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Natural products from marine invertebrates against *Leishmania* parasites: a comprehensive review

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Abstract Parasitic infections by *Leishmania* parasites remains a severe public health problem, especially in developing countries where it is highly endemic. Chemotherapy still remains a first option for the treatment of those diseases, despite the fact that available drugs exhibit a variety of shortcomings. Thus, innovative, less toxic more affordable and effective antileishmanial agents are urgently needed. The marine environment holds an immeasurable bio- and chemical diversity, being a valuable source of natural products with therapeutic potential. As invertebrates comprise about 60 % of all marine organisms, bioprospecting this class of organisms for antileishmanial properties may unravel unique and selective hit molecules. In this context, this review covers results on the literature of marine invertebrate extracts and pure compounds evaluated against *Leishmania* parasites mainly by in vitro methods. It comprises results obtained from the phyla Porifera, Cnidaria, Bryozoa (Ectoprota), Mollusca, Echinodermata, Annelida, Cetnophora, Platyhelminthes, sub phyla Crustacea (phylum Arthropoda) and Tunicata (phylum Chordata). Moreover, structure–activity relationships and possible mechanisms of action are mentioned, whenever available information is provided. About 70 species of marine invertebrates belonging to seven different phyla are included in this work. Besides a variety of crude extracts, a total of 140 pure compounds was tested against different *Leishmania* species. Although the research on the antileishmanial potential of marine invertebrates is in its early beginnings, promising results have been achieved that encourage further research. As more extracts and compounds are being screened, the possibility of finding active and selective antileishmanial molecules increases, rising new hope in the search for new treatments against leishmaniases.

Keywords Antileishmanial activity · Marine invertebrates · Parasitic infections · Structure–activity relationship (SAR)

Abbreviations

BuOH	Butanol
CC_{50}	Cytotoxic concentration that lysis 50 % of cells
CH_2Cl_2	Dichloromethane
CL	Cutaneous leishmaniasis
EtOAc	Ethyl acetate
Hex	Hexane

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IC_{50}	Inhibitory concentration that lysis 50 % of *Leishmania* parasites
MCL	Mucocutaneous leishmaniasis
MeOH	Methanol
SI	Selectivity index
SAR	Structure-activity relationship
VL	Visceral leishmaniasis

Introduction

This review gathers and summarizes the available information concerning antileishmanial activity of extracts and pure compounds from marine invertebrate, mainly using in vitro approaches. This work initiates with a description of leishmaniases and chemotherapeutic agents used for its management. Moreover, an overview of the potential of marine organisms as sources of novel drugs is provided, followed by the summary of the extracts and compounds tested for antileishmanial activity against promastigote, axenic amastigote and intracellular amastigote forms. It comprises and is divided according to marine phyla, namely Porifera, Cnidaria, Bryozoa (Ectoprota), Mollusca, Echinodermata, Annelida, Platyhelminthes, sub phylum Crustacea (phylum Arthropoda) and sub-phylum Tunicata (phylum Chordata). The structure–activity relationship (SAR) of compounds, as well as potential mechanisms of action are presented and discussed, whenever such information is available. The gathering of data, even that obtained at preliminary stages (i.e. extracts and fractions), aims to dereplicate data and improve research quality by encouraging an efficient search for anti-*Leishmania* hits and leads, from marine invertebrate organisms.

Leishmaniases

Leishmaniases are a group of neglected tropical diseases endemic in 98 countries and some Palestinian territories such as West Bank and Gaza Strip (WHO 2010; Alvar et al. 2012). It is estimated that leishmaniases affects about 12–14 million people worldwide, and that more than 350 million individuals are at risk of contracting this disease (WHO 2010). Leishmaniases are the ninth cause of disease burden among all infectious diseases, and thus remains a severe public health problem, especially in developing countries (Alvar et al. 2012).

Leishmania are protozoan parasites (order Kinetoplastida, family Tripanosomatidae) which are transmitted by the bite of female phlebotomine sand flies belonging to two genera, namely *Phlebotomus*, in the Old World, and *Lutzomyia*, in the New World (WHO 2010). Transmission can be classified as zoonotic or anthroponotic, according to the main reservoirs. For example, in the Mediterranean Basin the species *L. infantum* causes zoonotic visceral leishmaniasis, and the main reservoir is the domestic dog; hence humans are accidental hosts (Ruiz-Fons et al. 2013). Conversely, in India, the species *L. donovani* causes anthroponotic visceral leishmaniasis, and in this case humans play the most important role in the transmission of the disease (Singh et al. 2006). *Leishmania* alternates between two life stages: promastigote, inside the digestive tube of the vector where it differentiates into the infective form; amastigote, representing the clinical relevant stage of the parasite and occurring inside the mammalian host, after promastigotes are phagocytized by macrophages, dendritic cells and/or neutrophils (De Assis et al. 2012; Fig. 1).

The taxonomic classification of *Leishmania* parasites is complex where the sub-genus *Leishmania* and *Viannia* are further differentiated into species complexes, mainly based on genetic studies (WHO 2010; Fig. 2). Although *Leishmania* species usually exhibit tropism for certain organs, the outcome of infection also depends on host factors such as immunosuppression (Murray et al. 2005). Still, two major clinical forms of leishmaniasis are recognized, namely cutaneous (CL) and visceral (VL). The majority of *Leishmania* species can cause CL, thus making it the most common clinical form, which usually causes skin lesions and ulcers, which are frequently self-healing (WHO 2010). However, the extension of lesions to mucosal areas may lead to mucocutaneous leishmaniasis (MCL), which is often more associated to the species *L. braziliensis* and *L. panamensis* (WHO 2010). MCL can lead to partial or total facial disfiguration of nose and mouth membranes, being difficult to manage and potentially fatal (Desjeux 2004). VL is a systemic disease mainly caused by *L. donovani*, in Asia and Africa, and *L. infantum* (syn. *L. chagasi*), in southern Europe and South America

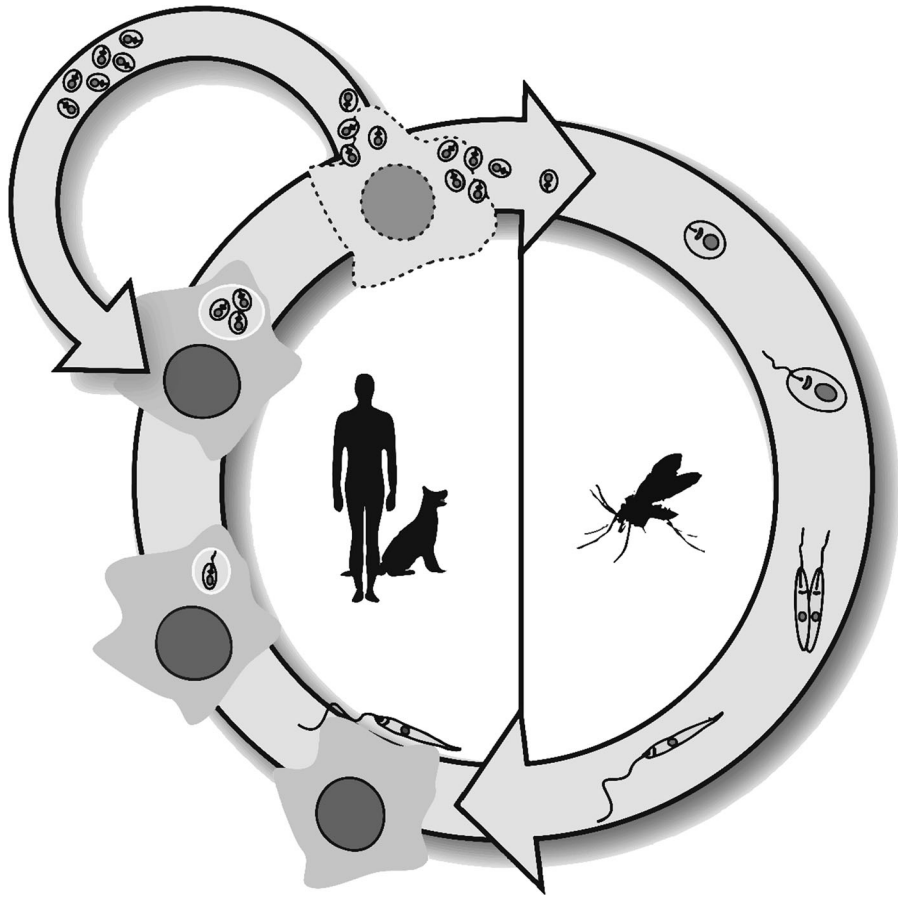


Fig. 1 Life cycle of *Leishmania* parasites. Inside the vector (on the *right*), parasites differentiate into metacyclic promastigotes and migrate to the phlebotomine proboscis. When the sand fly bites, it regurgitates promastigotes into blood vessels of the vertebrate mammalian host (on the *left*). Promastigotes infect mammalian cells and differentiate into oval amastigotes. Amastigotes multiply, eventually rupture the cell and reinvade other cells. The phlebotomine sand fly takes a blood meal and ingests amastigotes completing and also restarting the cycle (De Assis et al. 2012)

(WHO 2010). Visceral organs such as spleen, liver, bone marrow and lymphatic nodes are the main targets of the parasites. If left untreated, the VL mortality rate can reach 100 % in underdeveloped or in developing countries (Desjeux 2004; Mishra et al. 2009).

Chemotherapy

Currently used drugs exhibit several drawbacks such as high costs and high toxicity. However, chemotherapy still remains the first line choice for controlling all forms of leishmaniasis (Fig. 3). Moreover, antileisgmanial drugs depend on long-term administration, and its efficacy is declining due to the growing parasite resistance (Singh et al. 2012). For more than 60 years pentavalent antimonials, such as sodium stibogluconate and meglumine antimoniate, remained as first line drugs, administrated intramuscularly or intravenously at the average dose of 20 mg/kg (body weight)/day during 10–30 days depending on the leishmaniasis clinical form, region and *Leishmania* species, except in Bihar, India, where parasite resistances are already described (Croft and Olliaro 2011; Singh et al. 2012). Amphotericin B, a polyene antifungal agent and its liposomal formulations are second line drugs with high efficacy, being recommended for the management of VL cases (Singh et al. 2012). Amphotericin B is recommended for VL cases, applied intravenously daily or in alternate days at a

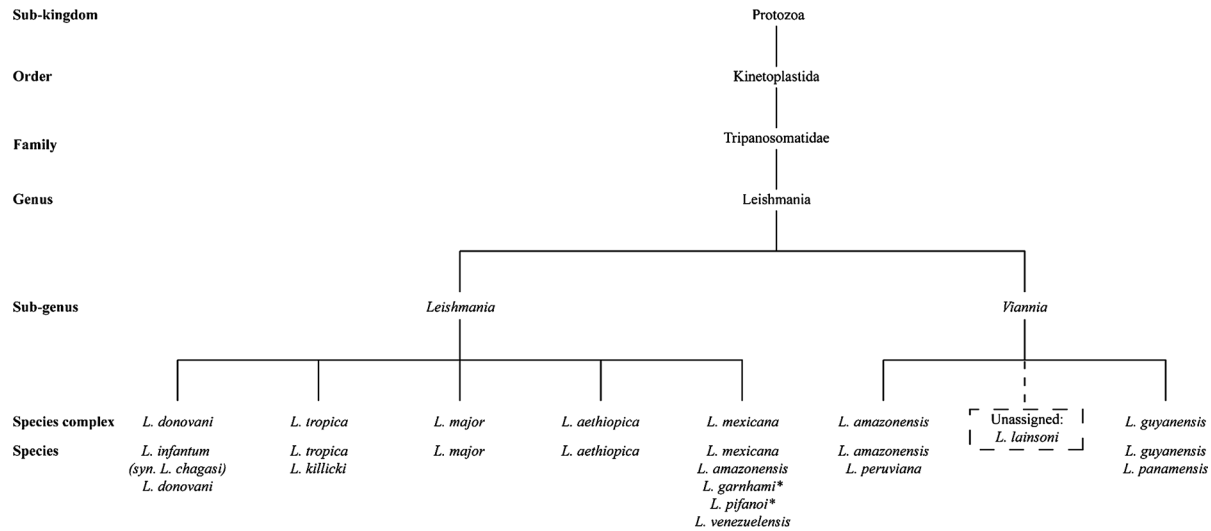


Fig. 2 Taxonomic classification of *Leishmania* parasites (Adapted from WHO 2010)

dose of 0.75 to 1 mg/kg (body weight)/day, for a total of 15–30 doses. Its liposomal formulations are endorsed at doses ranging from 2.5 (body weight) to 5 mg/kg, also by intravenous infusion during 3–10 days (WHO 2010). Amphotericin B binds to ergosterol, the major sterol present in *Leishmania* cell membranes, forming pores that will unbalance osmotic regulation and lead to parasite death (Ramos et al. 1996). However, amphotericin B causes acute adverse effects such as nausea, vomiting, fever, hypoxia, hypertension or hypotension; and chronic effects such as nephrotoxicity (Laniado-Laborín and Cabrales-Vargas 2009). Moreover, it requires long-term intravenous administration and it is also unaffordable in low income countries (Croft and Olliaro 2011; Singh et al. 2012). More recently, miltefosine, an alkyl-phosphocholine drug, was introduced into the market as the first oral drug to treat VL, despite its teratogenicity, long-term administration and high toxicity (Croft and Olliaro 2011; Singh et al. 2012). Recommended doses for VL treatment by miltefosine range from 2.5 mg/kg (body weight)/day for children and 50–150 mg/kg (body weight)/day for adults, orally administrated for a period of 28 days (WHO 2010). Although the mode of action of miltefosine is not fully understood, it is suggested that it causes an apoptosis-like death of *Leishmania* parasites (Paris et al. 2004). Both amphotericin B and miltefosine are associated to the emergence of parasite drug-resistance and thus, novel therapies are urgently needed to overcome this problem. Other drugs have also proved its efficacy alone or in combination with existing therapies, such as the aminoglycoside paromomycin, effective towards VL caused by *L. donovani*, and applied at the average dose of 15 mg/kg (body weight)/day, administrated intramuscularly for 21 days. Another examples include pentamidine, an aromatic diamine, which is used as a second line drug mainly against VL; and the aminoquinoline sitamaquine, developed against VL, which have showed cure rates above 80 % in phase II clinical trials in India and Kenya, using doses ranging from 1.75 to 3 mg/kg (body weight)/day, for a period treatment of 28 days (Croft and Olliaro, 2011; Singh et al. 2012). Having in mind the current panorama of leishmaniasis chemotherapy, efforts have been made to discover and develop new drugs which are less toxic, more affordable and effective to fight this vector borne disease (Figs. 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15).

Antileishmanial potential of marine organisms

The amount of studies focusing on the medicinal value of marine invertebrates is limited and therefore, their therapeutic potential still remains underestimated. About 15 years ago, Perry (2000) reported the occurrence of approximately 394 marine species with

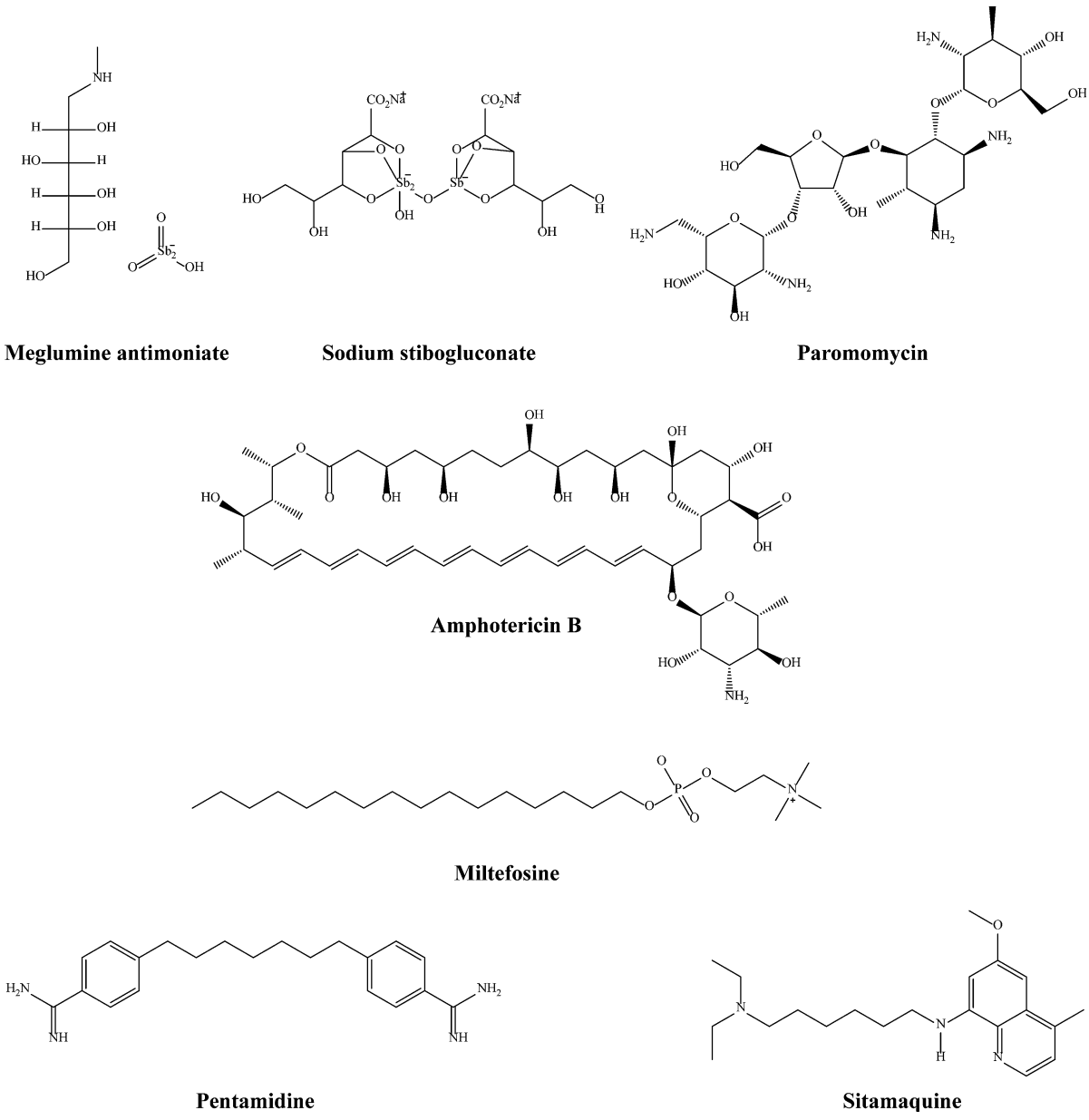


Fig. 3 Molecular structures of the main antileishmanial drugs currently used in the treatment of visceral and/or cutaneous leishmaniasis

medicinal properties worldwide, with marine invertebrates contributing to nearly half of those species. More recently, data on medicinal uses of marine invertebrates in ancient Greek world and early Byzantium was summarized by Voultsiadou (2010). From 38 marine invertebrate species reported to have therapeutic properties mainly against digestive, genitourinary and skin disorders, mollusks and crustaceans were the more active groups (Voultsiadou 2010). The latest review work of Alves and colleagues (2013) highlights 266 species of marine invertebrates with described traditional medical uses, from which approximately 88 % belong to Mollusca, Echinodermata and Crustaceans taxonomical groups. Having in

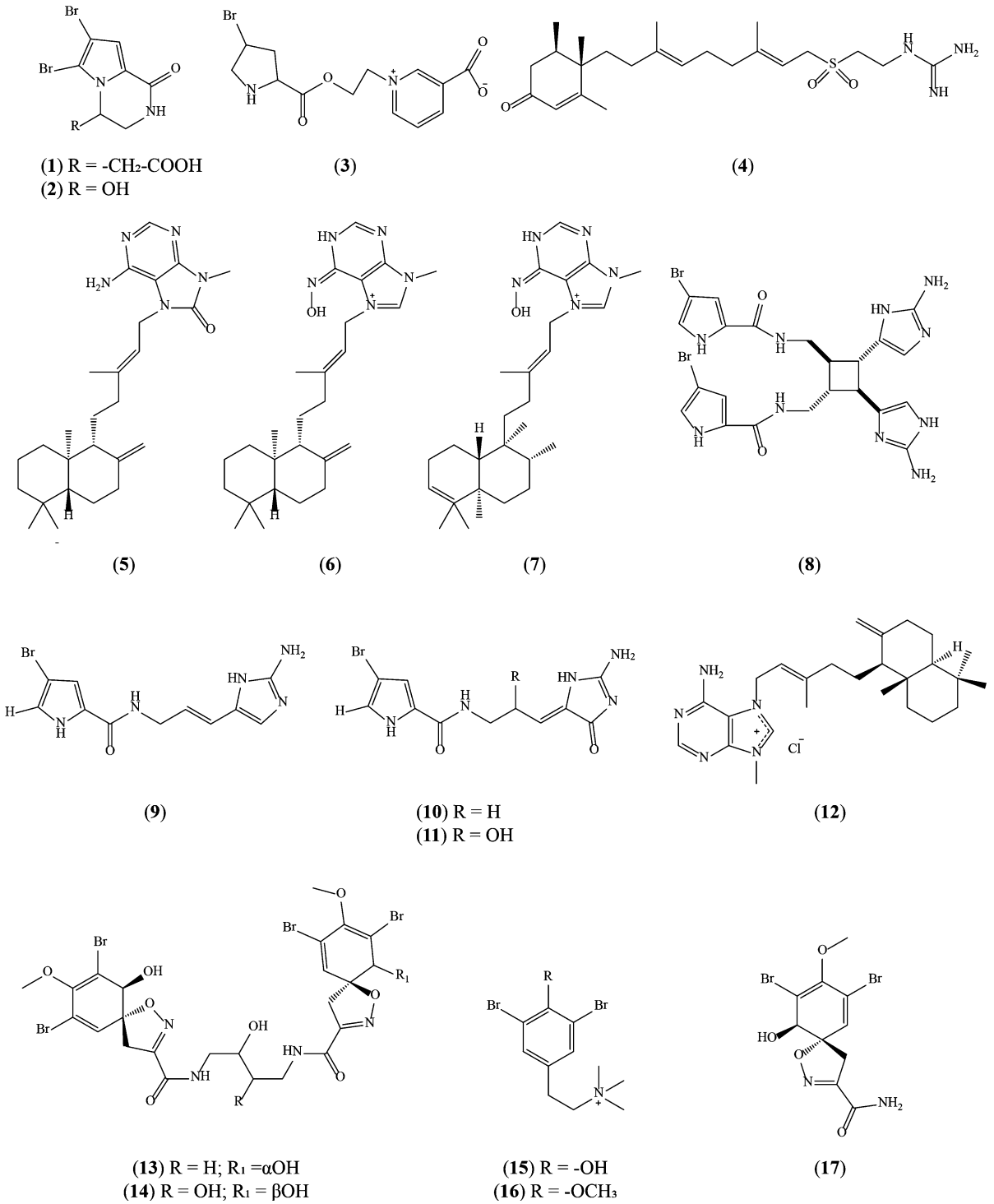
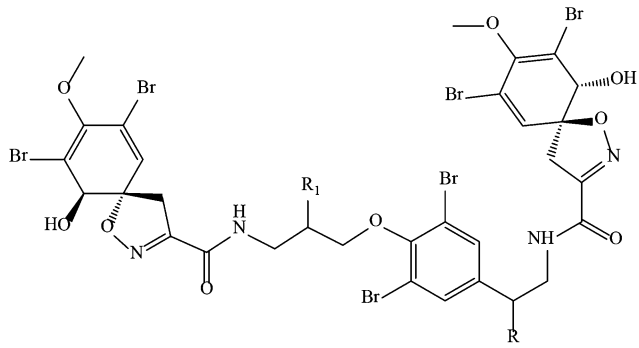
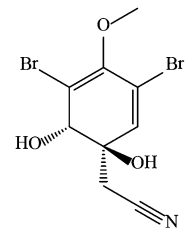


Fig. 4 Molecular structures of alkaloids isolated from marine sponges



(**18**) R = H; R_2 = —— OH
(**19**) R = OH; R_1 = ◥ OH



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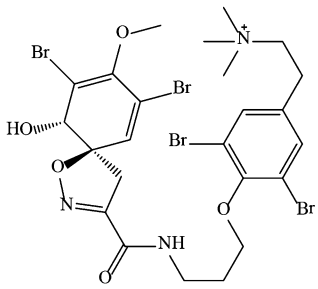

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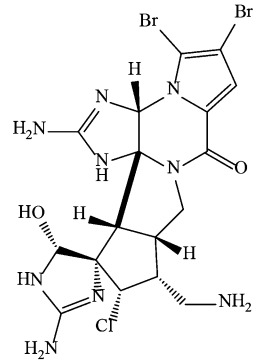

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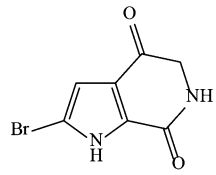

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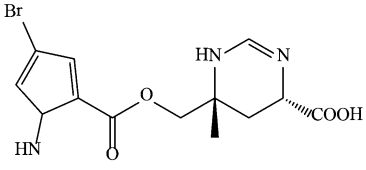

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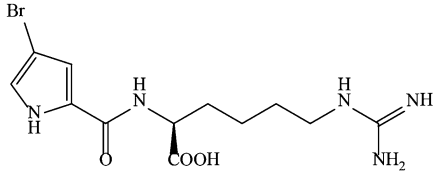

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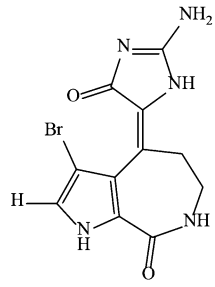

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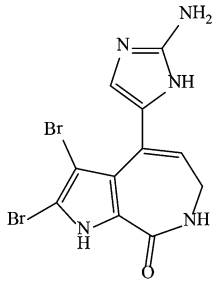

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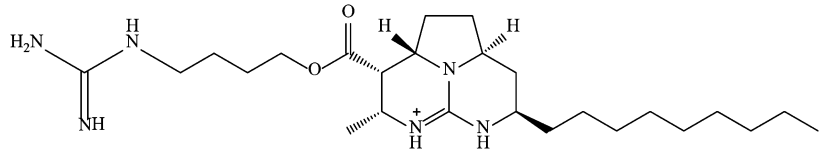
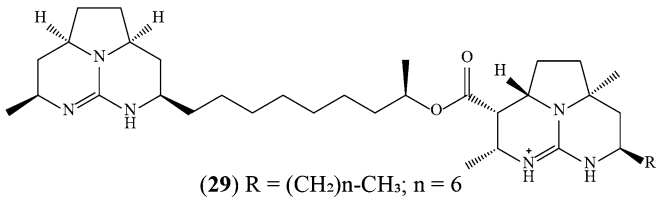
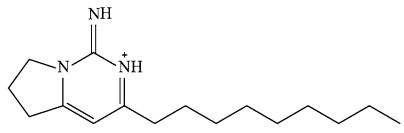


(**28**)

Fig. 4 continued



(**29**) R = $(CH_2)n\text{-}CH_3$; n = 6
(**30**) R = $(CH_2)n\text{-}CH_3$; n = 8
(**31**) R = $(CH_2)n\text{-}CH_3$; n = 7



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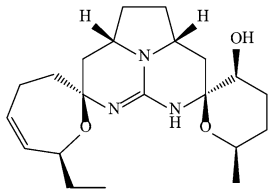

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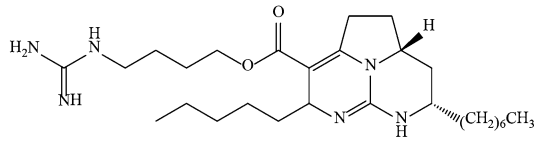

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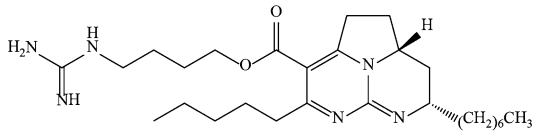

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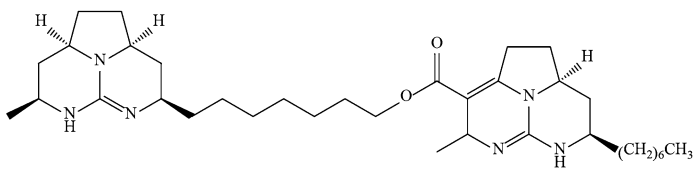

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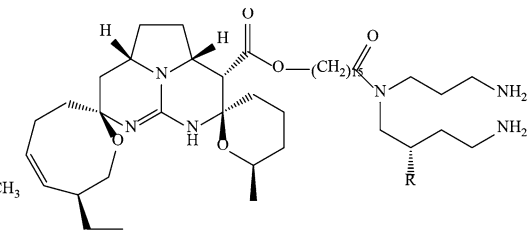
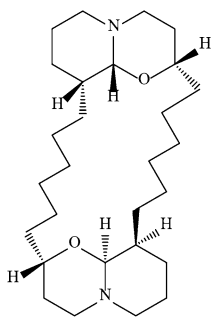


(**37**) R = OH
(**38**) R = H



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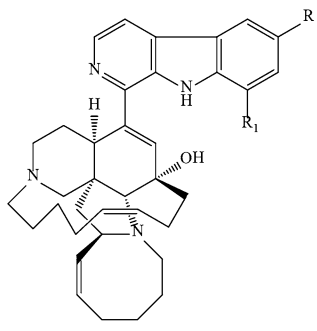
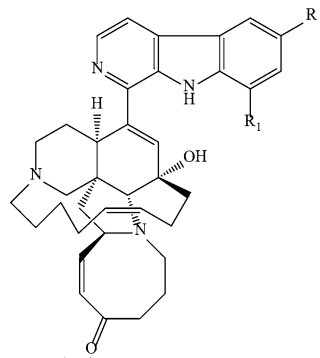


(**40**) R = R_1 = H
(**41**) R = OH; R_1 = H
(**42**) R = H; R_1 = OH



(**43**) R = R_1 = H
(**44**) R = OH; R_1 = H
(**45**) R = H; R_1 = OH

Fig. 4 continued

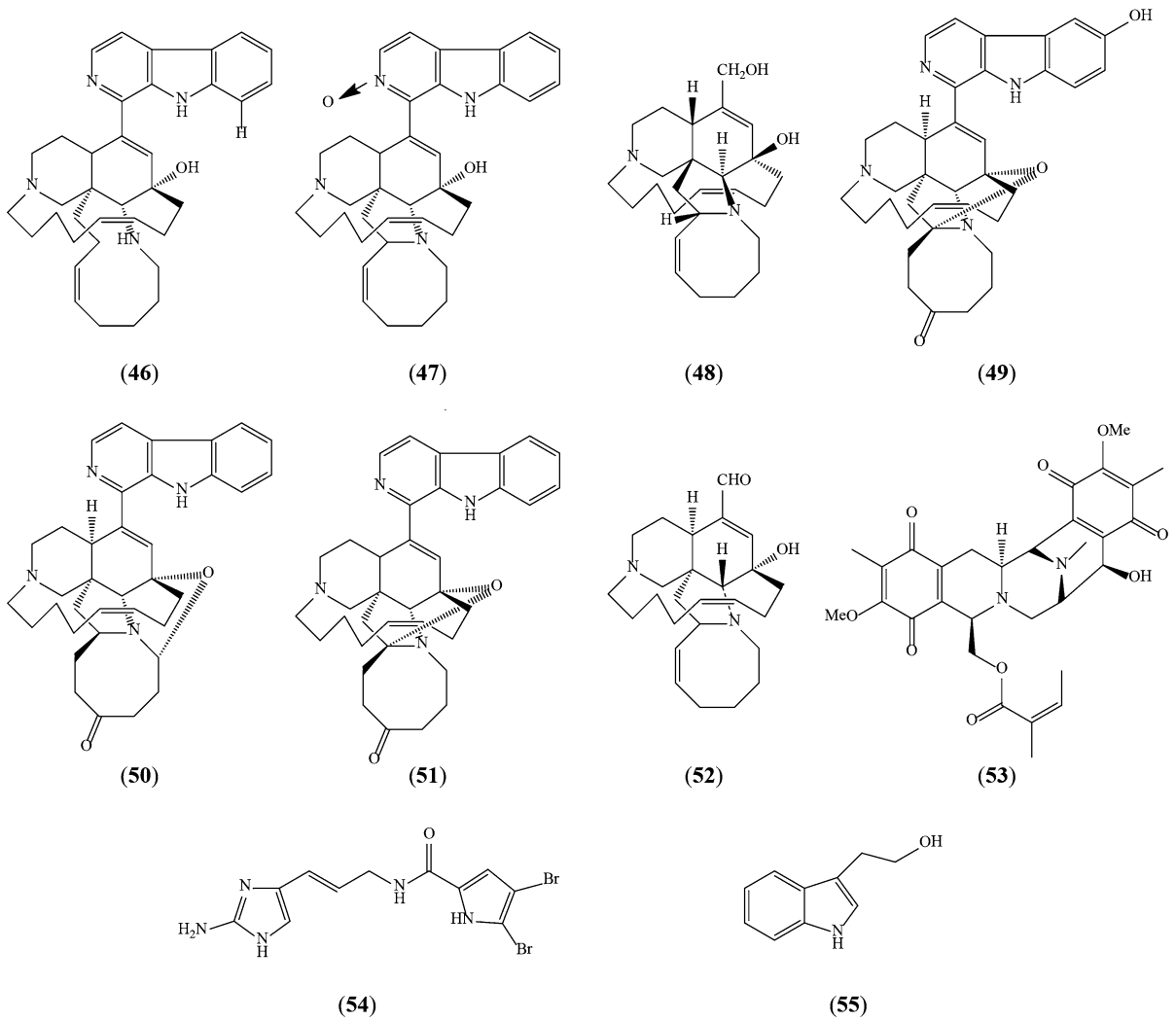
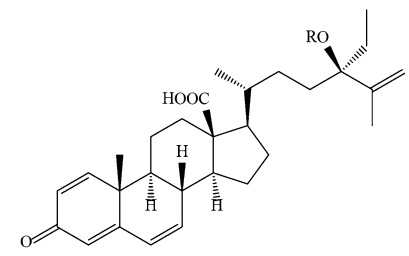


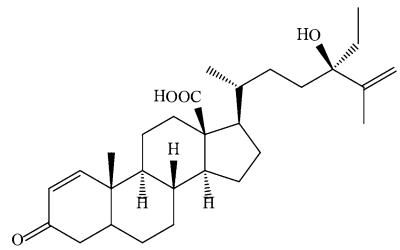
Fig. 4 continued

mind that the knowledge of the ethnopharmacological uses of different plants and animals has already provided new drugs to modern medicine (Achan et al. 2011; Weathers et al. 2011), the study of medicinal marine invertebrates may undoubtedly provide relevant and helpful information regarding the potential of marine natural products for different therapeutic uses against several diseases.

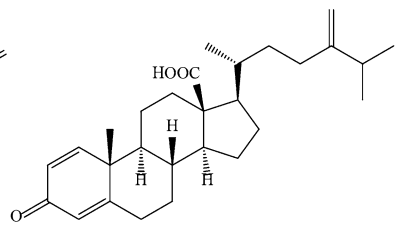
The discovery of the first marine natural products in the 1950s, namely the nucleosides spongothymidine and spongouridine isolated from the Caribbean sponge *Cryptotethya crypta* (Bergmann and Feeneyz 1951), boosted the finding of marine bioactive metabolites, which present an immeasurable chemical diversity. As more than 70 % of the Earth's surface is covered with water, holding an extensive biodiversity both in terms of photosynthetic organisms and animals, the marine environment is an irrefutable source of unique chemical scaffolds with promising biotechnological applications (Haefner 2003). Remarkably, marine invertebrates comprise 60 % of all marine diversity, belonging to phyla Porifera, Cnidaria, Bryozoa (Ectoprota), Mollusca, Arthropoda, Echinodermata, Annelida, Platyhelminthes, and sub-phylum Tunicata or Urochordata (Leal et al. 2012). From approximately 20,000 structurally novel marine natural products identified until now, almost 10,000 are derived from marine invertebrates (Martins et al.



(**56**) R = H
(**57**) R = AC



(**58**)



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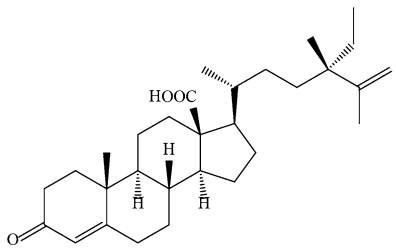

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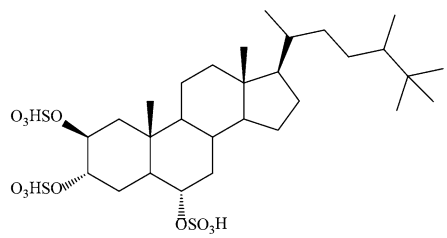
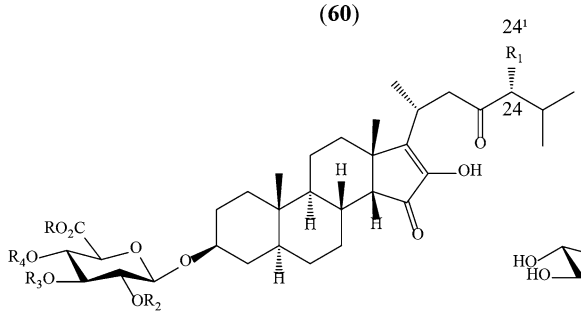
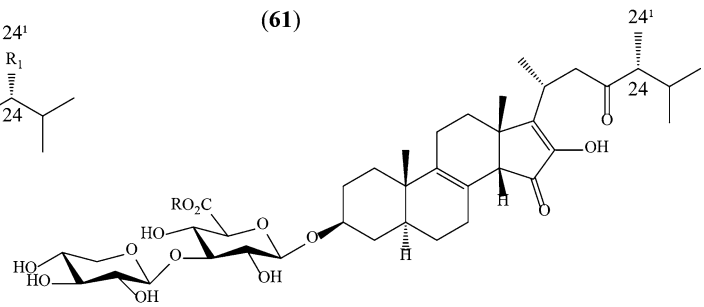


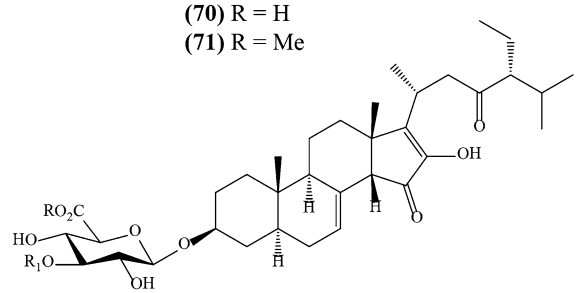
(**61**)





(**68**) R = H Δ24,24¹
(**69**) R = Me Δ24,24¹
(**70**) R = H
(**71**) R = Me

(**62**) R = H; R₁ = Et; R₂ = Glc; R₃ = H; R₄ = H
(**63**) R = Me; R₁ = Et; R₂ = Glc; R₃ = H; R₄ = H
(**64**) R = H; R₁ = H; R₂ = Glc; R₃ = H; R₄ =H
(**65**) R = Me; R₁ = H; R₂ = Glc; R₃ = H; R₄ =H
(**66**) R = H; R₁ = H; R₂ = H; R₃ = H; R₄ = H
(**67**) R = Me; R₁ = H; R₂ = H; R₃ = H; R₄ = H
(**74**) R = H; R₁ = Et; R₂ = H; R₃ = Xyl; R₄ = H
(**75**) R = Me; R₁ = Et; R₂ = H; R₃ = Xyl; R₄ = H
(**76**) R = H; R₁ = Et; R₂ = H; R₃ = H; R₄ = Rha
(**77**) R = Me; R₁ = Et; R₂ = H; R₃ = H; R₄ = Rha
(**78**) R = H; R₁ = Et; R₂ = Glc; R₃ = H; R₄ = H Δ24,24¹
(**79**) R = Me; R₁ = Et; R₂ = Glc; R₃ = H; R₄ = H Δ24,24¹



(**72**) R = H; R₁ =Xyl
(**73**) R = Me; R₁ = Xyl

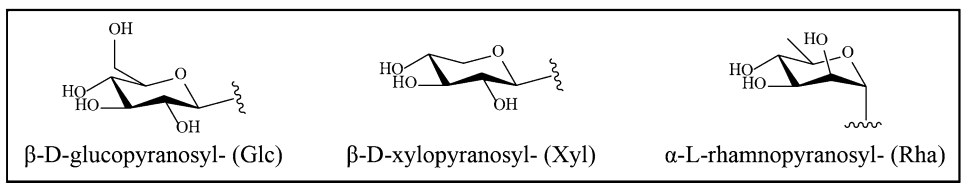
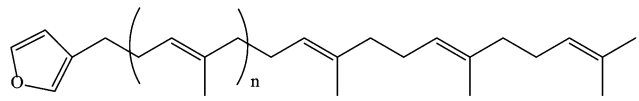
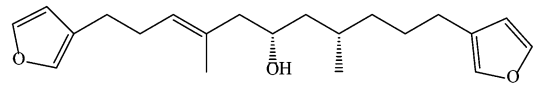


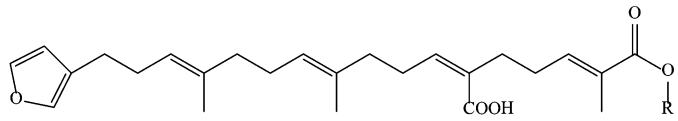
Fig. 5 Molecular structures of steroid-based compounds isolated from marine sponges



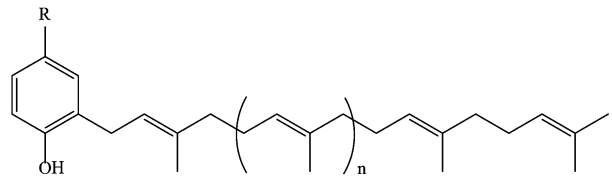
(**80**) n = 1
(81) n = 2



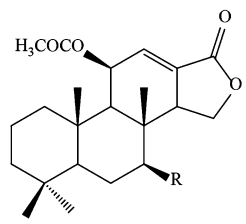
(**82**)



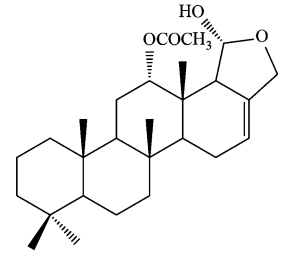
(**83**) R = CH_3
(84) R = H



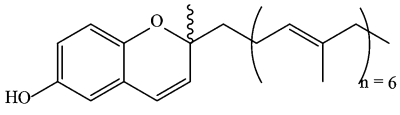
(**85**) R = COOH; n = 5
(86) R = CH_2-COOH; n = 1
(87) R = OH; n = 4



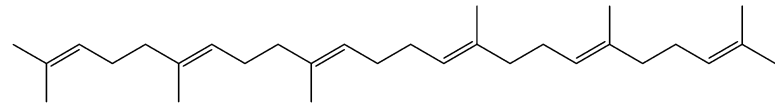
(**88**) R = $OCOCH_3$
(89) R = H



(**90**)

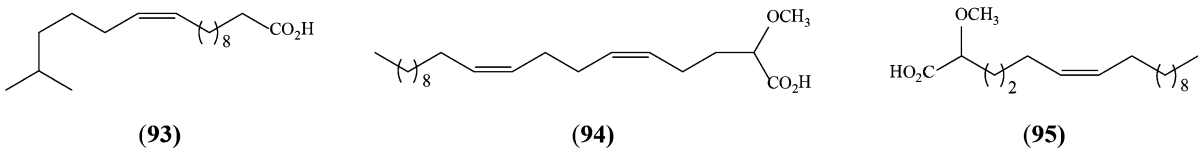


(**91**)



(**92**)

Fig. 6 Molecular structures of terpenoids isolated from marine sponges

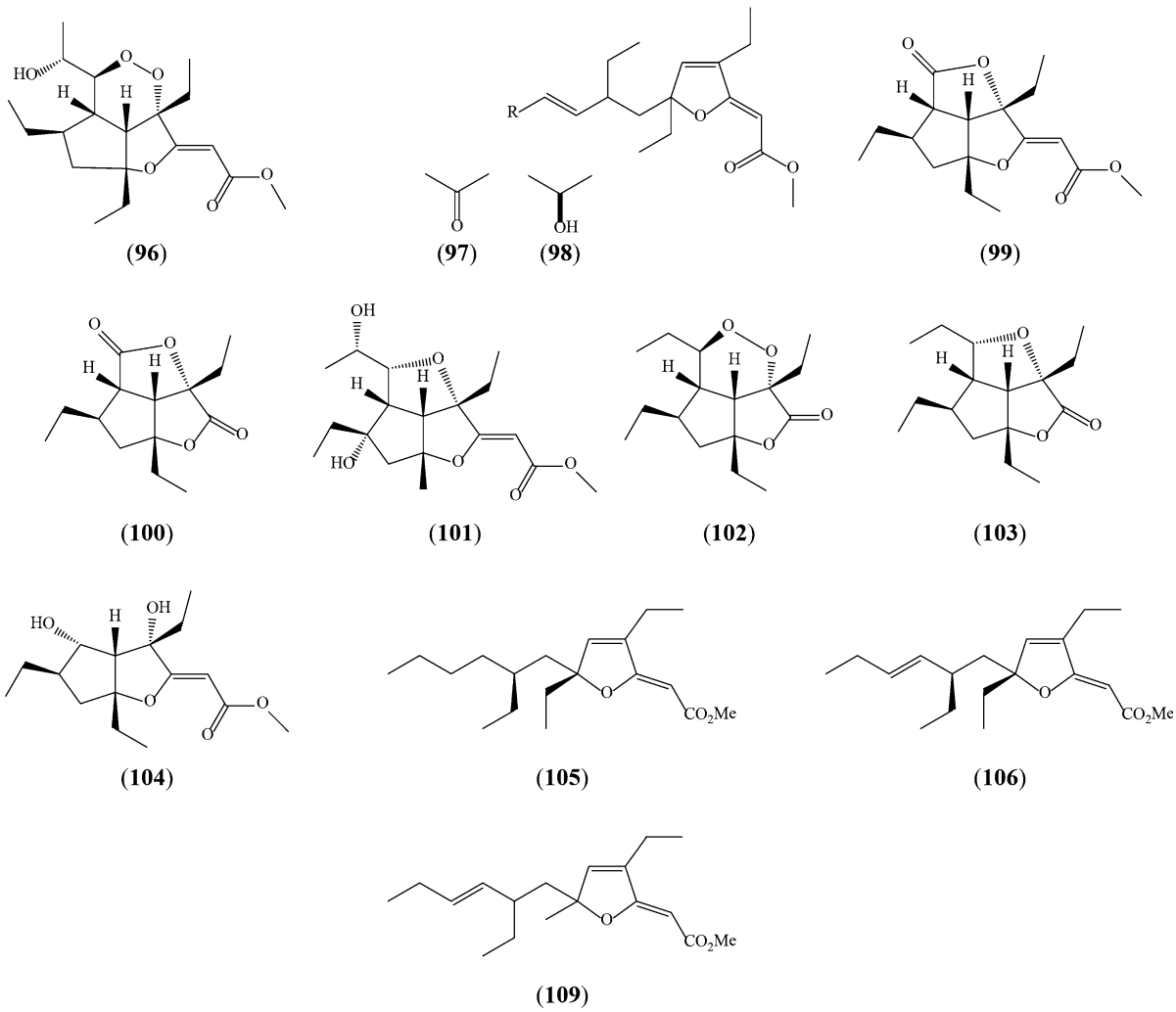


(93) (94) (95)

Fig. 7 Molecular structures of fatty acids isolated from marine sponges

2014; Leal et al. 2012). Interestingly, the high chemical variety of invertebrates have been linked to the high microbial diversity that symbiotically live in these organisms, unravelling these as the genuine sources of bioactive metabolites (Menezes et al. 2010).

Some reviews have addressed bioactive natural products from different marine invertebrate phyla (Rocha et al. 2011; Gomes et al. 2014). Other reviews have focused on natural products with antileishmanial activity (Rocha et al. 2005), and more specifically, marine algae extracts and derived compounds as



(96) (97) (98) (99) (100) (101) (102) (103) (104) (105) (106) (109)

Fig. 8 Molecular structures of polyketides isolated from marine sponges

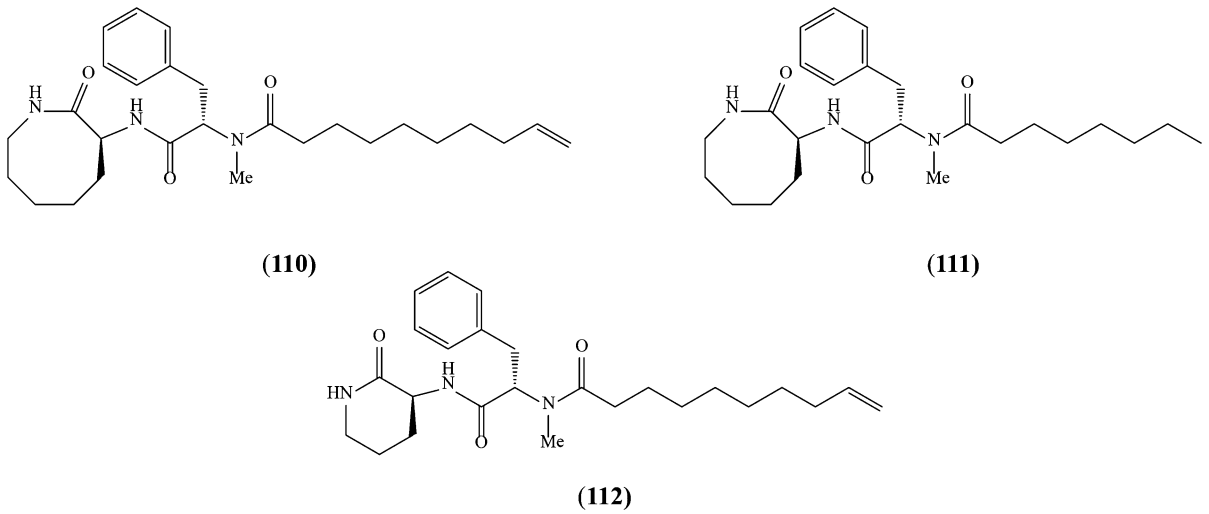


Fig. 9 Molecular structures of lipopeptides isolated from marine sponges

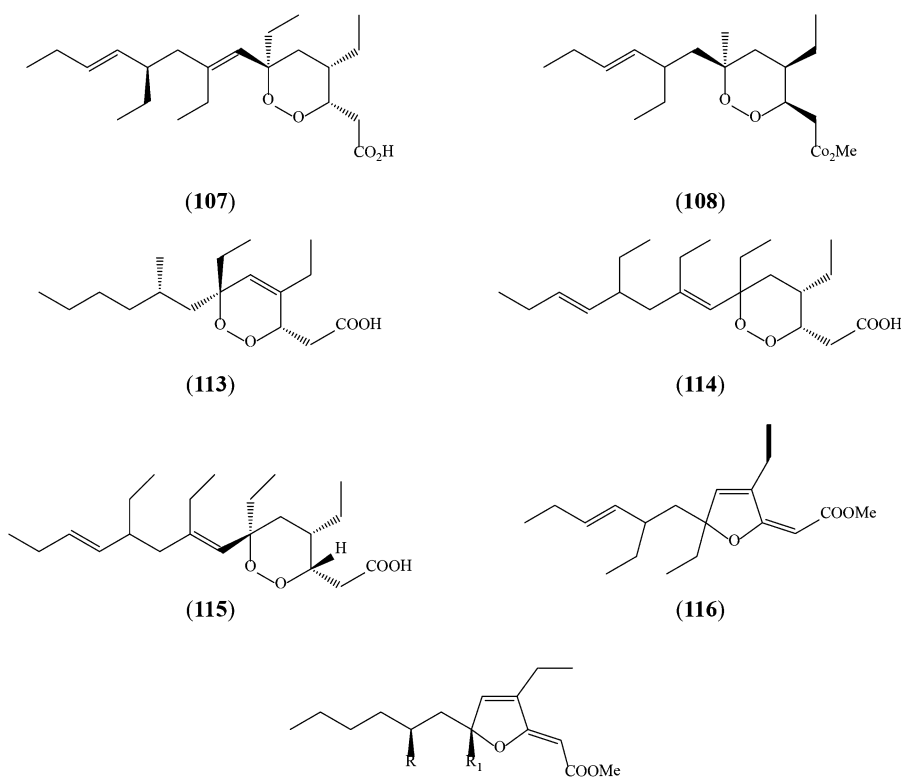


Fig. 10 Molecular structures of cyclic peroxides and furans isolated from marine sponges

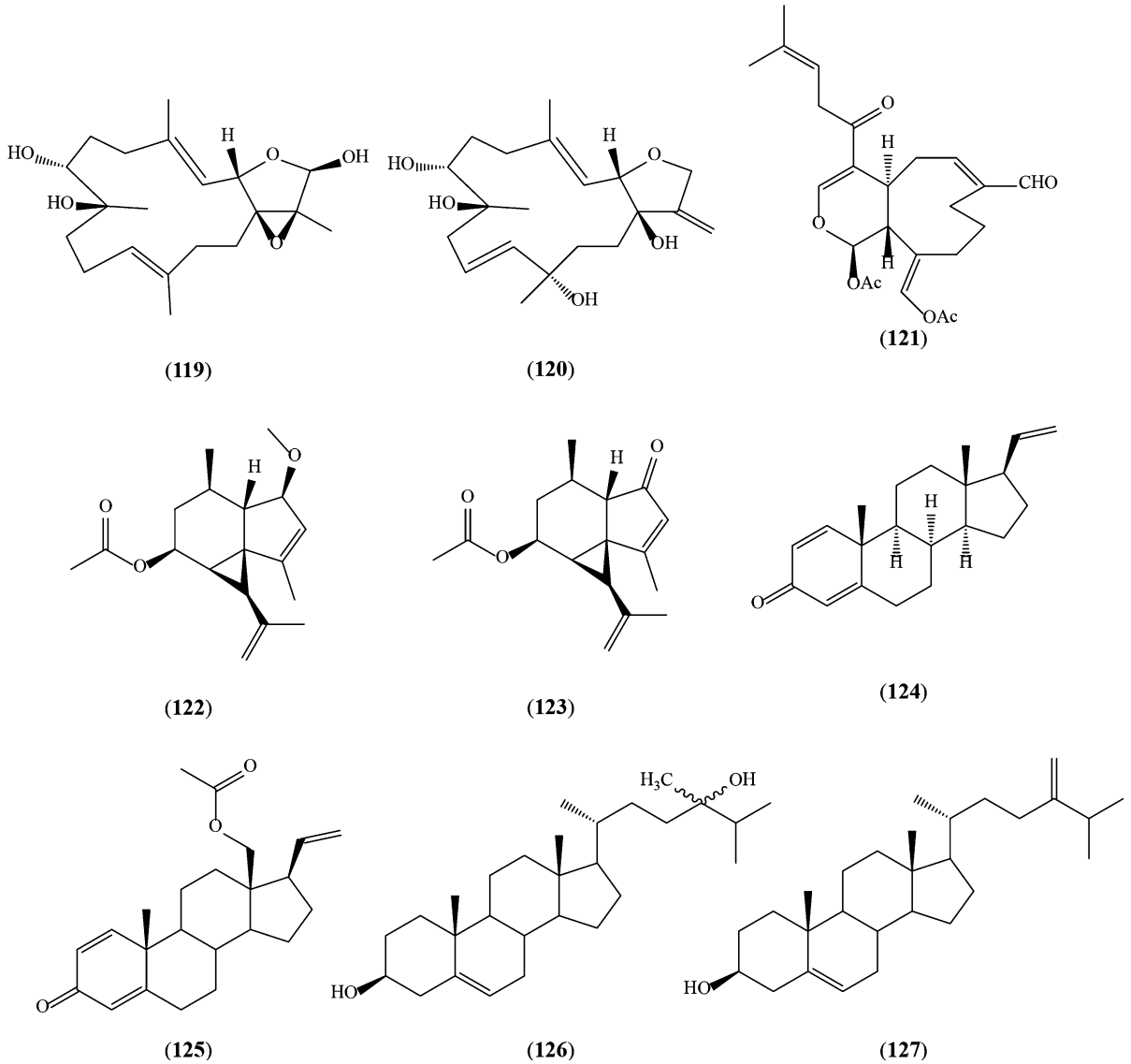


Fig. 11 Molecular structures of compounds isolated from marine cnidarians

antiprotozoal agents (Torres et al. 2014). However, a comprehensive review of molecules isolated from marine invertebrates with promising antileishmanial properties is still lacking. Systematic reviews have documented the evolution of identified marine natural products and its most promising biological activities (Faulkner 2001; Blunt et al. 2013, 2014; Mayer et al. 2011, 2013). From these reviews it is clear that research concerning the antileishmanial properties of marine compounds has increased and some encouraging results have been obtained.

Sponges (Phylum: Porifera)

The Phylum Porifera comprises about 5500 species of multicellular sessile invertebrates, and is considered the most prolific in terms of its pharmacological potential (Brusca and Brusca 2003; Laport et al. 2009). Until now, three sponge-derived compounds have reached the market, namely Cytosar-U® and Halaven® (antineoplastics) and Vira-A® (anti-viral). A high number of sponge-derived molecules is in preclinical and clinical trials, comparatively to other molecules

isolated from other marine organisms (Laport et al. 2009; Mayer et al. 2011). Alkaloids (Scala et al. 2010; Santos et al. 2015), steroids (Ma et al. 2009; Regalado et al. 2010), terpenoids (Gray et al. 2006; Orhan et al. 2010), fatty acids (Carballeira et al. 2011, 2012, 2013), polyketides (Kossuga et al. 2008; Festa et al. 2012), lipopeptides (Nakao et al. 2008), and glycoproteins (Le Pape et al. 2000) are some of the active molecules produced by sponges with a wide range of in vitro bioactivities, for example, against bacteria (Kossuga et al. 2007), viruses (Laport et al. 2009), parasites (Festa et al. 2012), neuroinflammation and cancer (Compagnone et al. 1998; Festa et al. 2012), amongst others.

Sponges are also the most studied group of marine invertebrates concerning the leishmanicidal activity of its extracts and compounds. From 21 species belonging to the class Demospongiae, 17 were able to reduce the viability of promastigotes and/or intracellular amastigotes of different *Leishmania* species (Table 1). The hexane extract of the Brazilian *Dragmaxia anomala* and the butanol fraction from the methanol extract of *Haliclona* (*Halichoclona*) sp., inhibited the growth of *L. braziliensis* promastigotes by 97.2 and 43.6 %, respectively, at the concentration of 50 μg/mL for 48 h. *Haliclona* sp. was the most selective towards intracellular amastigotes of *L. braziliensis*, when using the J774.G8 macrophage cell line (IC_{50} = 43.9 μg/mL; SI = 6.8; Bianco et al. 2013).

The methanol crude extract of Indian *H. exigua* was highly active against promastigotes and intracellular forms of *L. donovani*, with IC_{50} values of 18.6 and 47.2 μg/mL, respectively. Moreover, the application of this extract at the dose of 500 mg/kg (body weight) for 5 days on a VL hamster model infected with *L. donovani* resulted in a significant reduction of infection (72.2 %; Dube et al. 2007). When the crude methanol extract was fractionated, the *n*-butanol fraction reduced by 50 % the viability of both promastigotes and intracellular forms, when applied at the concentrations of 8.20 and 31.2 μg/mL, respectively. The same fraction was further evaluated in vivo and resulted in 60.9 % of parasite inhibition at a dose of 500 mg/kg, for a 5 days treatment period. The active component was identified as the alkaloid araguspongin C (**39**) (Dube et al. 2007), which is discussed in the section *Alkaloids*. Two fractions obtained from the hexane and dichloromethane extracts of the Jamaican sponge *Neofibularia nolitangere* were able to inhibit *L. donovani* promastigotes growth by more than 90 % at the concentration of 20 μg/mL (Thompson and Gallimore 2013).

The dichloromethane, ethyl acetate and aqueous extracts of the Tunisian *Sarcotragus sp.* allowed the best results against *L. major* promastigotes, during a treatment period of 72 h, with IC_{50} values lower than 9 μg/mL, followed by the ethyl acetate extract of *Ircinia spinulosa* (IC_{50} = 16.09 μg/mL; Kahla-Nakbi et al. 2010). The organic extract of the Japanese sponge *Aaptos ciliata* was able to reduce the viability of *L. major* promastigotes by 86 % when applied at the concentration of 10 μg/mL, and yielded three new lipopeptides, namely ciliatamides A, B and C (Nakao et al. 2008), which are further discussed in the section *Lipopeptides*.

Fractions from the methanol extract of *I. campana* had low IC_{50} values (2.6–3.9 μg/mL) against intracellular amastigotes of *L. panamensis*. The Ic2 fraction with the highest selectivity index (SI = 8.3) was only constituted by 5α,8α-epidioxysterols, suggesting a good antileishmanial potential of these compounds (Martínez et al. 2001). Curiously, If3 fraction obtained from *I. felix* also composed of 5α,8α-epidioxysterols and some sesterterpene tetronic acids was 4.6 times less selective than the 5α,8α-epidioxysterols enriched fraction from *I. campana* (Martínez et al. 2001). That difference was attributed to the presence of the sesterterpene molecules in the mixture (Martínez et al. 2001). Fractions A and B from *I. campana* enriched in 5α, 8α- epidioxysterols were also active against *L. panamensis* intracellular amastigotes ($IC_{50} \leq 30$ μg/mL; Márquez et al. 2007).

One hundred and twenty molecules isolated and identified from marine sponges were evaluated for in vitro antileishmanial activity (Table 2). To ease the comparison between similar structures, the molecules are numbered and discussed below according to its chemical class, namely alkaloids, steroid-based, terpenoids, fatty acids, polyketides, lipopeptides, peroxides and glycoproteins.

For an accurate interpretation of the activity parameters described for the different molecules in the following sections, the criteria proposed by TDR (2007) was used, i.e. compounds with in vitro activity against axenic amastigotes ($IC_{50} < 0.5$ μg/mL) or intracellular amastigotes ($IC_{50} < 1$ μg/mL) of *Leishmania*, with SI > 20 are considered as antileishmanial hits. These molecules are selected and move forward

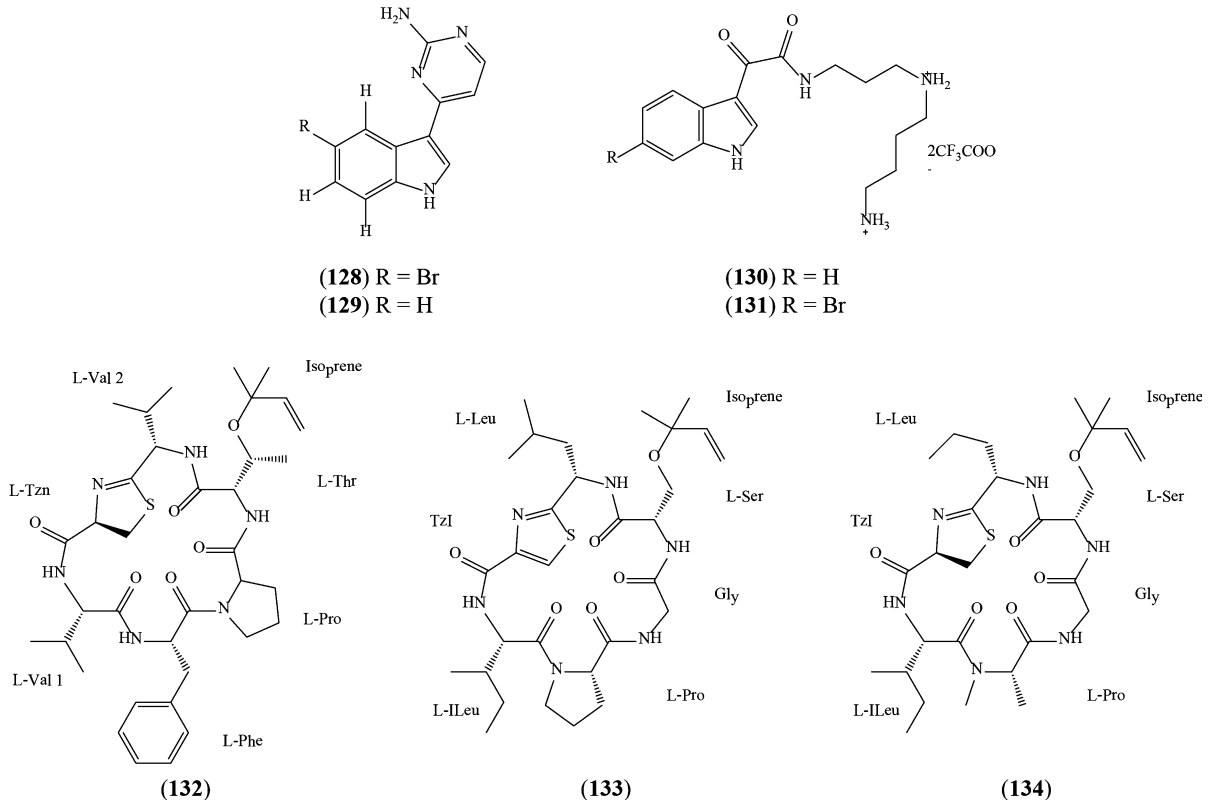


Fig. 12 Molecular structures of compounds isolated from marine tunicates

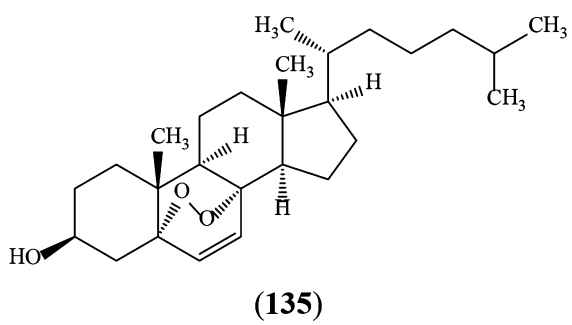


Fig. 13 Molecular structure of a compound obtained from marine mollusks

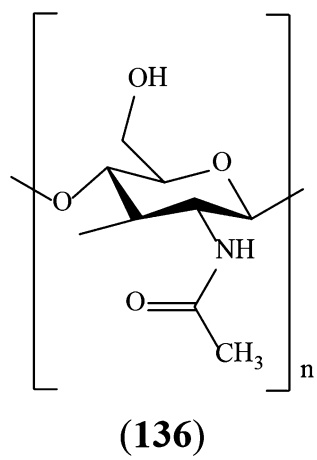


Fig. 14 Molecular structures of compounds isolated from marine crustaceans (adapted from Rinaudo 2006)

in the drug discovery pipeline, where they are evaluated for in vivo efficacy and safety.

Alkaloids

Alkaloids comprise about 47 % of all the anti-leishmanial compounds isolated from marine invertebrates, and were isolated from the Agelasidae, Aplysinidae, Axinellidae, Crambeidae, Halicloniidae, Niphatidae, Petrosiidae, Scopalinidae and Spongiidae/Irciniidae families. Compound **1** was highly active

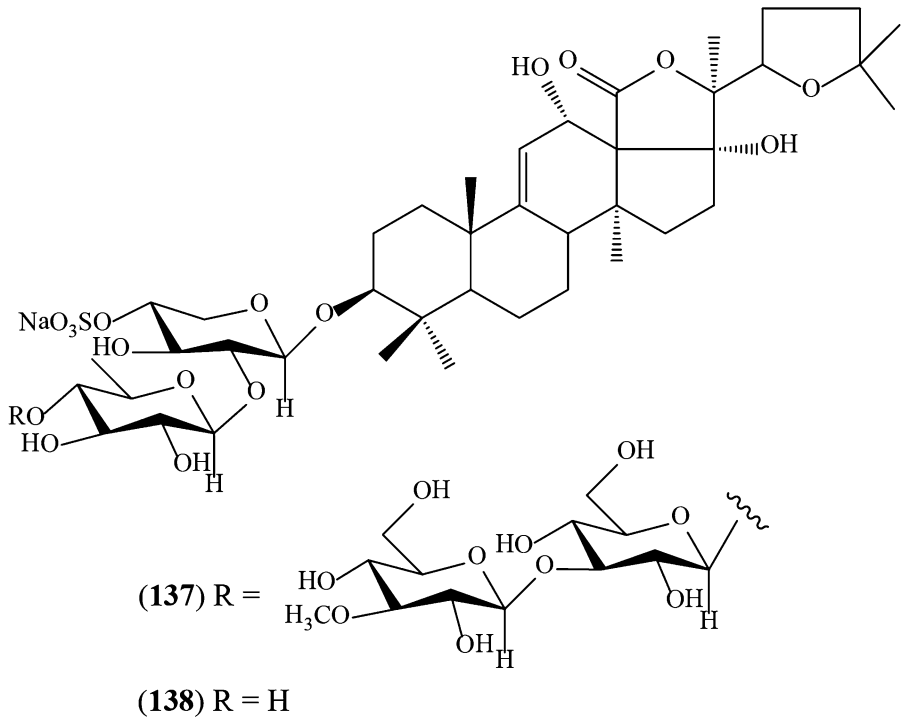


Fig. 15 Molecular structures of compounds isolated from marine echinoderms

against *L. donovani* amastigotes, with a IC_{50} value of 3.85 μg/mL. The difference between compound **1** and compound **2**, which was inactive, is that it has a methylene carboxylic acid instead of a hydroxyl group. Alkaloids **4** and **5** were not active against axenic amastigotes of *L. donovani*, while compounds **3**, **8** and **9** had similar activities with IC_{50} values ranging from 29.87 to 51.58 μg/mL. Compound **8** was also isolated from *Stylissa caribica* although it was not active against *L. donovani* promastigotes. Such difference in terms of activity is likely due to the biological and biochemical dissimilarities between the axenic promastigote and amastigote forms, which often results in different antileishmanial activities (Callahan et al. 1997). Compounds **6** and **7** were also able to reduce the viability of *L. donovani* promastigotes, with IC_{50} values of approximately 29 μg/mL, for a treatment period of 72 h. Compound **11** had moderate activity against *L. donovani* promastigotes (IC_{50} = 53.75 μg/mL) while **10** was inactive, probably due to the lack of the hydroxyl group, which is present in compound **11**. Moreover, the increase in conjugation of compound **9** resulted in about a twofold decrease in the IC_{50} value, in comparison to compound **11**. Compound **12** was highly active against intracellular amastigote forms of *L. infantum* (IC_{50} = 1.5 μg/mL), however it was also highly cytotoxic to human fetal lung fibroblast cells (MRC-5, CC_{50} = 6.7 μg/mL, SI = 4.47; Vik et al. 2009). Nine bromotyrosine derivatives (**13**–**21**) were applied during 72 h to axenic amastigotes of *L. panamensis*. All compounds were inactive, inhibiting less than 10 % of the growth of the axenic population at 20 μM. Moreover, compound **13** dimly decreased the intracellular growth of parasites at 10 μM (12.6 %), followed by **14** (2.1 %), which indicates their low potential as antileishmanial agents. Six bromopyrrole alkaloids were isolated from Axinellidae (**22**–**27**), and evaluated for their inhibitory activity towards *L. donovani* axenic amastigotes, after a period of incubation of 72 h. Although compound **22** had a remarkable low IC_{50} value (1.09 μg/mL), it was only 4.2 times more toxic to parasites than to L6 cells (Scala et al. 2010). When comparing compounds **26** and **27**, the addition of a carbonyl group to the cyclopentane ring and the substitution with a bromine atom, may be related to the 1.8 fold increase in the antileishmanial activity exhibited by compound **26**. From the eleven alkaloids obtained from *Monanchora arbuscula* (**28**–**38**), compounds **28** to **31** were able to reduce by

Table 1 In vitro antileishmanial activity of crude extracts and fractions from different species of sponges

Family/species	Extract/fraction	*Leishmania* species	Parasite form		Reference
			Axenic promastigote	Intracellular amastigotes	
Agelasidae					
Agelas clathrodes	MeOH	*L. panamensis*	NT	>282[a] (6.7[f])[f,a]	Martínez et al. (2001)
	MeOH	*L. panamensis*	NT	>103[a] (6.7[f])[f,a]	
Axinellidae					
Dragmacidon reticulatum	*n*-Hex	*L. braziliensis*	24.1[b] (0.1[g,e])	NT	Bianco et al. (2013)
	MeOH (aqueous residue)	*L. braziliensis*	13.8[b] (0.1[g,e])	NT	
	MeOH (BuOH)	*L. braziliensis*	19.8[b] (0.1[g,e])	NT	
Dragmaxia anomala	Hex	*L. braziliensis*	97.2[b] (0.1[g,e])	>15[a] (0.06[g,e])[g,a]	
Biemnidae					
Neofibularia nolitangere	Hex	*L. donovani*	10.96[c] (100[g,c])	NT	Thompson and Gallimore (2013)
	Hex (fractions 1–4)	*L. donovani*	0–91.77[c] (100[g,c])	NT	
	CH_2Cl_2	*L. donovani*	11.26[c] (100[g,c])	NT	
	CH_2Cl_2 (fractions 1–7)	*L. donovani*	2.53–93.31[c] (100[g,c])	NT	
Guitarridae					
Guitarra sepia	MeOH (aqueous-H_2O residue)	*L. braziliensis*	14.9[b] (0.1[g,e])	NT	Bianco et al. (2013)
	MeOH (BuOH)	*L. braziliensis*	12.5[b] (0.1[g,e])	NT	
Halicloniidae					
Haliclona sp.	MeOH (H_2O residue)	*L. braziliensis*	16.9[b] (0.1[g,e])	NT	
Halicloniidae					
Haliclona sp.	MeOH (BuOH fraction)	*L. braziliensis*	43.6[b] (0.1[g,e])	43.9[a] (0.06[g,e])	Bianco et al. (2013)
H. exigua	MeOH	*L. donovani*	18.6[a] (5.1[h,a])	47.2[a] (26.8[h,a])	Dube et al. (2007)
	(Hex fraction)	*L. donovani*	36.3[a] (5.1[h,a])	72.1[a] (26.8[h,a])	
	Chloroform fraction	*L. donovani*	>100[a] (5.1[h,a])	>100[a] (26.8[h,a])	
	n-BuOH insoluble fraction	*L. donovani*	>100[a] (5.1[h,a])	>100[a] (26.8[ha])	
	n-BuOH soluble fraction	*L. donovani*	8.20[a] (5.1[h,a])	31.2[a] (26.8[h,a])	
Ircinidae					
Ircina strobilina	MeOH	*L. panamensis*	NT	>145[a] (6.7[f,a])	Martínez et al. (2001)
I. campana	MeOH	*L. panamensis*	NT	>96.3[a] (6.7[f,a])	
	MeOH fraction 1–4	*L. panamensis*	NT	2.60–3.90[a] (6.7[f,a])	
	MeOH fraction A–B	*L. panamensis*	NT	25.7–30[a]	Márquez et al. (2007)
I. felix	MeOH	*L. panamensis*	NT	>71.3[a] (6.7[f,a])	Márquez et al. (2007)
	MeOH fraction 1–5	*L. panamensis*	NT	3.4–281[a] (6.7[f,a])	

Table 1 continued

Family/species	Extract/fraction	*Leishmania* species	Parasite form		Reference
			Axenic promastigote	Intracellular amastigotes	
I. spinosula	Aqueous	*L. major*	264[a] (56.64[g,a])	NT	Kahla-Nakbi et al. (2010)
	CH_2Cl_2	*L. major*	47.3[a] (56.64[ga])	NT	
	EtOAc	*L. major*	16.0[a] (56.64[g,a])	NT	
Sarcotragus sp.	Aqueous	*L. major*	3.02[a] (56.64[g,a])	NT	
	CH_2Cl_2	*L. major*	1.39[a] (56.64[g,a])	NT	
	EtOAc	*L. major*	8.49[a] (56.64[g,a])	NT	
Niphatidae					
Niphates erecta	MeOH	*L. panamensis*	NT	>328[a] (6.7[f,a])	Martínez et al. (2001)
Petrosiidae					
Xestospongia muta	MeOH	*L. panamensis*	NT	>240[a] (6.7[f,a])	
X. proxima	MeOH	*L. panamensis*	NT	>54.3[a] (6.7[f,a])	
Suberitidae					
Aaptos ciliata	Organic	*L. major*	86[d]	NT	Nakao et al. (2008)
Tedaniidae					
Tedania ignis	CH_2Cl_2	*L. braziliensis*	16.1[b] (0.1[g,e])	NT	Bianco et al. (2013)
	MeOH–Aqueous residue	*L. braziliensis*	19.1[b] (0.1[g,e])	NT	
	MeOH–EtOAc fraction	*L. braziliensis*	12.2[b] (0.1[g,e])	NT	
	MeOH–*n*-BuOH fraction	*L. braziliensis*	18.4[b] (0.1[g,e])	NT	

When available the activity of the positive control used in the study is included in brackets

NT not tested

[a] Concentration (μg/mL) able to inhibit the cellular growth by 50 % (IC_{50})

[b] Percentage of growth inhibition at the concentration of 50 μg/mL

[c] Percentage of growth inhibition at the concentration of 20 μg/mL

[d] Percentage of growth inhibition at the concentration of 10 μg/mL

[e] Concentration (μM) able to inhibit the cellular growth by 50 % (IC_{50})

[f] Glucantime®

[g] Amphotericin B

[h] Miltefosine

50 % the viability of *L. infantum* promastigotes after a 48 h treatment, at a range of 2 to 4 μM. Compound **30** also inhibited *L. donovani* promastigotes by 50 % at the concentration of 1.9 μg/mL. Compounds **30** and **31** were two times more effective than molecule **29**, thus suggesting that the increase in the length of the side carbon chain enhanced activity. Molecules **30** and **31** were able to modify the membrane permeability of *L. infantum* promastigotes, significantly inducing depolarization of the mitochondrial membrane potential and up-regulating reactive oxygen species production (Santos et al. 2015). These features are associated to an apoptosis like death mechanism on protozoan parasites such as *Plasmodium*, *Trypanosoma* and *Leishmania* (Rodrigues et al. 2006). Still, none of the alkaloids **28–32** were active against intracellular amastigotes of *L. infantum*, when applied for up to 120 h. Alkaloids **34–38** had similar IC_{50} values, ranging from 5.50 to 8.50 μg/mL, when applied to *L. donovani* promastigotes for 72 h. Thus, it is clear that the loss of the hydrogen atom from the dihydropyrimidine of molecule **34** to form a double bond in compound **35** had no effect on its activity. Likewise, the lack of a hydroxyl group in compound

Table 2 Compounds isolated from different species of sponges with in vitro antileishmanial activity

Family/species	Compound	*Leishmania* species	Parasite form			Reference
			Axenic promastigote	Axenic amastigote	Intracellular amastigote	
Agelasidae						
Agelas dispar	Longamide B (**1**)	*L. donovani*	NT	3.85[a] (0.206[j,a])	NT	Scala et al. (2010)
A. gracilis	Gracilioether A (**96**)	*L. major*	NA[c]	NT	NT	Ueoka et al. (2009)
	Gracilioether B (**97**)	*L. major*	68.0[c]	NT	NT	
	Gracilioether C (**98**)	*L. major*	NA[c]	NT	NT	
A. longissima	Longamide A (**2**)	*L. donovani*	NT	>90[a] (0.206[j,a])	NT	Scala et al. (2010)
	Agelongine (**3**)	*L. donovani*	NT	43.22[a]	NT	
A. mauritiana	(+)-2-Oxo-agelasidine C (**4**)	*L. donovani*	NA([k,i])	NT	NT	Yang et al. (2012)
	(−) 8′-Oxo-agelasine D (**5**)	*L. donovani*	NA ([k,i])	NT	NT	
	(−)-Ageloxime D (**6**)	*L. donovani*	29.2[a] ([k,i])	NT	NT	
	Ageloxime B (**7**)	*L. donovani*	28.5[a] ([k,i])	NT	NT	
Agelas sp.	Sceptrin (**8**)	*L. donovani*	NT	51.58[a] (0.206[j,a])	NT	Scala et al. (2010)
	Hymenidin (**9**)	*L. donovani*	NT	29.87[a] (0.206[j,a])	NT	
	Dispacamide B (**10**)	*L. donovani*	NT	>90[a] (0.206[j,a])	NT	
	Dispacamide D (**11**)	*L. donovani*	NT	53.75[a] (0.206[j,a])	NT	
	Agelasine D (**12**)	*L. infantum*	NT	NT	1.50[a] (0.24[j,a])	Vik et al. (2009)
Aplysinidae						
Verongula rigida	11-Hydroxyaerothionin (**13**)	*L. panamensis*	NT	0[d] (60.4[i,d])	12.6[e] (44.9[i,e])	Galeano et al. (2011)
	Dihydroxyaerothionin (**14**)	*L. panamensis*	NT	0.30[d] (60.4[i,d])	2.1[e] (44.9[i,e])	
Aplysinidae						
V. rigida	3,5-Dibromo-*N*,*N*,*N*-trimethyltyraminium (**15**)	*L. panamensis*	NT	0[d] (60.4[i,d])	NT	Galeano et al. (2011)
	3,5-Dibromo-*N*,*N*,*N*,*O*-tetramethyltyraminium (**16**)	*L. panamensis*	NT	0[d] (60.4[i,d])	NT	
	Purealidin R (**17**)	*L. panamensis*	NT	0[d] (60.4[i,d])	NT	
	19-Deoxyfistularin 3 (**18**)	*L. panamensis*	NT	0[d] (60.4[i,d])	NT	
	Fistularin-3 (**19**)	*L. panamensis*	NT	7.7[d] (60.4[i,d])	NT	
	Aeroplysinin-1 (**20**)	*L. panamensis*	NT	0[d] (60.4[i,d])	NT	
	Purealidin B (**21**)	*L. panamensis*	NT	1.60[d] (60.4[i,d])	0[e] (44.9[i,e])	

Table 2 continued

Family/species	Compound	*Leishmania* species	Parasite form			Reference
			Axenic promastigote	Axenic amastigote	Intracellular amastigote	
Axinellidae						
Axinella verrucosa	Dibromopalau'amine (**22**)	*L. donovani*	NT	1.09[a] (0.206[j,a])	NT	Scala et al. (2010)
	Bromoaldisin (**23**)	*L. donovani*	NT	>90[a] (0.206[j,a])	NT	
	Manzacidin A (**24**)	*L. donovani*	NT	75.83[a] (0.206[j,a])	NT	
	Non-oroidin bromopyrrolohomoarginin (**25**)	*L. donovani*	NT	34.49[a] (0.206[j,a])	NT	
	Spongiacidin B (**26**)	*L. donovani*	NT	41.59[a] (0.206[j,a])	NT	
	Stevensine (**27**)	*L. donovani*	NT	75.86[a] (0.206[j,a])	NT	
Dragmaxia undata	(Z)-16-Methyl-11-heptadecenoic acid (**93**)	*L. donovani*	165[b]	NT	NT	Carballeira et al. (2011)
Crambeidae						
Monanchora arbuscula	Batzelladine D (**28**)	*L. infantum*	2.00[b] (16[j,b])	NT	NA	Santos et al. (2015)
	Batzelladine F (**29**)	*L. infantum*	4.00[b] (16[j,b])	NT	NA	
Crambeidae						
Monanchora arbuscula	Batzelladine L (**30**)	*L. infantum*	2.00[b] (16[j,b])	NT	NA	Santos et al. (2015)
M. unguifera		*L. donovani*	1.90[a]	NT	NT	Hua et al. (2007)
M. arbuscula	Norbatzelladine l (**31**)	*L. infantum*	2.00[b] (16[j,b])	NT	NA	Santos et al. (2015)
	Monalidine A (**32**)	*L. infantum*	2.00[b] (16[j,b])	NT	NA	
M. unguifera	16-β-hydroxycrambescidin (**33**)	*L. donovani*	NA (1.7[k,a];1.8[i])	NT	NT	Hua et al. (2007)
	Batzelladine C (**34**)	*L. donovani*	5.50[a] (1.7[k,a];1.8[i,a])	NT	NT	
	Dehydrobatzelladine C (**35**)	*L. donovani*	5.70[a] (1.7[k,a];1.8[i,a])	NT	NT	
	Batzelladine M (**36**)	*L. donovani*	8.50[a] (1.7[k,a];1.8[i,a])	NT	NT	
	Crambescidine 800 (**37**)	*L. donovani*	6.80[a] (1.7[k,a];1.8[i,a])	NT	NT	
	Ptilomycalin A (**38**)	*L. donovani*	5.90[a] (1.7[k,a];1.8[i,a])	NT	NT	
Crellidae						
Crella sp.	Norselic acid A (**56**)	*Leishmania* sp.	2.50[b]	NT	NT	Ma et al. (2009)
	Norselic acid E (**57**)	*Leishmania* sp.	3.60[b]	NT	NT	
	Norselic acid B (**58**)	*Leishmania* sp.	2.40[b]	NT	NT	
	Norselic acid C (**59**)	*Leishmania* sp.	2.60[b]	NT	NT	
	Norselic acid D (**60**)	*Leishmania* sp.	2.00[b]	NT	NT	

Table 2 continued

Family/species	Compound	*Leishmania* species	Parasite form			Reference
			Axenic promastigote	Axenic amastigote	Intracellular amastigote	
Desmanthidae						
Petromica ciocalyptoides	Halistanol trisulfate A (**61**)	*L. chagasi*	NA ([k])	NT	NT	Kossuga et al. (2007)
Geodiidae						
Erylus goffrilleri	(5Z,9Z)-(±)-2-Methoxy-5-9-eicosadienoic acid (**94**)	*L. infantum*	260[b] (1.1[l,b])	NT	NT	Carballeira et al. (2013)
Pachymatisma johnstonii	Pachymatismin (**NS**)	*L. braziliensis resistant*	2.50[a]	NT	NT	Le Pape et al. (2000)
		L. braziliensis	1.00[a]	NT	NT	
		L. donovani	0.60[a]	NT	NT	
		L. mexicana	1.00[a]	1.20[a]	NT	
Halicloniidae						
Haliclona exigua	Araguspongin-C (**39**)	*L. donovani*	35.4[f] (100[j,c])	NT	21.6[f] (95.5[j,f])	Dube et al. (2007)
		L. donovani	61.2[g] (100[j,c])	NT	48.6[g] (95.5[j,f])	
Microcionidae						
Pandaros acanthifolium	Pandaroside A (**62**)	*L. donovani*	NT	19.7[b] (0.51[j,b])	NT	Regalado et al. (2010)
	Pandaroside A methyl ester (**63**)	*L. donovani*	NT	66.3[b] (0.51[j,b])	NT	
	Pandaroside C (**64**)	*L. donovani*	NT	>120[b] (0.51[j,b])	NT	
	Pandaroside C methyl ester (**65**)	*L. donovani*	NT	44.2[b] (0.51[j,b])	NT	
	Pandaroside D (**66**)	*L. donovani*	NT	31.0[b] (0.51[j,b])	NT	
	Pandaroside D methyl ester (**67**)	*L. donovani*	NT	13.7[b] (0.51[j,b])	NT	
	Pandaroside E (**68**)	*L. donovani*	NT	15.9[b] (0.51[j,b])	NT	
	Pandaroside E methyl ester (**69**)	*L. donovani*	NT	41.3[b] (0.51[j,b])	NT	
	Pandaroside F (**70**)	*L. donovani*	NT	4.30[b] (0.51[j,b])	NT	
	Pandaroside F methyl ester (**71**)	*L. donovani*	NT	26.8[b] (0.51[j,b])	NT	
	Pandaroside G (**72**)	*L. donovani*	NT	1.30[b] (0.51[j,b])	NT	
	Pandaroside G methyl ester (**73**)	*L. donovani*	NT	0.05[b] (0.51[j,b])	NT	
Microcionidae						
P. acanthifolium	Pandaroside H (**74**)	*L. donovani*	NT	46.7[b] (0.51[j,b])	NT	Regalado et al. (2010)
	Pandaroside H methyl ester (**75**)	*L. donovani*	NT	16.5[b] (0.51[j,b])	NT	
	Pandaroside I (**76**)	*L. donovani*	NT	36.0[b] (0.51[j,b])	NT	
	Pandaroside I methyl ester (**77**)	*L. donovani*	NT	28.7[b] (0.51[j,b])	NT	
	Pandaroside J (**78**)	*L. donovani*	NT	36.1[b] (0.51[j,b])	NT	
	Pandaroside J methyl ester (**79**)	*L. donovani*	NT	20.7[b] (0.51[j,b])	NT	

Table 2 continued

Family/species	Compound	*Leishmania* species	Parasite form			Reference
			Axenic promastigote	Axenic amastigote	Intracellular amastigote	
Niphatidae						
Amphimedon viridis	Alkaloidal substance (**NS**)	*L. mexicana*	10.0[a]	NT	NT	Marchán et al. (2000)
Petrosiidae						
Acanthostrongylophora sp.	Manzamine A (**40**)	*L. donovani*	0.90[a] (0.06[i,a]; 2.1[k,a])	NT	NT	Rao et al. (2004, 2006)
	(+)-8-Hydroxymanzamine A (**41**)	*L. donovani*	6.20[a] (0.06[i,a]; 2.1[k,a])	NT	NT	
	Manzamine Y (**42**)	*L. donovani*	1.60[a] (0.06[i,a]; 2.1[k,a])	NT	NT	
	Manzamine E (**43**)	*L. donovani*	3.80[a] (0.06[i,a]; 2.1[k,a])	NT	NT	
	6-Hydroxymanzamine E (**44**)	*L. donovani*	2.50[a] (0.06[i,a]; 2.1[k,a])	NT	NT	
	Manzamine F (**45**)	*L. donovani*	4.20[a] (0.06[i,a]; 2.1[k,a])	NT	NT	
	Manzamine J (**46**)	*L. donovani*	25.0[a] (0.06[i,a]; 2.1[k,a])	NT	NT	
	Manzamine A N-oxide (**47**)	*L. donovani*	1.10[a] (0.06[i,a]; 2.1[k,a])	NT	NT	
Petrosiidae						
Acanthostrongylophora sp.	Ircinol A (**48**)	*L. donovani*	0.90[a] (0.06[i,a]; 2.1[k,a])	NT	NT	Rao et al. (2004, 2006)
	12,34-Oxa-6-hydroxymanzamine E (**49**)	*L. donovani*	NA	NT	NT	
	12,28-Oxamanzamine E (**50**)	*L. donovani*	18.0[a] (0.06[i,a]; 2.1[k,a])	NT	NT	
	12-34-Oxamanzamine E (**51**)	*L. donovani*	NA	NT	NT	
	Ircinal A (**52**)	*L. donovani*	4.60[a] (0.06[i,a]; 2.1[k,a])	NT	NT	
Petrosiidae						
Neopetrosia sp.	Renieramycin A (**53**)	*L. amazonensis*	0.20[a] (0.42[i,a])	NT	NT	Nakao et al. (2004)
Phloeodictyidae						
Calyx podatypa	(±)-2-Methoxy-6Z-heptadecenoic acid (**95**)	*L. donovani*	404[b] (0.8[j,b])	NT	NT	Carballeira et al. (2012)
Scopalinidae						
Stylissa caribica	Sceptrin (**8**)	*L. donovani*	NA			Mohammed et al. (2006)
	Oroidin (**54**)	*L. donovani*	NA			
	Stevensine (**27**)	*L. donovani*	NA			
Spongiidae/Irciniidae						
Spongia sp./Ircinia sp.	Furospinulosin-1 (**80**)	*L. donovani*	NT	14.2[a] (0.20[j,a])	NT	Orhan et al. (2010)
	Furospinulosin-2 (**81**)	*L. donovani*	NT	>90[a] (0.20[j,a])	NT	
	Furospongin-1 (**82**)	*L. donovani*	NT	4.80[a] (0.20[j,a])	NT	
	Furospongin-4 (**83**)	*L. donovani*	NT	>90[a] (0.20[j,a])	NT	
	Demethylfurospongin-4 (**84**)	*L. donovani*	NT	10.2[a] (0.20[j,a])	NT	

Table 2 continued

Family/species	Compound	*Leishmania* species	Parasite form			Reference
			Axenic promastigote	Axenic amastigote	Intracellular amastigote	
Spongiidae/Irciniidae						
Spongia sp./Ircinia sp.	4-Hydroxy-3-octaprenylbenzoic acid (**85**)	*L. donovani*	NT	5.60[a] (0.20[j,a])	NT	Orhan et al. (2010)
	4-Hydroxy-3-tetraprenylphenylacetic acid (**86**)	*L. donovani*	NT	>90[a] (0.20[j,a])	NT	
	Heptaprenyl-p-quinol (**87**)	*L. donovani*	NT	18.9[a] (0.20[j,a])	NT	
	Dorisenone D (**88**)	*L. donovani*	NT	>90[a] (0.20[j,a])	NT	
	11β-acetoxyspongi-12-en-16-one (**89**)	*L. donovani*	NT	0.75[a] (0.20[j,a])	NT	
	12-epi-Deoxoscalarin (**90**)	*L. donovani*	NT	>90[a] (0.20[j,a])	NT	
	2-(Hexaprenylmethyl)-2-methylchromenol (**91**)	*L. donovani*	NT	15.9[a] (0.20[j,a])	NT	
	Squalene (**92**)	*L. donovani*	NT	>90[a] (0.20[j,a])	NT	
	Tryptophol (**55**)	*L. donovani*	NT	9.60[a] (0.20[j,a])	NT	
Suberitidae						
Aaptos ciliata	Ciliatamide A (**110**)	*L. major*	50.0[c]	NT	NT	Nakao et al. (2008)
	Ciliatamide B (**111**)	*L. major*	45.5[c]	NT	NT	
	Ciliatamide C (**112**)	*L. major*	NA[c]	NT	NT	
Plakinidae						
Plakinastrella mamillaris	Gracilioether E (**99**)	*L. infantum*	NT	159[b]	NT	Festa et al. (2012)
	Gracilioether F (**100**)	*L. infantum*	NT	396[b]	NT	
	Gracilioether G (**101**)	*L. infantum*	NT	136[b]	NT	
	Gracilioether H (**102**)	*L. infantum*	NT	78.8[b]	NT	
	Gracilioether I (**103**)	*L. infantum*	NT	64.1[b]	NT	
	Gracilioether J (**104**)	*L. infantum*	NT	320[b]	NT	
Plakinidae						
Plakortis angulospiculatus	(2E,6R,8S)-Methyl 3,6-epoxy-4,6,8-triethyldodeca-2,4-dienoate (**105**)	*L. chagasi*	3.90[a] (0.1[k,a])	NT	3.40[a] (21[h,a])	Kossuga et al. (2008)
	(2Z,6R,8R,9E)-Methyl 3,6-epoxy-4,6,8-triethyl-2,4,9-dodecatrienoate (**106**)	*L. chagasi*	8.50[a] (0.1[k,a])	NT	1.60[a] (21[h,a])	
	Plakortide P (**107**)	*L. chagasi*	1.90[a] (0.1[k,a])	NT	0.50[a] (21[h,a])	
	Plakortine (**108**)	*L. chagasi*	6.00[a] (0.1[k,a])	NT	NT	
	Spongosoritin A (**109**)	*L. chagasi*	2.50[a] (0.1[k,a])	NT	3.10[a] (21[h,a])	

Table 2 continued

Family/species	Compound	*Leishmania* species	Parasite form			Reference
			Axenic promastigote	Axenic amastigote	Intracellular amastigote	
Plakortis sp.	(3S6R8S)-4,6-Diethyl-3,6-epidioxy-8-methyldodeca-4-enoic acid (**113**)+	*L. mexicana*	0.29[a] (0.06[m,a])	NT	NT	Compagnone et al. (1998)
	3,6-Epidioxy-4,6,8,10-tetraethyltetradeca-7,11 -dienoic acid (**114**)+	*L. mexicana*	1.00[a] (0.06[m,a])	NT	NT	
	ent-3,6-Epidioxy-4,6,8,10-tetraethtyltetradeca-7,11-dienoic acid (**115**)	*L. mexicana*	1.00[a] (0.06[m,a])	NT	NT	Lim et al. (2006)
	ent-[3,5-Diethyl-5-(2-ethyl-hex-3-enyl)-5H-furan-2-yliidene]-acetic acid methyl ether (**116**)	*L. mexicana*	23.0[a] (0.06[m,a])	NT	NT	
	Methyl (2Z,6R,8S)-3,6-epoxy-4,6,8-triethyldodeca-2,4-dienoate (**117**)+	*L. mexicana*	2.71[a] (0.06[m,a])	NT	NT	Compagnone et al. (1998)
	Methyl (2Z,6R,8S)-4,6-diethyl-3,6-epoxy-8-methyldodeca-2,4-dienoate (**118**)+	*L. mexicana*	1.86[a] (0.06[m,a])	NT	NT	

When available the activity of the positive control used in the study is included in brackets

NT not tested, *NS* no structure provided

[a] Concentration (μg/mL) able to inhibit the cellular growth by 50 %

[b] Concentration (μM) able to inhibit the cellular growth by 50 %

[c] Percentage of growth inhibition at the concentration of 10 μg/mL

[d] Percentage of growth inhibition at the concentration of 20 μM

[e] Percentage of growth inhibition at the concentration of 10 μM

[f] Percentage of growth inhibition at the concentration of 50 μg/mL

[g] Percentage of growth inhibition at the concentration of 100 μg/mL

[h] Glucantime®

[i] Amphotericin B

[j] Miltefosine

[k] Pentamidine

[l] Camptothecin

[M] Ketoconozole

37, in comparison to compound **38**, does not affect its activity. The alkaloid **39** was isolated from *H. exigua* and had moderate in vitro activity against promastigotes (35.40 % inhibition at the concentration of 50 μg/mL) and intracellular amastigotes (48.60 % of inhibition at 100 μg/mL) forms of *L. donovani*, and was not toxic towards J774A.1 macrophages (Dube et al. 2007). Nevertheless, compound **39** had low efficacy in vivo (Dube et al. 2007). Thirteen alkaloids (**40**–**52**) were applied for 72 h to *L. donovani* promastigotes. Comparing compounds **40** and **42**, it appears that substitution with a hydroxyl group had no influence in the observed antileishmanial activity, as they had comparable IC_{50} values (0.9 and 1.6 μg/mL, respectively). On the other hand, the hydroxyl group in molecule **41** might be responsible for the decrease of activity, comparatively to **42**. This assumption is reinforced by the intramolecular hydrogen bonding in the indole observed in **42**, which results in increased stability of the molecule. Compounds **40** and **47** were highly active, with IC_{50} values of 0.90 and 1.10 μg/mL, respectively. The only difference between these two compounds is the presence of the n-oxide in **47**, which suggests that it is not essential for the activity. Nonetheless, the 25 fold less activity of molecule **46**, compared to compounds **40** and **47**, clearly indicates that the bond forming the cyclooctane ring in the latter compounds is of major importance for the activity.

The alkaloid **53** isolated from *Neopetrosia* sp. had a very low IC_{50} value (0.20 μg/mL) against promastigote forms of *L. amazonensis*, after a treatment period of 72 h. The indole alkaloid **55** inhibited half of the *L. donovani* amastigote population at the concentration of 9.60 μg/mL, being about 6.6 times more selective to parasites than to L6 cells (Orhan et al. 2010).

Steroid-based compounds

Several steroid-based compounds were isolated from Crellidae, Desmanthidae and Microcinidae families. Norselic acids (**56**–**60**) had similar activities against *Leishmania* promastigotes with IC_{50} values ranging from 2.0 to 3.6 μM. Compound **57** is the acetate derivative of **56**, which may be responsible for the decrease in activity. Steroid **61** had no effect of the viability of *L. chagasi* promastigotes. Concerning the pandarosides and its methyl ester derivatives (compounds **62** to **79**) isolated from the Microcinidae family, we can observe that in general methyl esters were more active against *L. donovani* promastigotes, for a treatment period of 72 h, except for the **68/69** and **79/71** pairs. Regalado and colleagues (2010) suggested that this pattern could be related to the fact that methyl esters may act as prodrugs, being hydrolyzed to its respective acid after entering the cells. All compounds had antileishmanial activity, except for molecule **64** ($IC_{50} > 120$ μM). Comparing compounds **68** and **70** we can observe that the lack of Δ 24,24[1] in molecule **70** enhances its activity. Compounds **70**, **72** and **73** allowed the lowest IC_{50} values: 4.3, 1.3 and 0.05 μM, respectively. However, they were also the most toxic against L6 cells (CC_{50} = 10.8, 5.4 and 0.22 μM, respectively; Regalado et al. 2010). Compound **73** meets the hit activity criteria defined previously (TDR 2007), as it exhibited a 19.6 times lower IC_{50} value than the one required for a hit (<1 μM). Conversely, its selectivity is 5 times lower than the required (SI = 4.3; Regalado et al. 2010).

Terpenoids

Thirteen terpenoids (**80**–**92**) were isolated from species belonging to the Spongiidae and Irciniidae families and were evaluated against *L. donovani* axenic amastigotes. Linear furanoterpenes **80** and **81** differ only in an additional isoprene unit present in molecule **81**, which is probably the cause for its loss of activity against amastigotes and increased cytotoxicity towards L6 cells (CC_{50} = 34.0 μg/mL; Orhan et al. 2010). Furoterpene **82** was more active, with an IC_{50} value of 4.8 μg/mL, and was 5.7 times more toxic to parasites than to L6 cells (CC_{50} = 27.45 μg/mL; Orhan et al. 2010). Contrary to what was saw for the steroids **62**–**79** in which an increased toxicity towards promastigotes with the methylation of the carboxylic group was observed, compound **83** (methylated) is 9 fold less active than compound **84** (non-methylated carboxylic group; IC_{50} = 10.2 μg/mL). Meroterpenes **85**–**87** are structurally similar; however the presence of a longer isoprenyl chain in compound **85** may be responsible for the increased leishmanicidal potential, when comparing to molecules **86** and **87**. Moreover, the substitution on the aromatic ring in compound **86** does not seem to improve its activity against *Leishmania* parasites. Similarly, the substitution in the cyclohexane ring in compound **88** appears to deactivate the molecule ($IC_{50} > 90$ μg/mL) since the non-

substituted compound **89** was more active (IC_{50} = 0.75 μg/mL), although less selective (SI = 4.43; Orhan et al. 2010).

Fatty acids

Three fatty acids (**93–95**) were isolated from the Axinellidae, Geodiidae and Phloeodictyidae families. However, these molecules were ineffective against the promastigote forms of *L. donovani* and *L. infantum* ($IC_{50} \geq 165$ μM).

Polyketides

Fourteen polyketides were obtained from the Agelasidae and Plakinidae families. Oxygenated polyketides **96** to **98** were isolated from *Agelas gracilis* but only compound **97** had antileishmanial activity, suppressing 68 % of *L. major* promastigote viability at the concentration of 10 μg/mL, when applied for 72 h, while compound **98** presenting a hydroxyl instead of a ketone group was non-toxic. Moreover, molecules **99–104** were tested against axenic amastigotes of *L. infantum* and the majority was devoid of significant activity ($IC_{50} > 136.8$ μM). However, molecules **102** and **103** had moderate toxicity towards parasites, with IC_{50} values of 78.8 and 64.1 μM, respectively. In another study, three polyketides obtained from *Plakortis angulospiculatus* (**105**, **106** and **109**) were tested against *L. chagasi* promastigotes, for 48 h, and allowed promising IC_{50} values ranging from 2.5 to 8.5 μg/mL.

Lipopeptides

Three new lipopeptides (**110–112**) were isolated from the organic extract of *A. ciliata*. Compounds **110** and **111** inhibited about 50 % of the promastigotes growth when applied at the concentration of 10 μg/mL, while **112** was not active against *L. major* promastigotes. Nakao and colleagues (2008) suggested that other bioactive compounds or synergistic effects could be present in the extract, since it inhibited 86 % of the viability of promastigote forms of *L. major* at 10 μg/mL.

Cyclic peroxides and furans

From the Plakinidae family 5 peroxides and 3 furans (**107** to **108** and **113** to **118**) were also isolated. Besides its high activity towards *L. chagasi* promastigotes (IC_{50} = 1.9 μg/mL), compound **107** had an IC_{50} value of 0.5 μg/mL against amastigotes, no hemolytic activity and a high selectivity index (SI = 31.7), being considered a hit (Kossuga et al. 2008). Molecule **107** induced severe ultrastructural alterations on promastigotes morphology after a period of incubation of 3 h, but no significant nitric oxide (NO) production by peritoneal macrophages was observed, suggesting other related leishmanicidal mechanism rather than macrophage activation (Kossuga et al. 2008). In contrast, peroxide **108** was active towards *L. chagasi* promastigotes (IC_{50} = 6.00 μg/mL), but was not tested towards amastigotes due to its high cytotoxicity (CC_{50} = 4.7 μg/mL; Kossuga et al. 2008).

The peroxide **113** allowed the lowest IC_{50} value (0.29 μg/mL). Its application resulted in a significant reduction of the motility of *L. mexicana* promastigotes after only 30 min of treatment (Compagnone et al. 1998). Peroxide **114** was highly active, similar to furan **115** (IC_{50} = 1 μg/mL). Furans **117** and **118** had similar activities (IC_{50} = 2.71 and 1.86 μg/mL) suggesting that the presence of either an ethyl or a methyl group does not greatly influences the antileishmanial activity. Although cytotoxicity studies were not performed to allow an interpretation of its selectivity, marine sponge peroxides were undoubtedly highly active against *Leishmania* parasites. In fact, these compounds are well known for their antiprotozoal potential, especially as potent antimalarial drugs (e.g. artemisinin; Slack et al. 2012).

Glycoproteins

Pachymatismin, a compound isolated from *Pachymastisma johnstonii*, was highly active against promastigote forms of *L. donovani*, *L. mexicana* and *L. braziliensis*, against a pentavalent antimonial resistant strain of *L. braziliensis* and also on axenic amastigotes of *L. mexicana* (IC_{50} values ranging from 0.6 to 2.5 μg/mL). Data concerning its cytotoxicity was not reported, however, pachysmatismin was previously reported to be toxic to different cell lines (Zidane et al. 1996). Moreover, it induced morphological alterations in *Leishmania* promastigotes, mainly in terms of cell shape and flagellum, and increased the activity of phospholipases A2, which are enzymes involved in macrophage invasion, suggesting that its activity may be related to a calcium-modulated mechanism or

apoptosis like death (Le Pape et al. 2000). This was the only study reporting the simultaneous susceptibility of different *Leishmania* species and strains to pure compounds. Since in some highly endemic regions more than one species may be present, is of major relevance to obtain this information, having in mind that an ideal drug should be efficient towards more than one *Leishmania* species.

Cnidarians (Phylum: Cnidaria)

The phylum Cnidaria contains about 10 000 species including jellyfish, soft corals, sea pens, anemones, hydroids, sea wasps and box jellyfishes, from which over 2000 natural products have been described in the last decade (Brusca and Brusca 2003; Rocha et al. 2011). Cnidarians are considered the second most prolific source of marine natural products, having already yielded alkaloids, sterols, sesquiterpenes, diterpenes, terpenoids, steroids and icosanoids (Rocha et al. 2011; Blunt et al. 2013). These compounds are not only active against infectious diseases such as HIV, malaria and tuberculosis, but also have other relevant biological activities, including anti-inflammatory, antifouling and antitumor (Rocha et al. 2011; Blunt et al. 2013). The antileishmanial activity of cnidarians has recently started to be explored, and so far, only few extracts and compounds were evaluated for such purpose. The methanol extracts of *Heterogorgia uatumani* (Plexauridae), *Carijoa riisei* (Clavulariidae) and *Macrorhynchia philippina* (Aglaopheniidae) had promising IC_{50} values of 4.40, 2.84 and 15.37 μg/mL, respectively, whilst that of *Leptogorgia punicea* (Gorgoniidae) was only moderately active (IC_{50} = 93.30 μg/mL) against *L. chagasi* promastigotes (Reimão et al. 2008). The methanol extracts of *Aiptasia pallida* (Aiptasiidae), *Physalia physalis* (Physaliidae), *Palythoa caribaeorum* (Sphenopidae) and *Zoanthus sociatus* (Zoanthidae) were inactive towards *L. chagasi* promastigotes (Reimão et al. 2008). The octocoral *C. riisei* was the most studied and is considered the most promising cnidarian species. Hexane and *n*-butanol fractions were obtained from an active methanol extract of *C. riisei*, and were applied at the concentration of 50 μg/mL to *L. braziliensis* promastigotes, resulting in a reduction of cellular viability of 35.9 and 14.6 %, respectively. Additionally, the hexane extract had an IC_{50} value of 43.3 μg/mL on intracellular amastigotes of *L. braziliensis* (Bianco et al. 2013). Likewise, the application of hexane and butanol fractions obtained from the ethanol extract of that species on *L. braziliensis* amastigotes for 48 h resulted in a cell viability inhibition of 35.9 and 14.2 %, respectively (Almeida et al. 2012). From the *n*-hexane fraction a pregnane steroid (**124**) was purified and its activity is discussed below (Almeida et al. 2012). From the results reported for *C. riisei* it is clear that this species is endowed with compounds able to reduce *Leishmania* parasites viability.

Several terpenoids isolated from marine cnidarian species have been evaluated against *Leishmania* parasites and the majority of the tested compounds had remarkable strong antileishmanial activities. Lobocrasol A (**119**) and C (**120**), obtained from the soft coral *Lobophytum crissum* (Alcyoniidae) were highly active and selective against *L. donovani* axenic amastigotes ($IC_{50} < 0.2$ μM; SI = 310.83 and 237.94, respectively; Thao et al. 2015). The diterpenoid cristaxenicin A (**121**) isolated from the deep-sea gorgonian *Acanthoprimnoa cristata* (Primnoidae) was extremely active against *L. amazonensis* promastigotes (IC_{50} = 0.088 μM; Ishigami et al. 2012). In addition, compound **121** was 20 to 50 times more active towards parasites than to P388 and HeLa cells (CC_{50} = 4.7 and 2.1 μM, respectively; Ishigami et al. 2012). The tryiclic sesquiterpenes shagene A (**122**) and B (**123**) were isolated from an unidentified Antartic octocoral (Von Salm et al. 2014). Compound **122** was 10 times more toxic to intracellular amastigotes (IC_{50} = 5 μM) than to axenic amastigote forms (IC_{50} = 54 μM) of *L. donovani*, and was not toxic towards J774.A1 macrophages (CC_{50} = 345 μM; Von Salm et al. 2014). However, molecule **123** was not active, thus suggesting the relevance of having the methoxy substituent at the C8 position (Von Salm et al. 2014).

The pregnane steroid **124** was isolated from an extract of *C. riisei* (Clavulariidae). Although this molecule inhibited half of *L. braziliensis* promastigotes viability at 50 μM, it was inactive against intracellular amastigote forms ($IC_{50} > 100$ μM; Almeida et al. 2012). Another steroid, 18-acetoxipregna-1,4,20-trien-3-one (**125**), previously isolated from *C. riisei* (Kossuga et al. 2007), was able to reduce promastigotes viability by 50 % at the concentration of 5.51 μg/mL, after a 24 h treatment. Although it was also active towards the amastigote stage after 96 h

(IC_{50} = 16.88 μg/mL), and had no significant hemolytic activity, it was not selective as it was highly toxic towards peritoneal macrophages (CC_{50} = 10.68 μg/mL, SI < 1; Reimão et al. 2008).

The (24R,S)-24-hydroxy-24-methylcholesterol (**126**) and 24-methylenecholesterol (**127**), isolated from the coral *Palythoa variabilis* (Sphenopidae) had comparable activities against three different strains of *L. donovani*, with IC_{50} values of 3.0 and 4.5 μM, respectively (Bazin et al. 2006). However, the cytotoxic activity of those compounds toward mammalian cell lines was not evaluated.

Tunicates (Phylum: Chordata; Sub-phylum: Urochordata = Tunicata)

Ascidians are the most abundant group of the subphylum Tunicata and is represented by approximately 3000 species (Brusca and Brusca 2003). Around 35 % of all tunicate-derived compounds were isolated from the Didemnidae family, which is recognized as the most prolific family of bioactive molecules (Schmidt et al. 2012). Till date, the antineoplastic alkaloid trabectedin (Yondelis®, Ecteinascidin-743,ET-743) initially isolated from the sea squirt *Ecteneiscidia turbinata*, is the only EU-approved tunicate-derived compound, recommended for the treatment of soft tissue sarcoma and ovarian cancer (Martins et al. 2014). Interestingly, this compound was not subjected to any chemical modifications until its launch in the market. Still, other tunicate-derived molecules are currently under phases II and III of clinical trials (Martins et al. 2014; Atmaca and Bozkurt 2015). Regarding *Leishmania* parasites, only one study evaluated the anti-*L. braziliensis* activity of extracts and fractions from the ascidian *Didemnum granulatum* (Didemnidae). The ethyl acetate and the butanol partitions obtained from the methanol crude extract inhibited 15.7 and 17.9 % of the viability of *L. braziliensis* promastigotes, respectively, at 50 μg/mL (Bianco et al. 2013). Only seven tunicate-derived molecules were so far evaluated against *Leishmania*: two indole-based alkaloids, meridianin C (**128**) and G (**129**), previously identified in *Aplidium meridianum* (Polyclinidae), were evaluated against *L. donovani* promastigotes (Bharate et al. 2013). Compound **129** was inactive and differed from **128**, which was active (IC_{50} = 64.86 μM), by a bromine atom in the cyclohexane ring of the indole (Bharate et al. 2013). Furthermore, didemnidine A (**130**) and B (**131**) were isolated from the New Zealand ascidian *Didemnum* sp. (Didemnidae; Finlayson et al. 2011). Both compounds were inactive (IC_{50} > 160 μM), thus the presence of a bromine atom in compounds **131** and **130** does not influences its activity (Finlayson et al. 2011). Also from the Didemnidae family, two cyclic hexapeptides mollamide B (**132**) and C (**133**) and a known peptide keenamide A (**134**) were obtained from the Indonesian *Didemnum molle* (Donia et al. 2008). Although **133** and **134** were not active against *L. donovani*, compound **132** inhibited 50 % of the parasite growth at the concentration of 18 μg/mL (Donia et al. 2008).

Mollusks (Phylum: Mollusca)

The Phylum Mollusca is one of the biggest marine phyla comprising about 50,000 species, including sea snails, sea slugs, clams, oysters and octopuses (Brusca and Brusca 2003).

The isolation of ω-conotoxin, a peptide from the venom of the sea snail *Conus magus*, led to the synthesis of Ziconotide (Prialt®), one of the few molecules that did not suffer any modification from the original scaffold, reaching the market for the treatment of severe chronic pain associated with cancer (Martins et al. 2014). More recently, Adcetris® successfully reached the market as a medication for Hodgkin and systemic anaplastic large cell lymphoma. Adcetris® is a derivative of the natural dolastatin 10, linked to an antibody, initially isolated from the sea hare *Dolabella auricularia*, but found to be synthesized by cyanobacteria present in the sea hare diet (Martins et al. 2014). In fact, mollusks include in their diets a high variety of other invertebrates such as sponges, and also algae or cyanobacteria from which they absorb specific metabolites (Garson 2010). Thus, bioactive compounds obtained from these animals may in fact be synthetized by other marine organisms, from lower food chain levels.

So far only one compound, namely 5α,8α-epidioxycholest-6-en-3β-ol (**135**) was tested against *L. donovani* parasites (Clark et al. 2013). This molecule was identified in the digestive gland of the clam *Dolabrifera dolabrifera* (Aplysiidae) and had an IC_{50} value of 4.9 μM against amastigote forms, showing no citotoxicity (CC_{50} = 281 μM) and thus, a high

selectivity index (SI = 57.3; Clark et al. 2013). Compound **135** had no activity against other protozoan parasites such as *P. falciparum* and *Trypanosoma cruzi*, which suggests its selectivity towards *Leishmania* (Clark et al. 2013).

Crustaceans (Phylum: Arthropoda; Sub-phylum: Crustacea)

The sub-phylum Crustacea holds a massive biodiversity as it includes about 68, 000 species belonging to different Classes, namely Remipedia, Cephalocarida, Branchiopoda, Malacostraca and Maxillopoda (Brusca and Brusca 2003).

Chitin (**136**) isolated from the shell of the shrimp *Parapenaeus longirostris* (Penaeidae) was evaluated against promastigotes of a Glucantime® sensitive strain of *L. infantum*. Compound **136** was able to suppress 50 and 100 % of *L. infantum* promastigotes at the concentrations of 600 and 5000 μg/mL, respectively, which indicates its low antileishmanial potential (Salah-Tazdaït et al. 2014).

Echinoderms (Phylum: Echinodermata)

About 7000 species of radially symmetrical organisms comprise the Echinodermata Phylum which is divided in five classes: Crinoidea (sea lilies and feather stars); Asteroidea (sea stars); Ophiuroidea (brittle stars and basket stars); Echinoidea (urchins and sand dollars) and Holothuroidea (sea cucumbers; Brusca and Brusca 2003). Echinoderms are well-known producers of different bioactive metabolites, as extensively addressed in other reviews (Blunt et al. 2013; Gomes et al. 2014). Nevertheless, few reports describe the antileishmanial potential of these organisms. Indeed, only results concerning the leishmanicidal potential of two compounds, beside a small number of extracts, are found in the literature.

The methanolic extract of the sea star *Echinaster* (*Othilia*) *echinophorus* (Echinasteridae) collected in Cuba had a two times folder increase in activity on the intracellular model of *L. amazonensis* (IC_{50} = 37.5 μg/mL) comparatively to the extracellular form, and was not toxic against peritoneal macrophages from BALB/c mice (CC_{50} = 348.6 μg/mL, SI = 9.3; Parra et al. 2010). Moreover, in vivo studies showed that the extract was not toxic to mice when administered intraperitoneally at the dose of 100 mg/kg for 15 days, since no mortality and weight loss (less than 10 %) was observed. Moreover, it significantly reduced the parasite burden and lesion size in infected mice (Parra et al. 2010). Dichloromethane/methanol (1:1) extracts of *Actinopyga crasa, A. mauritiana, Bohadschia cousteaui, B. tenuissima, Holothuria atra, H. fuscogilva, H. leucospilota, H. nobilis* (Holothuriidae) and *Stichopus hermanni* (Stichopodidae), collected along the Red Sea, had reduced or nil antileishmanial activity (Lawrence et al. 2009). In the same study, different dichloromethane/methanol extracts of *A. mauritiana, H. atra*, *B. vitiensis* and *Pearonothuria graeffei* (Holothuriidae) were moderately active, with IC_{50} values ranging from 85 to 462 μg/mL, confirming that intraspecific variation in bioactive metabolites production may occur by collecting organisms in different habitats (Lawrence et al. 2009). The crude methanol extract of the coral reef sea cucumber *Actinopyga lecanora* (Holothuriidae) and its fractions were tested against promastigote and intracellular amastigote forms of *L. donovani*, during 96 and 72 h, respectively. The crude extract was able to reduce 88.50 and 72.45 % of the promastigotes and amastigotes population, respectively, at the concentration of 100 μg/mL (Singh et al. 2008). When applied at the same concentration, the ethyl acetate soluble fraction was poorly active, inhibiting less than 22.0 % of both parasites forms. In contrast, the butanol soluble fraction reduced 98.5 and 76.4 % of the promastigote and amastigote growth (Singh et al. 2008). Furthermore, at a 500 mg/kg dose it was able to reduce parasite burden to 26 % in *L. donovani* infected hamsters (Singh et al. 2008). Two glycosides, namely holothurin A (**137**) and B (**138**) were isolated and identified from the *n*-butanol fraction (Singh et al. 2008).

Compound **138** was found to be more active than **137**, both in vitro and in vivo, and when applied at the concentration of 50 μg/mL, both were able to reduce the viability of intracellular amastigotes by 45 % (**137**) and 57.65 % (**138**). In *L. donovani* infected hamsters, the application of molecule **138** at a dose of 100 mg/kg/day for 5 days resulted in a reduction of the parasite burden in 71.5 %, in contrast to compound **137** that reduced approximately 50 % (Singh et al. 2008). Even at a lower dose (50 mg/kg/day for 5 days) molecule **138** was significantly more efficient than

137, reducing the parasite burden in 40 %, comparatively to compound **138** that only allowed a reduction of 20 % (Singh et al. 2008). It is likely that the increase in the glicosyl groups, observed in molecule **137**, leads to a decrease in the in vitro and in vivo antileishmanial activity. Data concerning the in vitro and in vivo toxicity of both compounds was not reported.

Bryozoans (Phylum: Ectoprocta = Bryozoa)

Bryozoans (also known as sea mates or sea mosses) are sessile colonial invertebrates comprising more than 8000 species that inhabit freshwater and marine environments (Sharp et al. 2007). Bryozoans are clearly understudied regarding their composition in bioactive metabolites, comparatively to all other marine invertebrates, although the number of reports describing their possible biotechnological applications is rising (Faulkner 2001; Blunt et al. 2013). Some of the activities reported for bryozoan-derived compounds such as alkaloids, sterols and lactones include antiparasitic, antibacterial, antineoplastic and anti-Alzheimer's (Blunt et al. 2013). To the best of our knowledge, only one study evaluated the anti-leishmania activity of hexane, dichloromethane and methanol extracts made from the marine bryozoan *Bugula neritina* (Bugulidae; Bianco et al. 2013). The active methanol extract was partitioned into 3 fractions, namely ethyl acetate, butanol and water. The hexane extract and the butanol and water fractions were active at the concentration of 50 μg/mL against *L. braziliensis* promastigotes, with inhibition values of 66, 47 and 30.7 %, respectively. The hexane extract was poorly effective against intracellular amastigotes ($IC_{50} > 50$ μg/mL; Bianco et al. 2013). To our knowledge, no antileishmanial compounds were isolated so far from bryozoan species.

Conclusions and perspectives

This review covered the literature from 1998 to 2015, and 45 references are cited. In the last two decades, approximately 70 species of marine invertebrates were evaluated for antileishmanial activity, belonging to nearly 40 families. About 140 compounds were identified and tested in vitro against *Leishmania* parasites. Having in mind that about 10,000 compounds were already described from marine invertebrates, roughly 1.4 % has been prospected for their antileishmanial properties.

The phylum Porifera was unquestionably the most studied for antileishmanial activity. From the 120 compounds tested, about 40 % had IC_{50} values lower or similar to 10 μg/mL or 10 μM. However, based on the cytotoxicity data available, only one met the criteria of a hit, namely plakortide P (**107**), obtained from the sponge *P. angulospiculatus*, which was highly active and selective against intracellular amastigotes of *L. chagasi* ($IC_{50} = 0.5$ μg/mL, SI = 31.6; Kossuga et al. 2008).

Despite the reduced number of compounds tested, the phylum Cnidaria was the most promising, as the majority of molecules had lower IC_{50} values and higher selectivity indexes. In fact, from 9 cnidarian-derived promising compounds, 30 % were considered antileishmanial hits, namely lobocrasol A (**119**: $IC_{50} = 0.18$ μM, SI = 310.8), lobocrasol C (**120**: $IC_{50} = 0.17$ μM, SI = 237.9) and the diterpenoid cristaxenicin A (**121**: $IC_{50} = 0.088$ μM; SI = 20).

All together the phyla Mollusca, Echinodermata and subphyla Crustacea and Tunicata yielded 11 promising molecules; no antileishmanial compounds were described from Bryozoa, Ctenophora, Annelida and Platyhelminthes phyla.

Although a high number of molecules did not meet the hit activity criteria suggested by TDR (2007), there were some compounds with promising low IC_{50} values and/or with high selectivity indexes. Such molecules should not be discarded, since modifications on their structures may increase their activity/selectivity. Thus, the participation of medicinal chemists should be highly encouraged in the drug discovery process, since they can ascertain the chemical and physical properties of the active molecule, establish SAR and unravel novel compound analogues to be retested. It is worth mentioning that during the traditional process of discovery of natural products, supply issues and difficulties on the synthesis of the molecule of interest are frequently encountered, as revised by Martins et al. (2014), restraining future work.

Several constraints are faced from the collection to the isolation of the pure compound, which makes the natural products discovery time-consuming, challenging and laborious. Difficulties to access deep sea or small organisms (that may yield low biomass

quantities), the lack of taxonomic knowledge and scarce information regarding the traditional medicinal uses of marine invertebrates, which have only been roughly described (Perry 2000; Voultsiadou 2010; Alves et al. 2013), have led to strategies characterized by random collection of samples to explore its bio- and chemodiversity (Martins et al. 2014). Without a well-established pre-defined approach based on the identification of organisms that are more likely to synthesize bioactive metabolites, the search for new natural compounds with therapeutic potential in general, and to be used as antileishmanial agents in particular, can be exhausting and frustrating. Many researchers use only one type of solvent for extraction (Martínez et al. 2001) whilst others use solvents with different polarities (Bianco et al. 2013). The use of different polarity solvents is recommended when the nature of the target bioactive molecule is not known, since compounds belonging to diverse chemical classes were active on *Leishmania* parasites. Moreover, as crude extracts are a mixture of compounds, synergistic or antagonistic effects may occur resulting in an over- or underestimated antileishmanial activity, respectively. Thus, this strategy may lead to false negatives, i.e., missing potential hits because the compound was present in low quantities.

Although differences in drug susceptibility of intracellular amastigotes and axenic promastigote and amastigote forms are described (Callahan et al. 1997), axenic forms are frequently used, since they are an easier and more affordable model for primary drug screening (Tempone et al. 2011). However, some of the extracts and compounds that are active against axenic promastigote and amastigote forms are not always further studied against the clinical relevant stage of the parasites, i.e. the intracellular amastigotes. This can be problematic since compounds can be extremely active towards axenic forms, but lack the capacity to pass the host cell barrier and/or to inactivate its defense machinery. In this sense, the use of a suitable in vitro model is of extreme importance for an adequate screening. Along with the use of different parasite in vitro models, other factors prevent a proper comparison of results.

Different methodologies are employed for the evaluation of the anti-promastigote/amastigote activity, and the most popular are the Alamar Blue or resazurin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and gene reporter technologies i.e. GFP- or luciferase-transfected parasites. Generally researchers evaluate the effects of samples on intracellular amastigotes through cell counting after Giemsa staining, but some apply flow cytometry for this purpose. However, another issue arises, which is the period of incubation of the samples being tested with the parasites that differs significantly between studies (from 18 to 120 h). Additionally, in general no information is given regarding the toxicity of the samples towards mammalian cell lines.

In summary, the possibility of finding bioactive compounds from marine invertebrate organisms that may lead to novel antileishmanial drugs is increasing as more species, extracts and pure molecules are being screened. Undoubtedly, marine invertebrates have a high potential as sources of novel bioactive molecules to be used in the treatment of leishmaniasis. However, the standardization of methodological parameters used would allow a better comparison among results of different research groups, thus contributing for the dereplication of results, erroneous interpretations and consequently, would lead to a more efficient search for antileishmanial hits and leads.

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