in 2006 was the derivative nabilone (**11**, Cesamet®) approved for the treatment of chemotherapy-induced nausea. In 2008, two long-known naturally-derived drugs came under the spotlight, namely methylnaltrexone bromide (**12**), a morphine-derivative drug (Relistor®) introduced onto the market to treat opioid-induced constipation, and tetrabenazine (**13**, Xenazine®), a benzylisoquinoline alkaloid derived from emetine (**14**), designed for the treatment of Huntington's disease. In 2009, two plant-derived drugs were made commercially available. More specifically, one of these drugs is the new antimalarial chemical entity artemether (**15**, Coartem®), derived from the known antimalarial artemisinin (**16**). Three natural plant product derivatives were approved as drugs by the FDA in 2010, among which is the new paclitaxel (**6**, Taxol®)-derivative named cabazitaxel (**17**, Jevtana®). Paclitaxel is a diterpene isolated from *Taxus brevifolia* and *T. baccata,* considered to be one of the most important drugs discovered in the last century and which became a blockbuster in the anticancer pharma market. Paclitaxel is a very complex molecule and its successful history has served as inspiration for medicinal chemistry recently. The FDA approved paclitaxel for the treatment of drug-refractory metastatic ovarian cancer in 1992. It has also furnished promising results in clinical trials for the treatment of lung, breast, and head and neck cancers, as well as AIDS-related Kaposi's sarcoma.[7]

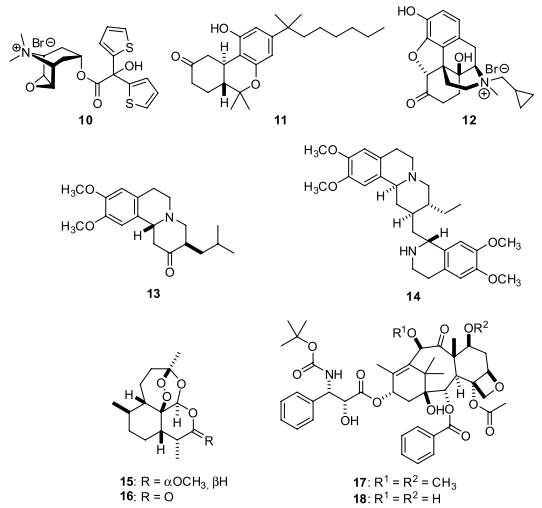


Figure 2. *Natural products and natural product-derived drugs*

Researchers seek continuous improvement in drugs, aiming to achieve greater bioavailability of the commercially-available drugs. Before the development of Jevtana®, another important formulation in which paclitaxel is bound to albumin had been designed and sold under the trademark Abraxane®.[19,20]

Docetaxel (**18**, Taxotere®) is a semisynthetic analogue of paclitaxel, but is more active than the natural product and has better pharmacokinetic properties, such as improved water solubility.[7] The FDA approved docetaxel in 2006 for the treatment of head and neck, breast, gastric, and prostate cancers.[21]

A long list of many drugs could be cited in this text as natural products, semisynthetic derivatives, or total synthesis pharmaceuticals, all of which were inspired by natural molecules isolated from biodiversity, especially plant species. However, the Earth's biodiversity remains underexplored (Table 1).[22] There are thousands of molecules yet to be discovered, and among these will be several that are completely novel from both biological and chemical standpoints.

Table 1. Estimated number of species described in Brazil and in the world. Adapted from ref. 22

Taxon	Known Brazilian species	Species known worldwide
VERTEBRATES		
Mammals	394	4,327
Birds	1,573	9,672
Reptiles	468	6,550
Amphibians	502	4,000
Freshwater fish	2,000	4,000
INVERTEBRATES		
Pseudoscorpions	40	3,000
Opiliones	581	3,500
Isoptera	500	2,000
Formicidae	2,233	10,000
Carabidae	5,000	40,000
PLANTS		
Angiospermae	55,000	250,000

ALTERNATIVE SOURCES OF NATURAL PRODUCTS: A HOPE FOR THE DISCOVERY OF NEW DRUGS?

Besides plant species (Angiosperms and Gymnosperms), there are several other organisms to be studied on Earth. Terrestrial biodiversity includes many biomes that remain largely underexplored or entirely unexplored, even though they represent potentially rich sources of new molecular models for synthesis, medicinal chemistry, and pharmacological and toxicological evaluations.[23]

Unlike terrestrial biodiversity, marine biodiversity has a shorter history when it comes to the use of its huge medicinal potential. However, the extensive biodiversity to be found in the oceans constitutes a novel alternative for drug discovery, since oceans cover 70% of the planet.[24] Many secondary metabolites with unique structural features have been isolated from only a small number of ocean organisms.[25] Therefore, oceans must contain huge supplies of structurally unique secondary metabolites that can be useful as new chemical templates for drug discovery studies, as seen in Table 2 and Figure 3.[25] These few examples demonstrate that the oceans indeed represent an unexploited source of novel compounds with promising applications in medicinal chemistry.

A good example of a product derived from a natural marine compound is ziconotide (**19**, Prialt®). This is a synthetic analog of ω-conotoxin MVIIA, which in turn is a peptide toxin consisting of

Table 2. Some marine drugs and potential natural compound-derived drugs

Metabolite	Source	Ref.
Ziconotide (**19**)	Cone shell, *Conus magus*	8,26-28
Bryostatin 1 (**20**)	Bryozoa, *Bugula neritina*	28-30
Halichondrin B (**21**)	Sponge, *Halichondria okadai*	28,31
Ara-A (**22**)	Sponge, *Tethya crypta*	28,32
Dolastatin 10 (**23**)	Mollusk, *Dolabella auricularia*	28,30

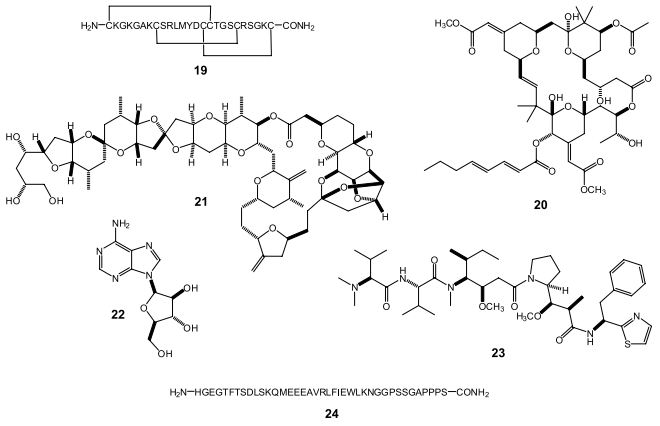


Figure 3. *Drugs obtained from marine sources (**19**-**23**) and from Gila monster (**24**). Abbreviations for amino acids in peptide structures: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; Y, Tyr; W, Trp*

25 amino acids. This toxin was isolated from *Conus magus* (syn. *C. fulvobullatus*), a sea snail gastropod mollusk (Conidae taxon).[8,26-28] This new natural product sold under the trademark Prialt® by Elan Pharmaceuticals is a novel non-opioid drug recently approved by the FDA for treatment of chronic pain. It acts by binding and inhibiting the presynaptic calcium channel, thereby preventing the release of neurotransmitters.[27] The therapeutic benefit of this drug stems from its potent and selective blockade of neuronal-type voltage-sensitive calcium channels, so this compound represents a new class of *N*-type calcium channel blocker agent. This drug may represent an alternative option for patients with refractory pain syndromes and will be made commercially available more than 200 years after the discovery of morphine (**1**), one of the major alkaloids from *Papaver somniferum* presently used for the treatment of refractory pain syndromes.

In addition to the enormous biodiversity found in the seas, there are several ecosystems that can be considered alternative sources in the search for new biologically active natural products (Table 2). Toxins from insects, lizards, snakes, animals, and microorganisms can also be valuable sources of potential new drugs. Several organisms adapted to extraordinary environments have accumulated unusual secondary metabolites generated via special biosynthetic pathways and have become an important source of New Molecular Entities (NMEs).[33]

A further example showing the importance of searching for natural products from alternative sources is the recent drug launched onto the market as Byetta®, a 39-amino acid peptide designated exendin-4 (**24**, exenatide), which was originally isolated from the salivary secretions of *Heloderma suspectum* (Gila monster).[34] Exendin-4 interacts with the newly described exendin receptor, thereby increasing pancreatic acinar cAMP. This is the first agent described within the new class of incretin mimetics approved by the FDA in 2005 for the treatment of patients with type 2 diabetes mellitus.[35] This compound is a glucagon-like peptide 1 (GLP-1) receptor agonist with high potency. Truncated exendin-(9–39)-amide is an antagonist at the glucagon-like peptide 1-(7–36)-amide receptor of insulin-secreting β-cells that possesses multiple glucoregulatory effects, including enhancement of glucose-dependent insulin secretion, reduction of glucagon secretion, reduction of food intake, and slowing of gastric emptying.[36] Additionally, bio-engineering techniques aimed at reducing production costs have also been described.[37]

Nojirimycin (**25**) is another example of a drug for the treatment of diabetes that has been inspired in natural products. This substance is a natural constituent first isolated as an antibiotic from fermentation broths of several strains of *Streptomyces* such as *Str. roseochromogenes*, *Str. Lavendulae*, and *Str. nojiriensis* in 1966.[38] The structure of **25** is a glucose analog comprising an endocyclic nitrogen instead of the pyranosidic oxygen in glucose. This drug was shown to be a potent inhibitor of both α- and β-glucosidase in the 1970s.[39] Although it presents relevant activity, the hydroxyl group at C–1 makes the compound unstable.[40] This drawback has prompted studies regarding structural modification, with a view to improving the stability of the compound. Reduction of **25** by catalytic hydrogenation or sodium borohydride afforded the analog 1-deoxynojirimycin (**26**), a more stable and potent glucosidase inhibitor *in vitro*. Later, it was verified that its *in vivo* activity was only moderate and produced side effects.[41] In 1994 isofagomine (**27**) was reported as a new potent β-glucosidase inhibitor[42] that mimics the transition state of glycoside cleavage in its protonated form. It is noteworthy that **27** has been described to inhibit β-glucosidase, glucoamylase, and isomaltase more strongly than **26**.[43] However, its lack of selectivity has been shown to cause problems and side effects in therapeutic applications.[44]

The search for more potent iminosugars has led to the preparation of *N*-alkylated analogs, which were generally found to be stronger glycosidase inhibitors than the corresponding non-alkylated derivatives.[41,45] *N*-butyl-1-deoxynojirimycin (**28**, Zavesca®) and *N*-hydroxyethyl-deoxynojirimycin (**29**, miglitol) (Figure 4) are especially remarkable for their high biological activity. Clinical trials for type 1 Gaucher disease and lysosomal storage disorder have been successfully completed for these compounds.[44,46] Zavesca potently inhibits the activity of glycosidase enzymes, whereas miglitol is a potent sucrase inhibitor and antidiabetic drug that has been available since 1996.[45]

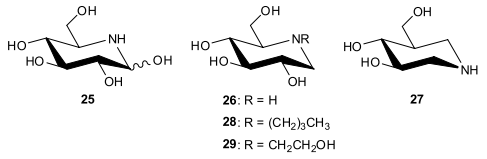


Figure 4. *Glucosidase inhibitors **25**-**29***

Therefore, modification of a known iminosugar has been established as a positive strategy for the attainment of stronger and more selective glycosidase inhibitors of therapeutic interest. The therapeutic potential of *N*-alkylated derivatives has attracted increased interest and motivated the development of new methodologies for their synthesis and biological evaluation. To this end, a number of structural modifications in the basic skeleton of the original nojirimycin have been made, namely presence/absence of hydroxyl groups, and introduction of different alkyl substituents into the ring containing the nitrogen atom.[45]

CURRENT DRUG DISCOVERY SCENARIO: NATURAL PRODUCTS IN THE LIMELIGHT OR A POWERFUL SOURCE OF RAW MATERIAL TO BE EXPLORED OTHERWISE?

The process of drug discovery, which starts from a compound's basic science through to drug development and clinical trials, is very

complex, expensive, and time-consuming. However, the statistically estimated number of species described to date, especially in the case of tropical and equatorial biomes (Table 1), shows that the Earth's biodiversity is a treasure that has yet to be uncovered, mainly with regard to secondary metabolites produced by countless organisms that have never been studied. So far, biodiversity, including those of extreme habitats, has been the most sophisticated source for the discovery of NMEs. One of the main challenges for the rational exploration of this remarkable chemical and biological diversity concerns methodologies for the separation and structural elucidation of natural compounds. Fortunately, there have been recent advancements in HPLC, MS, and NMR techniques. New approaches have been developed, in order to minimize the problems inherent to the analysis of complex mixtures containing structurally intricate molecules, thus allowing for the rapid identification of new biologically-active compounds.[47,48]

A recent commentary published in Nature Chemical Biology[49] highlights that the international economic crisis has predominantly affected the pharmaceutical industry in developed countries, mainly because of the low number of new drugs that have been recently approved and the relatively high costs involved in the Research and Development (R&D) of new targets (Figure 5).

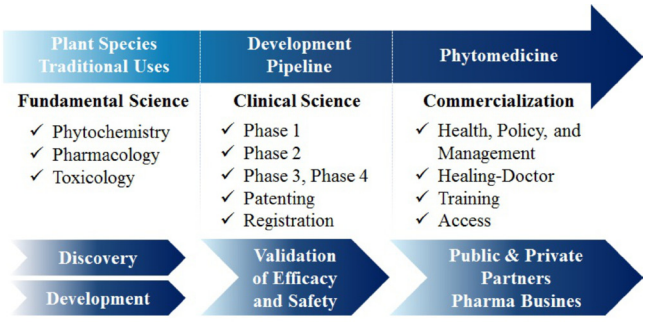


Figure 5. *Data from multinational pharmaceutical companies in the period 2005-2010, including data from FDA-approved NMEs versus R&D spending (in million US Dollars) for nine big companies (AstraZeneca, Bristol-MeyersSquibb, Eli Lilly, GlaxoSmithKline, Merck, Novartis, Pfizer, Roche, and Aventis. Adaptation of ref. 49*

An additional challenge in drug discovery today is expanding the number of new categories of drugs. Natural products have been substantiated in ca. 87% of all the categorized medicines for the treatment of human diseases, prioritizing antibacterial, anticancer, anticoagulant, antiparasitic, and immunosuppressant agents.[15] However, a glimpse at all the research and development accomplished by pharmaceutical companies from 1981 to 2002 shows that they did not launch any new drugs based on or derived from natural products for the seven categories, namely anesthetic, antianginal, antihistaminic, anxiolytic, chelating and antidote, diuretic, and hypnotic.[50] This outcome serves as a further motivation for medicinal chemistry based on natural products, since they establish complex molecules, most of which have not yet been pharmacologically and toxicologically analyzed.[51]

The use of natural products as a source of novel therapeutic agents reached its peak in the 1990s in the Western pharmaceutical industry. Since then, their use has declined dramatically, largely because of a shift in paradigms among the drug companies. The reason for this change is the fact that the pharmaceutical industry has decided to invest in combinatorial synthesis, which is capable of quickly producing thousands of compound libraries (Figure 6). However, natural products remain the most sophisticated source for drug discovery. Indeed, they have gained a prominent position in pharmaceutical discovery research since the dawn of medicine.[52]

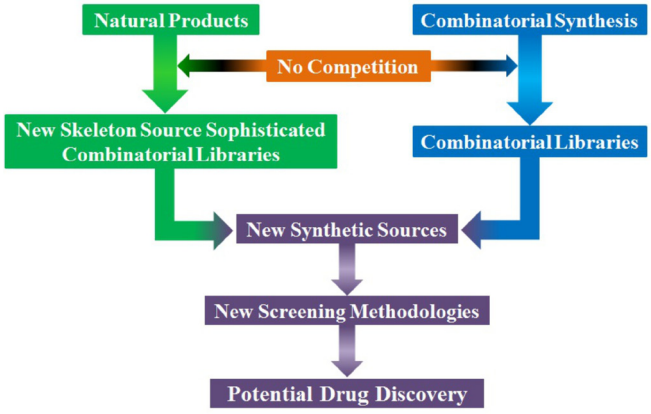


Figure 6. *Illustrative scheme of natural products and combinatorial synthesis, two important complementary sources of new biologically-derived compounds and valuable therapeutic agents*

Medicines based on natural products have also been prosperous in the Eastern world. The Ayurveda, written in around 900 BC, constitutes a seminal document describing several plants with medical use, which must be investigated by means of new analytical tools such as metabolomic methodologies.[53] Traditional Chinese Medicine (TCM) has been one of the most famous uses of medicinal plant species since the beginning of this civilization. The medicinal book *Wu Shi Er Bing Fang* translates how to treat several diseases with plants and is one of the oldest documents dedicated to the utilization of medicinal plants used as medications.[51,54,55]

Pharmaceutical research into herbal medicines has increased dramatically worldwide (Figure 7) and is largely based on traditional practices such as TCM. This traditional knowledge can represent a therapeutic alternative especially in the case of countries that do not have pharmaceutical companies with R&D expertise, since it involves lower costs and reduced development time when compared to the identification of new NMEs.[55]

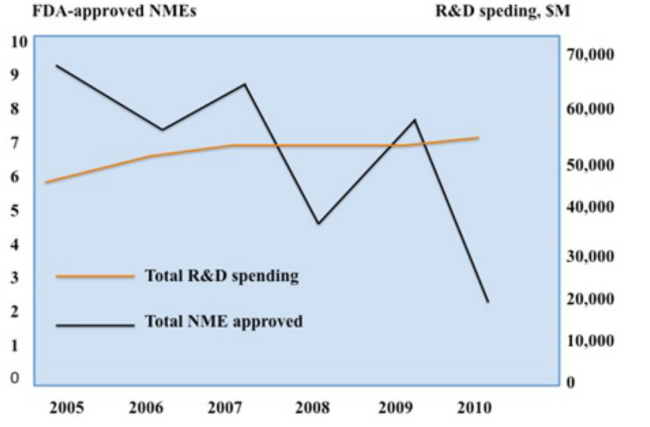


Figure 7. *Platform for the development of phytotherapic drugs (herbal medicines), which should require less time from the discovery of the active compound to its commercial availability. An alternative for pharmaceutical companies to invest in developing countries*

In a country such as Brazil, which is home to one of the richest sources of biodiversity and has a traditional history of native tribes, herbal medicines represent a powerful source for investigation by the academic community and pharmaceutical industries. This remarkable chemical diversity remains completely unexplored, even though it encompasses new sources of drug leads to be used in drug discovery.[56]

In view of the many challenges faced in the area of medicinal chemistry and drug discovery, the question raised by today's researchers worldwide is "what hope is there for drug discovery?". In this scenario, the title of this review is very pertinent: tropical biodiversity - has it been a potential source of secondary metabolites useful for medicinal chemistry? The answer can be evidenced by the old and new examples described herein and in many reviews on drug discovery published over the last decade.[6,8,9,14,31]

BRAZILIAN BIODIVERSITY: AN UNKNOWN POTENTIAL SOURCE FOR DRUG DISCOVERY

The vast and biologically-rich Neotropical Region represents an outstanding opportunity for the development of biodiversity science in many different domains, including drug discovery. Covering a total area of 8,511,996 km^2, Brazil hosts huge biodiversity, which is distributed across several important biomes and ecosystems and contains 10 to 20% of the world's known living species, many of which have not been described. It has been estimated that around 2 million plant, animal, and microorganism species exist in Brazil.[22] The most important Brazilian biomes are the Amazonian rainforest and the deciduous forests in the North, the Eastern coastal forest (known as the Atlantic Forest), the savannah areas (Cerrado) in the center of the country, the thorn forest (Caatinga scrubs) in the Northeast and North, the Pantanal wetlands in the mid-west, and the pine forests and Pampa fields in the South. Some of these great biomes have been heavily damaged by the encroachment of human activities, as in the case of the Atlantic Forest and the Southern pine forests.[22]

A classic example of a Brazilian natural product used as a drug or model for drug design is tubocurarine (**30**) (Figure 8), a *bis*-benzyltetrahydroisoquinoline alkaloid that is the most important constituent of the curare poison prepared from *Chondondendron tomentosum*. This substance has been applied to arrows by the South American Indians during their hunt for animals. Curare is only active by contact with the bloodstream, but is harmless if taken orally.[57] The main medical application for tubocurarine is the relaxation of abdominal muscles during the pre-operative period, thereby allowing for the use of lower levels of general anesthetics. Tubocurarine has been used for many years, but its undesirable side effects have driven the development of new anesthetics (Figure 8). Analogs of this natural product such as decamethonium (**31**), suxamethonium (**32**), and atracurium (**33**) have now been designed. The tubocurarine-like effect is preserved in these compounds containing two quaternary nitrogens separated by a polymethylene chain.[58]

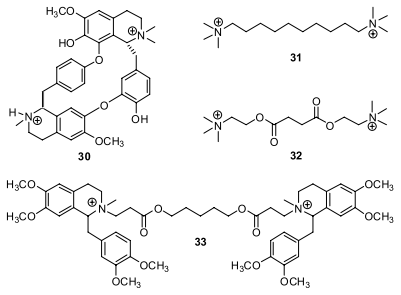


Figure 8. *Tubocurarine-derived anesthetics* ***30-33***

Another very successful example has emerged from studies on the venom of *Bothrops jararaca*, a common snake species in Brazil. These investigations have culminated in the discovery of bradykinin (**34**) (Figure 9), a peptide inhibitor of the angiotensin-converting enzyme (ACE), an important target in the therapy of hypertensive disease,[59] and have contributed to the design of a class of hypertension regulators, the ACE inhibitors, represented by captopril (**35**).[60] The molecular design of **35** was based on simple peptide units, created on the basis of knowledge acquired from structure-activity studies on synthetic analogs of venom peptides. These investigations have improved understanding of the active site of ACE and revealed that the optimal carboxy-terminal amino acid sequence of inhibitors for binding to the enzyme was Phe-Ala-Pro. Also on the basis of these studies, the exchange of the carboxyl for a sulfhydryl group in the D-2-methylsuccinyl-Pro (**36**) derivative, with the purpose of favoring interaction with the zinc present in the active site of the enzyme, has resulted in compound **35** and led to a 1000-fold increase in inhibitory potency. Indeed, **35** has been proven to be one of the most potent competitive inhibitors known to date and is the first really useful antihypertensive drug designed for binding to the active site of ACE.[61] Specific interactions between this type of inhibitor and the active site of ACE have been proposed and confirmed by measuring the inhibitory potency of structural analogs.[61]

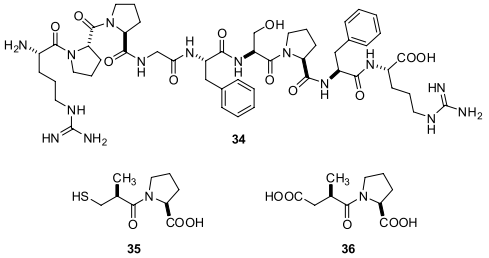


Figure 9. *ACE inhibitors* ***34-36***

Within this scenario, the State of São Paulo Research Foundation/FAPESP[62] launched the BIOTA/FAPESP Program: The Virtual Institute of Biodiversity,[63] in March 1999. Among the several objectives of this program, one goal is the search for bioactive compounds from São Paulo State's biodiversity, aiming to find new lead molecules from the main Cerrado and Atlantic Forest biomes, which remain chemically and biologically underexplored. Some examples of lead molecules obtained by means of this collaborative program may highlight the importance of tropical biodiversity for drug discovery and will be further described.

Several extracts of *Casearia* species have been the focus of study by groups working mainly in Japan and Brazil. *Casearia sylvestris* Swartz (Salicaceae) is a tree that grows practically throughout the entire Brazilian territory, from the Northern state of Amazonas (Tapajós River region) to the Southern state of Rio Grande do Sul.[64] It has been utilized for the treatment of snake bites, wound healing, and also as an anti-ulcer, anti-pyretic, and topical antiseptic agent in both popular and traditional medicine. *C. sylvestris* extract is also currently sold as capsules. Studies on *C. sylvestris* extract have confirmed its antiulcerogenic,[65] anti-inflammatory,[66] antimicrobial, antivenom,[67] and cytotoxic activities.[68,69] Moreover, no significant toxicological effects have been observed after oral administration of ethanolic extracts of *C. sylvestris* leaves to animals.[70] Additionally, no genotoxicity was detected when the extracts were assayed against hepatoma tissue and lung fibroblast V-79 cell cultures.[71]

Phytochemical investigations of the leaves of *C. sylvestris* have led to the identification of bioactive clerodane diterpenes, namely the casearins and casearvestrins (Figure 10).[64] Several diterpenes have been shown to possess very interesting structural features and pharmacological properties. One of the foremost examples is paclitaxel, a market leader in anticancer drugs. There are numerous examples of potential antitumor clerodane diterpenes.[68,69,72] A crude bioactive ethanolic extract of the twigs of *C. obliqua* afforded two clerodane diterpenes, caseobliquins A (**37**) and B (**38**), while *rel*-6β-hydroxyzuelanin-2β-benzoate (**39**) and *rel*-2α-hydroxyzuelanin-6β-benzoate (**40**), as

a mixture, as well as 2β-hydroxyzuelanin-6β-cinnamate (**41**), have been isolated from the *n*-hexane extract of the leaves of this species. The cytotoxic activities of these compounds have been evaluated *in vitro* against HL-60 (human myeloblastic leukemia), HCT-8 (human colon carcinoma), MDA/MB-435 (human melanoma), and SF-295 (human glioblastoma) cells. The mixture of **39** and **40** showed significant cytotoxicity activity against the four cancer cell lines, with IC_{50} values ranging from 0.13 to 1.00 μM, similar to that of the positive control, doxorubicin.[73]

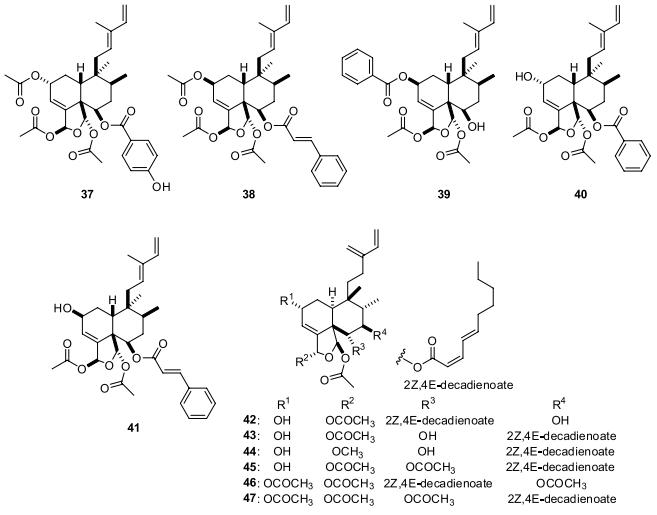


Figure 10. *Bioactive clerodane diterpenes (**37-45**) isolated from C. obliqua and C. rupestris and semi-synthetic derivatives (**46** and **47**)*

Recently, four new clerodane diterpenes, the casearupestrins A–D (**42-45**), have been isolated from the leaves of *C. rupestris* (Figure 10). Casearupestrins A (**42**), B (**43**), and D (**45**) exhibited significant cytotoxicity against the same four cell lines described above, with IC_{50} values ranging from 0.10 to 1.30 μM, being 0.10 μM superior to that of the standard drug doxorubicin. This result was obtained when **42** was evaluated against the cancer cell line HL-60. To complete a more comprehensive study, acetylation of **42** and **45** with Ac_2O–pyridine was carried out, which yielded two new clerodane diterpenes derivatives, namely 2,7-di-*O*-acetylcasearupestrin A (**46**) and 2,6-di-*O*-acetylcasearupestrin D (**47**). The acetyl derivatives **46** and **47** exhibited decreased cytotoxicity, thus indicating that the hydroxyl group at C–2 is important for the cytotoxicity of these compounds.[74]

An additional study on the antitumor activity of casearins has revealed that the ethanolic extract of *C. sylvestris* at low concentrations protects DNA against damage, which was carefully estimated by using the micronucleus test and comet assay. Interestingly, the same ethanolic extract was also able to cause DNA damage at higher concentrations. The isolated compound caseargrewiin F (**48**) (Figure 11) has been demonstrated to be both mutagenic and genotoxic at the highest dose; however, this same compound protects DNA against damage at low concentrations.[75]

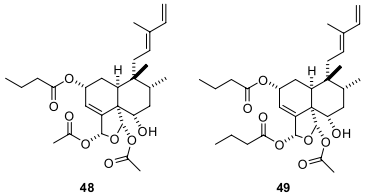


Figure 11. *Bioactive clerodane diterpenes (**48** and **49**) isolated from C. sylvetris*

Further studies on **48** and casearin X (**49**) (Figure 11) have been conducted, in order to evaluate their respective cytotoxicity against the four human tumor cell lines MOLT-4 (leukaemia), MDA-MB-435 (melanoma), HCT-8 (colon), and SF-295 (glioblastoma), as well as against L-929 (normal fibroblasts). Caseargrewiin F (**48**) exhibited stronger cytotoxicity against all the investigated tumor cell lines (IC_{50} < 0.20 μM) when compared to normal L-929 cells (IC_{50} = 1.09 μM), and showed 12.10-fold higher selectivity toward MOLT-4 cells as compared to L-929. These values are similar to those obtained for the positive control doxorubicin, which presented IC_{50} values ranging from 0.02 to 0.83 μM for tumor cells, and 1.20 μM for L-929 cells, which corresponds to 24-fold higher selectivity. Casearin X (**49**) has been demonstrated to be less cytotoxic, exhibiting good antiproliferative potential against tumor cells (IC_{50} < 1.00 μM). Moreover, none of the examined compounds causes hemolysis, even at the highest concentration (200 mg mL^{-1}). This suggests that the cytotoxicity mechanism is probably related to a more specific pathway.[71]

A study to establish whether the growth inhibition elicited by **49** is related to the induction of apoptosis or necrosis has been recently accomplished using the cell line HL-60. Cells treated with **49** were analyzed, and the effects of lower doses of this compound were intact membrane integrity, mitochondrial depolarization, DNA fragmentation, and PS externalization, all of which are typical of apoptosis. Increasing concentrations of **49** culminated in necrosis features such as loss of membrane integrity, loss of mitochondrial depolarization, and highly activated caspase 3/7.[64]

Analysis of the numerous secondary metabolites with some interesting biological activity published in the past and current literature evidence few examples of detailed experimental data on the mechanism of action, even in preliminary assays. This scenario also reveals a good starting point for further studies in the search for natural products such as new NMEs. There is no well-established mechanism of action for any of the casearins described to date. Hence, our collaborative research group is currently focused on determining the interaction of these compounds with some important proteins.

The labdane diterpenes are another class of secondary metabolites that we have selected from Brazilian biodiversity for their bioactivity. Three derivatives, namely 6α-acetoxymanoyl oxide (50), 6α-malonyloxymanoyl oxide (51), and 6α-malonyloxy-*n*-butyl ester manoyl oxide (52) (Figure 12), have been isolated from the aerial parts of the ethanolic extract of *Stemodia foliosa* previously described in the literature.[76] *Stemodia foliosa* Benth is widespread in Brazil and grows predominantly in the Northeastern region. It is popularly used as a bioinsecticide and also employed for treatment of respiratory infections. The diterpenoids **50**, **51**, and **52** have been tested for their antibiotic activity toward the bacterial strains *Staphylococcus aureus*, *Bacillus cereus*, *B. subtilis*, *B. anthracis*, *Micrococcus luteus*, *Mycobacterium smegmatis*, and *M. phlei*. Compound **51** exhibited moderate antibiotic activity (MIC values in the range 7.0-20.0 μg mL^{-1}), as compared to the positive control clarithromycin (MIC values in the range 0.5-2.0 μg mL^{-1}). The malonyloxy function at C-6 seems to be an important feature for the antibacterial activity, as this is the only difference between the active **51** and the other inactive compounds **50** and **52**. Additionally, the antibacterial activity of **51** corroborated the popular use of this plant for the treatment of infectious respiratory diseases.[76]

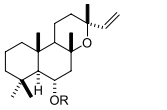


Figure 12. *Labdane diterpenes (**50–52**) isolated from S. foliosa*

During the ongoing search for bioactive natural products from the equatorial and tropical Brazilian flora, a new neolignan skeleton named chimarrhinin (**53**) (Figure 13) was isolated from an extract of the leaves of *Chimarrhis turbinata*, a Rubiaceae plant species. ^{13}C NMR spectrometric techniques including 1D and 2D experiments as well as HRMS provided structural confirmation of this new C6.C3 skeleton type. The relative configuration of **53** was established by 2D ^{1}H–H analysis and *J* couplings, while its conformation was evaluated through molecular modeling using the RM1 semi-empirical method, with the aid of coupling constants obtained by NMR analysis. The radical scavenging effects observed for compound **53** (IC_{50} 7.50 ± 0.50 μM), as assayed with DPPH, produced the best results, with an IC_{50} value lower than that of the standard antioxidant BHT (IC_{50} 62.50 ± 0.60 μM) and chlorogenic acid (IC_{50} 20.00 ± 0.20 μM), used as positive controls.[77]

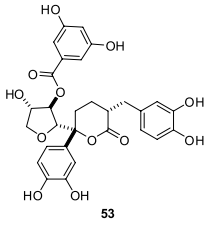
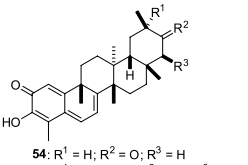


Figure 13. *New neolignan (**53**) isolated from C. turbinate*

Quinonemethide sesquiterpenes (Celastraceae and Hippocrateaceae) (Figure 14) are also another interesting class due to their bioactivities.[78,79] Some of these triterpenes, namely maytenin (**54**), pristimerin (**55**), 22β-hydroxymaytenin (**56**), 20α-hydroxymaytenin (**57**), and celastrol (**58**), are associated with a range of biological activities such as anti-tumor, antimicrobial, antimalarial,[80] antibiotic, and antioxidant action.[81]

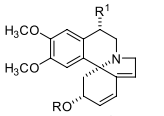


54: R^1 = H; R^2 = O; R^3 = H
55: R^1 = CO_2CH_3; R^2 = 2H; R^3 = H
56: R^1 = H; R^2 = O; R^3 = OH
57: R^1 = OH; R^2 = O; R^3 = H
58: R^1 = CO_2H; R^2 = 2H; R^3 = H
59: R^1 = CO_2CH_3; R^2 = 2H; R^3 = OH

Figure 14. *Quinonemethide sesquiterpenes **54**-**59***

The *in vitro* evaluation of 22β-hydroxypristimerin (**59**), **54** and **55**, revealed strong cytotoxic activity against peripheral blood mononuclear cells (PBMC), and leukemia cells in the same concentration range, thus, no selectivity was observed.[81,82] The mechanism involved in the cytotoxic effect of **55** has been studied, but not yet completely explored. Treatment of human breast cancer cells (MDA/MB-231) with **55** caused release of cytochrome c from the mitochondria as well as decrease in the mitochondrial membrane potential, culminating in apoptosis.[83] The effect of this compound on human leukemia cell lines, as well as the underlying mechanism of action, have been investigated based on changes in cell viability and morphology (induction of apoptosis and/or necrosis), and it has been concluded that the mechanism involved in cell death is apparently apoptosis.[82] DNA synthesis was also affected by treatment with **55** at all concentrations, in a dose-dependent manner. Current cancer chemotherapy drugs such as intercalating agents and topoisomerase inhibitors can also inhibit DNA synthesis. Furthermore, **55** was unable to inhibit the activity of human topoisomerase I, which is an indication that the antiproliferative mechanism of this compound probably includes alterations to another pathway.[82]

Another very well-known plant species in Brazil is *Erythrina mulungu* (EM), due to its popular medicinal uses. A tincture prepared from the leaf or bark decoction from this plant is used to calm agitation and other disorders of the nervous system. Commercial EM preparations are available in Brazil and in the USA as herbal medicine.[84,85] Several *Erythrina* species have been studied, and studies on *E. mulungu* conducted by our group have resulted in the isolation of three erythrinian alkaloids, (+)-11α-hydroxyerythravine (**60**), (+)-erythravine (**61**), and (+)-α-hydroxyerysotrine (**62**) (Figure 15), as well as confirmation of the anxiolytic properties of these erythrinian alkaloids. The effects of these alkaloids associated with the low toxicity of one standard mixture are currently under study by a Brazilian Pharmaceutical Co. that aims to launch a herbal medicine whose effects could be similar to those produced by diazepam, a classic anxiolytic drug.[84,86] These results suggest that the erythrinian alkaloids isolated from *E. mulungu* play a major role in the anxiolytic effects of EM and support the popular use of the tincture as an anxiolytic medicine.[85]



60: R = H, R^1 = OH
61: R = R^1 = H
62: R = CH_3, R^1 = OH

Figure 15. *Erythrinian alkaloids (**60**-**62**) isolated from E. mulungu*

In Brazil, *Senna* species are known for several applications, including their ornamental use given their beautiful yellow blossoms. Our group has systematically studied *Senna spectabilis, syn. Cassia spectabilis,* because this species accumulates rare piperidine alkaloids with DNA repair properties.[87] The first chemical study of the leaves of this species led to the isolation of seven alkaloids, namely (–)-spectaline (**63**), leptophyllin A (**64**), 3-*O*-acetil-leptophyllin A (**65**), leptophyllin B (**66**), (–)-spectalinine (**67**), carnavaline (**68**), and iso-6-carnavaline (**69**) (Figure 16). Compounds **63**, **67**, and **68** exhibited selective cytotoxic activity against *Saccharomyces cerevisiae* strains.[87]

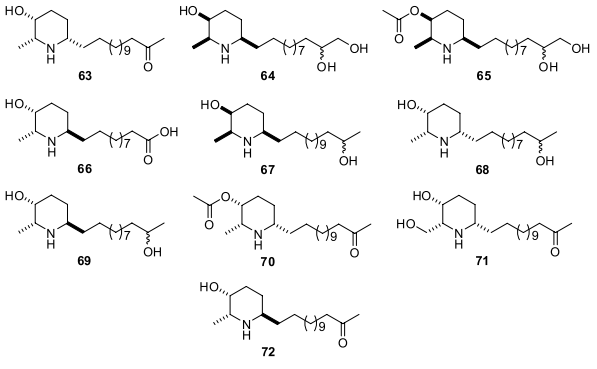


Figure 16. *Piperidine alkaloids (**63**-**72**) isolated from S. spectabilis*

Further studies on the flowers of *S. spectabilis* have resulted in the isolation of three new alkaloids, more specifically (–)-3-*O*-acetylspectaline (**70**), (–)-7-hydroxy-spectaline (**71**), and *iso*-6-spectaline (**72**) (Figure 16).[88] In addition, the presence of a homologous series of novel piperidine alkaloids has been detected in the fruits of *S. spectabilis* by means of tandem mass spectrometry with electrospray ionization.[89] A detailed analysis of the structural features of **70** has led to identification of an acetylcholine (ACh) subunit internalized in this

molecule (Figure 17), and led to the design of several semi-synthetic derivatives, including (–)-3-*O*-acetyl-spectaline hydrochloride (**73**), prepared from **63**. This derivative displays cholinergic activity both *in vitro* and *in vivo* (IC_{50} = 7.32 μM).[90] Kinetic studies aimed at elucidating the mechanism of cholinesterase inhibition elicited by this derivative have demonstrated noncompetitive cholinesterase inhibition as well central nervous selectivity with few peripheral side effects.[91]

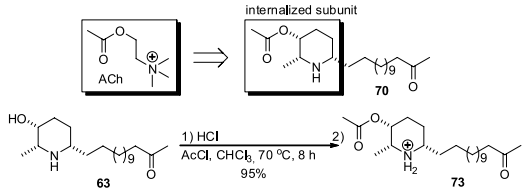


Figure 17. *Structural design of the semi-synthetic AChE inhibitor* ***73***

Taking these results into account, **70** and tacrine (**74**), a potent acetylcholinesterase inhibitor (AChEI), were selected for molecular hybridization (Figure 18), which furnished a range of isosteric pyridinic and pyrazinic compounds represented by ethyl 2-[(2,3-dihydro-2-oxo-1H-pyrido[2,3-b][1,4]thiazin-6-yl)sulfanyl] acetate (**75**). These substances were assayed for their nematostatic and anthelmintic activities, since reversible and irreversible AChEIs have been employed in numerous applications including the control of parasites. All the compounds exhibited anthelmintic activity in the assays against the gastrointestinal parasitic nematode *Nippostrongylus brasiliensis*, and the synthetic intermediate ethyl 2-[(6-chloropyrazin-2-yl)sulfanyl] acetate (**76**) was highly potent in the screening assay against the phytopathogen *Meloidogyne incognita*, inducing immobilization in 98% of the nematodes. In addition, the accurate oral toxicity of a representative compound, namely diethyl 2,2'-[(3-nitropyridine-2,6--diyl) bissulfanediyl] diacetate (**77**), was evaluated in rats, and this drug was shown to be non-toxic at a dose of 2000 mg/kg. These results highlight the potential use of this class of compounds as anthelmintic or nematicidic agents.[92]

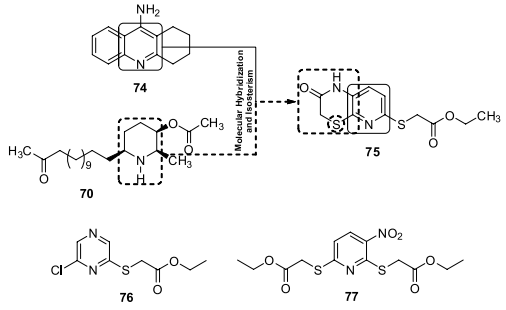


Figure 18. *Lead compounds derived from natural products*

The few examples from our recent search for bioactive natural products are highly encouraging. One prototype has been selected, demonstrating our efforts to continue the search for bioactive compounds from Brazilian biodiversity.

FUTURE PERSPECTIVE AND CONCLUSIONS

Drug discovery critically depends on access to thousands of small molecules available in synthetic or natural product libraries, which can be active in biomolecules such as receptors and proteins, among others. In this context, natural products can definitely be recognized as an excellent starting point for the discovery of NMEs. Several studies have been published recently regarding a set of descriptors that are able to identify natural products from those obtained by synthesis based on their Shannon Entropy.[93] With this parameter, it is possible to identify several new natural scaffold architectures that have not yet been described and that could be useful in medicinal chemistry and drug design.[94]

In this sense, natural products will continue to inspire drug discovery. However, the identification and functional analysis of this treasure will imply the use of different strategies, such as data set methodologies capable of bridging secondary metabolites and metabolomics by means of sophisticated HPLC-MS or HPLC-NMR, hyphenated technologies and computational algorithms, which can aid analysis and identification of bioactive compounds in complex unfractionated mixtures of extracts.[95]

More recently, the dereplication methodology has been employed in order to accelerate the preliminary step of the process, which has been greatly facilitated by recent advances in analytical technologies such as hyphenated HPLC-mass spectrometry (LC-MS) and HPLC-NMR spectrometry.[96,97] The powerful experiments capable of rapidly determining physical-chemistry data at very low concentrations of bioactive compounds allied with the convenience of high-throughput screening will enable the biological assays (receptors, enzymes, etc.) to be related to bioactive compounds in the area of pharmaceutical drug discovery development.

Also, the need for new drugs for the treatment of diseases presently without an effective cure, such as Alzheimer's and Parkinson's, is clear, as is the need to find medications for the treatment of conditions such as malignant cancer and infectious diseases caused by resistant bacteria, for which currently available pharmaceuticals are not fully effective. Furthermore, the discovery of new applications for old drugs is significant, mainly those that have inherent side effects, which might now be minimized or removed by structural modifications of existing drugs. The gap in some drug categories (anesthetic, antianginal, antihistaminic, anxiolytic, antidote, diuretic, and hypnotic agents) highlighted between 1981 and 2005 shows that the discovery of novel drugs is of extreme importance, especially considering that new medicines for these purposes are translated into well-being for mankind.

In the commentary reported by Bunnage,[49] it is evident that the current strategies for drug discovery must be revised, considering the productivity of the pharmaceutical industry from 2006 to 2010, irrespective of the drug origin (synthetic or natural). Even against this rather negative outlook, natural products will continue to open doors and yield new ideas for medicinal chemistry research because they open up possibilities for the design of countless new structures, which would be quite impossible through combinatorial synthetic strategies alone.[98]

Other aspects regarding the use of the remarkable chemical diversity of natural products in this new perspective are the selection of a good target and the time involved in choosing lead natural products. At present, the great challenge with respect to drug discovery from natural products lays in the limitations imposed by the existing separation technology and the application of robust methods for the identification and characterization of a biologically active compound in a complex mixture.[52]

Within this perspective, natural products will never lose their place in the spotlight, since they remain a source of completely new structural models, which are essential for the design of novel drugs.

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