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ORIGINAL RESEARCH

Design, synthesis and effect of triazole derivatives against some toxic activities of *Bothrops jararaca* venom

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Abstract

According to the World Health Organization, snakebite envenoming is a neglected disease that affects around 5.4 million people worldwide each year. In Brazil, in 2019 there were 29,000 cases of accidents, with 104 deaths. The genus *Bothrops* was responsible for 90% of reported envenomations, mainly the species *B. jararaca*. The current therapy is performed with an intravenous injection of antivenom, be it monovalent or polyvalent. However, this treatment has high manufacturing costs, may induce side effects, and it does not effectively neutralize tissue necrosis. The latter issue may lead to deformity or amputation of the affected limb. Therefore, new treatments are needed to aid or improve the efficacy of antivenoms. In this study, nine triazole compounds (AM11–AM19) were chemically synthesized, characterized using infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy analyses, and tested against some in vitro (hemolysis, coagulation, and proteolysis) and in vivo (hemorrhaging, lethal, and edema) activities of *B. jararaca* venom. Each compound was incubated with *B. jararaca* venom (incubation protocol) or injected after the venom (treatment protocol), and then, biological assays were performed. As a result, all the compounds inhibited the toxic activities of *B. jararaca* venom with different potencies in the incubation protocol, while the compound AM13 inhibited hemorrhaging in the treatment protocol. In addition, the compounds were devoid of toxicity, as shown through admetSAR analysis or in vitro cytotoxicity test. Thus, these compounds may be an important tool for the development of antivenom molecules to improve serum therapy for recovering patients envenomed by *B. jararaca* venom.

Keywords Synthesis · Triazole compounds · Antivenom · Snake · *Bothrops jararaca* venom · Neutralization

Introduction

Snakebite envenoming is a serious public health problem worldwide, but with the highest incidence in the Americas. According to the World Health Organization (WHO), snakebite envenoming is a neglected disease, affecting 5.4 million people, with 100,000 deaths each year [1]. However, the exact number of cases is unknown [2]. Besides deaths, 300,000 amputations and other permanent disabilities occur due to snakebites. Thus, more efficient treatments at impairing local effects of the venom should be considered.

In Brazil, most cases of envenoming are caused by the *Bothrops* genus (87%), followed by *Crotalus* (8%) and *Lachesis* (2%) [3]. Within the *Bothrops* genus, the species *B. jararaca* is responsible for the majority of accidents, around 54%, and has a lethality index of 0.7% [4, 5]. *B. jararaca* is a highly venomous pit viper, located in southern Brazil, reaching a maximum length of 160 centimeters, with typical envenomation symptoms including local hemorrhaging, inflammation, pain, massive tissue necrosis, bleeding, renal and cardiac failure, and death [6, 7]. Besides death, morbidities can occur as well.

Intravenous injection of antivenom is the official treatment used to block toxic effects of venoms. In general, antivenoms have been produced by hiperimmunization of

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horses, yielding polyvalent or monovalent products, which are efficient to protect patients from death. However, antivenoms are unsatisfactory in blocking local effects, like tissue necrosis, and this may lead to amputation or disabilities of the affected limb [8, 9]. Antivenoms may induce fever or anaphylactic reactions and their production and manufacturing have high costs [10]. Thus, seeking for alternative therapies that efficiently inhibit local effects and have lower costs deserve more efforts. Natural products derived from plants [11, 12] or seaweed [13] have been tested, and some of them showed promising results. However, molecules derived from organic synthesis have not been deeply investigated as antivenoms. Triazole compounds belong to a five-membered ring family with two carbons and three nitrogen atoms, and are widely studied as a group of interest for developing active molecules against diseases [14], including some reported by our group: thrombosis [15, 16] or antivenom for the snakes *Lachesis muta* or *B. jararaca* [17, 18]. Moreover, many biological effects have been described elsewhere, like anti-malaria [19], antifungal [20], anticancer [21], antiviral [22], and antibacterial activity [23], and some of them have become medicinal drugs, like fungicides [24].

As part of our experiment and strategy to evaluate triazole derivatives with antivenom potential, in this study, we synthesized a novel family of 1,2,3-triazoles able to inhibit coagulant, hemolytic, proteolytic, hemorrhagic, and edematogenic activities of *B. jararaca* venom and we also evaluated their predicted toxicity using the tool admetSAR.

Materials and methods

Venom, antivenom, animals, and reagents

Lyophilized *B. jararaca* venom or antibothropic polyvalent serum (antivenom) was provided by Fundação Ezequiel Dias, Belo Horizonte, MG, Brazil, and stored at −20 °C until the time for its use in assays. Snake venom collection was conducted under the authorization of the Brazilian National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN) (Process number A39CD4E). Swiss mice (18–20 g) were obtained from the Animal Care Laboratory of the Federal Fluminense University (UFF), and housed under constant light and temperature (24 ± 1 °C) conditions. All in vivo experiments were approved by the UFF Institutional Committee for Ethics in Animal Experimentation (protocol number 847), which is in accordance with the guidelines from the Brazilian Committee for Animal Experimentation (COBEA). Dimethylsulfoxide (DMSO) and azocasein were purchased by Sigma-Aldrich (San Louis, Missouri, USA), and all the organic solvents or reagents are of the best grade available.

Chemistry and synthetic compounds

Reagents specific for compound synthesis were used as purchased, from Sigma-Aldrich or Bio-Grade, without further purification. Column chromatography was performed with F60 silica gel (Silicycle 40–65 μm). Analytical thin-layer chromatography (TLC) was performed with silica gel plates (Silicycle, TLC silica gel 60F-254), and the plots were visualized under ultraviolet light or developed by immersion in an ethanolic solution of vanillin. Yields refer to chromatographically and spectroscopically homogeneous materials. Melting points (mp) were obtained on a Fisatom apparatus (430 D model). Infrared spectral data were recorded from KBr pellets on a Thermo Scientific model Nicolet 6700-FTIR spectrophotometer calibrated relative to the 1601.8 cm^{-1} absorbance of polystyrene. Elemental analysis was used to ascertain purity > (95%) of all compounds for which biological data were determined. CHN elemental analyses were performed on a Perkin-Elmer 2400 CHN elemental analyzer. NMR spectra were recorded on a Bruker AVHD 9.40 T (400.13 MHz ^{1}H e 100.61 MHz ^{13}C) and AVIII 11,75 T (500.13 MHz ^{1}H e 125.76 MHz ^{13}C) system in $CDCl_3$ solutions using tetramethylsilane as the internal reference standard (0.0 ppm). Coupling constants (*J*) are reported in hertz and refer to apparent peak multiplicities.

General procedure for obtaining aromatic azides from aromatic amines (2a-i)

An aqueous solution of 1.5 mmol of sodium nitrite ($NaNO_2$) in 2.5 mL of distilled water was slowly added under vigorous stirring to an Erlenmeyer flask containing 1 mmol of aromatic amine (1a-i) and 1 mL of 6 M hydrochloric acid solution (50%) in an ice bath (maintaining temperature between 0–5 °C). Thereafter, stirring was continued at a low temperature for 30 min. Subsequently, a 4 mmol solution of sodium azide (NaN_3) in 5 mL of distilled water was slowly added while maintaining the temperature between 0–5 °C. The reaction was maintained at room temperature for the necessary time until completion. Then, the mixture was extracted with ethyl acetate, and the organic phase was washed with saturated sodium bicarbonate solution and water and dried with anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to obtain aromatic azides (1a-i). The residual crude product was used directly without purification.

1-azido-2-trifluoromethyl-4-chlorobenzene (1a)

Compound 1a was obtained as a brown liquid with 83% yield. Rf = 0.60 (hexane/ethyl acetate: 7/3). IR (KBr pellet) $\nu(cm^{-1})$: 3059 (C-H sp^2), 2100 (-N=N=N). ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ: 7.11 (d, 1H, *J* = 2.5 Hz, H-Ar),

7.36 (d, 1H, $J = 2.3$ Hz, H-Ar), 7.65 (s, 1H, H-Ar). ^{13}C NMR ($CDCl_3$, 125.76 MHz) δ: 113.00 (C-Ar), 118.46 (C-Ar), 120.47 (C-Ar), 125.90 (C-Ar), 129.45 (C-Ar), 142.05 (C-Ar), 147.18 (q, $J = 2.93$ Hz, C-CF_3), 163.38 (q, $J = 245.5$ Hz, CF_3).

1-azido-3-methylbenzene (1b)

Compound 1b was obtained as a brown liquid with 60% yield. Rf = 0.61 (hexane/ethyl acetate: 7/3). IR (KBr pellet) ν(cm^{-1}): 3060 (C-H sp^2), 2945 (C-H sp^3), 2105 (-N=N=N). ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ: 2.34 (s, 3H, CH¬3), 6.80–6.84 (m, 2H, H-Ar), 6.5 (s, 1H, H-Ar), 7.19–7.25 (m, 1H, H-Ar). ^{13}C NMR ($CDCl_3$, 125.76 MHz) δ: 21.52 (CH_3), 116.32 (C-Ar), 119.79 (C-Ar), 125.95 (C-Ar), 129.74 (C-Ar), 140.08 (C-Ar).

1-azido-2-methoxybenzene (1c)

Compound 1c was obtained as a brown liquid with 88% yield. Rf = 0.69 (hexane/ethyl acetate: 7/3). IR (KBr pellet) ν(cm^{-1}): 3066 (C-H sp^2), 2940 (C-H sp^3), 2113 (-N=N=N). ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ: 3.87 (s, 3H, OCH_3), 6.89 (d, 1H, $J = 8.0$ Hz, H-Ar), 6.9 (t, 1H, $J =$ 7.8 Hz, H-Ar), 7.01 (d, 1H, $J = 7.8$ Hz, H-Ar), 7.10 (t, 1H, $J = 7.9$ Hz, H-Ar). ^{13}C NMR ($CDCl_3$, 125.76 MHz) δ: 56.07 (OCH_3), 112.25 (C-Ar), 120.46 (C-Ar), 121.47 (C-Ar), 125.84 (C-Ar), 128.49 (C-Ar), 152.05 (C-Ar).

1-azido-4-hydroxybenzene (1d)

Compound 1d was obtained as a brown liquid with 44% yield. Rf = 0.36 (hexane/ethyl acetate: 7/3). IR (KBr pellet) ν (cm^{-1}): 3075 (C-H sp^2), 2110 (-N=N=N). ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ: 7.12 (d, 2H, $J = 2.4$ Hz, H-Ar), 7.25 (d, 2H, $J = 2.3$ Hz, H-Ar).^{13}C NMR ($CDCl_3$, 125.76 MHz) δ: 120.60 (C-Ar), 126.82 (C-Ar), 145.85 (C-Ar), 145.90 (C-Ar).

1-azido-2-chlorobenzene (1e)

Compound 1e was obtained as a brown liquid with 89% yield. Rf = 0.74 (hexane/ethyl acetate: 7/3). IR (KBr pellet) ν(cm^{-1}): 2135 and 2106 (-N=N=N), 1585 (C=C). ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ: 7.06–7.10 (m, 1H, H-Ar), 7.18 (dd, 1H, $J = 8.0$ Hz, H-Ar), 7.28–7.31 (m, 1H, H-Ar), 7.38 (dd, 1H, $J = 8.0$ Hz, H-Ar). ^{13}C NMR ($CDCl_3$, 125.76 MHz) δ: 119.89 (C-Ar), 125.21 (C-Ar), 125.85 (C-Ar), 128.09 (C-Ar), 130.96 (C-Ar), 137.41 (C-Ar).

1-azido-2,5-dichlorobenzene (1f)

Compound 1 f was obtained as a brown liquid with 75% yield. Rf = 0.56 (hexane/ethyl acetate: 7/3). IR (KBr pellet) ν(cm^{-1}): 2117 and 2113 (-N=N=N), 1579 (C=C). ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ: 7.06 (dd, 1H, $J = 2.3$ Hz and $J = 8.5$ Hz, H-Ar), 7.15 (d, 1H, $J = 2.3$ Hz, H-Ar), 7.30 (d, 1H, $J = 8.6$ Hz, H-Ar). ^{13}C NMR ($CDCl_3$, 125.76 MHz) δ: 119.99 (C-Ar), 123.66 (C-Ar), 125.99 (C-Ar), 131.69 (C-Ar), 133.68 (C-Ar), 138.60 (C-Ar).

1-azido-4-nitrobenzene (1g)

Compound 1 g was obtained as a yellow solid with 90% yield. Rf = 0.45 (hexane/ethyl acetate: 7/3). IR (KBr pellet) ν(cm^{-1}): 3056 (C-H sp^2), 2127 (-N=N=N), 1517 and 1351 (N=O). ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ: 7.12 (d, 2H, $J =$ 2.1 Hz, H-Ar), 8.25 (d, 2H, $J = 2.3$ Hz, H-Ar). ^{13}C NMR ($CDCl_3$, 125.76 MHz) δ: 119.60 (C-Ar), 125.82 (C-Ar), 144.85 (C-Ar), 147.08 (C-Ar).

1-azido-4-chlorobenzene (1 h)

Compound 1 h was obtained as a brown liquid with 74% yield. Rf = 0.61 (hexane/ethyl acetate: 7/3). IR (KBr pellet) ν(cm^{-1}): 3054 (C-H sp^2), 2130 (-N=N=N). ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ: 6.95 (d, 1H, $J = 2.0$ Hz, H-Ar), 7,30 (d, 2H, $J = 2.1$ Hz, H-Ar). ^{13}C NMR ($CDCl_3$, 125.76 MHz) δ: 120.48 (C-Ar), 130.05 (C-Ar), 130.44 (C-Ar), 138.88 (C-Ar).

1-azido-3-chlorobenzene (1i)

Compound 1i was obtained as a brown liquid with 44% yield. Rf = 0.62 (hexane/ethyl acetate: 7/3). IR (KBr pellet) ν(cm^{-1}): 3068 (C-H sp^2), 2103 (-N=N=N). ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ: 6.90 (d, 1H, $J = 2.3$ Hz, H-Ar), 7.02 (s, 1H, H-Ar), 7.12 (d, 1H, $J = 2.2$ Hz, H-Ar), 7.25 (t, 1H, $J = 2.0$ Hz and 2.1 Hz, H-Ar). ^{13}C NMR ($CDCl_3$, 125.76 MHz) δ: 117.45 (C-Ar), 119.53 (C-Ar), 125.27 (C-Ar), 130.85 (C-Ar), 135.65 (C-Ar), 141.68 (C-Ar).

General method for preparing (1-phenyl-1H-1,2,3-triazol-4-yl-)methanol compounds from aromatic azides (AM11-AM19)

5 mmol of propargyl alcohol (3), 0.1 mmol of $CuSO_4.5H_2O$ and 0.2 mmol of sodium ascorbate were added to a solution containing 1 mmol of the appropriate aromatic azide (2a-i) in dichloromethane (1 mL) and water (1 mL). The resulting suspension was maintained in reflux for 2 hr. After this time, the reaction was quenched with $NaHCO_3$ to neutralize the solution. The mixtures were diluted with 5 mL of dichloromethane and 5 mL of water. The organic phases were separated, dried with anhydrous sodium sulfate, and concentrated under reduced pressure, furnishing the 1,2,3-triazole compounds, which were analyzed by ^{1}H and

^{13}C NMR spectroscopy and IR spectroscopy. When isolated as crude material, the compounds were subjected to purification prior to biological evaluation to ensure that no residual metals or other organic impurities were present. Filtration was performed in a flash column, using an elution gradient of hexane/ethyl acetate.

(1-(2-trifluoromethyl-4-chlorophenyl)-1H-1,2,3-triazol-4-yl) methanol (AM11)

Compound AM11 was obtained as a white powder with 80% yield. mp 110–115 °C; Rf = 0.34 (hexane/ethyl acetate: 1/1). IR (KBr pellet) $\nu(cm^{-1})$: 3280 (O-H), 3120 (C-H sp^2), 1600 (C=C). ^1H NMR ($CDCl_3$, 500.13 MHz) δ 4.70 (s, 2H, CH_2OH), 5.40 (s, 1H, OH), 7.30 (d, 1H, $J = 3.9$ Hz H-Ar), 7.41 (d, 1H, $J = 3.7$ Hz H-Ar), 7.65 (s, 1H, H-Ar), 8.08 (s, 1H, CHtriazole). ^{13}C NMR ($CDCl_3$, 125.76 MHz) δ 53.5 (CH_2OH), 120.3 (CHtriazole), 121.6 (C-Ar), 123.38 (q, $J = 240.5$ Hz, CF_3), 124.9 (C-Ar), 130.0 (C-Ar), 131.6 (C-Ar), 132.3 (C-Ar), 134.6 (C-Ar), 144.2 (Cq-triazole). Anal. Calcd for $C_{10}H_7ClF_3N_3O$: C, 43.26; H, 2.54; N, 15.14. Found: C, 43.20; H, 2.50; N, 15.13.

(1-(2-methylphenyl)-1H-1,2,3-triazol-4-yl)methanol (AM12)

Compound AM12 was obtained as a white powder with 82% yield. mp 58–59 °C; Rf = 0.54 (hexane/ethyl acetate: 1/1). IR (KBr pellet) $\nu(cm^{-1})$: 3266 (O-H), 3112 (C-H sp^2), 2935 (C-H sp3), 1498 (C=C). ^1H NMR ($CDCl_3$, 500.13 MHz) δ 2.25 (s, 3H, CH_3), 4.63 (d, 2H, $J = 5.4$, CH_2OH), 5.45 (s, 1H, OH), 7.40–7.49 (m, 4H, H-Ar), 8.23 (s, 1H, CHtriazole). ^{13}C NMR ($CDCl_3$, 125.76 MHz) δ 17.8 (CH3), 55.5 (CH_2OH), 124.6 (C-Ar), 126.2 (C-Ar), 127.3 (CHtriazole), 130.0 (C-Ar), 131.6 (C-Ar), 133.3 (C-Ar), 136.6 (C-Ar), 148.2 (Cq-triazole). Anal. Calcd for $C_{10}H_{11}N_3O$: C, 63.48; H, 5.81; N, 22.21. Found: C, 63.40; H, 5.89; N, 22.20.

(1-(2-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methanol (AM13)

Compound AM13 was obtained as a white powder with 85% yield. mp 110–113 °C; Rf = 0.34 (hexane/ethyl acetate: 1/1). IR (KBr pellet) $\nu(cm^{-1})$: 3278 (O-H), 3124 (C-H sp^2), 2939 (C-H sp^3), 1602 (C=C). ^1H NMR ($CDCl_3$, 500.13 MHz) δ 3.75 (s, 1H, OH), 3.86 (s, 3H, OCH_3), 4.89 (s, 2H, CH_2OH), 7.06–7.09 (m, 2H, H-Ar), 7.41 (t, 1H, $J =$ 7.9 Hz H-Ar), 7.73 (d, 1H, $J = 7.7$ Hz, H-Ar), 8.11 (s, 1H, CHtriazole).^{13}C NMR ($CDCl_3$, 125.76 MHz) δ 56.09 (OCH_3), 56.50 (CH_2OH), 112.42 (C-Ar), 121.37 (C-Ar), 124.24 (CHtriazole), 125.69 (C-Ar), 126.43 (C-Ar), 130.34 (C-Ar), 147.25 (Cq-triazole), 151.35 (C-Ar). Anal. Calcd for $C_{10}H_{11}N_3O_2$: C, 58.53; H, 5.40; N, 20.48. Found: C, 58.50; H, 5.42; N, 20.47.

(1-(4-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)methanol (AM14)

Compound AM14 was obtained as a white powder with 65% yield. mp 125–128 °C; Rf = 0.20 (hexane/ethyl acetate: 1/1). IR (KBr pellet) $\nu(cm^{-1})$: 3350 (O-H), 3060 (C-H sp^2), 1241 (C-O). ^1H NMR ($CDCl_3$, 400.13 MHz) δ 4.60 (d, 2H, $J = 5.7$, CH_2OH), 5.40 (s, 1H, OH), 7.60 (d, 2H, $J =$ 9.0, H-Ar), 7.90 (d, 2H, $J = 9.1$, H-Ar), 8.50 (s, 1H, CHtriazole). ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ 54.61 (CH_2OH), 121.00 (CHtriazole), 123.75 (2C-Ar), 130.97 (2C-Ar), 131.87 (Cq-Ar), 137.95 (Cq-Ar), 149.00 (Cq-triazole). Anal. Calcd for $C_9H_9N_3O_2$: C, 56.54; H, 4.75; N, 21.98. Found: C, 56.53; H, 4.79; N, 21.90.

(1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl)methanol (AM15)

Compound AM15 was obtained as a brown powder with 72% yield. mp 83–86 °C; Rf = 0.44 (hexane/ethyl acetate: 1/1). IR (KBr pellet) $\nu(cm^{-1})$: 3294 (O-H), 3134 (C-H sp^2). ^1H NMR ($CDCl_3$, 500.13 MHz) δ 3.80 (s, 1H, OH), 4.91 (s, 2H, CH_2OH), 7.42–7.48 (m, 2H, H-Ar), 7.56–7.60 (m, 2H, H-Ar), 8.00 (s, 1H, CHtriazole). ^{13}C NMR ($CDCl_3$, 125.76 MHz) δ 56.38 (CH_2OH), 124.15 (CHtriazole), 127.94 (C-Ar), 128.09 (C-Ar), 128.80 (C-Ar), 130.92 (C-Ar), 130.99 (C-Ar), 134.97 (C-Ar), 147.74 (Cq-triazole). Anal. Calcd for $C_9H_8ClN_3O$: C, 51.57; H, 3.85; N, 20.05. Found: C, 51.53; H, 3.89; N, 20.00.

(1-(2,5-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methanol (AM16)

Compound AM16 was obtained as a white powder with 90% yield. mp 114–116 °C. Rf = 0.26 (hexane/ethyl acetate: 1/1). IR (KBr pellet) $\nu(cm^{-1})$: 3282 (O-H), 3140 (C-H sp^2), 1240 (C-O). ^1H NMR ($CDCl_3$, 400.13 MHz) δ 3.04 (s, 1H, OH), 4.92 (s, 2H, CH_2OH), 7.44 (dd, 1H, $J =$ 8.6 Hz, H-Ar), 7.52 (d, 1H, $J = 8.7$ Hz, H-Ar), 7.67–7.68 (m, 1H, H-Ar), 8.02 (s, 1H, CHtriazole). ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ 56.61 (CH_2OH), 123.88 (CHtriazole), 126.89 (C-Ar), 127.97 (C-Ar), 131.00 (C-Ar), 131.87 (C-Ar), 133.95 (C-Ar), 147.94 (Cq-triazole). Anal. Calcd for $C_9H_7Cl_2N_3O$: C, 44.29; H, 2.89; N, 17.22. Found: C, 44.26; H, 2.89; N, 17.30.

(1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methanol (AM17)

Compound AM17 was obtained as a white powder with 90% yield. mp 200–203 °C; Rf = 0.34 (hexane/ethyl acetate: 1/1). IR (KBr pellet) $\nu(cm^{-1})$: 3283 (O-H), 3138 (C-H sp^2), 1237 (C-O). ^1H NMR ($CDCl_3$, 400.13 MHz) δ 4.63 (s, 2H, CH_2OH), 5.39 (s, 1H, OH), 8.23 (d, 1H, $J = 9.1$ Hz, H-Ar), 8.44 (d, 1H, $J = 9.1$ Hz, H-Ar), 3.04 (s, 1H, H-10), 4.92 (s, 2H, H-9), 7.44 (dd, 1H, $J = 8.6$ Hz, H-Ar), 7.52 (d,

1H, $J = 8.7$ Hz, H-Ar), 8.00 (s, 1H, CHtriazole). ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ 54.61 (CH_2OH), 120.88 (CHtriazole), 120.95 (C-Ar), 124.90 (2C-Ar), 131.00 (2C-Ar), 145.87 (Cq-Ar), 149.95 (Cq-Ar), 147.94 (Cq-triazole). Anal. Calcd for $C_9H_8N_4O_3$: C, 49.09; H, 3.66; N, 25.45. Found: C, 49.10; H, 3.60; N, 25.43.

(1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methanol (AM18)

Compound AM18 was obtained as a white powder with 90% yield. mp 144–146 °C; Rf = 0.46 (hexane/ethyl acetate: 1/1). IR (KBr pellet) $\nu(cm^{-1})$: 3320 (O-H), 3070 (C-H sp^2), 1239 (C-O). ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ 4.66 (d, 2H, $J = 5.6$, CH_2OH), 5.37 (t, 1H, $J = 5.6$, OH), 7.66 (d, 2H, $J = 9.1$, H-Ar), 7.95 (d, 2H, $J = 9.1$, H-Ar), 8.71 (s, 1H, CHtriazole). ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ 55.61 (CH_2OH), 122.00 (CHtriazole), 122.65 (2C-Ar), 129.97 (2C-Ar), 132.87 (Cq-Ar), 135.95 (Cq-Ar), 149.94 (Cq-triazole). Anal. Calcd for $C_9H_8ClN_3O$: C, 51.57; H, 3.85; N, 20.05. Found: C, 51.50; H, 3.89; N, 20.02.

(1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)methanol (AM19)

Compound AM19 was obtained as a white powder with 83% yield. mp 95–97 °C; Rf = 0.34 (hexane/ethyl acetate: 1/1). IR (KBr pellet) $\nu(cm^{-1})$: 3264 (O-H), 3090 (C-H sp^2), 1236 (C-O). ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ 4.61 (d, 2H, $J = 4.6$, CH_2OH), 5.35 (t, 1H, $J = 5.6$, OH), 7.55–7.58 (m, 1H, H-Ar), 7.64 (t, 1H, $J = 8.0$, H-Ar), 7.90–7.93 (m, 1H, H-Ar), 8.03–8.07 (m, 1H, H-Ar), 8.71 (s, 1H, CHtriazole). ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ 55.01 (CH_2OH), 118.54 (CHtriazole), 119.65 (C-Ar), 122.00 (C-Ar), 128.32 (C-Ar), 130.97 (C-Ar), 133.87 (Cq-Ar), 137.95 (Cq-Ar), 149.04 (Cq-triazole Anal. Calcd for $C_9H_8ClN_3O$: C, 51.57; H, 3.85; N, 20.05. Found: C, 51.50; H, 3.80; N, 20.01.

Biological activities

Coagulating activity of *B. jararaca* venom

Coagulant activity of *B. jararaca* venom was determined using a digital Amelung coagulometer, model KC4A (Labcon, Germany). Plasma (obtained from the blood bank of the UFF Hospital) was maintained for 1 min at 37 °C, and then different concentrations of *B. jararaca* venom (5–45 µg/mL) were added to the reaction medium and plasma coagulation time was registered in seconds. The amount of venom (µg/mL) capable of clotting plasma around 60 sec was designated as the minimum coagulation concentration (MCC). Then, this venom concentration (25 µg/mL) was incubated for 30 min at 25 °C with saline or 0.9% v/v DMSO (positive controls) or with 250 µg/mL of each compound (AM11–AM19). After incubation, each mixture was added to plasma, and coagulation was monitored. Negative control groups were performed by incubating each compound, saline or DMSO with plasma, in the absence of venom.

Proteolytic activity of *B. jararaca* venom

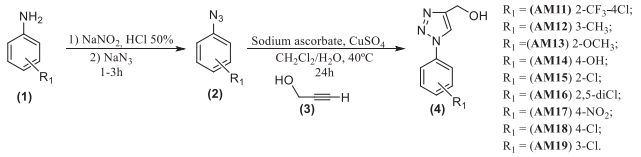
Proteolytic activity of *B. jararaca* venom was determined according to Garcia et al. [25], using azocasein as a substrate (0.2% w/v, in 20 mM Tris-HCl, 8 mM $CaCl_2$, pH 8.8), with minor modifications. Different concentrations of *B. jararaca* venom (2–20 µg/mL) were incubated with azocasein, and the amount of venom (µg/mL) that achieved 70–80% of maximum activity was considered to be 100% proteolytic activity. This concentration of *B. jararaca* venom (9 µg/mL), called the effective concentration (EC), was incubated with 90 µg/mL of each compound (AM11–AM19), saline or 0.9% DMSO (positive controls) for 30 min at 25 °C. Finally, an aliquot of the mixture was added to the reaction medium, and proteolytic activity was evaluated. Negative control experiments were performed by adding solely compounds, saline or DMSO to the reaction medium, instead of the venom, while the positive control was performed by incubating *B. jararaca* venom with saline or DMSO.

Hemolytic activity of *B. jararaca* venom

The degree of hemolysis caused by *B. jararaca* venom was determined by the indirect hemolytic test, using washed human red blood cells and hen's egg yolk emulsion as the substrate [26]. After performing a concentration-response curve of *B. jararaca* venom (1–30 µg/mL), the amount of venom able to induce 100% hemolysis was called the minimum indirect hemolytic concentration (MIHC). Then, one MIHC of *B. jararaca* venom (10 µg/mL) was incubated with each compound (100 µg/mL), saline or DMSO (positive controls) for 30 min at 25 °C, followed by the hemolytic test. Negative control experiments were performed by adding compounds or solvents to the reaction medium, instead of venom.

Hemorrhagic activity of *B. jararaca* venom

Hemorrhagic lesions produced by *B. jararaca* venom were quantified using a procedure described by Kondo et al. [27], with modifications. *B. jararaca* venom was injected subcutaneously (s.c.) into the abdominal skin of mice. Two hours later, the animals were euthanized, and the abdominal skin was removed, stretched, and inspected for visual changes in its internal aspect in order to localize hemorrhagic spots. A minimum hemorrhagic dose (MHD) was defined as the dose of venom (µg/mouse) able to produce a



(1) (2) (3) (4)

Scheme 1 Synthetic route for the preparation of 1,2,3-Triazoles

hemorrhagic halo of 10 millimeters (mm), which was 16 µg/mouse. The effect of compounds (AM11–AM19) on *B. jararaca* venom-induced hemorrhaging was analyzed through two protocols, incubation and treatment. In the incubation protocol, compounds or solvents (saline or DMSO) were incubated with 2 MHD of *B. jararaca* venom (32 µg/mouse) for 30 min at 25 °C. Then, an aliquot of the mixture was injected s.c. into mice, and the hemorrhage halo of the venom was measured and compared to that produced in the absence of the venom. In the treatment protocol, *B. jararaca* venom was injected s.c. into mice, and after 10 min, compounds or solvents were administered s.c. at the same site as venom injection. Instead of compounds, after injecting the venom, 100 µL of antivenom or saline were injected intravenously (i.v.), as well. Negative control experiments were performed by injecting solely solvents or compounds s.c. or injecting antivenom i.v., instead of venom. The total volume of sample injection into the mice was 100 µL.

Edematogenic activity of *B. jararaca* venom

Edema-inducing activity of *B. jararaca* venom was determined according to Vishwanath et al. [28]. Groups of mice received a single s.c. sub plantar injection of *B. jararaca* venom into the right paw, and the left one received an injection of saline or DMSO. Then, 1 h after injection, edema was evaluated and expressed as the percent increase in the weight of the right paw compared to the left one. After that, *B. jararaca* venom (16 µg/mouse) was mixed with 160 µg/mouse of compounds (AM11–AM19) or solvents for 30 min at 25 °C, and then, the mixture was injected s.c. into animals, and edema was evaluated, as described. Negative control mice received a single injection of either compounds or solvents, instead of venom. The total sample volume injected into the mice was 50 µL.

Lethal activity of *B. jararaca* venom

An intraperitoneal (i.p.) injection of *B. jararaca* venom (130 µg/mouse) mixed with solvents (saline or DMSO) into mice killed them in 60 min. This dose of *B. jararaca* venom was incubated with each compound (AM11–AM19) or antivenom for 30 min at 25 °C, and then, an aliquot of mixture was injected i.p. into mice, and survival time of the mice was recorded. Solvents, compounds or antivenom were injected i.p. into mice in the absence of *B. jararaca* venom. The number of dead or alive mice was counted after 6 h of experimentation. The total sample volume injected into the mice was 100 µL.

Toxicity of compounds

Prediction of pharmacological and toxicological properties by admetSAR

The ADMET parameters of the nine compounds (AM11–AM19) were determined using the AdmetSAR online tool (http://lmmd.ecust.edu.cn/admetsar2) [29].

Cytotoxicity

Toxicity of the compounds (AM11–AM19) was evaluated by the hemocompatibility test, according to Bauer et al. [30], with modifications. All the compounds (600 µg/mL) or saline (negative control) were incubated with a 13 % (v/v) of red blood cell suspension for 3 h at 37 °C. After, samples were centrifuged for 3 min at 1800 × *g* and lysis of cells was detected by measuring hemoglobin at A578 nm, using a Microplate Reader (SpectraMax, Model M4, Molecular Devices, California, USA). 100 % of hemolysis (positive control) was achieved by adding Triton X-100 (1%, v/v) or water to the red blood cell suspension.

Statistical analysis

Results are expressed as means ± standard deviation (SD) of number of mice or experiments. Statistical significance of differences among experimental groups was evaluated using the ANOVA test. $*p$ values < 0.05 were considered significant.

Results

Chemistry

1,2,3-triazoles substituted with a methanol at position 4 were reported by Boechat et al. [29] and the methodology described in the paper was followed in this study (Scheme 1). The first step involved the preparation of aromatic azides from aromatic amines and sodium nitrite, which were appropriately combined to form diazonium salts; then, with the addition of an aqueous sodium azide solution, aromatic azides were obtained in good yields, ranging from 44–90%. With the azides properly characterized, it was possible to obtain the desired triazoles through a 1,3-dipolar cycloaddition reaction between propargyl alcohol and aromatic

azides catalyzed by Cu (I). Copper sulfate ($CuSO_4$) and sodium ascorbate guided region selectivity [31]. For the formation of triazole, it is necessary that Cu(I) species be present as a catalyst; however, the system used uses Cu (II), so sodium ascorbate has the function of reducing this Cu(II) ion to Cu(I), generating these ions in situ. The first stage of the triazole formation mechanism consists of the π complexation between copper and the terminal alkyne, which reduces the pKa value of the alkyne, favoring the deprotonation of the species without the need for a strong base in the reaction medium, enabling the formation of copper acetylide. This acetylide then forms a complex with azide, which favors the formation of a metallocycle, since copper increases the electrophilicity of the azide's terminal nitrogen and the nucleophilicity of the β-vinylidene carbon. This complex leads to the formation of copper triazolyl, which is protonated, leading to obtaining the final products and regenerating the Cu (I) ion. It was observed that the use of dichloromethane and heating at 40 °C, replacing tert-butanol at room temperature proved to be more efficient in terms of reaction time and low formation of by-products [29]. Triazole compounds were obtained as white solids with yields ranging between 65–90%.

The chemical structures of the azides and triazoles (AM11–AM19) were confirmed by analyzing the crude product using FTIR, NMR of ^{1}H and ^{13}C. Analysis showed a strong absorption band around 2100 cm^{-1}, referring to the stretch vibrations of N_3 at IR of azides and the absence of stretching vibrations of the azide group and the presence of bands related to axial deformation of the OH bond at IR of triazoles. In the ^{1}H NMR spectrum, the signals of the respective protons of the synthesized compounds were verified based on their chemical shifts, multiplicities, and coupling constants. The characteristic proton signal for identifying the triazolic nucleus can be observed as a singlet around 8 ppm. All data obtained and analyzed were consistent with data from the literature for this family of compounds.

Furthermore, all the compounds (AM11–AM19) were tested against some in vitro or in vivo toxic activities of *B. jararaca* venom, followed by theoretical toxicity using the tool admetSAR. The compounds (AM11–AM19), at a concentration of 600 μg/mL did not lyze red blood cells evaluate by the hemocompability test (data not shown). Thus, compounds were not cytotoxic.

Effect of the compounds against of *B. jararaca* venom-induced plasma coagulation

The concentration of *B. jararaca* venom capable of coagulating plasma in around 60 s was designated as the minimum coagulant concentration (MCC). One MCC of *B. jararaca* venom (25 μg/mL) was incubated with the compounds (250 μg/mL) or solvents for 30 min at 25 °C. After incubation, the mixture was added to plasma, and coagulant activity was evaluated. As seen in Fig. 1, the compounds AM12, AM14, AM15, and AM16 impaired coagulation induced by *B. jararaca* venom, when compared to the positive group (*B. jararaca* + saline or *B. jararaca* + DMSO). The other compounds, AM11, AM13, AM17, and AM19, did not inhibit coagulation (Fig. 1). None of the compounds (250 μg/mL) or DMSO alone (at 0.9%, final concentration) induced plasma coagulation nor interfered with the coagulation test.

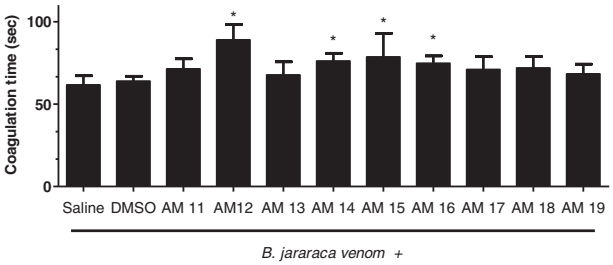


Fig. 1 Effect of compounds on *B. jararaca* venom-induced coagulation

B. jararaca venom (25 μg/mL) was incubated for 30 min at 25 °C with 250 μg/mL of compounds (AM11–AM19), saline or DMSO (0.9%, final concentration). Then, an aliquot of the mixture was added to plasma and coagulation time was measured, as described in the methods section. Results express the means ± SD of three individual experiments ($n = 3$). *$p < 0.05$ in relation to positive controls (*B. jararaca* venom + saline or *B. jararaca* venom + DMSO).

Effect of compounds against of *B. jararaca* venom-induced proteolysis

B. jararaca venom incubated with saline or DMSO (positive controls) hydrolyzed azocasein in a concentration-dependent manner, and reads of 0.2 at absorbance of 420 nm was called the effective concentration (EC). One EC of *B. jararaca* venom (9 μg/mL) was incubated for 30 min at 25 °C with compounds (90 μg/mL), and then, the mixture was added to the reaction medium, proteolytic activity was evaluated, and result is shown as inhibition of proteolysis (Fig. 2). The compounds AM15 (32%) or AM19 (53%) had the highest values of inhibition against the proteolytic activity of *B. jararaca* venom; while the compounds AM14, AM16, and AM18 achieved the lowest, below 20% (Fig. 2). The compounds AM11, AM12, AM13, and AM17 inhibited approximately 20% (Fig. 2).

B. jararaca venom (9 μg/mL) was incubated with 90 μg/mL of each compound (AM11–AM19) for 30 min at 25 °C. After that, the mixture was added to the reaction medium, proteolytic activity was evaluated, and inhibition of proteolysis was determined. Results express the means ± SD of three individual experiments ($n = 3$).

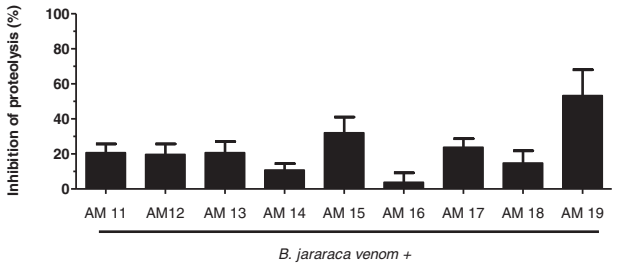


Fig. 2 Inhibition by compounds of proteolysis induced by *B. jararaca* venom

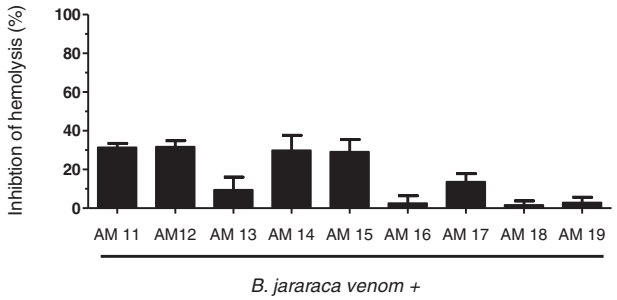


Fig. 3 Inhibition by compounds of *B. jararaca* venom-induced hemolysis

Effect of compounds against *B. jararaca* venom-induced hemolysis

Incubation of *B. jararaca* venom with saline or DMSO (1%, final concentration) lysed a suspension of red blood cells, with a similar CMHI of 10 µg/mL. Then, this venom concentration was incubated with the compounds (100 µg/mL) for 30 min at 25 °C. After that, an aliquot of the mixture was removed and added to the reaction medium, and a hemolytic activity test was performed (Fig. 3). The compounds AM11, AM12, AM14, and AM15 inhibited ~30% of *B. jararaca* venom-induced hemolytic activity, while the others inhibited less than 15% of hemolysis (Fig. 3). None of the compounds alone lysed red blood cells.

B. jararaca venom (10 µg/mL) was incubated with 100 µg/mL of compounds (AM11–AM19) for 30 min at 25 °C. After that, the mixture was added to red blood cells, hemolytic activity was evaluated, and inhibition of hemolysis was determined. Results express the means ± SD of three individual experiments ($n = 3$).

Effect of compounds against of *B. jararaca* venom-induced hemorrhaging

A single injection of *B. jararaca* venom (16 µg/mouse) into mice produced a hemorrhagic halo of 10 mm which represents one MHD. Compounds (320 µg/mouse), saline or DMSO were incubated for 30 min at 25 °C with 2 MHD of *B. jararaca* venom (32 µg/mouse). Then, an aliquot of the mixture was injected s.c. into mice, and hemorrhaging was analyzed, as described. This approach was the incubation protocol. As seen in Fig. 4a, the compounds AM11 (11%), AM13 (22%), AM18 (4%), and AM19 (17%) inhibited the hemorrhagic activity of *B. jararaca* venom. Thus, the treatment protocol was employed using compounds AM11, AM13, AM18, and AM19. *B. jararaca* venom (32 µg/mouse) was injected s.c. into mice, and after 10 min, each of these compounds (300 µg/mouse) was injected s.c. (at the same site as venom injection), followed by an intravenous (i.v.) injection of commercial antivenom. As seen in Fig. 4b, the compound AM13 inhibited 32% of *B. jararaca* venom-induced hemorrhaging, while the other compounds did not protect the mice from hemorrhaging (Fig. 4b). The inhibitory percentage of AM13 (20%) in the absence of antivenom was similar in the incubation (Fig. 4a) and treatment protocols (Fig. 4b). On the other hand, inhibition of AM13 was enhanced to 32 % in the presence of antivenom (Fig. 4b). Moreover, administration of antivenom i.v. after administration of *B. jararaca* venom did not inhibit hemorrhage as well as a cocktail using the derivatives AM11, AM13, AM18, and AM19 did not enhance inhibitory percentage (data not shown). Injection of solvents (saline or DMSO), antivenom or the compounds alone did not induce hemorrhaging in mice, regardless of the protocol employed.

Effect of compounds against *B. jararaca* venom-induced edema

The effect of compounds on edema caused by *B. jararaca* venom was investigated. *B. jararaca* venom (16 µg/mouse) was incubated for 30 min at 25 °C with 160 µg/mouse of each compound (AM11–AM19), saline or DMSO (0.9% v/v, final concentration), and then the mixture was injected s.c. into the paw of the animals and edema was analyzed. All of the compounds inhibited the edematogenic activity from *B. jararaca* venom with similar inhibitory percentages: AM11 (48%), AM12 (37%), AM13 (44%), AM14 (47%), AM15 (44%), AM16 (39%), AM17 (39%), AM18 (39%), and AM19 (45%) (Fig. 5). None of the compounds induced edema in the mice.

B. jararaca venom (16 µg/mouse) was incubated with 160 µg/mouse of compounds (AM11–AM19) for 30 min at 25 °C. Then, an aliquot of the mixture was injected into the mice, edema was evaluated as described, and inhibition of edema was determined. Results are expressed as the means ± SD of three individual experiments ($n = 3$).

Effect of compounds against lethality of *B. jararaca* venom

Intraperitoneal (i.p.) injection of *B. jararaca* venom (130 µg/mouse) incubated with saline or DMSO (0.9% v/v,

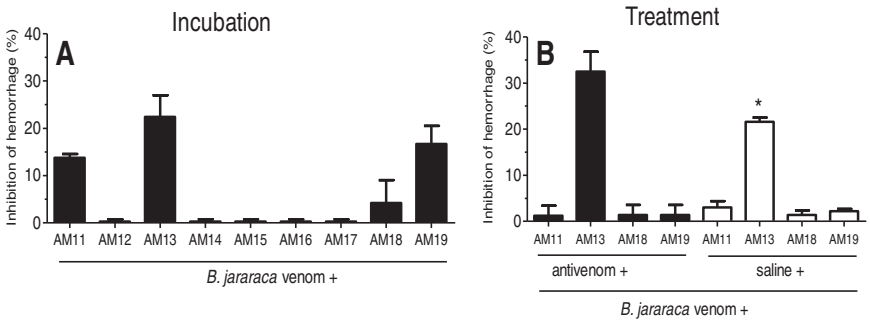


Fig. 4 Inhibition by compounds on hemorrhaging from *B. jararaca* venom. **a** Incubation protocol: *B. jararaca* venom (32 µg/mouse) was incubated with 320 µg/mouse of each compound (AM11–AM19) for 30 min at 25 °C. Then, the mixture was injected s.c. into mice, and hemorrhaging was analyzed. **b** Treatment protocol: *B. jararaca* venom (32 µg/mouse) was administered s.c. into mice, and 10 min later, 320 µg/mouse of the compounds AM11, AM13, AM18, and AM19 were injected s.c., followed by i.v. injection of antivenom (black columns) or saline (white columns). After 2 h of *B. jararaca* venom injection, hemorrhaging was analyzed, as described. Results are shown as inhibition of hemorrhage, and expressed as the means ± SD of three individual experiments ($n = 3$). *$p < 0.05$ in relation to *B. jararaca* venom + antivenom + AM13

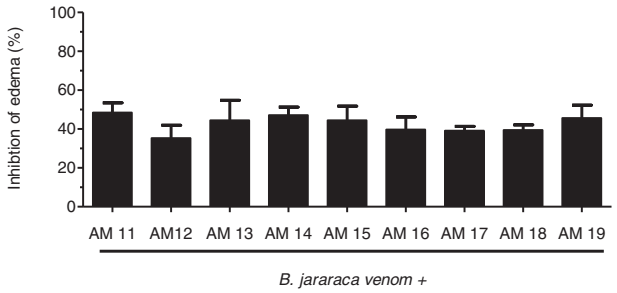


Fig. 5 Inhibition by compounds on edema from B. jararaca venom

final concentration) killed the mice in 60 min (Table 1). This dose of *B. jararaca* venom was incubated with 320 µg/mouse of compounds (AM11–AM19) or antivenom for 30 min at 25 °C; then, the mixture was injected i.p., and survival time of the mice was observed and compared to the positive control groups (*B. jararaca* + saline or *B. jararaca* + DMSO). As seen in Table 1, the compounds AM11 and AM16 prolonged survival time of the mice. Moreover, antivenom fully protected mice from *B. jararaca* venom-induced lethality (Table 1). The maximal period of observation of mice was 360 min, and i.p. injection of compounds or antivenom alone did not kill the mice (data not shown).

B. jararaca venom (130 µg/mouse) was incubated for 30 min at 25 °C with saline, DMSO, 320 µg/mouse of compounds (AM11–AM19) or with antivenom. After incubation, the mixture was injected i.p. into mice, and survival time of the mice was recorded. Results are expressed as the means ± SD of two individual experiments ($n = 6$). *$p < 0.05$ when compared to *B. jararaca* + DMSO or to *B. jararaca* + saline (positive controls). Maximal observation of the mice was 360 min.

Table 1 Effect of compounds on lethality from *B. jararaca* venom

Groups	Survival time (min)
B. jararaca + saline	60 ± 6
B. jararaca + DMSO	61 ± 8
B. jararaca + AM11	100 ± 11*
B. jararaca + AM12	69 ± 5
B. jararaca + AM13	68 ± 5
B. jararaca + AM14	57 ± 20
B. jararaca + AM15	76 ± 19
B. jararaca + AM16	154 ± 11*
B. jararaca + AM17	66 ± 3
B. jararaca + AM18	73 ± 10
B. jararaca + AM19	71 ± 6
B. jararaca + antivenom	360 ± 10*

Theoretical studies of toxicity and bioavailability

In silico analysis of the compounds (AM11–AM19) was investigated using the software ADMET, and the results are shown in Table 2. Some parameters, such as absorption, distribution, metabolism, excretion, and toxicity were investigated (Table 2). Upon analysis of the results through this software, we observed that the compounds interfered with the following absorption parameters: Blood-Brain Barrier, Human Intestinal Absorption, and Caco-2 Permeability; but did not interfere with P-glycoprotein Substrate and P-glycoprotein Inhibitor. In the parameters of distribution and metabolism, all the compounds acted on CYP450 1A2 Inhibitor, and only derivative AM11 acted on CYP450 3A4 Substrate. The compounds did not act on the CYP450 2C9

Table 2 Pharmacokinetic and toxicological studies of the compounds AM11–AM19 using the tool AdmetSAR

Model	Lead compound								
Absorption	AM11	AM12	AM13	AM14	AM15	AM16	AM17	AM18	AM19
Blood-brain barrier (BBB)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Human intestinal absorption (HIA)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Caco-2 permeability	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
P-glycoprotein substrate	No	No	No	No	No	No	No	No	No
P-glycoprotein inhibitor	No	No	No	No	No	No	No	No	No
Distribution and metabolism									
CYP450 2C9 substrate	−	−	−	−	−	−	−	−	−
CYP450 2D6 substrate	−	−	−	−	−	−	−	−	−
CYP450 3A4 substrate	+	−	−	−	−	−	−	−	−
CYP450 1A2 inhibitor	+	+	+	+	+	+	+	+	+
CYP450 2C9 inhibitor	−	−	−	−	−	−	−	−	−
CYP450 2D6 inhibitor	−	−	−	−	−	−	−	−	−
CYP450 2C19 inhibitor	−	−	−	−	−	−	−	−	−
CYP450 3A4 inhibitor	−	−	−	−	−	−	−	−	−
CYP inhibitory promiscuity	L	L	L	L	L	L	L	L	L
Excretion and toxicity									
Human Ether-a-go-go-related gene inhibition	−	−	−	−	−	−	−	−	−
AMES toxicity	−	−	−	−	−	−	−	−	−
Carcinogen	−	−	−	−	−	−	−	−	−
Fish toxicity	+	−	−	+	+	+	−	+	+
Honey bee toxicity	−	−	−	−	−	−	−	−	−
Biodegradation	−	−	−	−	−	−	−	−	−
Acute oral toxicity	III	III	III	III	III	III	III	III	III
Carcinogen (three-class)	−	−	−	−	−	−	−	−	−
DMET predicted profile (regression)									
Absorption									
Water solubility (LogS)	−3.270	−1.429	−1.659	−1.860	−2.628	−2.628	−1.804	−2.445	−2.445
Plasma protein binding (PPB) (100%)	0.746	0.725	0.564	0.551	0.729	0.560	0.493	0.681	0.804
Acute oral toxicity Kg/mol	3.21	2.49	2.16	1.51	2.10	2.40	1.54	2.40	2.30
Tetrahymena pyriformis (plGC50, μg/L)	1.202	0.241	0.220	0.212	0.552	0.784	0.057	0.521	0.454

+ or − means positive or negative in silico toxicity profile, respectively, L means low, and acute oral toxicity III means slightly toxic and a slight irritant

Substrate, CYP450 2D6 Substrate, CYP450 2C9 Inhibitor, CYP450 2D6 Inhibitor, CYP450 2C19 Inhibitor, CYP450 3A4 Inhibitor nor CYP Inhibitory Promiscuity. According to the excretion and toxicity parameters, the compounds did not act on Human Ether-a-go-go-Related Gene Inhibition, AMES Toxicity, Carcinogens, Honey Bee Toxicity, Biodegradation or Carcinogenicity. The compounds showed level III Acute Oral Toxicity which indicates slight toxicity and a slight irritant. On the other hand, the compounds AM11, AM14, AM15, AM16, AM18, and AM19 showed toxicity against fish, but not to honey bee. Thus, the compounds are favorable to become drugs according to their absorption parameters.

Discussion

Snakebite envenoming by the species *B. jararaca* induces a range spectrum of clinical alterations in victims. On the other hand, it is worth saying that snake venoms have intriguing compositions and actions in victims; they may induce toxic effects in victims or they can be used to develop medicines and save lives. A good example of this positive effect of venom is the drug known as angiotensin converting enzyme inhibitor (ACEI). ACEI was developed from a peptide isolated from *B. jararaca* venom and is currently used to treat hypertension and some types of heart failure [32].

Antivenom is the official treatment for snakebites worldwide, and in Brazil, it is produced by three major public research centers: Vital Brazil Institute (Niterói, RJ), Butantan Institute (São Paulo, SP), and the Ezequiel Dias Foundation (Belo Horizonte, MG). The earliest studies related to antivenom therapy were carried out at the end of the 1800s [33], but only in 1901 did Vital Brazil develop the first antivenom [34]. Antivenom binds to venom and neutralizes its toxic effects. Thus, it is effective for preventing death; however, it has some drawbacks. To date, manufacturing processes of antivenoms have not been improved to overcome these limitations. Therefore, due to these limitations, new treatments to aid conventional antivenoms are being investigated. Natural products derived from plants or seaweed have been used by citizens or healers to treat many diseases, since ancient civilization. However, in some cases, such products were chemically modified to enhance efficacy, earn faster profits, diminish side effects or attend to market demand [35, 36]. The rational planning of compounds with lower costs of production and side effects are an important challenge in medicinal chemistry, and, because of that, the number of new compounds has increased dramatically [37].

Among strategies to synthesize new compounds to become drugs, the target-oriented synthesis strategy is relevant. A synthetic compound can be used either to discover a target protein or as a modulating ligand. In this case, it is quite common to screen collections of molecules with low molecular weights [37]. 1,2,3-triazoles or 1,2,4-triazoles triazole derivatives are widely used in medicine for drug development because of their structure and low toxicity [18, 20, 38, 39, 41, 42].

Thus, here, we synthesized a novel family of 1,2,3-triazoles able to inhibit some in vitro or in vivo activities of *B. jararaca* venom. The 1,2,3-triazoles substituted with methanol at position 4 were first described elsewhere, and we followed the methodology described in that paper [29]. The first step involved the preparation of aromatic azides from aromatic amines and sodium nitrite which were appropriately combined to form diazonium salts; then, with the addition of the aqueous sodium azide solution, aromatic azides were obtained in good yields, ranging from 44–90%. With the azides properly characterized, it was possible to obtain the desired triazoles through a 1,3-dipolar cycloaddition reaction between propargyl alcohol and aromatic azides catalyzed by Cu (I). Copper sulfate ($CuSO_4$) and sodium ascorbate guided the regioselectivity. It was observed that the use of dichloromethane and heating at 40 °C, replacing tert-butanol at room temperature, proved to be more efficient in terms of reaction time (change from 3 h to 30 min) and low formation of by-products [29]. Only for compounds AM11 and AM14, was purification with flash column filtration necessary to remove by-products.

The chemical structures of the azides and triazoles were confirmed by analyzing the product using FTIR, NMR of ^{1}H, and ^{13}C. Analysis showed a strong absorption band around 2100 cm^{-1}, referring to the stretch vibrations of N_3 at IR of azides and the absence of stretching vibrations of the azide group and the presence of bands related to axial deformation of the OH bond at IR of triazoles. In the ^{1}H NMR spectrum, the signals of the respective protons of the synthesized compounds were verified based on their chemical shifts, multiplicities, and coupling constants. The characteristic proton signal for identifying the triazolic nucleus can be observed as a singlet around 8 ppm. All data obtained and analyzed were consistent with the literature for this family of compounds.

The new 1,2,3-triazole compounds inhibited the proteolytic, coagulant, hemolytic, edematogenic, lethal, and hemorrhagic activity of *B. jararaca* venom. These assays cover most of the active enzymes (phospholipase A_2 (PLA_2), snake venom metalloproteinases (SVMPs), and snake venom serine proteases (SVSPs)) of this species as well as the other venomous snakes. These enzymes are responsible for most of the toxic symptoms of envenomation, but it is very difficult to correlate one enzyme with a specific symptom or effect observed in victims, because snake venoms are a complex mixture of active proteins. SVMPs comprise a large group of zinc-dependent enzymes with a domain enriched with histidine residues that induce tissue damage, inflammation or disorders in the coagulation cascade [43] and impair regeneration of the affected skeletal muscle [44]. SVSPs exhibit multiple toxic activities, such as the induction of plasma coagulation, hemorrhaging, and effects on platelet aggregation, while the PLA_2 enzymes may induce edema, hemolysis, and tissue necrosis [45, 46]. Therefore, the neutralization of these enzymes is extremely important in order to treat patients and help them recover faster. As has been described in the literature, commercial antivenom fails to fully inhibit these enzymes [47], even following the standard guidelines recommended by authorities, which is performed by incubating commercial antivenom with venoms (incubation protocol). On the other hand, antivenom efficiently inhibited *B. jararaca* venom lethality but failed to inhibit hemorrhaging [18]. Thus, if antivenom is mixed with venom, rather than administered after the venom, neutralization is more effective. Indeed, the time of administration of antivenom should also be taken into consideration, and some studies postulate that antivenom should be given around 60 min after a snakebite to avoid death; however, the local effects of venoms are not inhibited [45].

The literature has shown that the experimental strategy of potency tests is performed by incubating antivenom with venoms (incubation protocol). However, this approach does not reflect how a snake accident really occurs, since a

venomous snake injects venom into victims, and later, victims receive antivenom intravenously. Here, all the 1,2,3-triazoles inhibited toxic activities of *B. jararaca* venom through the incubation protocol, but only the compound AM13 inhibited hemorrhaging in the treatment protocol. This is a promising result and may turn, mainly compound AM13, into a candidate molecule for neutralization of *B. jararaca*-induced hemorrhaging, since its envenomation is one of the most toxic of the *Bothrops* species. Moreover, hemorrhaging is responsible for amputation or morbidities of victims.

In silico studies are an important virtual tool capable of analyzing theoretical toxicity, enabling the screening of molecules more efficiently and quickly, reducing the costs of drug development, reducing the use of animals, elucidating mechanisms of action or determining safety or side effects to humans or the environment. The software ADMET (absorption, distribution, metabolism, excretion, and toxicity) is of vital importance in drug discovery studies through the analysis of some parameters, such as water solubility, sites of cytochrome P450 (CYP) metabolism or metabolites, mutagenicity, toxicity, ability to cross the blood-brain barrier (BBB), and gastrointestinal absorption [48]. However, like any other technique, there are limitations with this process [49]. Some programs use the structure-activity relationship to preview the physical-chemical structure of a molecule for possible biological functions.

ADMET properties of the lead compounds were calculated; admetSAR was used to predict some chemical properties in order to investigate 1,2,3-triazole derivatives (AM11–AM19) as potential molecules for the development of antivenoms. According to the parameters in Table 2 (Caco-2 permeability, HIA or P-glycoprotein), the compounds AM11–AM19 have good penetrability across the blood-brain barrier and can be easily absorbed by the human intestine, due to their chemical structure and non-polarity. This is in accordance with the chemical structure of triazoles [50]. High absorption of these compounds (AM11–AM19) meant that they can be given orally to patients, resulting in some advantages (they can be self-administered without suffering pain or discomfort and they can have relatively lower prices) or disadvantages (patient must be conscious, molecules suffer first-pass effect, may produce irritation to gastric mucosa or may be destroyed by the action of digestive enzymes or extreme pH values). According to ADMET distribution and metabolism, the compounds (AM11–AM19) do not suffer liver metabolism or renal clearance, and this fact may facilitate the desired effect as antivenoms, or indeed, maintain an effective concentration of them in the plasma longer. On the other hand, one negative consequence of circulating longer is their non-specific interaction with plasma proteins, which means the possibility of increasing side effects on any tissues. However, the compounds have low acute oral toxicity (below 3) and low percentage (below 1%) of unbound to plasma proteins (PPB parameter) that reduce even more, appearance of undesirable effects. Moreover, LogS values above -4 of the compounds indicate good water solubility and distribution through human body that in turn is facilitated by their low molecular weight (below 450 Kda). The compounds (AM11–AM19) inhibited some of the enzymes of cytochrome P450, the superfamily that is responsible for metabolized drugs or xenobiotics [49]. The inhibitory action of triazoles on the enzymatic activity of cytochrome P450 has been well established [49]. All the compounds (AM11–AM19) are devoid of toxicity or mutagenicity, since they did not inhibit the isoform CYP1A2 that is responsible for metabolizing polycyclic aromatic hydrocarbons (PAHs) to activate carcinogenic products. Moreover, these compounds fell into category III of toxicity which means they produce mild toxicity or slight irritation. According to WHO [51], toxicity of a chemical compound is classified into four categories (I–IV); category I has the lowest toxicity and category 4, the highest. Another result that reinforces the lack of toxicity of the compounds was the inability of them to lyse red blood cells, evaluated by the in vitro hemocompatibility test that is regularly used to test drugs or any molecule that enter into circulating blood.

Therefore, according to favorable data, 1,2,3-triazole derivatives, especially AM13, may be a good molecule candidate for use as antivenom, because it did neutralize hemorrhagic, edematogenic, and lethal activities of *B. jararaca* venom and it is devoid of toxicity. Moreover, a simple and easy method to synthesize such derivatives and reinforce the interest to study the mechanism of action and develop pharmaceutical formulations using these derivatives.

Conclusion

Thus, these new compounds derived from 1,2,3-triazoles could be used as adjuvants of commercial antivenom to enhance the effectiveness of neutralizing the local effects of *B. jararaca* venom. However, an in-depth scientific investigation is imperative to evaluate other approaches for deriving these therapeutically effective compounds against symptoms of snakebites.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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