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# BROMOTYROSINE DERIVATIVES FROM MARINE SPONGES INHIBIT THE HIV-1 REPLICATION *IN VITRO*

## BROMOTIROSINAS DERIVADAS DE ESPONJAS MARINAS INHIBEN LA REPLICACIÓN *IN VITRO* DEL VIH-1

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### ABSTRACT

**Background:** Human immunodeficiency virus type 1 (HIV-1) infection and Acquired immunodeficiency syndrome are mayor global public health issues. HIV-1 infection is now manageable as a chronic disease thanks to the development of antiretroviral therapy; however, the existence of HIV drug resistance and collateral effects have increased the search for therapeutic alternatives. Compounds of marine resources have been studied for their antiviral potential. **Objectives:** To evaluate the antiviral activity of isolated bromotyrosine-derivative compounds from the Colombian marine sponges, *Verongula rigida* and *Aiolochoira crassa* against HIV-1 infection *in vitro*. **Methods:** Cytotoxicity of 11 bromotyrosine-derivative compounds was determined by the MTT assay. Inhibition of HIV-1 replication was performed using the U373-MAGI cell line, which was infected with recombinant green fluorescent protein (GFP)-expressing viruses pseudotyped, in the presence or absence of the compounds. The percentage of infected cells was evaluated by flow cytometry. In addition, the inhibition of reverse transcription and nuclear import was determined by quantification of early and late reverse transcription products and 2-LTR circles, respectively, using quantitative PCR. **Results:** Aeroplysinin-1, purealidin B and 3-bromo-5-hydroxy-O-methyltyrosine inhibited the HIV-1 replication in a dose-dependent manner, with a median maximum percentage of inhibition of 74% (20  $\mu$ M), 57% (80  $\mu$ M) and 47% (80  $\mu$ M), respectively. Importantly, none of these concentrations were cytotoxic. Aeroplysinin-1, 19-deoxyfistularin 3, purealidin B, fistularin 3 and 3-bromo-5-hydroxy-O-methyltyrosine inhibited the nuclear import efficiently; while 3,5-dibromo-N,N,N,O-tetramethyltyraminium, aeroplysinin-1, purealidin B, fistularin 3 and 3-bromo-5-hydroxy-O-methyltyrosine inhibited X4 HIV-1 cell entry with a median maximum percentage of inhibition ranging between 2 to 30%. **Conclusions:** Aeroplysinin-1, 19-deoxyfistularin 3, purealidin B, fistularin 3 and 3-bromo-5-hydroxy-O-methyltyrosine inhibited HIV replication at different steps. This study opens the possibility of chemically synthesizing these compounds and evaluating them as alternative therapies against HIV-1.

**Keywords:** HIV-1, marine resources, antiviral activity, bromotyrosine, marine sponge.

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## RESUMEN

**Antecedentes:** La infección por el virus de la inmunodeficiencia humana tipo 1 (VIH-1) y el síndrome de inmunodeficiencia adquirida son los principales problemas de salud pública mundial. En la actualidad, la infección por el VIH-1 se maneja como una enfermedad crónica gracias al desarrollo de la terapia antiretroviral; sin embargo, la aparición de cepas virales resistentes y el efecto colateral de los medicamentos, han incrementado la búsqueda de alternativas terapéuticas. Colombia cuenta con una gran biodiversidad de recursos marinos, la cual es materia de estudio en búsqueda de compuestos antivirales. **Objetivo:** Evaluar la actividad antiviral de los compuestos, derivados de la bromotirosina, aislados de las esponjas marinas Colombianas, *Verongula rigida* y *Aiolochoira crassa* contra la infección por el VIH-1 *in vitro*. **Métodos:** La citotoxicidad de 11 compuestos derivados de la bromotirosina se determinó por medio del ensayo MTT. La actividad anti-VIH (AAV) se determinó en la línea celular U373-MG infectada con virus recombinantes que expresaban la proteína verde fluorescente en presencia/ausencia de los compuestos. El porcentaje de células infectadas se determinó mediante citometría de flujo. Adicionalmente, se evaluó la inhibición de la transcripción reversa (TR) e importe nuclear del ADN viral mediante la cuantificación por PCR en tiempo real de los transcritos tempranos y tardíos de la TR y los círculos 2-LTR virales. **Resultados:** La evaluación de la AAV demostró que Aeroplysinina-1, purealidina B y 3-bromo-5-hidroxy-O-methyltyrosina inhiben la replicación del VIH-1 de manera dosis dependiente, con una inhibición máxima del 74% (20  $\mu$ M), 57% (80  $\mu$ M) y 47% (80  $\mu$ M), respectivamente, sin citotoxicidad importante. Aeroplysinina-1, 19-deoxyfistularina 3, purealidina B, fistularina 3 y 3-bromo-5-hidroxy-O-methyltyrosina inhibieron el importe nuclear eficientemente; mientras que los compuestos 3,5-dibromo-N,N,N,O-tetramethyltyraminium, aeroplysinina-1, purealidina B, fistularina 3 y 3-bromo-5-hidroxy-O-methyltyrosina inhibieron la entrada de cepas X4 del VIH-1 con un rango de inhibición del 2 al 30%. **Conclusiones:** Los compuestos aeroplysinina-1, 19-deoxyfistularina 3, purealidina B, fistularina 3 and 3-bromo-5-hidroxy-O-methyltyrosina inhibieron la replicación del VIH en diferentes pasos del ciclo. Este estudio abre la posibilidad de realizar la síntesis química de estos compuestos y su posterior evaluación como terapia alternativa contra el VIH-1.

**Palabras clave:** VIH-1, recursos marinos, actividad antiviral, bromotirosina, esponja marina.

## INTRODUCTION

Since its appearance, the pandemic of human immunodeficiency virus type 1 (HIV-1) has become a major public health problem in the world. Indeed, since the beginning of the epidemic, over 60 million individuals have been infected with HIV-1 and 25 million people have died from related causes. Recent epidemiological data indicated that in 2011, 34 million persons were living with HIV-1 in the world and approximately 2.5 million new infections and 1.7 million persons died of diseases linked to the Acquired immunodeficiency syndrome (AIDS) (1).

During the past 30 years the scientific community has focused its efforts on the fight against HIV-1 infection; to date, over 24 antiretroviral drugs inhibit or block HIV-1 replication, enhancing the quality and life expectancy of infected individuals. However, the nature of the virus and its high mutation rate favors the generation of drug-resistant virus (2). In addition, there are unwanted side

effects such as hypersensitivity reactions to some antiretrovirals (3), reducing the options that can be used to inhibit viral replication efficiently. Collateral effects of drugs also include changes in the profile of antioxidant enzymes and increased oxidative stress caused by reactive oxygen species, that can adversely affect the immune response (4), favoring the development of opportunistic infections and malignancies (5).

Marine sponges have been the source of several compounds with anti HIV-1 activity, promoting the constant search for molecules with such biological activity from this marine resource. Among these products are papuamides A, B, C and D (6); haplosamates A and B (7); crambescidin 826 (8); homophymine A (9); dehydrofurodendin (10); neamphamide A (11); petrosins (12); koshikamides F and H (13); celebeside A and theopapuamides (14); mirabamides E, F, G and H (15), and baculiferins (16).

Recently, Galeano *et al.* reported isolation of bromotyrosine-derivative compounds from marine

sponges of the *Aplysinidae* family, found in the marine ecosystem of the Gulf of Uraba (Colombia), specifically of the *Verongula rigida* and *Aiolochoiria crassa* species. Bromotyrosines are brominated type amino acid chemical compounds, found in various tissues of animals and plants, that have previously been shown to possess cytotoxic (17), antimicrobial (18), anti-angiogenic (19) and anti-parasitic (20, 21) activities, among others. Earlier studies described bioactive compounds such as mololipids derived from bromotyrosines exhibiting HIV-1 activity (22). Further studies reported *in vitro* anti-HIV activity of mololipids with EC<sub>50</sub> to 52.2 mM, exhibiting no cytotoxicity in human lymphocytes (IC<sub>50</sub> > 100 µM) (23). These studies underlined the importance of exploring these natural products as possible sources of bioactive compounds in view of controlling HIV-1 infection.

This work evaluated the *in vitro* anti-HIV-1 activity of eleven bromotyrosine-derivative compounds isolated previously from marine sponges of Colombia. Viruses with different tropisms were used in a cellular model with a single round replication; additionally, the phase of the HIV-1 replication cycle in which these compounds exerted their antiviral action was explored.

## MATERIALS AND METHODS

### Cells, vectors and reagents

U373-MAGI cells were obtained through the AIDS Research and Reference Reagent Program, NIAID, NIH, from M. Emerman and A. Gaballe (24, 25). 293T cells were purchased from the American Tissue Culture Collection (ATCC). Cell lines were maintained in Dulbecco modified Eagle medium (DMEM, Invitrogen), supplemented with 10% fetal bovine serum (FBS) at 37°C with 5% CO<sub>2</sub>. The plasmids pVSV-G expressing the G protein of Vesicular stomatitis virus (VSV) and the pNL4-3 delta env GFP (HIV-GFP), encoding the full-length NL4-3 HIV-1 proviral DNA with a frameshift in the *env* gene and expressing GFP instead of *nef*, were kindly provided by Johnny He (26).

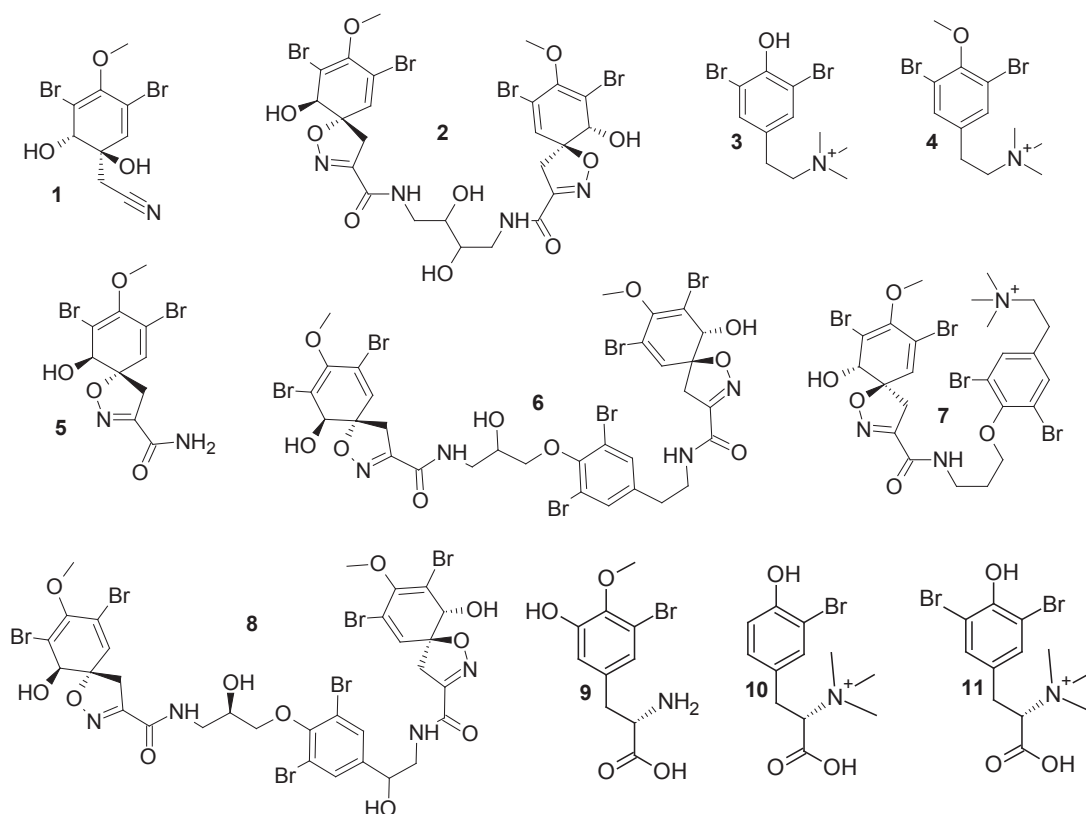
### Virus production

Pseudotyped HIV-1 with VSV-G envelope were prepared using the following procedure: 293T cells were seeded at 4x10<sup>5</sup> cells/well in 6-well plates; 24 h later the cells were cotransfected with 1.6 µg of HIV-GFP reporter plasmid and 0.4 µg of pVSV-G expression plasmid per well, using the calcium phosphate precipitation method, as previously reported (27). Cell culture supernatants were collected 48 h after changing the transfection medium and centrifuged at 700 x g for 4 min, followed by filtration (0.22 µm pore) and precipitation with PEG 8000 (Sigma-Aldrich, St Louis, MO, USA) for 48 h. Finally, the PEG fraction was centrifuged at 13000 x g for 30 min at 4°C, and the viral pellet was suspended in DMEM and stored as virus stock at -70°C.

### Tested compounds

Tested compounds were: Aeroplysinin-1 [compound 1], dihydroxyaerotherionin [2], 3,5-dibromo-*N,N,N*-trimethyltyraminium [3], 3,5-dibromo-*N,N,N,O*-tetramethyltyraminium [4], purealidin R [5], 19-deoxyfistularin 3 [6], purealidin B [7], fistularin-3 [8], 3-bromo-5-hydroxy-*O*-methyltyrosine [9], 3-bromo-*N,N,N*-trimethyltyrosinium [10] and 3,5-dibromo-*N,N,N*-trimethyltyrosinium [11]. Compounds 1-8 and 9-11 were isolated from marine sponge *Verongula rigida* and *Aiolochoiria crassa*, respectively (20, 21). The chemical structure is shown in Figure 1. These compounds were donated by A. Martinez and E. Galeano from the Marine Natural Products Research Group, University of Antioquia, Medellín, Colombia.

Aeroplysinin-1 [compound 1], dihydroxyaerotherionin [2], 3,5-dibromo-*N,N,N*-trimethyltyraminium [3], 3,5-dibromo-*N,N,N,O*-tetramethyltyraminium [4], purealidin R [5], 19-deoxyfistularin 3 [6], purealidin B [7], fistularin-3 [8], 3-bromo-5-hydroxy-*O*-methyltyrosine [9], 3-bromo-*N,N,N*-trimethyltyrosinium [10] and 3,5-dibromo-*N,N,N*-trimethyltyrosinium [11].



**Figure 1.** Chemical structures of bromotyrosine-derivative compounds isolated from marine sponges.

### Cytotoxicity assay

To determine the cytotoxicity of each compound, the cell line U373-MAGI was used. After 24 h of culture, double dilutions of each bromotyrosine, and production medium (vehicle control) were added in triplicate in a final volume of 200  $\mu$ L. Forty-eight hours after treatment, the cytotoxic effect was evaluated by the MTT assay. This assay, determined at what concentration each treatment is capable of killing 50% of the cells ( $CC_{50}$ ). This concentration plus two dilutions below that value were used to evaluate the antiviral effect. The cytotoxicity percentage was calculated as follows:  $((OD_{cm} - OD_x) / OD_{cm}) \times 100$ , in which  $OD_{cm}$  is the mean optical density of the control, and  $OD_x$  is the optical density obtained for each concentration of each treatment.

### Inhibition of viral replication

U373-MAGI cells were plated at a density of  $10^4$  cells/well, in a 96-well plate and allowed to grow for at least 24 h. They were treated or not for 1 h with the bromotyrosine dilutions, at three concentrations chosen according to the MTT assay; AZT

(zidovudine) at  $3.7 \mu$ M was used as a positive control. Cells were then infected with 100 ng of gag p24 HIV-GFP-VSV-G reporter virus in the presence of 8  $\mu$ g/ml polybrene. After 3 h, the virus was removed, the cells were extensively washed, and then fresh medium with or without the bromotyrosine dilutions, was added. Percentage of inhibition was calculated as follows:  $100 - (\text{infection percentage in treated cells} \times 100 / \text{percentage in control infection})$ .

### Inhibition of reverse transcription

Inhibition of reverse transcription was evaluated by detecting and quantifying early and late products by qPCR. Briefly, cells were cultured, treated and infected as above. After 3 h, the virus was removed, the cells were extensively washed and fresh medium devoid of, or containing the bromotyrosines was added. Forty-eight hours later, DNA was extracted using cell lysis solution and protein precipitation solution (Qiagen, GentraPuregen), following the manufacturer's protocol. Each 20  $\mu$ L of qPCR mixture consisted of 2  $\mu$ L of DNA, 1X of Maxima SYBR Green/qPCR Master Mix (Fermentas), and primers (0.4  $\mu$ M each). Primer sequences for early and late reverse transcripts were

previously reported (28). GAPDH DNA was used to normalize the DNA content in each preparation. The primer sequences for GAPDH were: Fw: 5'ACCATTGAGAACTCCAGGATTGTC3', Rv: 5'CTCATGCGCAGAGCCTGTT3'. The cycling profile was: 95°C for 10 min followed by 45 cycles of 95°C for 10 sec and 63°C for 60 sec. A melting curve to confirm the specificity of the PCR products was included. All qPCR amplifications and data acquisitions were performed using the CFX96 real-time system (Bio-Rad, Hercules, CA), and the software CFX Manager Version: 1.5.534.0511 (Bio-Rad). Relative expression was calculated by the  $\Delta C_t$  method (29). The results are given as average relative expression units of triplicate assays.

### Statistical analysis

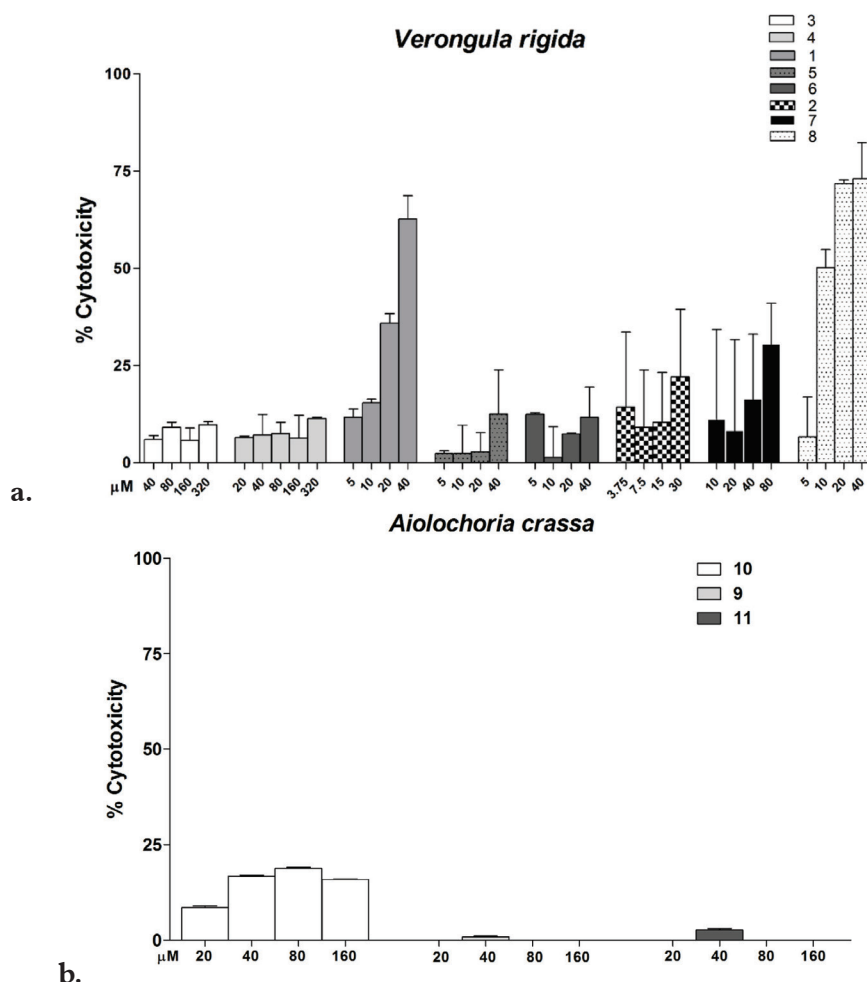
All statistical analyses were carried out with GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). Statistical differences between AZT and each concentration of bromo-

tyrosine-derivative compounds, and between concentrations within each treatment were assessed by the Mann-Whitney U test. All tests were two-sided, and a  $p < 0.05$  was considered statistically significant.

## RESULTS

### Cytotoxicity of the bromotyrosine-derivative compounds

Cytotoxicity of the compounds was determined by the MTT assay, using the cell line U373-MAGI. Figure 2 shows that as the compound concentration increased, there was an increase in the cytotoxicity in a dose-dependent manner. Only the compounds 3,5-dibromo-N,N,N,O-tetramethyltyraminium [compound 4] (40  $\mu$ M) and fistularin 3 [8] (20 - 40  $\mu$ M) showed a cytotoxicity above 50%; other concentrations of different compounds exhibited a cytotoxicity below 45% (Figure 2). For the following experiments,  $CC_{50}$  and either two or three lower concentrations were used.



**Figure 2.** Cytotoxic effect of the bromotyrosine-derivative compounds.

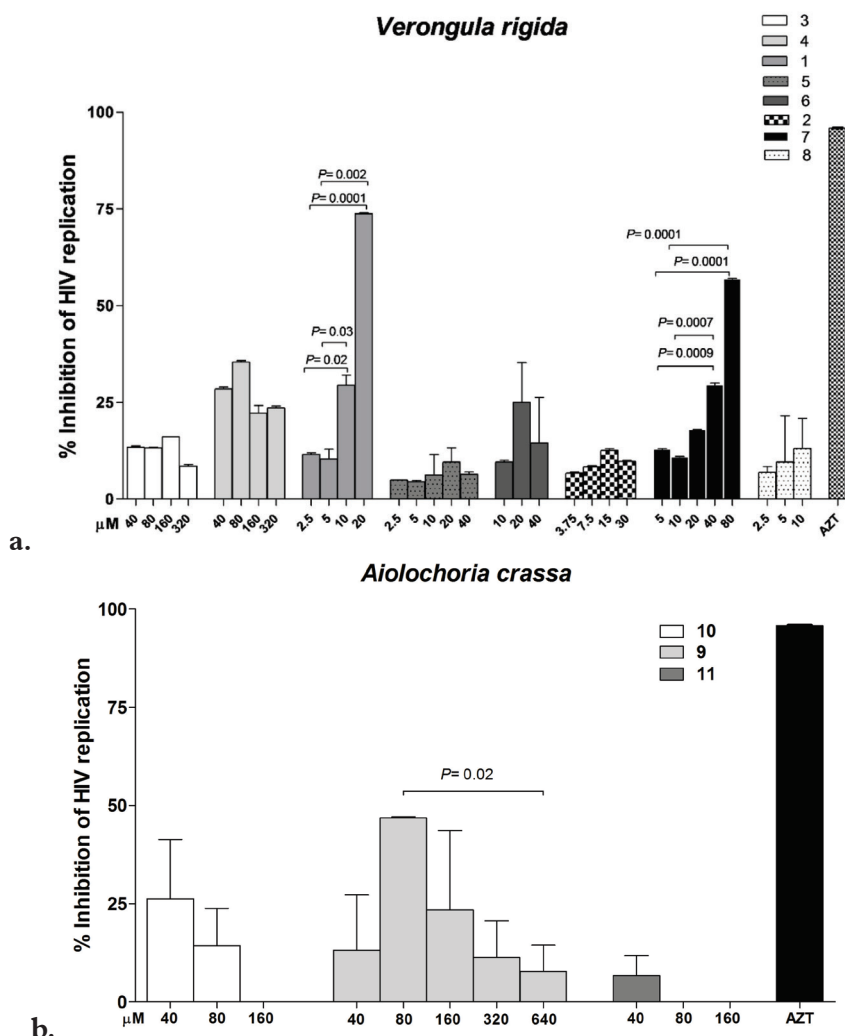


The U373-MAGI cell line was treated with increasing concentration of bromotyrosine-derivative compounds from *V. rigida* (a) and *A. crassa* (b). AZT was used as control. Forty-eight hours post-treatment, the cytotoxic effect was determined by the MTT assay. The figures represent the median of three independent experiments performed in triplicate. Aeroplysinin-1 [compound 1], dihydroxyaerotherionin [2], 3,5-dibromo-*N,N,N*-trimethyltyraminium [3], 3,5-dibromo-*N,N,N,O*-tetramethyltyraminium [4], purealidin R [5], 19-deoxyfistularin 3 [6], purealidin B [7], fistularin-3 [8], 3-bromo-5-hydroxy-*O*-methyltyrosine [9], 3-bromo-*N,N,N*-trimethyltyrosinium [10] and 3,5-dibromo-*N,N,N*-trimethyltyrosinium [11].

### Bromotyrosine-derivative compounds inhibit HIV-1 replication

The percentage of inhibition of HIV-1 replication obtained by flow cytometry is shown in the

Figure 3. Most concentrations of the bromotyrosine-derivative compounds inhibited over 20% of virus replication. The positive control of inhibition, AZT, inhibited 97.7% of viral replication. Aeroplysinin-1 [compound 1] and purealidin B [7] inhibited HIV-1 replication in a dose-dependent manner, with statistically significant differences among the medians of the percentages of inhibition (Figure 3a). A median maximum percentage of inhibition of 74% was also observed for aeroplysinin-1 [1] at 20  $\mu$ M and 57% for purealidin B [7] at 80  $\mu$ M, respectively (Figure. 3a). No statistically significant differences were found between the inhibition obtained at such maximum concentrations and the inhibition by AZT ( $p < 0.05$ ). Likewise, the median maximum percentage of 47% inhibition was obtained with 80  $\mu$ M of 3-bromo-5-hydroxy-*O*-methyltyrosine [compound 9] (Figure 3b). Importantly, none of these concentrations exhibited a significant cytotoxic effect.



**Figure 3.** HIV-1 replication is inhibited by bromotyrosine-derivative compounds.

The U373-MAGI cell line was infected with HIV-1-GFP, and treated with or without the compounds of *V. rigida* (a) and *A. crassa* (b). AZT was used as control. Forty-eight hours post-treatment, the percentage of infected cells was determined by flow cytometry for GFP. The values from the X axis are given as  $\mu\text{M}$ . The graphics represent the median of three independent experiments performed in triplicate. Aeroplysinin-1 [compound 1], dihydroxyaerotherionin [2], 3,5-dibromo-*N,N,N*-trimethyltyraminium [3], 3,5-dibromo-*N,N,N,O*-tetramethyltyraminium [4], purealidin R [5], 19-deoxyfistularin 3 [6], purealidin B [7], fistularin-3 [8], 3-bromo-5-hydroxy-*O*-methyltyrosine [9], 3-bromo-*N,N,N*-trimethyltyrosinium [10] and 3,5-dibromo-*N,N,N*-trimethyltyrosinium [11].

### Reverse transcriptase and nuclear import is altered by bromotyrosine-derivative compounds

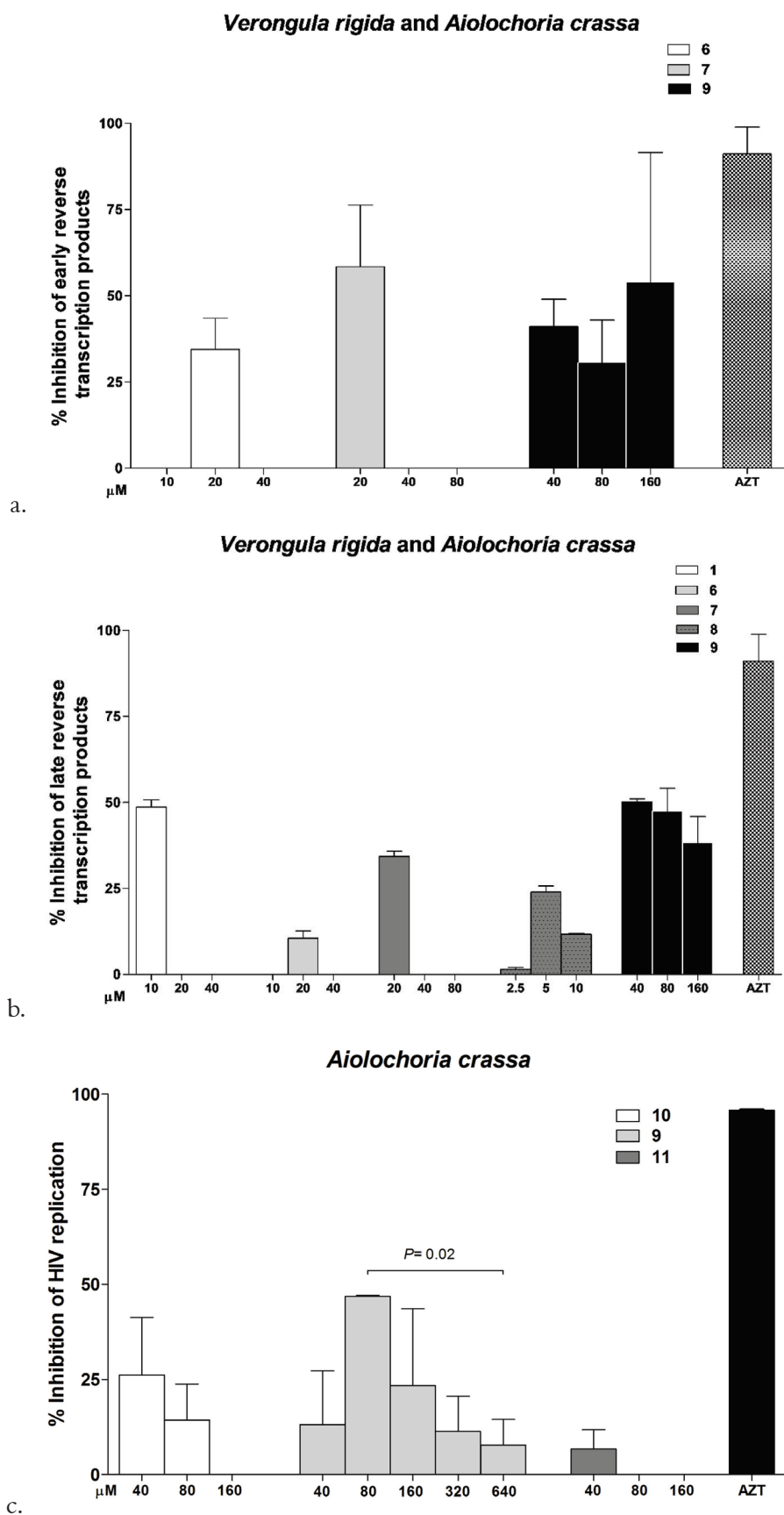
Previous studies have shown an inhibitory effect on reverse transcription (RT) by different compounds extracted from marine sponges (32). The inhibitory effect of the compounds on the levels of early and late transcripts was evaluated, in order to associate their effect on the process of reverse transcription of HIV-1. In addition, the level of inhibition of nuclear import was determined by quantifying the amount of 2-LTR circle transcripts produced. Relative units of early, late and 2-LTR transcripts were evaluated by qPCR and based on these results, the percentage of inhibition of reverse transcription and nuclear import were calculated and are graphed in Figure 4. 19-deoxyfistularin 3 [compound 6], purealidin B [7] and 3-bromo-5-hydroxy-*O*-methyltyrosine [9] inhibited the syn-

thesis of early transcripts with a median maximum percentage of inhibition of 35% (20  $\mu\text{M}$ ), 58% (20  $\mu\text{M}$ ) and 54% (160  $\mu\text{M}$ ), respectively (Figure 4a). In addition, aeroplysinin-1 [compound 1], 19-deoxyfistularin 3 [6], purealidin B [7], fistularin 3 [8] and 3-bromo-5-hydroxy-*O*-methyltyrosine [9] inhibited late transcript production with a median maximum percentage of inhibition of 48% (10  $\mu\text{M}$ ), 11% (20  $\mu\text{M}$ ), 34% (20  $\mu\text{M}$ ), 24% (5  $\mu\text{M}$ ) and 50% (40  $\mu\text{M}$ ), respectively (Figure 4b). However, statistically significant differences between the inhibition obtained by AZT and each one of these compounds were found ( $p < 0.05$ ); indicating that this inhibition is not comparable to AZT inhibition.

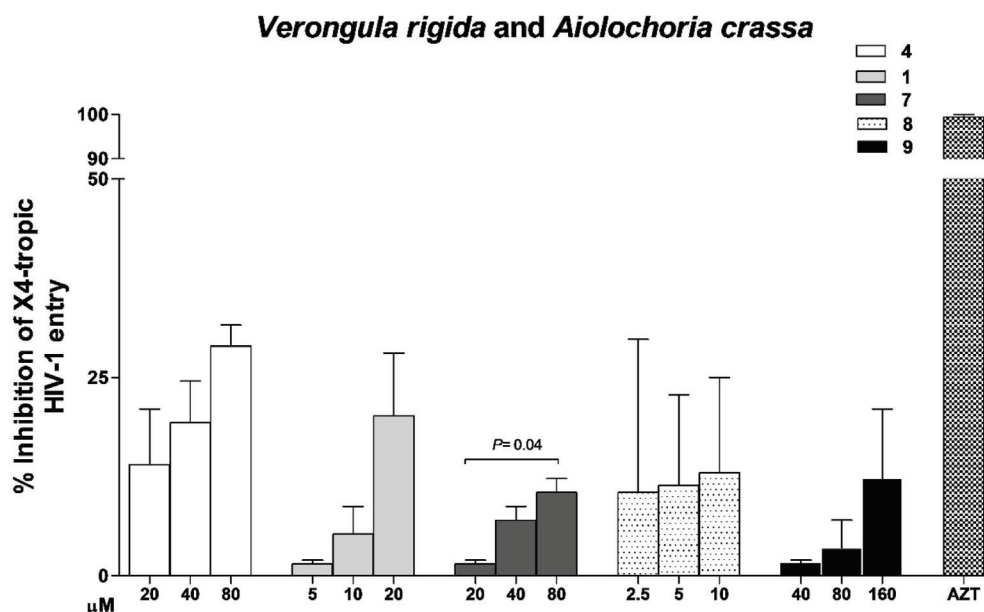
Additionally, aeroplysinin-1 [compound 1], 19-deoxyfistularin 3 [6], purealidin B [7], fistularin 3 [8] and 3-bromo-5-hydroxy-*O*-methyltyrosine [9] efficiently inhibited nuclear import (Figure 4c). Aeroplysinin-1 inhibited nuclear import with median maximum percentage of 67% at 10  $\mu\text{M}$ ; 19-deoxyfistularin 3 did so with 62% at 20  $\mu\text{M}$ ; purealidin B with 66% at 20  $\mu\text{M}$ ; fistularin 3 with 47% at 10  $\mu\text{M}$  and 3-bromo-5-hydroxy-*O*-methyltyrosine [9] with 73% at 80  $\mu\text{M}$  (Figure 4c). Again, no statistically significant differences between the inhibition obtained at such maximum concentrations and the inhibition by AZT were not found ( $p < 0.05$ ).

The DNA of U373-MAGI cells, infected with HIV-1-GFP and treated or not with the compounds, was isolated 48 hours post-treatment. AZT was used as control. The DNA was analyzed by qPCR for early (a), late (b) and 2-LTR circle (c) transcripts. The values from the X axis are given as  $\mu\text{M}$ . The graphics represent the median of three independent experiments performed in triplicate.





**Figure 4.** Reverse transcription and nuclear import are inhibited by bromotyrosine-derivative compounds.



**Figure 5.** Inhibition of HIV-1 entry.

### HIV entry is blocked by bromotyrosine-derivative compounds

As seen in Figure 5, aeroplysinin-1 [compound 1], 3,5-dibromo-*N,N,N,O*-tetramethyltyraminium [4], purealidin B [7], fistularin-3 [8] and 3-bromo-5-hydroxy-*O*-methyltyrosine [9] inhibited the X4-tropic HIV-1 entry, in a dose dependent manner, with a median maximum percentage of inhibition ranging between 14 to 30% for compound 3,5-dibromo-*N,N,N,O*-tetramethyltyraminium, 2 to 20% for aeroplysinin-1, 2 to 11% for purealidin B, 11 to 13% for fistularin 3 and 2 to 12% for 3-bromo-5-hydroxy-*O*-methyltyrosine (Figure 5). The compounds did not show any inhibitory effect against R5-tropic HIV-1 (data not shown).

Inhibition of HIV-1 entry was evaluated in an *in vitro* single-round, recombinant-based viral infectivity assay. U373. MAGI cells were treated or not with the compounds, and the cells were then infected with HIV-GFP plus X4 envelope to evaluate the entry inhibition by flow cytometry. AZT was used as a positive control of HIV-1 replication inhibition. Three experiments were performed in triplicate. The results are shown as percentage of viral entry inhibition. The values from the X axis are given as μM.

### DISCUSSION

Bromotyrosine-derivatives have been shown to be strong bioactive compounds, but their studies as new potential compounds against HIV-1 have only been scarcely evaluated. In particular, mololipids (bromotyrosine-derived lipids) isolated from a Hawaiian sponge of the order *Verongida*, with similar chemical structure to these bromotyrosine-derived compounds, were found to be active against HIV-1 *in vitro* (23).

Using an MTT assay and determining the CC<sub>50</sub> of each compound we ruled out a cytotoxic effect as potential mechanism to explain viral inhibition.

Bromotyrosine-derivative compounds (Figure 1) exhibited a variable ability to inhibit HIV-1 replication *in vitro*; in particular aeroplysinin 1 [compound 1] and purealidin B [7] compounds from *V. rigida* inhibited HIV-1 replication in a dose-dependent manner by more than 50%; in fact, this level of inhibition, although lower, was statistically similar to the inhibition caused by AZT. The dose-dependent effect may indicate that the compounds exert a maximum inhibition at certain concentrations; therefore, the effect of saturation in cell cultures can be counterproductive for the evaluation of the biological effect.

Aeroplisinin 1 has been previously reported to have antiangiogenic (19) and antibacterial activity (18), purealidin B shows antibacterial and antiparasitic activity (20); however, there are not reports regarding their antiviral activities. In addition, 3-bromo-5-hydroxy-*O*-methyltyrosine [compound 9] showed anti-HIV-1 activity up to 47% of inhibition of virus replication *in vitro*, this activity had not been previously reported for this compound.

Similar compounds, such as bromotyrosine-derived lipids called mololipids, isolated of *Psammoplysilla purpureas* from Hawaii, have shown activity against HIV-1 and Herpes simplex virus type 2 without cytotoxicity against human peripheral blood mononuclear cells; however, the mechanism of action has not been explored (22, 23). Additionally, sulfated polysaccharide isolated of the marine sponge *Erylus discophorus* showed potent HIV-1 inhibitory activity, mainly at the step of viral entry, preventing HIV adsorption and fusion with the lymphocytic cell (37). Likewise, other sulfated compounds derived from the *Iotrochota baculifera* and *Clathria* species have shown anti-HIV-1 activity and reverse transcription inhibition, respectively (16, 35). Finally, the following compounds have shown anti-HIV-1 effect during replication, entry and reverse transcription: avarol isolated from *Disidea avara*; papuamides isolated from *Theonella mirabilis* and *Theonella swinhoei*; microspinosamide, from the sponge *Sidonops microspinosus*; dragmacidin F from a marine sponge of the genus *Halicortex* and Manzamine A isolated from *Halidona* sp. These findings support all the potential anti-HIV-1 activity of compounds isolated from marine sponges found in this work.

Previous reports have shown that different compounds from marine sponges inhibit the RT of the virus (30–32). In the qPCR experiments, the bromotyrosine-derivative compounds decreased the early and late transcripts with a range of median maximum percentage of inhibition from 11% to 58%. Since the early and late transcripts result from an efficient reverse transcription process, these results suggest that the compounds have an effect on the RT process.

Several compounds derived from marine sponges inhibit the reverse transcriptase by blocking the RNA-dependent DNA polymerase activity and the RNase H activity (33, 34). This effect can also be explained because the bromotyrosine-

derived compounds show structural similarities with the reverse transcriptase inhibitors, whereas AZT is a nucleoside reverse transcriptase inhibitor, bromotyrosine-derived compounds are similar to non-nucleoside reverse transcriptase inhibitors. This conclusion is based on the following structural characteristics: i) halogenated substitutions on the benzene rings in efavirenz (chlorine and fluorine) and etravirine (bromine); ii) rings of five elements including nitrogen and oxygen and iii) higher amount of nitrogen in all structures.

Interestingly, bromotyrosine-derivative compounds inhibited nuclear import efficiently with a range of median maximum percentage from 47% to 73%. This effect has not been reported previously; therefore, the mechanism causing this inhibition is still not clear. It is also plausible that the inhibition of the RT described above could also be influencing the inhibition of nuclear import.

Additionally, aeroplisinin-1 [compound 1], 3,5-dibromo-*N,N,N,O*-tetramethyltyraminium [4], purealidin B [7], fistularin-3 [8] and 3-bromo-5-hydroxy-*O*-methyltyrosine [9] demonstrated anti-HIV-1 activity by blocking entry of X4 viruses; it is noted that this activity was significant, peaking at 30% for 3,5-dibromo-*N,N,N,O*-tetramethyltyraminium [compound 4]. Previously, it was shown that adociavirin (35) and crambescidin 826 (8) inhibited HIV-1 entry; binding the cellular receptor CD4 and the viral envelope glycoprotein gp120 has been proposed as a mechanism of action for Adociavirin (35). A similar mechanism might explain the action of the bromotyrosines.

These results indicate that bromotyrosine-derivative compounds obtained from marine sponges, particularly, 3,5-dibromo-*N,N,N,O*-tetramethyltyraminium [compound 4], aeroplisinin-1 [1], 19-deoxyfistularin 3 [6], purealidin B [7] and fistularin 3 [8] of *V. rigida* and 3-bromo-5-hydroxy-*O*-methyltyrosine [compound 9] of *A. crassa* inhibit HIV replication by acting at the steps of entry, reverse transcription and nuclear import of retroviral DNA. This is the first report indicating that these eleven bromotyrosine-derivatives act as HIV-1 inhibitors *in vitro*. Although with the limited number of compounds tested in this work there is no possibility to do conclusive structure-activity relationships, it is interesting to note the common presence of methoxyl group with two vicinal bromine atoms in most active compounds like compounds

1, 4, 6, 7, 8 and 9. This chemical characteristic is absent in compounds like 3, 10 and 11.

These results underline the importance of further research, particularly in unraveling the mechanisms of action of these compounds. It is also important to evaluate other steps of the virus cycle such as viral integration, protein translation and assembly, since structural similarities were also observed with molecules such as integrase and protease inhibitors (36). Given that the synthetic production of these compounds is under process in Colombia, it will be imperative continuing this line of research in order to develop new therapeutic strategies against HIV-1 infection.

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