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Antiparasitic Activity of Methanol Extracts and Isolated Fractions from Caribbean Sponges

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ABSTRACT

Methanol extracts from eight Caribbean sponges (class Demospongiae, *phylum* Porifera) were evaluated against selected parasites. Sponges studied were *Ircinia strobilina, Ircinia felix, Ircinia campana, Xestospongia proxima, Xestospongia muta, Agelas conifera, Agelas clathrodes* and *Niphates erecta*. Parasites used were *Trypanosoma brucei, Trypanosoma cruzi, Leishmania infantum, Leishmania panamensis, Plasmodium falciparum* Itg2 and *Plasmodium falciparum* Ghana. The most active extracts were the obtained from sponges *I. campana, I. felix* and *X. proxima*. Chromatographic fractionations of methanol extracts from sponges *I. campana* and *I. felix* led to isolation of several fractions from which the most active ones against *Leishmania panamensis* amastigotes contain mixtures of at least 20 5α,8α-epidioxysterols, according to ^{1}H-NMR and HPLC analysis. The most active fraction against *Plasmodium falciparum* Itg2 was a fraction of *I. felix* whose NMR spectral analysis shows no evidence for the presence of 5α, 8α-epidioxysterols and sesterterpene tetronic acids.

KEYWORDS: *Marine sponges, Antiparasitic substances, Ircinia, Epidioxysterols*

RESUMEN

Se evaluaron contra varios parásitos seleccionados, los extractos metanólicos de ocho esponjas Caribeñas (Clase Demospongia, Filo Porifera). Las esponjas estudiadas fueron *Ircinia strobilina, Ircinia felix, Ircinia campana, Xestospongia proxima, Xestospongia muta, Agelas conifera, Agelas clathrodes* y *Niphates erecta*. Los parásitos utilizados como bioindicadores fueron *Trypanosoma brucei,*

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***Trypanosoma cruzi, Leishmania infantum, Leishmania panamensis, Plasmodium falciparum* Itg2 y *Plasmodium falciparum* Ghana. Los extractos más activos fueron los obtenidos de las esponjas *Ircinia felix, Ircinia campana y Xestospongia proxima.* Los fraccionamientos cromatográficos de los extractos metanólicos de las esponjas *Ircinia felix* e *Ircinia campana*, permitieron aislar varias fracciones, de las cuales las que mostraron mayor actividad contra amastigotes de *Leishmania panamensis*, contienen mezclas complejas de 5α,8α-epidioxiesteroles, de acuerdo con los análisis por RMN-^{1}H y HPLC. Por otro lado, la fracción más activa contra *Plasmodium falciparum* Itg2 fue una fracción obtenida del extracto de *I. felix*, la cual no mostró evidencia de la presencia de 5α,8α-epidioxiesteroles ni de ácidos tetrónicos sesterterpénicos.**

PALABRAS CLAVES: *Esponjas marinas, Sustancias antiparasitarias, Ircinia, Epidioxiesteroles.*

INTRODUCTION

Malaria and Leishmaniasis are two tropical diseases widely distributed in the world. In the case of Malaria, some 300 – 500 million of the world's people are in risk of infection, presenting over 120 million clinical cases annually. It is estimated that between 1,5 and 2,7 million people die because of malaria every year (Casteel, 1997). Similarly, Leishmaniasis affects an estimated 350 million people in the equatorial Asia, Africa and Central and South America. As malaria, Leishmaniasis is found in many of the world's poorest countries, where it is estimated that 1,5 to 2,0 million people are infected each year (Compagnone *et al.,* 1998). Because of the rapidly increasing threat worldwide of malaria epidemics resistant to alkaloid drugs such as chloroquine, there is an urgent global need to isolate new compounds from natural sources and/or synthesize new classes of antiparasitic compounds (Compagnone *et al.,* 1998). For Leishmaniasis, the most common drugs for the treatment (Pentostam® and Glucantime®) contain pentavalent antimonials that have cardiotoxic effects at the recommended doses. The urgent need for alternative treatments has led to programs to screen natural products for their potential use in the malaria or Leishmaniasis therapy. In this direction, there are two natural sources: terrestrial plants and marine organisms. In the first case, researchers are encouraged to isolate natural substances from "medicinal plants" based on previous ethnobotanical and ethnopharmacological studies. In the later case, the recent development of the chemistry from marine natural products led to demonstrate how the sea is a very promising source of new drugs, and some new bioactive substances have been isolated from organisms as sponges, coelenterates, algaes, microorganisms, etc. However, there are few literature reports about the antimalarial and antileishmanial activity of marine organisms. In 1996, Wright *et al.,* reviewed and outlined an approach to the isolation of potential antimalarial agents and leads[3]. These authors isolated 15 different diterpenes from the sponge *Cymbastela hooperi*, being the compound number 1 (Figure 1) the most active (IC_{50} 4.7 and 4.3 ng/ml against *Plasmodium falciparum* clones D6 and W2, respectively). The authors conclude that natural substances containing –NC, -NCS and –NCO funcional groups must be judiciously subjected to bioassays to search new antimalarial lead compounds not only active against the parasite but also with a good selectivity index. Recently, two indol compounds from the sponge *Hyrtios cf. erecta,* the homofascaplysin (2, Figure 1), (IC_{50} 14 and 24 ng/ml against *Plasmodium falciparum* K1 and NF54 strains, respectively), and the fascaplysin (3, figure 1) (IC_{50}= 50 and 34 ng/ml, respectively) were isolated (Kirsch *et al.,* 2000). Other class of antimalarial compounds include the synthetic peroxyketals as compound 4 (Figure 1) which was developed on the basis of the known antimalarial drug artesunate (5, Figure 1) (Posner *et al.,* 1976). The cyclic C-O-O-C moiety

(named endoperoxy) is associated with its antimalarial action. This moiety is found in several bioactive marine metabolites, which include compound 6 isolated from the Palauan *sponge Plakortis aff. angulospicatus*. This compound has antileishmanial activity (LD_{50} 0.29 μg/mL) (Compagnone *et al.,* 1998).

In this paper we describe the results of preliminary antiparasitic screening for extracts from the Caribbean sponges *Agelas conifera*, *Agelas clathrodes*, *Ircinia campana*, *Ircinia felix*, *Ircinia strobilina*, *Xestospongia muta*, *Xestospongia proxima* and *Niphates erecta*. The preliminary chemical characterization of the leishmanicidal fractions indicate that *I. felix* and *I. campana* contain a mixture of epidioxy steroids, a class of natural compounds until now unknown as antiparasitic. The bioactive fraction of *I. felix* also contains the mixture of known sesterterpene tetronic acids.

MATERIALS AND METHODS

Animal Material

The marine sponges were collected at a depth of 10 – 20 m in Punta Betín on the Colombian Caribbean Coast. The sponges were identified as *Ircinia strobilina, Ircinia felix, Ircinia campana, Xestonpongia proxima, Xestonpongia muta, Agelas conifera, Agelas clathrodes,* and *Niphates erecta* by Dr. S. Zea at the Instituto de Investigaciones Marinas-INVEMAR, Punta Betín, Santa Marta-Colombia. One specimen of each sponge was deposited in the reference collection of INVEMAR.

Extraction and Isolation of fractions

All sponges were frozen, cut and freeze-dried. Dried samples were milled and extracted with methanol. The methanol extracts were then evaporated under reduced pressure and evaluated for their activity against *Leishmania (V) panamensis* parasites. The most active extracts were then subjected to bioassay guided isolation. Briefly, one specimen of *Ircinia campana* was freeze-dried and weighed (63.986 g). This material was extracted with methanol and evaporated under reduced pressure until obtaining a residue of 11.0169 g. This residue was then extracted with ethyl acetate. This new extract was again evaporated under reduced pressure until obtaining a residue of 0.498 g. Under UV light (254 nm), the thin layer chromatography analysis of this residue showed four regions of absorption which were extracted by preparative column chromatography (silica gel, n-hexane-ethyl acetate 2:1) and named *Ic1, Ic2, Ic3* and *Ic4*. The *Ircinia felix* specimen was treated by the same manner. However, five regions were observed in the absorption spectrum. These fractions were named *If1, If2, If3, If4* and *If5*. All nine fractions were tested against their cytotoxic and antileishmanial activities. The pharmaceutical formulation of the Antimony of Meglumine was used (Glucantime®, Specia, Rhone-Poulenc Rorer, France).

Preliminary antiparasitic bioassay for crude extracts

Methanol extracts were tested against different parasites, including *Trypanosoma brucei, Trypanosoma cruzi, Leishmania infantum,* and *Plasmodium falciparum* (Ghana) by by R. Pink and K. Zbinden at the World Health Organization (WHO-TDR/DDR). Cytotoxicity was evaluated on the fibroblastic MRC-5 and L6 cell lines. Chloroquine, Nifurtimox, PX-6518 and Suramin were used as standard drugs against *P. falciparum*, *T. cruzi*, *L. infantum* and *T. brucei*, respectively.

Cytotoxic activity

Cytotoxicity of Caribbean sponges was evaluated on the human promonocytic U-937 cell line (Sundstrom and Nilsson, 1976). To estimate 50% lethal doses (LD_{50}), the 3-(4,5-dimethyithiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) enzymatic micromethod was used (Sereno and Lemesre, 1996). Briefly, the U-937 cells were cultured in suspension in com-

plete RPMI 1640 medium (Gibco BRL, Grand Island, New York) containing 10% heat-inactivated fetal calf serum (FCS) in the presence of 5% CO_2 at 37°C. Medium was renewed at 2-day intervals. Cells were harvested and washed by centrifuging for 10 min at 400 g, then counted and adjusted to a final concentration of 1 x 10^6 cell/ml. 100 µl were seeded in 96-well-flat-bottom microplates (Falcon, Becton Dickinson, Franklin Lakes, New Jersey). One hundred µl of corresponding extract or fraction were added at a concentration ranged from 3 to 100 µg/ml. Cells were then incubated at 37°C with 5% CO_2. After 48 h of incubation medium was changed and cells were incubated again in presence of the same concentrations of extract or fraction. After 96 h of incubation, 10 µl of MTT (10 mg/ml) was added to each well. Plates were further incubated for 3 h. The enzymatic reaction was then stopped by addition of 100 µl of 50% isopropanol – 10% sodium dodecyl sulfate solution. The plates were incubated for an additional 30 min under agitation at room temperature. The optical density at 570 nm using an ELISA plate reader (Bio-Rad Laboratories, Hercules, California) measured production of formazan. Cells cultivated in absence of treatment but maintained under the same conditions were used as control. Three independent experiments in triplicate were performed for the determination of toxicity of each extract or fraction. Results were expressed as LD_{50} calculated by Probit (Finney, 1978).

In vitro assay for antileishmanial activity

Effect of extracts and isolated fractions was evaluated on intracellular amastigotes as described (Robledo *et al.,* 1999). Antileishmanial activity was measured on intracellular amastigotes from *Leishmania (Viannia) panamensis* (M/HOM//87/UA140 strain), a strain isolated at The Programa de Estudio y Control de Enfermedades Tropicales – PECET, Universidad de Antioquia) from one patient having localized cutaneous Leishmaniasis. The sample was criopreserved in liquid nitrogen until use. To maintain the virulence of parasite and therefore, to obtain a good *in vitro* infection, parasites were maintained by passage in golden hamsters (*Messocrycetus aureatus*) (Rey *et al.,* 1990). Periodically, lesions were aspirated and the material obtained was cultivated in NNN medium (Novy, Nicolle and Mc Neal) until stationary phase growth of promastigotes.

After 48 h of growth, the U-937 cells were washed twice with Dulbecco's phosphate buffer saline (DPBS) (Gibco BRL). One hundred thousand cells were added to each well of a 24-well plate (Falcon) containing sterile round 12-mm glass coverslip (Fisher Scientific, Pittsburg, Pensnsylvania) and were exposed to stationary phase growth promastigotes at a ratio of 25:1 parasite:cell. Infected cell cultures were incubated for 2 h with 5% CO_2 at 34°C. Free parasites were removed by washing twice with warm DPBS. After 24 h of incubation with 5% CO_2 at 34°C in RPMI 1640 medium containing 10% FCS, the medium was replaced with complete RPMI 1640 medium containing the corresponding concentration of extract or fraction. The range of concentration varied between 0.1 and 10 µg/ml, depending of the LD_{50} for each extract or fraction. Thereafter, the medium was renewed every 2 days. After 96 h of incubation in the presence of extract or fraction, cells were washed, fixed with methanol (Fisher) for 20 min and stained with Giemsa (Sigma-Aldrich Chemical Co, St Louis, MO). Similarly, infected cells cultured in absence of extract or fraction served as control of infection. Three independent experiments in triplicate were performed for the determination of leishmanicidal activity of each extract or fraction. All assays were evaluated blindly. For each test 200 cells/well were examined at random; the number of infected and uninfected cells were recorded. Percentage of infection was calculated by dividing the number of infected cells obtained in presence of each extract or

fraction by the number of infected cells obtained in absence of treatment. Results were expressed as 50% Effective Dose (ED_{50}) which was calculated by Probit analysis (Finney, 1978).

In vitro assay for antimalarial activity

The procedure for *in vitro* antimalarial evaluation of the extracts and fractions was carried out according to Rieckman *et al,* 1978, Cruz-Mancipe and Fuenmayor 1989. Briefly, *Plasmodium falciparum* (Itg2 strain) were maintained in continuous culture as described by Trager and Jensen, 1976, 1978. Parasites were maintained in RPMI 1640 medium containing Hepes, hypoxanthine and gentamicine complemented with human serum to obtain 10% of pool of recalcified plasma ABO, 5% sodium bicarbonate and reduced glutation (pH 7.2-7.4) and 5% hematocrite of O+ human erythrocytes. Parasites were incubated at 37°C in a dissecator under a gas atmosphere of 7% of CO_2, 5% of O_2 and balanced nitrogen. Every four days it was made a dilution with fresh red globules O+ and 5% hematocrite.

In each experiment, two microplates were used to evaluate the extract/fraction and the negative control (culture of erythrocytes with PVP-10 and infected with *P. falciparum* (Itg2 strain) and one microplate was used to evaluate the chloroquine (positive control) and the negative control. Ten doses (double dilutions) of each extract/fraction or chloroquine were evaluated in each microplate by duplicate. The response of chloroquine was evaluated for concentrations under 16 ppm using ten double dilutions. The parasitemia percentage was measured in every well as percentage of parasited erythrocytes after 24 hours of treatment.

Chemical characterization of isolated fractions

All nine isolated fractions were analyzed by ^{1}H-NMR spectrometry (Bruker AMX300 spectrometer, 300 MHz, all samples dissolved in $CDCl_3$). Fraction Ic2 was also analyzed by Reverse Phase-High Performance Liquid Chromatography (Shimadzu LC-6A Liquid chromatograph, Shiseido Capcell-pak C-18 column 250 x 4.6 mm d.i., a mixture of acetonitrile-methanol 2:1 was used as isocratic mobile phase, 0.5 ml/min, Shimadzu SPD-6A UV-Visible detector, 215 nm, room temperature).

RESULTS

Preliminary antiparasitic bioassays for crude extracts

The activity of the methanol extracts from the Caribbean marine sponges against different parasites is shown in Table 1. The methanol extract from *Ircinia campana* was active against *T. brucei, T. cruzi* and *P. falciparum* (Ghana strain). It is very interesting that its activity is obtained at concentrations lower than toxic concentration for MRC-5 cells. Activity of *Ircinia felix* against *T. brucei* was low. *Xestospongia proxima* is active against *T. cruzi*, but its toxicity is two times higher. All the other extracts were inactive to concentrations below 32 μg/ml.

Antileishmanial activity of methanol extracts from sponges

The activity of the methanol extract from Caribbean sponges against *Leishmania (V) panamensis* is shown in Table 2. Results agree clearly that the most active antiparasitic extracts are those obtained from *I. felix*, *I. campana* and *X. proxima* sponges.

Antileishmanial activity of fractions isolated from I. campana and I. felix

Bioactivity against intracellular amastigotes of *Leishmania (V) panamensis* for the isolated fractions is shown in Table 3. Both toxicity and activity was lower in the isolated fractions than methanol extracts.

Chemical characterization of bioactive fraction Ic2

Fraction *Ic2* isolated from the methanol extract from *I. campana* (Rf: 0.21, n-hexane-ethyl acetate 2:1) was analyzed by RP-HPLC and ^{1}H-NMR. The HPLC analysis showed that it is a complex mixture of at least 20 compounds, because 20 peaks were seen.

^{1}H-NMR spectrum shows 5 different regions, such as: a methyl region between 0.5 and 1 ppm; a very complex region between 1.0 and 2.2 ppm. The regions 1 and 2 are characteristic of steroid compounds. The third region corresponds to a multiplet near to 4.0 ppm; the fourth region corresponds to a complex mixture of signals between 5.0 and 5.8 ppm. The last region corresponds to two sharp doublets centered in δ 6.25 (1H, J=8.6 hz) and 6.52 (1H, J=8.7 hz) which are characteristic of a 5α,8α-epidioxysterols (Figure 1, Structure 7, R is an alkyl chain) (Sera *et al.,* 1999). These results let us establish that bioactive fraction *Ic2* is a complex mixture of at least 20 5α,8α-epidioxysterols.

Chemical characterization of the bioactive fraction If

The *If3* fraction isolated from the methanol extract from *I. felix* (Rf: 0.21, n-hexane-ethyl acetate 2:1) was analyzed by ^{1}H-NMR. When compared with *Ic2* fraction, the ^{1}H-NMR spectrum of *If3* fraction shows similar complex signal regions; however, the characteristic signals for 5α,8α-epidioxysterols were seen again: one region that is a multiplet near to 4.0 ppm and other region that corresponds to two sharp doublets centered in δ 6.25 (1H, J=8.6 hz) and 6.52 (1H, J=8.7 hz). Moreover, several signals characteristic for sesterterpene tetronic acids were also seen: δ 6.27 (bs, 1H), 7.20 (bs, 1H) and 7.33 (bs, 1H) (Martinez *et al.,* 1997). These results made evident that *If3* fraction contains a mixture of 5α,8α-epidioxysterols and sesterterpene tetronic acids. Related sesterterpene tetronic acids were evaluated against *Plasmodium* but they resulted inactive (Höller et al., 1997).

Antileishmanial activity of Ic2 *and* If3 *fractions*

Comparison of bioactivity against amastigotes of *L. (V) panamensis* for the *Ic2* and *If3* fractions (Table 3) shows clearly that *Ic2* fraction is 8 times more active and 4.8 times more selective than *If3* fraction. This result suggests that the antileishmanial activity in these sponges is associated with 5α,8α-epidioxysterols rather than sesterterpene tetronic acids because of the *Ic2* fraction contains only these compounds whereas the *If3* fraction contains a mixture of both classes of compounds.

Antimalarial activity of isolated fractions from I. felix and I. campana

Comparison of the *in vitro* bioactivity of isolated fractions from *I. campana* and *I. felix* against *Plasmodium falciparum* (Table 3) indicates that *If4* fraction is the most active and with the higher selectivity index; however, when compared with more antileishmanial active fractions *Ic2* and *If3*, by ^{1}H-NMR spectrometry, there are no evidences for presence of 5α,8α-epidioxysterols or sesterterpene tetronic acids in fraction *If4*.

DISCUSSION

In this paper we describe the preliminary antiparasitic screening for extracts from the Caribbean sponges *Agelas conifera*, *Agelas clathrodes*, *Ircinia campana*, *Ircinia felix*, *Ircinia strobilina*, *Xestospongia muta*, *Xestospongia proxima* and *Niphates erecta*. Preliminary chemical characterization of the leishmanicidal fractions from *I. campana* and *I. felix* and the results of *in vitro* bioassays against *Leishmania panamensis* amastigotes and *Plasmodium falciparum* are also included.

The most active antiparasitic extracts obtained from *I. felix*, *I. campana* and *X. proxima* sponges and the lower cytotoxicity of extracts from *I. felix* and *I. campana* prompted us to study

the chemical components of the methanol extracts from these sponges. Both toxicity and activity was lower in the isolated fractions than methanol extracts suggesting that evaluation of cytotoxicity and antileishmanial activity of pure fractions, in which some toxic components have been eliminated, is a necessary process in order to define their potential as therapeutic alternatives.

Ic2 fraction was more active and more selective than *If3* fraction. This result suggests that the antileishmanial activity in these sponges is associated with 5α,8α-epidioxysterols rather than sesterterpene tetronic acids because of the *Ic2* fraction contains only these compounds whereas the *If3* fraction contains a mixture of both classes of compounds. These results and the previous results for antileishmanial cyclic peroxides from the Palauan sponge *Plakortis aff. Angulospiculatus* (Compagnone *et al.,* 1998), suggest that cyclic peroxide moiety maybe a chemical clue for antileishmanial activity of this class of compounds. A literature survey shows that epidioxysterols have been little studied about its biological activity. However, there are several reports about antifouling (Sera *et al.,* 1999), sulfatase inhibition, anticomplementary, antitumor and antiviral activities (Kim *et al.,* 2000), but this is the first time antiparasitic activity is reported for this class of compounds.

Comparison of the *in vitro* bioactivity of isolated fractions from *I. campana* and *I. felix* against *Plasmodium falciparum* indicates that *If4* fraction is the most active and more selective; however, when compared with more antileishmanial active fractions *Ic2* and *If3* by ^{1}H-NMR spectrometry, there are no evidences for presence of 5α,8α-epidioxysterols or sesterterpene tetronic acids in fraction *If4*. These results suggest in a preliminary pointview, that the sponge *I. felix* contains distinct fractions with antileishmanial and antimalarial activity.

We concluded that *I. campana* and *I. felix* sponges contain antileishmanial and antimalarial fractions with a complex mixture of 5α, 8α-epidioxysterols. Actually, we are trying to establish the relationship between cyclic peroxide moiety and antiparasitic activity of compounds from *I. campana*, which according to results here published contains active substances against *Leishmania. panamensis* amastigotes, *P. falciparum* (Ghana and Itg2 strains), *T. brucei* and *T. cruzi*. Also we are trying to establish the chemical stability, and a possible artifact origin from 5,7-diunsaturated sterols which are reported for this sponge genus.

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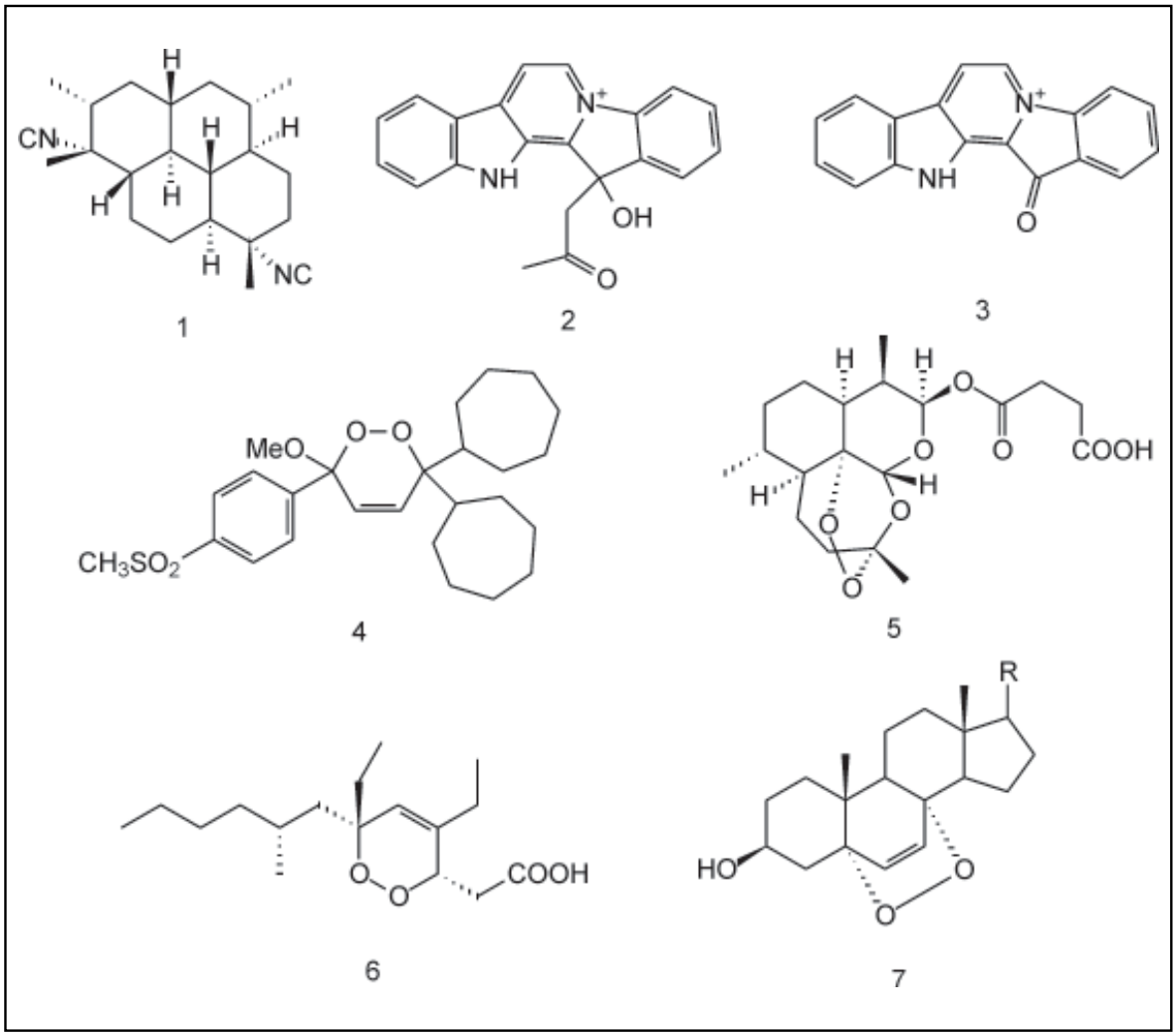
Figure 1.


H
CN
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1
N+
NH
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N+
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MeO
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CH3SO2
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COOH
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5
COOH
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7

Table 1. Citotoxicity and antiparasitic activity of methanol extracts from marine sponges

Extract	LD_{50} (µmg/ml)	ED_{50} (µmg/ml)				SI [a]			
		Tb	Tc	Li	Pf	Tb	Tc	Li	Pf
Ircinia strobilina	*>32*	*>32*	*>32*	*>32*	*>32*	*>1*	*>1*	*>1*	*>1*
Niphates erecta	*>32*	*>32*	*>32*	*>32*	*>32*	*>1*	*>1*	*>1*	*>1*
Agelas clathrodes	*>32*	*>32*	*>32*	*>32*	*>32*	*>1*	*>1*	*>1*	*>1*
Ircinia felix	*27*	*16*	*>32*	*>32*	*>32*	*1.7*	*0.84*	*0.84*	*0.84*
Ircinia campana	*>32*	*4*	*7*	*>32*	*20*	*8.0*	*4.57*	*>1*	*1.6*
Xestospongia proxima	*2*	*>32*	*4*	*>2*	*>32*	*0.06*	*0.5*	*ND*	*0.06*
Xestospongia muta	*>32*	*>32*	*>32*	*>32*	*>32*	*>1*	*>1*	*>1*	*>1*
Agelas clathrodes	*>32*	*>32*	*>32*	*>32*	*>32*	*>1*	*>1*	*>1*	*>1*
Chloroquine					*0.036*				
Nifurtimox			*0.83*						
PX-6518				*0.01*					
Suramin		*0.59*							

Tb: Trypanosoma brucei, Tc: Trypanosoma cruzi, Li: Leishmania infantum, Pf: Plasmodium falciparum. ND: No Data
a Cytotoxicity was evaluated on fibroblastic cell line MRC-5
b Selectivity Index (SI) = LD_{50}/ ED_{50}

Table 2. Citotoxicity and antileishmanial activity of methanol extracts from Colombian Caribbean marine sponges

Extract	LD_{50} (µg/ml)	ED_{50} (µg/ml)	SI [a]
Ircinia strobilina	*72.7*	*>145.3*	*>0.5*
Niphates erecta	*164.3*	*>328.7*	*>0.5*
Agelas clathrodes 18m	*141.4*	*>282.9*	*>0.5*
Ircinia felix	*35.7*	*> 71.3*	*>0.5*
Ircinia campana	*48.1*	*> 96.3*	*>0.5*
Xestospongia proxima	*27.2*	*> 54.3*	*>0.5*
Xestospongia muta	*120.3*	*>240.5*	*>0.5*
Agelas clathrodes 4.5m	*51.6*	*>103.1*	*>0.5*

a Selectivity Index (SI) = LD_{50}/ ED_{50}

Table 3. Citotoxicity, antileishmanial and antiplasmodial activity of isolated fractions from *Ircinia felix* and *I. campana*

Fraction	*LD_{50} (µg/ml)*	*L.* (V) *panamensis*		*P. falciparum*	
		ED_{50} (µg/ml)	*SI* [a]	*ED_{50} (µg/ml))*	*SI*
[b]Ic1	*27.9*	*3.6*	*7.8*	*21.3*	*1.3*
Ic2	*21.5*	*2.6*	*8.3*	*19.0*	*1.1*
Ic3	*17.8*	*2.6*	*6.8*	*8.2*	*2.2*
Ic4	*10.8*	*3.9*	*2.8*	*ND*[d]	*ND*
[c]If1	*83.0*	*79.0*	*1.1*	*ND*	*ND*
If2	*8.9*	*281.0*	*0.03*	*8.2*	*1.1*
If3	*37.0*	*21.0*	*1.8*	*20.9*	*1.8*
If4	*31.2*	*23.0*	*1.4*	*3.8*	*8.2*
If5	*2.0*	*3.4*	*0.6*	*ND*	*ND*
Glucantime	*399.6*	*6.7*	*59.6*	*NA*	*NA*
Chloroquine	*<0.03*	*NA*	*NA*	*4.0*	*<0.0075*

a Selectivity Index (SI).
b *Ic* : *Ircinia campana.*
c *If* : *Ircinia felix.*
d ND.: No data available. NA: No apply.