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Natural products from Brazilian biodiversity as a source of new models for medicinal chemistry*

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Abstract: Natural products are the inspiration for many valuable therapeutic agents and attest to biodiversity being a rich source of new molecular structures. Their value as templates for medicinal chemistry remains undisputed, even after the growth of the combinatorial chemistry era. Tropical environments, such as Brazilian biomes, offer a particularly rich potential for biologically active compounds with unique structures and continue to contribute toward modern drug discovery. Our bioprospecting of plant species of the *Cerrado* and Atlantic Forest biomes has yielded promising bioactive secondary metabolites, and we describe some of these molecules and semisynthetic derivatives as potential acetylcholinesterase (AChE) inhibitors.

Keywords: Brazilian biomes; drug discovery; medicinal chemistry; natural products; tropical biodiversity.

INTRODUCTION

Plants are the most ancient source of medicine for mankind, and until recently, they have continued to directly contribute to the development of new drugs despite modern advances in organic synthesis and combinatorial chemistry. This statement can be substantiated by examining the actions of the Food and Drug Administration (FDA), which approved 16 new plant-derived drugs during the period 2001 to 2010 [1]. Furthermore, research in this field not only affords natural bioactive compounds but also continues to provide new ideas and routes to new molecular structures that serve as models for drug design [1,2]. Given the number of plant species on Earth and the numerous biosynthetic pathways capable of producing such extraordinary chemical diversity, biodiversity in tropical and equatorial environments obviously offers a particularly rich potential for biologically active compounds that can be used as templates for medicinal chemistry and drug discovery (Fig. 1).

Obstacles in the search for potential drug leads from plants or other organisms include the complexity of crude extracts and the need for documentation of the many constituents and for robustness of high-throughput screening (HTS) methods. However, the search for natural product hits has recently

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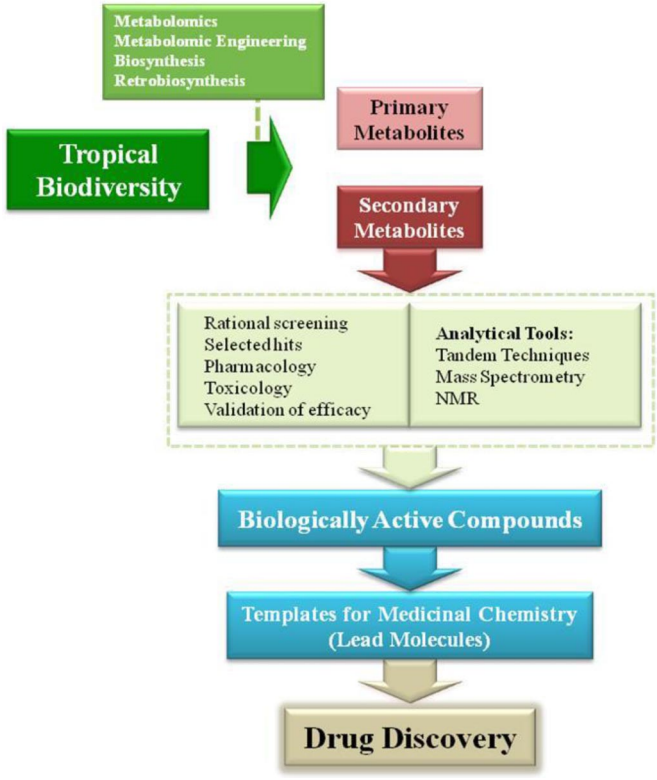


Fig. 1 The use of tropical biodiversity as templates for medicinal chemistry and drug discovery.

been facilitated by the development of more efficient technology for screening based on several modern analytical methods for isolation and structure elucidation [3,4]. These developments have reinvigorated several bioprospecting initiatives on different continents, especially those that target tropical rainforests. Examples include the Tropical Botanic Garden and Research Institute (TBGRI), which undertook ethnobotanical studies in the Western Ghat region of Kerala in India, the Bioresources Development and Conservation Program (BDCP), a multi-ethnic international nongovernmental organization (NGO) based in Nigeria, the Cooperative Drug Development Group (CDDG), the National Institutes of Health (NIH), and Biota-Fapesp [5].

A biotechnological approach has been recognized as a rapid and efficient method for the preservation and sustainable exploitation of plant species and can contribute to economic development in non-industrialized countries [6]. This strategy implies the possibility of producing bioactive compounds through in vitro cultivation and/or micropropagation of selected species. Moreover, studies of the biosynthetic steps involved may provide information about rate-limiting steps for the formation of these metabolites, which introduces the possibility of steering metabolic pathways to increase the yield of desired compounds or of designing their production through the addition of appropriate biosynthetic precursors. The isolation and characterization of the genes encoding for the key enzymes in the biosynthesis of these compounds form the basis of new strategies for the regulation of metabolic pathways through the production of transgenic plants.

One of the major obstacles during research on natural products arises from the limitations of the methodologies for separation and structural elucidation. Recent advances in high-performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) techniques have greatly facilitated the more rapid identification and isolation of new biologically active molecules

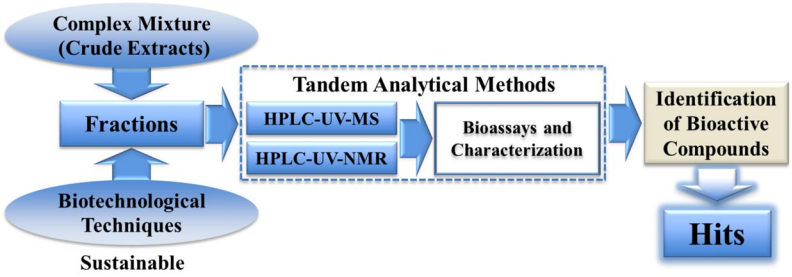


Fig. 2 Methodologies for the identification of natural compounds.

[3,7] (Fig. 2). Furthermore, dereplication methodology also contributes to accelerating the preliminary steps of the biodiscovery process [8,9].

BRAZILIAN SCENARIO ON DRUG DISCOVERY

Brazil is one of the most biodiverse countries in the world. It has a territorial area of 8 511 996 km^2 and an Atlantic coast length of 7491 km. Brazil encompasses several important biomes, such as the Amazonian rainforest, the Atlantic tropical forest, and the savannah area called *Cerrado*. Brazilian biodiversity is therefore enormously rich and diverse. Brazil contains 10–20 % of the world's known living species, and may have many more that have not yet been discovered. Brazil has been estimated to contain approximately 2 million plant, animal, and microorganism species [10].

Most of the Brazilian plant species have not yet been studied, which represents an economic potential to be explored in the identification of new bioactive agents [11]. The present rate of extinction of some plant species in Brazil will cause not only the loss of valuable therapeutic compounds, but also of genes encoding enzymes with potential for plant improvement in the biosynthesis of new natural products. A strategy for the development of Brazil is to protect and promote a rational exploitation of the plant biodiversity as a source for new substances [11,12]. In view of this strategy, the State of São Paulo Research Foundation (FAPESP), one of Brazil's main agencies for the funding of scientific and technological research has played an exceptional role in establishing mechanisms for the conservation of the biodiversity in São Paulo. This agency created, for example, the Biota Program, which we will further discuss in this paper. The high urbanization rate in São Paulo state, which was followed by extraordinary deforestation of the *Cerrado* and Atlantic Forest ecosystems, demands measures that are able to identify and preserve the biodiversity in the remaining areas [13,14].

The huge biodiversity present in Brazil is not consistent with the few examples of new drugs developed in this country. There are only a few natural compounds isolated from Brazilian biodiversity that have inspired the design of new drugs. A traditional example is tubocurarine (**1**) (Fig. 3). This compound is a poisonous alkaloid and is the main constituent of curare, a preparation of *Chondodendron tomentosum* traditionally used by the South American Indians on arrows for hunting. This poison has an interesting characteristic in that it is only poisonous when taken intravenously, but not when taken orally; the Indians could therefore still eat the hunted animals [15]. Tubocurarine has been used for many years for the relaxation of abdominal muscles during the pre-operative period. Because of its undesirable side effects, new analogues such as decamethonium (**2**), suxamethonium (**3**), and atracurium (**4**) have been designed. These analogues contain two quaternary nitrogen atoms separated by a polymethylene chain, which preserves the tubocurarine-like effect [16].

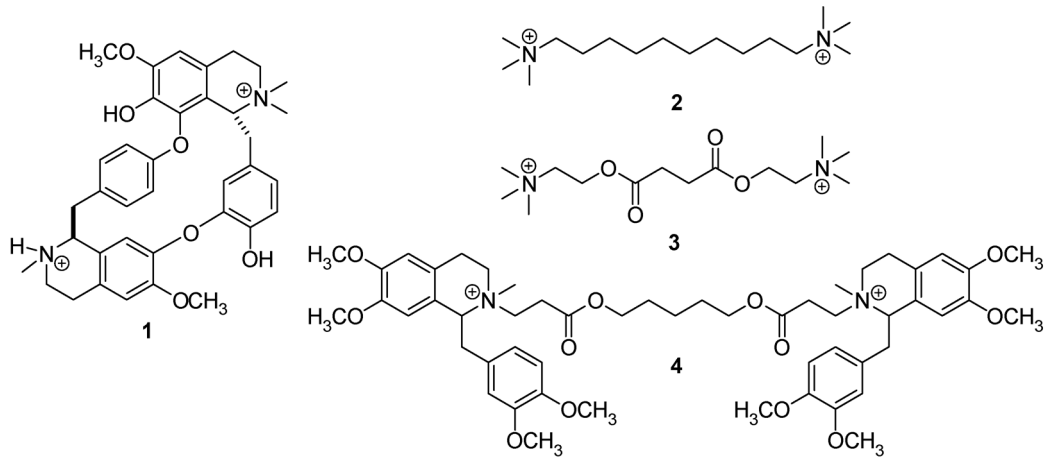


Fig. 3 Tubocurarine (**1**) and analogues used as anesthetic adjuvants (**2–4**).

Another representative Brazilian discovery was the peptide bradykinin (**5**) (Fig. 4), which was isolated from the viper *Bothrops jararaca* venom [17]. This peptide is an inhibitor of angiotensin-converting enzyme (ACE), which is responsible for the conversion of angiotensin I to angiotensin II during its passage through pulmonary circulation and an important target for the therapy of hypertensive disease. Further investigations have improved the understanding of the active site of ACE and have led to the design of new ACE inhibitors, a class of hypertension regulators predominantly represented by captopril (**6**, Capoten®) [18]. This drug was designed using bradykinin as a model and the revolutionary concept of structure-based drug design associated with quantitative structure–activity relationship (QSAR)-based modification. Captopril was one of the most potent competitive inhibitors identified to date and is specifically designed for binding to the active site of ACE; it is also the first ACE inhibitor approved to treat hypertension [19]. Further studies regarding the specific interactions between this type of inhibitor and the active site of ACE have been proposed and confirmed through measurements of the inhibitory potency of structural analogues [20].

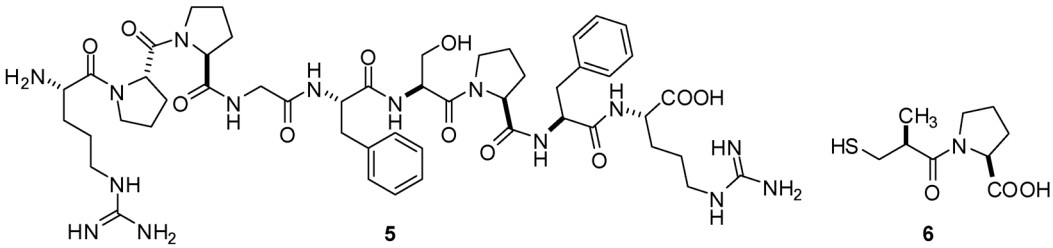


Fig. 4 ACE inhibitors bradykinin (**5**) and captopril (**6**).

The alkaloid pilocarpine (**7**) (Fig. 5), isolated from *Pilocarpus* sp. (Rutaceae), is another lead compound isolated from Brazilian plant species that has led to the development of Salagen®. This drug is a pilocarpine hydrochloride derivative and was designed as a cholinergic agonist for the treatment of dry mouth from salivary gland hypofunction caused by radiotherapy in patients with head and neck cancers [21].

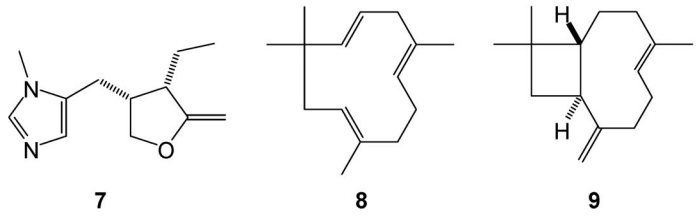


Fig. 5 Pilocarpine (**7**) and the sesquiterpenoids α-humulene (**8**) and *trans*-caryophyllen (**9**), Brazilian natural products used as medicines.

In addition, several examples exist of herbal medicines available from Brazilian plant species that show great potential. However, the complex development of drug discovery remains incipient in Brazil, and the transfer from basic research to preclinical and clinical research is quite new. Even with the latest advances in science and technology, few national pharmaceutical companies are involved in the research and development of new medicines from Brazilian natural resources. The topical anti-inflammatory Acheflan® is an interesting example of a medicine researched and developed completely in Brazil. It was introduced to the market by the pharmaceutical company Aché Laboratórios S.A. as an herbal medicine and contains a standardized mixture of the sesquiterpenes α-humulene (**8**) and *trans*-caryophyllen (**9**) as the active component. This mixture is present in the essential oil of *Cordia verbenacea* D.C. [22]. This topical medicine is currently very successful in Brazil, and, following FDA approval, has been introduced in the USA, Canada, and Japan.

Another example of an herbal medicine recently launched on the market is Fitoscar®. It is a mixture of phenolic derivatives present in standardized dry extract of *Stryphnodendron adstringens* (Mart.) Coville, which was developed by Apsen Farmacêutica. This product is the first example of an herbal medicine developed from a plant from the Brazilian *Cerrado* biome [23], a genuine Brazilian ecosystem considered a hotspot of biodiversity [24].

THE CONTRIBUTIONS OF THE BIOTA RESEARCH PROGRAM

FAPESP launched the BIOTA Program in March 1999. One of the main objectives of this collaborative program was to prospect and map the São Paulo state biodiversity and to find new bioactive compounds and lead molecules from the main underexplored *Cerrado* and Atlantic Forest biomes. Since the beginning of this research project, more than 2000 plant extracts have been prepared and evaluated in preliminary bioassays for a diverse range of activities, including antifungal, antioxidant, antimalarial, and anticancer activities [6,25].

The selectivity, accuracy, and convenience of these bioassays have already demonstrated the potential of the program in screening extracts from collected plants because it has allowed the isolation of more than 700 compounds. Among these compounds were found DNA-damaging piperidine alkaloids from *Senna spectabilis* (D.C.) H.S. Irwin and R.C. Barneby [26], cytotoxic casearins from *Casearia sylvestris* Swartz [27], antitumor guanidine alkaloids from *Pterogyne nitens* Tul. [28], indole alkaloids from *Chimarrhis turbinata* D.C. [29], and pyridine alkaloids from *Senna multijuga* (Rich.) H.S. Irwin and R.C. Barneby [30]. These examples of lead molecules obtained by means of the BIOTA Program highlight the importance of tropical biodiversity for drug discovery.

Studies on the indole alkaloids isolated from *Chimarrhis turbinata*

As part of studies on the acetylcholinesterase (AChE) inhibition activity of several plant species from the Brazilian rainforest, potential bioactive compounds were detected in *C. turbinata* (Rubiaceae) extract. Species of Rubiaceae have been used around the world as medicinal plants. In another study,

nine indole alkaloids were isolated and submitted to a preliminary thin-layer chromatography (TLC) screen for AChE inhibition. The alkaloids turbinatine (**10**) and desoxycordifoline (**11**) (Fig. 6) inhibited AChE at 0.1 and 1.0 μM, respectively. Alkaloid **10** showed moderate activity (IC_{50} = 1.86 μM) compared to the standard compound galanthamine (IC_{50} = 0.92 μM) in an in vitro rat brain assay. New potential AChE inhibitors are of great interest, especially with the discovery of galanthamine, an AChE inhibitor alkaloid with long-acting, selective, reversible, and competitive activity in the treatment of Alzheimer's disease [29].

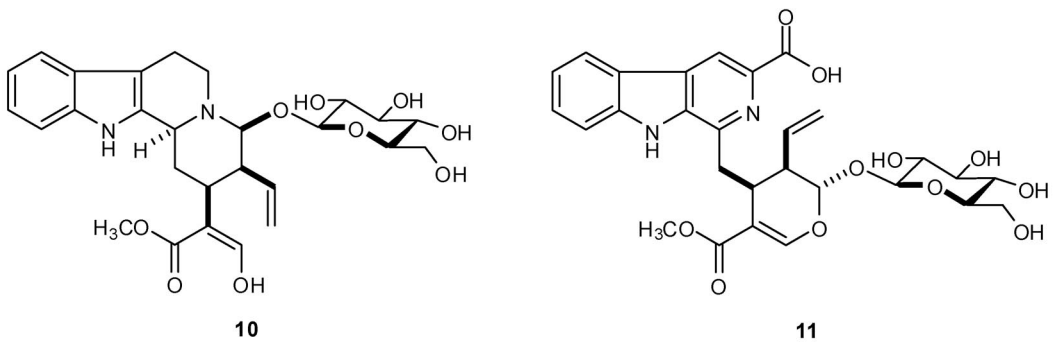


Fig. 6 Indole alkaloids (**10** and **11**) isolated from *C. turbinata.*

Studies on the piperidine alkaloids isolated from *Senna spectabilis*

Senna species are known in Brazil for their efficacy in several traditional applications, including antimicrobial, laxative, antiulcerogenic, analgesic, and anti-inflammatory uses [26]. Our group has systematically studied *S. spectabilis* (syn. *Cassia spectabilis*) because of the rare piperidine alkaloids with bioactive properties accumulated in this species. An investigation of the flowers of *S. spectabilis* yielded three new piperidine alkaloids, (–)-3-*O*-acetylspectaline (**12**), (–)-7-hydroxyspectaline (**13**), and *iso*-6-spectaline (**14**), together with the known (–)-spectaline (**15**) (Fig. 7) [26]. Additional studies described the presence of a homologous series of novel piperidine alkaloids detected in the flowers and fruits of *S. spectabilis* via tandem MS with electrospray ionization [31]. The alkaloids **12** and **14** exhibited DNA-damaging activity using a mutant yeast (*Saccharomyces cerevisiae*) assay [26]. Further investigation of the bioactivity of these alkaloids identified an acetylcholine (ACh) subunit internalized in (–)-3-*O*-acetylspectaline (**12**) (Fig. 7).

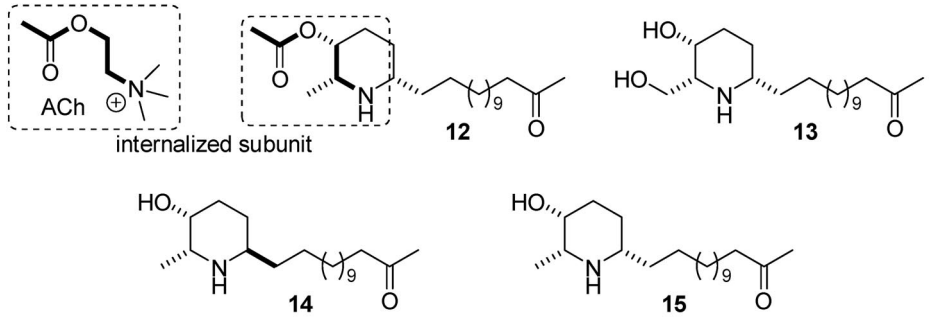
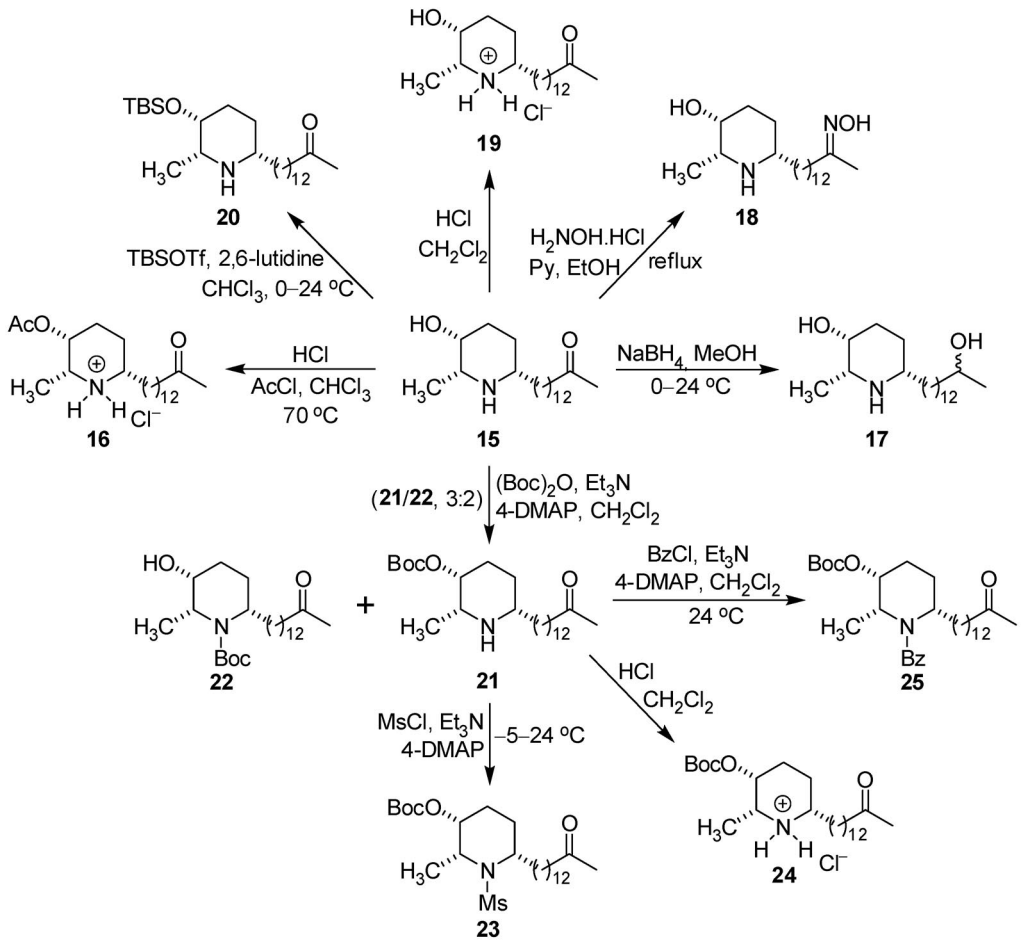


Fig. 7 Piperidine alkaloids (**12–15**) isolated from *S. spectabilis* and a view of the internalized subunit of ACh in (–)-3-*O*-acetylspectaline (**12**).

This observation led to the design of several semisynthetic derivatives (**16–25**) (Scheme 1), of which (–)-3-*O*-acetylspectaline hydrochloride (**16**) exhibited AChE inhibitory activity both in vitro and in vivo (IC_{50} = 7.32 μM) [32]. To elucidate the mechanism of cholinesterase inhibition of derivative **16**, kinetic studies were performed and demonstrated noncompetitive cholinesterase inhibition. This result was in disagreement with the first idea of a competitive mechanism suggested by the internalized ACh unit. These further studies also revealed central nervous system selectivity with few peripheral side effects [33]. This lead compound is currently under preclinical development [34].



Scheme 1 Semisynthetic derivatives (**16–25**) prepared from (–)-spectaline (**15**).

Considering these results, we selected compound **12** and tacrine, a potent AChE inhibitor, for molecular hybridization and synthesized a range of 27 pyridinic and pyrazinic derivatives [34]. Because AChE inhibitors have been used in the control of parasites, 24 of these compounds were assayed for their nematostatic and anthelmintic activities. All of the compounds tested exhibited anthelmintic activity against the gastrointestinal parasitic nematode *Nippostrongylus brasiliensis*, which revealed a new potential use for this chemical class. In addition, one of the synthetic intermediates, ethyl 2-[(6-chloropyrazin-2-yl)sulfanyl] acetate (**26**) (Fig. 8), was active against the phytopathogen *Meloidogyne incognita*, and induced immobilization in 98 % of the nematodes. Additional studies in rats on the oral

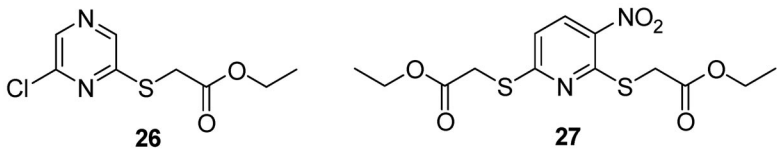


Fig. 8 New lead compounds (**26** and **27**) designed by molecular hybridization of (–)-3-*O*-acetylspectaline (**12**) and tacrine.

toxicity of the representative compound, diethyl 2,2'-[(3-nitropyridine-2,6-diyl) bissulfanediyl] diacetate (**27**) (Fig. 8), showed the compound to be nontoxic at a dose of 2 g/kg [35].

CONCLUSIONS

Tropical regions hold outstanding biodiversity and, therefore, present a great chemical diversity that could be better explored for medicinal purposes. Despite the enormous biodiversity in Brazil, only a few drugs originating from Brazilian plant species have been developed. The major bottleneck that continues to affect natural-product drug discovery in Brazil is poor collaborative research and the isolation and purification of active compounds from complex mixtures in amounts sufficient for further pharmacological and toxicological tests. Often, only a few milligrams of a natural lead molecule is isolated, and even with the latest advances in analytical technology, drug discovery stops in the initial stages. In addition, Brazilian pharmaceutical companies are not well established in research and development.

Biodiversity provides unique chemical scaffolds that have been used as templates for medicinal chemistry. Natural products such as tubocurarine (**1**) and bradykinin (**5**) are good examples of lead compounds from Brazilian biodiversity used as models for the design of more potent drugs and have encouraged scientists to turn their attention to biodiversity. Specifically, Brazil has launched some herbal drugs onto the market but not drugs from isolated or synthesized compounds, which illustrates the enormous underexplored potential for medicinal chemistry studies. Thus, the few examples of herbal medicine from Brazilian plant species serve as a starting point for the development of more potent drugs.

Our biodiscovery collaborative research project supported by Biota-FAPESP and a partnership with a national pharmaceutical company has encouraged us to establish some strategies for drug discovery from Brazilian biodiversity. One of the most successful results, accomplished after the screening of more than 2000 extracts, are the studies on the piperidine alkaloids isolated from *S. spectabilis*, which represent a significant example of the rational exploration of biodiversity. These alkaloids have shown interesting pharmacological properties and attractively simple chemical structures, which have already served as inspiration for the synthesis of new bioactive compounds. Despite the encouraging studies on these molecules, numerous pharmacological studies are needed to launch a drug to market. Toward this end, medicinal chemistry studies have been performed to investigate new semisynthetic derivatives capable of binding to the same biological targets as the natural product to optimize bioactivity. The results presented herein are exciting and highlight that natural products from Brazilian biomes are an impressive source of bioactive compounds and model compounds for medicinal chemistry.

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