APLYSFISTULARINE: A NOVEL DIBROMOTYROSINE DERIVATIVE ISOLATED FROM Aplysina fistularis*

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The new dibromotyrosine derivative 3,5-dibromo-4-[3’dimethylamonium]-N,N,N-trimethylethanamonium, here referred to as aplysfistularine (1), was isolated from the marine sponge Aplysina fistularis along with 2-(3,5-dibromo-4-methoxyphenyl)-N,N,N-trimethylethanamonium (2), aplysterol (3) and 24,28-didehydroaplysterol (4). Their identification was performed by mass spectrometry, infrared, $^1$H and $^{13}$C NMR, and by comparison with literature data. Compound 2 and the mixture of 3 and 4 were tested in vitro (inhibitory activity) with supercoiled DNA relaxation techniques, and showed inhibitory activity on human DNA topoisomerase II-α. Compound 1 was not tested due to paucity of the material.

Keywords: Aplysina fistularis; aplysfistularine; topoisomerase activity.

INTRODUCTION

A review of recent research reveals that the quest for new drugs is changing direction. Given the ever growing number of natural marine products discovered, researchers have recognized the promising potential of the sea for the chemistry of natural products.1,3 Despite the obstacles to effective development of marine organism-derived pharmaceutical agents, the interest in marine organisms as a new drug source has increased in recent years.7,10 Marine sponges are a prolific source of a huge variety of secondary metabolites.11-13 Sponges of the order Verongida, and the family Aplysinaidae, characterized by the absence of terpenes and the production of steroids, produce a wide diversity of bromotyrosine-containing metabolites with interesting biological properties.16 The richest sources of biogenetically, tyrosine-derived bromo-containing amines, are members of the Verongida order, and the genus Aplysina.15-20 Previous and recent reports of Aplysina fistularis have documented the presence of a large number of brominated metabolites including: fistularines, aerothionines, ceratinamines, aplysamines, anamonianes and psammaplysines.21-23 The diversity of biological activity found in compounds isolated from marine sponges is due to the presence of bromotyrosine derivatives. In the case of the order Verongida, many of the species produce compounds with antimicrobial, antibacterial, cytoxic and antitumor activity.23,33

Nuclear enzymes that control and modify the topological states of DNA are known as topoisomerases. In mammalian cells, they are classified into types I and II, according to their mechanisms and physical properties. Topoisomerase II (Topo II), a dimer composed of or isoforms with a total size of 170 KDa, is responsible for separating the double DNA helix, leading to events such as DNA release, transcription, chromosome condensation and recombination.25-28 During cell proliferation, topoisomerases take part in DNA maintenance and replication. When these functions are deactivated, cells become vulnerable. Furthermore, the expression of DNA Topo I and II is higher in tumors than in normal cells.29 Topoisomerase II inhibitors with anticancer and antiviral potential are important targets in the development of new drugs.30 In an attempt to discover new topoisomerase inhibitors, many classes of natural products have been tested and described in the literature, including flavonoids,31 biflavonoids,32 diterpenes,33 triterpenoids,34 estilbenoids,35 alkaloids,36-39 naphthodianthrones,40 naphtoquinones,41 binaphtoquinones,42 polysaturated fatty acids,43 derivatives of the chromone nucleus, and many substances isolated from plants.44 In medicine, compounds from the anthracycline and epipodophytoxin classes stand out as potent topoisomerase II inhibitors. These act by inhibiting DNA rebinding, and inducing the binding of proteins at breaks, constituting part of first line chemotherapy for a large variety of solid and hematological tumors. Etoposide, a semisynthetic derivative of the lignan podophyllotoxin, plays an important role in clinical treatments as a chemotherapeutic agent for a variety of tumors, including carcinomas, testicular cancer and lymphomas.45

According to Rhee et al.46 one of the main structural requirements for Topo II inhibition is the presence of a planar chromophore in aromatic rings. Substances with this kind of chromophore can intercalate with DNA causing blockage or enzymatic reading errors during the replication process. Metabolite 2 has called our attention for presenting planar chromophores in aromatic rings, and this structural feature might confer inhibitory activity for the topoisomerase enzyme.

The work with A. fistularis led to the isolation of four substances: the dibromotyrosine derivatives 3,5-dibromo-4-[3’dimethylamonium] propoxyphenyl]-N,N,N-trimethylethanamonium also known as aplysfistularine (1) and 2-(3,5-dibromo-4-methoxyphenyl)-N,N,N-trimethylethanamonium (2), along with aplysterol (3) and 24,28-didehydroaplysterol (4).
RESULTS AND DISCUSSION

Structural analysis and determination

All the substances were identified by means of their NMR, mass, and infrared spectroscopic data, as well as by comparison with the literature (Figure 1).

In vitro assay for inhibitory activity against human DNA topoisomerase II-α

The presence of the planar chromophore, due to the aromatic ring, confers compound 2 the possibility of interacting with the Topo II-α enzyme. Due to this structural feature, we evaluated the possible action of 2 on the human DNA Topo II-α from DNA plasmid relaxation assays. Compound 1 was not tested for Topoisomerase II-α activity due to the paucity of the material for the experiments.

Figure 2 shows the catalytic activity inhibition for the enzyme DNA topoisomerase II-α, observed in vitro with plasmid DNA (pBR322) relaxation in the presence of ATP and Mg2+. Both the steroid mixture (aplysterol/24,28-didehydroaplysterol), and compound 2 exhibited complete Topo II-α inhibition at 100 µM concentrations, as can be seen on lanes 5 in Figures 2B and 2C, respectively. In Figure 2C, no Topo II-α inhibition is evident at 25, 12 and 1 µM concentrations. This result was compared with etoposide, a well-known inhibitor specific to Topo II-α, which was used as a control (100 µM), and presents a similar profile to that observed for the steroid mixture and compound 2 (Figures 2B and 2C). The minimum concentration for inhibitory activity was determined as 50 µM for the steroid mixture tested (lane 4 of Figure 2B).

Compound 2 and the steroid mixture showed inhibitory activity against human DNA-Topo II-α, and would be a good prototype for future investigations for new anti-tumor agents.

EXPERIMENTAL

Instruments

Infrared (IR) spectra were registered in KBr pellets, on a Bomem model MB 100 spectrophotometer. Mass spectra were obtained on a Q-TOF-Micromass mass spectrometer with analysis by Electrospray Ionization (+) on a hybrid Quadrupole Time of Flight (QTOF) device. Samples were dissolved and diluted in a methanol: H2O (1:1) solution with formic acid at 0.01% to the concentration of 1.0 µg mL-1. The spectra were obtained in positive ion mode. The injection flow was 1.0 mL min-1. One and two-dimensional NMR of 1H and 13C spectra were obtained on a Bruker spectrometer NMR (DRX 500), and Varian System spectrometer NMR (500) operating at 500 MHz (1H).
Aplysfistularine: a novel dibromotyrosine derivative isolated from Aplysina fistularis

Collection, processing and fractionation of Aplysina fistularis

The sponge A. fistularis was collected in the sea canyons of the State of Paraíba, Brazil. The species were registered under numbers 63 and 65, and deposited in the Paulo Yang Marine Invertebrates Collection, at the Department of Systematics and Ecology of the Universidade Federal da Paraíba. As soon as they were collected, the specimens were preserved in ethanol. The crude ethanol extract was equivalent to 16.65% of the dry weight of the sponges. This extract was subjected to a liquid-liquid partition with hexane, dichloromethane and ethyl acetate. The fractions containing the dibromotyrosine derivatives (detected by TLC under UV light 254 nm) were purified by column chromatography over silica gel using a gradient of methanol:dichloromethane or methanol:ethyl acetate. The chromatographic fractionation of the ethanol extract of the sponge A. fistularis yielded the newly isolated 1, the known substance 2 and a mixture of the steroids 3 and 4, at a 1:1 proportion.

Aplysfistularine (1)

Amorphous yellow solid; Solubility: chloroform; C_{26}H_{28}O_{12}Br, Mol. wt.: 532.08 u.m.a; IR (KBr) ν_{max} 3426, 3003, 2976, 2938, 2862, 2816, 2335 1300-1100, 1259, 1440-1600 cm\(^{-1}\); \(^{1}\)H and \(^{13}\)C NMR data, Table 1; HRESIMS: m/z 425.0204; 426.0095. 2-(3,5-Dibromo-4-methoxyphenyl)-N,N,N-trimethylethanammonium (2)

Amorphous yellow solid; Solubility: methanol; C_{26}H_{28}Br,O, Mol. wt.: 532.08 u.m.a; IR (KBr) ν_{max} 3426, 3003, 2976, 2938, 2862, 2816, 2335, 1440-1600; 1300-1100, 1259 (cm\(^{-1}\)); \(^{1}\)H and \(^{13}\)C NMR data, Table 1; HRESIMS: m/z 349.9877; m/z 351.9876 (molecular

Table 1. NMR data of \(^{1}\)H (500 MHz) and \(^{13}\)C (125 MHz) for compounds 1 and 2 (δ in ppm)

<table>
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<tr>
<th></th>
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<th>2 (Measured in CD_{2}OD)</th>
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<td>HMBC</td>
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<tr>
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<tr>
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<td>3’</td>
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<td>-</td>
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<tr>
<td>MeO</td>
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<td>-</td>
<td>61.35</td>
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</table>

Figure 2. Inhibitory activity of Human DNA Topoisomerase II-α by chemical constituents of the marine sponge Aplysina fistularis. (A) 0.125 μg/mL of DNA supercoiled plasmid pBR322 electrophoresed in 1% agarose gel alone (lane 1A); 0.125 μg/mL of Human DNA with 1.0 unit of Topo II-α enzyme (lane 2A), negative control or treated with both 0.125 μg/mL of Human DNA, 1.0 unit of Topo II-α enzyme and its inhibitor 100 μM Etoposide (lane 3A) as positive control. Plasmid incubated with enzyme and several concentrations of steroids mixture (Aplysterol and 24-28-didehydroaplysterol) (B) or Compound 2 (C), from A. fistularis. Concentrations from 1 to 100 μM for each inhibitor candidate. With exception of lane 1A, all lanes contain 0.125 μg/mL of the plasmid DNA pBR322 and 1.0 unit of TopoII-α enzyme and at 125 MHz (\(^{13}\)C). Deuterated solvents from Cambridge Isotope Laboratories were used (CIL) (CDCl\(_3\), CD_{2}OD).

Table 1. NMR data of \(^{1}\)H (500 MHz) and \(^{13}\)C (125 MHz) for compounds 1 and 2 (δ in ppm)
inhibitory activity on the human topoisomerase II-α DNA enzyme.

SUPPLEMENTARY MATERIAL

1H and 13C NMR spectra, COSY, HMQC, HMBC, NOESY spectra, and HRESIMS spectra of compounds 1 and 2 as well as the HSQC-TOCSY spectra of compound 1 are available at http://quimicanova.sbq.org.br, in PDF file, with free access.

ACKNOWLEDGEMENTS

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Figure 1S. HRESIMS spectrum of compound 1

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The approximate Relative intensities of the ions (with a difference of two mass units), containing two bromine atoms (isotopes 79 and 81), are marked in **bold**.

**Figure 2S.** NMR proposed fragmentation for the molecule of compound 1

**Figure 3S.** NMR $^{13}$C-APT spectrum of compound 1 (CD$_2$OD, 125 MHz)
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**Figure 4S.** $^1$H NMR spectrum data of compound 1 (CD$_3$OD, 500 MHz)

**Figure 5S.** Expansion of $^1$H NMR spectrum at the region of 4.1 – 2.9 of compound 1 (CD$_3$OD, 500 MHz)
Figure 6S. $^1$H x $^1$H-COSY correlation spectrum of compound 1 (CD$_3$OD, 500 MHz)

Figure 7S. Expansion of $^1$H x $^1$H-COSY correlation spectrum of compound 1 (CD$_3$OD, 500 MHz)
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**Figure 8S.** $^1$H $^1$C-HMQC correlation spectrum of compound I (CD$_3$OD, 500 and 125 MHz respectively)

**Figure 9S.** Expansion of $^1$H $^1$C-HMQC correlation spectrum of compound I (CD$_3$OD, 500 and 125 MHz respectively)
Figure 10S. $^1$H x $^{13}$C-HMBC correlation spectrum of compound 1 (CD$_2$OD, 500 and 125 MHz respectively)

Figure 11S. Expansion of $^1$H x $^{13}$C-HMBC correlation spectrum of compound 1 (CD$_2$OD, 500 and 125 MHz respectively)
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**Figure 12S.** $^1$H x $^1$H-NOESY spatial correlation spectrum of compound 1 (CD$_3$OD, 500 MHz)

**Figure 13S.** $^1$H x $^{13}$C-HSQC-TOCSY spatial correlation spectrum of compound 1 (CD$_3$OD, 500 and 125 MHz respectively)
Figure 14S. Expansion of $^1$H x $^{13}$C-HSQC-TOCSY spatial correlation spectrum of compound 1 (CD$_3$OD, 500 and 125 MHz, respectively)

Figure 15S. HRESIMS spectrum of compound 2
Figure 16S. Proposed fragmentation for the molecule of compound 2

Figure 17S. NMR $^{13}$C-APT spectrum of compound 2 (CD$_3$OD, 125 MHz)
Figure 18S. $^1H$ NMR spectrum data of compound 2 (CD$_2$OD, 500 MHz)

Figure 19S. Expansion of $^1H$ NMR spectrum at the region of 3.0 – 3.6 of compound 2 (CD$_2$OD, 500 MHz)
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**Figure 20S.** $^1$H × $^1$H-COSY correlation spectrum of compound 2 (CD$_3$OD, 500 MHz)

**Figure 21S.** $^1$H × $^{13}$C-HMQC correlation spectrum of compound 2 (CD$_3$OD, 500 and 125 MHz respectively)
Figure 22S. $^1$H x $^{13}$C-HMBC correlation spectrum of compound 2 (CD$_2$OD, 500 and 125 MHz respectively)

Figure 23S. $^1$H x $^1$H-NOESY spatial correlation spectrum of compound 2 (CD$_2$OD, 500 MHz)