Bioorganic & Medicinal Chemistry 17 (2009) 7429–7434

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis, biological, and theoretical evaluations of new 1,2,3-triazoles against the hemolytic profile of the *Lachesis muta* snake venom

Vinícius R. Campos [a], Paula A. Abreu [b], Helena C. Castro [b], Carlos R. Rodrigues [c], Alessandro K. Jordão [a], Vitor F. Ferreira [a], Maria C. B. V. de Souza [a], Fernanda da C. Santos [a], Laura A. Moura [b], Thaisa S. Domingos [b], Carla Carvalho [b], Eládio F. Sanchez [d], André L. Fuly [b,*], Anna C. Cunha [a,*]

[a] *Universidade Federal Fluminense, Departamento de Química Orgânica, Programa de Pós-Graduação em Química, Outeiro de São João Batista, s/n°, Niterói 24020-141, Rio de Janeiro, Brazil*
[b] *Departamento de Biologia Celular e Molecular, Instituto de Biologia, Outeiro de São João Baptista, 24020-141 Universidade Federal Fluminense, Niterói, RJ, Brazil*
[c] *Laboratório de Modelagem Molecular e QSAR (ModMolQSAR), Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, CEP 21941-590, RJ, Brazil*
[d] *Fundação Ezequiel Dias, Centro de Pesquisa e Desenvolvimento, Belo Horizonte, MG, Brazil*

ARTICLE INFO

Article history:
Received 13 July 2009
Revised 16 September 2009
Accepted 17 September 2009
Available online 24 September 2009

Keywords:
Triazoles
Diazo compounds
Snake venoms
Biological activities
Lachesis muta

ABSTRACT

The current treatment used against envenomation by *Lachesis muta* venom still presents several side effects. This paper describes the synthesis, pharmacological and theoretical evaluations of new 1-arylsulfonylamino-5-methyl-1*H*-[1,2,3]-triazole-4-carboxylic acid ethyl esters (**8a–f**) tested against the hemolytic profile of the *L. muta* snake venom. Their structures were elucidated by one- and two-dimensional NMR techniques (^{1}H, APT, HETCOR $^1J_{CH}$ and $^nJ_{CH}$, $n = 2, 3$) and high-resolution electrospray ionization mass spectrometry. The series of triazole derivatives significantly neutralized the hemolysis induced by *L. muta* crude venom presenting a dose-dependent inhibitory profile ($IC_{50} = 30–83$ μM) with 1-(4′-chlorophenylsulfonylamino)-5-methyl-1*H*-[1,2,3]-triazole-4-carboxylic acid ethyl ester **(8e)** being the most potent compound. The theoretical evaluation revealed the correlation of the antiophidian profile with the coefficient distribution and density map of the Highest Occupied Molecular Orbitals (HOMO) of these molecules. The elucidation of this new series may help on designing new and more efficient antiophidian molecules.

1. Introduction

The family **Viperidae**, represented by three main genera *Crotalus* (rattlesnakes), *Bothrops* (lance-heads), and *Lachesis* (bushmaster, in Brazil popularly known as surucucu), consists in the most important group of snakes regarding public health issues since they are responsible for most of the serious ophidian accidents reported not only in Brazil but also in other Western countries.[1–4] This latter genus comprises two subspecies: *Lachesis muta muta,* found in the Amazon tropical forest, and *Lachesis muta rhombeata*, distributed in the Atlantic forest of the eastern regions of Brazil.[3,4]

Envenomation by snakes of the family *Lachesis* usually causes local tissue damage, including edema, hemorrhage, and myonecrosis as well as systemic disorders such as nausea, vomiting, diarrhea, hypotension and bradycardia, coagulation disturbs, and renal malfunction.[4] The venom of *Lachesis muta* contains several components such as highly active enzymes, arginyl-ester hydrolase and thrombin-like, hemorrhagic and neurotoxic substances, and fibrinogenase and kininogenase substances.[2,5] Among the bioactive proteins, phospholipases A2 (sPLA2s) isoforms[6–8] constitute the major toxic components of the snake venoms. They exhibit a wide range of pharmacological effects, that is, pre- or postsynaptic neurotoxicity, cardiotoxicity, myotoxicity, effects on platelet function, edema, hemolysis, anticoagulation, convulsion and hypotension, and their catalytic activity on cell membranes of specific tissues suggests an important role in venom toxicity.[9,10]

Nowadays, the regular treatment for ophidic accidents is the parentheral use of antiserum obtained from hyperimmunized equines. However, this treatment presents drawbacks including poor availability in distant regions, refrigerated storage need and some allergic reactions. In addition, despite systemic and lethal effects are usually reversed or avoided, local tissue damages are not, which lead to a high morbidity.[11] Therefore, the search for new natural and synthetic compounds that can prevent the toxins from reaching the mammalian targets and acting as cytotoxic agents is still relevant.[12]

Several natural compounds with activity against snake venoms have been reported in the literature.[13,14] For example, koninginins[15] (**1–2**), rosmarinic acid[16] (**3**), and pterocarpans[12] (**4**) are snake venom phospholipase A_2 inhibitors isolated from *Trichoderma koningii*, *Cordia verbenacea*, and cabenegrina A–I, respectively.

* Corresponding authors. Tel.: +55 21 26292148; fax: +55 21 26292135 (A.C.C.).
E-mail address: annac@vm.uff.br (A.C. Cunha).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.09.031

Suramin (**5**) is a polysulfonated napthylurea that was originally developed for the treatment of African trypanosomiasis and onchocerciasis.[17–19] This charged compound also prevents muscle necrosis induced by some snake venoms, inhibiting their myotoxicity and in vitro neuromuscular blocking activities of Lys49 phospholipases A_2 from *Bothrops* species. The benzoyl phenyl benzoate **6** is an effective inhibitor of phospholipase A_2 and hyaluronidase enzymes in several snake families (e.g., *Elapidae*, *Viperidae*, and Cr*otalidae*).[20] The imidazolic compound **7** exhibits a significant PLA_2 enzyme inhibitory activity against group II PLA_2[7] (Fig. 1).

Literature has described different classes of 1,2,3-triazole compounds with different pharmacological profiles. There are reports of triazole analogs as antiplatelet agents,[21] dopamine D2 receptor ligands for treating schizophrenia,[22] tripanocidal,[23] antimycobacterial,[24] and leishmanicidal diseases.[25] 1,2,3-Triazole has been elected as a lead compound group for designing active molecules against different pathologies such as thrombosis, including recently by our group.[26] In this previous work we synthesized several 1,2,3-triazole derivatives based on *N*′-[(4′-bromophenyl)methylene)]-1-(*p*-chlorophenyl)-1*H*-[1,2,3]-triazole-4-carbohydrazide and identified them as significant platelet aggregation inhibitors.[21,26]

As part of an ongoing research program on the synthesis of new biologically active triazoles and on the basis of our experience in the field of the use of diazo compounds in organic synthesis, herein we synthesized a novel family of 1-arylsulfonylamino-5-methyl-1*H*-[1,2,3]-triazole-4-carboxylate derivatives, and evaluated their ability to neutralize *L. muta* snake venom hemolytic activity and their structure–activity relationship (SAR) by using a molecular modeling approach.

2. Results and discussion

2.1. Chemistry

The synthesis of new 1-arylsulfonylamino-5-methyl-1*H*-[1,2,3]-triazole-4-carboxylic acid ethyl esters **8a–f** is shown in Scheme 1. The ethyl 2-diazoacetoacetate **12**, prepared in 70% yield by the method of Danheiser et al.,[27] was condensed with arylsulfonylhydrazides **10a–f**, giving the corresponding diazo-hydrazone intermediates **14a–f**, which underwent 1,5-electrocyclization leading to the new 1,2,3-triazole derivatives **8a–f** (Scheme 1). The known sulfonylhydrazides **10a–f** were prepared in excellent yields by adding the corresponding arylsulfonyl chlorides **9a–f** dissolved in THF to a slightly excess of hydrazine hydrate solution 80%, according to the procedure described in the literature[28] (Scheme 1). The reaction yields and the melting points of new triazole derivatives are listed in Table 1.

2.2. Antihemolytic effect and structure–activity relationship analysis

The biological assay revealed that all triazole derivatives **8a–f** were able to completely inhibit the *L. muta* crude venom-induced hemolysis at 130 μM. In fact, these compounds presented a dose-dependent inhibitory profile with IC_{50} that ranges from 30 to 83 μM (Fig. 2). Importantly, those compounds did not interfere on the integrity of neither rabbit nor human erythrocytes when tested alone, even up to 390 μM, inferring that they are not toxic to such cells.

Since the new synthesized triazole derivatives presented this promising antiophidian effect in a dose-dependent manner, we performed a structure–activity relationship (SAR) analysis to correlate the stereoelectronic properties of the molecules with their antihemolytic profile (Table 2).

The cross-correlation matrix showed that energy of HOMO, electrostatic charge of the substituted carbon atom in phenyl ring, lipophilicity and water solubility are the most correlated descriptors to the experimental IC_{50} (Table 3). The most active compounds 8a, **8b**, **8e**, and **8f** showed higher lipophilicity as well as lower water solubility. In addition, low LUMO energies values are also apparently related to these derivatives biological profile where the most active compounds presented the lowest values for these orbital energies (Table 2).

The analysis of HOMO density maps and coefficient distribution showed a different distribution in the most active compounds with a higher HOMO density concentrated in the triazole ring, whereas in the less active derivatives, HOMO orbitals were concentrated in the phenyl ring. Since the frontier orbitals are usually important for ligand–target interaction, probably the location of HOMO in the

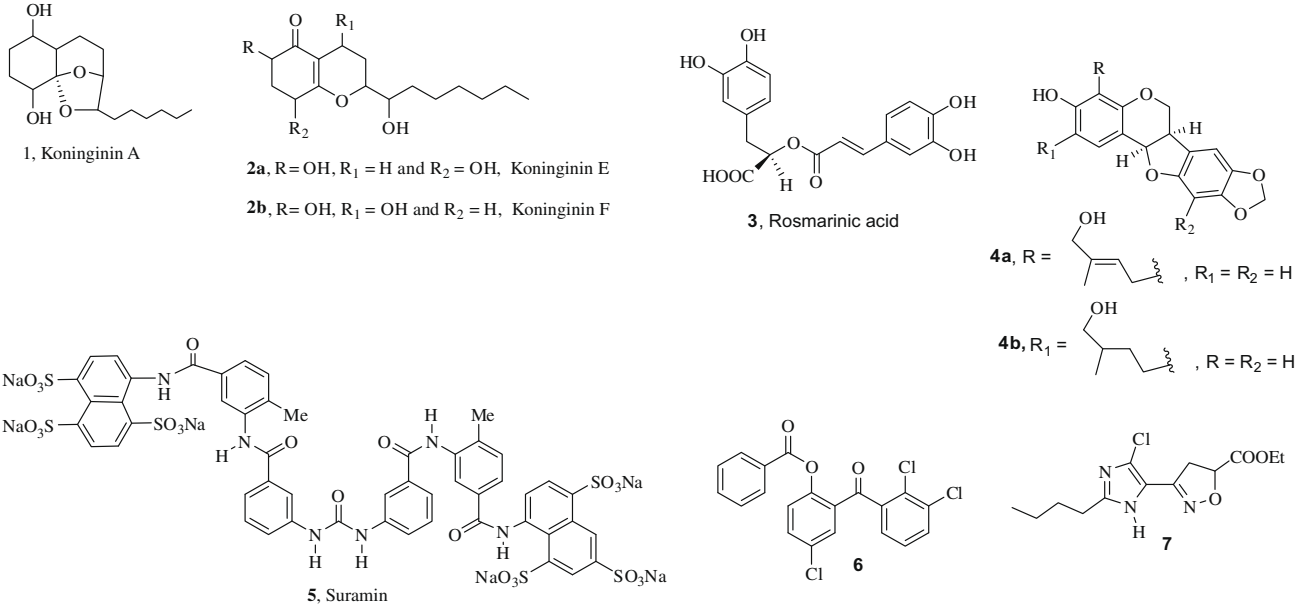
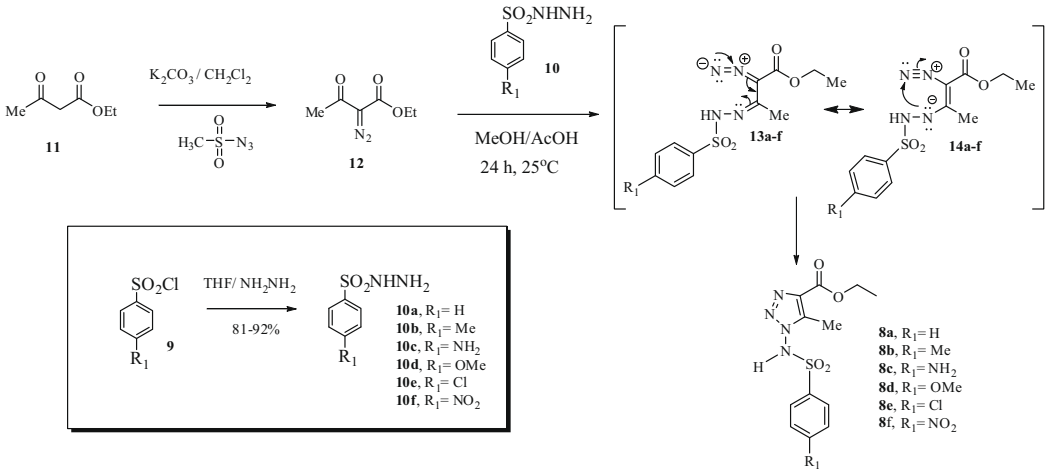


Figure 1. Natural **1–4** and synthetic compounds **5–7** active against snakebites.



Scheme 1. Synthetic pathways used for **8a–f**.

Table 1
Yields and melting points (mp) of the 1-arylsulfonylamino-5-methyl-1*H*-[1,2,3]-triazole-4-carboxylic acid ethyl esters **8a–f**

Sulphonylhydrazides	1,2,3-Triazole derivatives	R_1	Mp (°C)	Yield (%)
10a	**8a**	H	151–152	60
10b	**8b**	Me	140–141	61
10c	**8c**	NH_2	200–203	55
10d	**8d**	OMe	173–175	60
10e	**8e**	Cl	155–157	60
10f	**8f**	NO_2	195–197	63

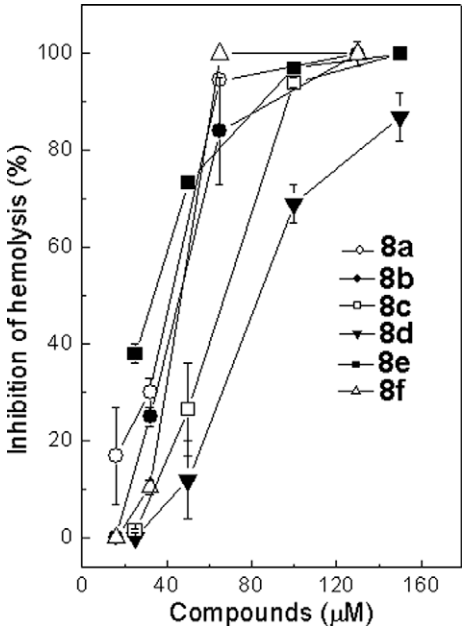


Figure 2. Effect of 1,2,3-triazoles derivatives **8a–f** on *L. muta*-induced hemolysis. Different concentrations of sulfonamide derivatives (16–130 μM) were pre-incubated with *L. muta* snake venom (90 μg/mL) for 30 min and then hemolytic test performed. Data are expressed as mean ± SD of three individuals experiments (n = 3).

triazole group may be contributing to a better biological profile (Fig. 3).

The electrostatic charge of the substituted carbon of phenyl ring varied among the triazoles probably due to the nature of the substituents. However, these values were not directly correlated to the biological activity observed (Table 2). As these substituents may be interacting with the unknown target through noncovalent interaction, apparently their nature is more important for the biological activity than their influence on phenyl carbon to which is attached.

In this work, we submitted the new series of triazole derivatives to the analysis[29] of 'Lipinski Rule of Five' that indicates if a chemical compound could be an orally active drug in humans. The 'Rule of Five' states that a compound violating any two of the following rules is likely to be poorly absorbed:[29] (1) molecular weight less than 500 Da; (2) number of hydrogen bond donors (OH or NH groups) equal to or less than 5; (3) number of hydrogen bond acceptors less than 10; and (4) calculated *c*log *P* less than 5. Our results showed that all compounds fulfilled this rule (molecular weight = 296.31–341.30, *c*log *P* = 2.6–3.4, *n*HBA = 8–11 and *n*HBD = 1–3) pointing for a good theoretical biodisponibility of this series (Table 2).

3. Conclusion

In summary, a novel family of 1-arylsulfonylamino-5-methyl-1*H*-[1,2,3]-triazole-4-carboxylic acid ethyl esters **8a–f** has been synthesized and evaluated for its ability to neutralize *L. muta* snake venom's hemolytic activity. All the compounds were able to neutralize hemolytic property of venom. Based on literature,[30,31] the triazole derivatives could be possibly affecting the *L. muta* venom phospholipase A_2 that is involved in the hemolytic profile of this snake venom. Since triazoles **8a–f** presented a promising antihemolytic profile, these compounds may be useful as prototypes for designing new antiophidian molecules to improve the current treatment used for *L. muta* bites.

4. Experimental

Chemical reagents and all solvents used in this study were purchased from Merck AG (Darmstadt, Germany) and VETEC LTDA (Rio de Janeiro, Brazil). Melting points were determined with a Fisher-Johns instrument and are uncorrected. Infrared (IR) spectra were recorded on Perkin–Elmer FT-IR model 1600 senes spectrophotometer, in KBr pellets. NMR spectra, unless otherwise stated, were obtained in deuterated $CDCl_3$ using a Varian Unity Plus 300 MHz spectrometer. Chemical shifts (δ) are expressed in ppm and the coupling constants (J) in hertz. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was performed in positive ion mode on a Waters-Micromass Q-Tof Micro instrument. The progress of all reactions was monitored by TLC performed on 2.0 cm × 6.0 cm aluminum sheets precoated with

Table 2
Comparison of HOMO and LUMO energies (E_{Homo} and E_{Lumo}), water solubility (log *S*), electrostatic charge of the substituted carbon of phenyl ring (Elect C), and Lipinski profile, including lipophilicity (clog *P*), molecular weight (MW) and the number of hydrogen bond donor and acceptor groups (HBD and HBA, respectively) of the triazole derivatives

#	R_1	IC_{50} (μM)	Energy (eV)		Elect C	Log *S*	Lipinski Rule of Five			
			HOMO	LUMO			*c*Log *P*	MW (Da)	*n*HBA	*n*HBD
8a	H	41 ± 4.1	−10.10	1.27	−0.047	−2.42	2.78	310.33	8	1
8b	Me	44 ± 5.8	−10.05	1.33	0.547	−2.76	3.1	324.36	8	1
8c	NH_2	63 ± 3.4	−9.29	1.23	0.329	−2.5	2.06	325.35	9	3
8d	OMe	83 ± 5.5	−9.75	1.27	0.665	−2.44	2.68	340.36	9	1
8e	Cl	30 ± 0.57	−10.19	1.19	0.235	−3.16	3.4	344.78	8	1
8f	NO_2	44 ± 4.5	−10.36	0.4	0.038	−2.88	2.65	355.33	11	1

Table 3
Cross-correlation matrix of the experimental biological activities (pIC_{50}) for the triazole derivatives (**8a–f**) and the calculated parameters: E_{Homo} and E_{Lumo} (HOMO and LUMO energies, Ev), electrostatic charge of the substituted carbon of phenyl ring (Elect C), water solubility (log *S*), μ (molecular dipole moment, Debye), MSA (molecular surface area, $Å^2$), MW (molecular weight, Da), *c*log *P* (calculated octanol/water partition coefficient), *c*log *S* (calculated water solubility), and *n*HBA (number of hydrogen bond acceptors, that is, number of N and O atoms) and *n*HBD (number of hydrogen bond donors)

	IC_{50}	E_{Homo}	E_{Lumo}	Elect C	Log *S*	*c*Log *P*	MW	*n*HBA	*n*HBD	μ	MSA	PSA
IC_{50}	1.00											
E_{Homo}	0.67	1.00										
E_{Lumo}	0.20	0.50	1.00									
Elect C	0.64	0.45	0.49	1.00								
Log *S*	0.69	0.59	0.36	0.16	1.00							
*c*Log *P*	−0.61	−0.72	0.14	0.02	−0.67	1.00						
MW	0.02	−0.36	−0.70	0.07	−0.62	0.17	1.00					
*n*HBA	0.21	−0.15	−0.91	−0.22	−0.07	−0.45	0.68	1.00				
*n*HBD	0.31	0.85	0.16	0.06	0.32	−0.77	−0.24	0.07	1.00			
μ	0.49	0.78	0.90	0.56	0.63	−0.29	−0.72	−0.66	0.46	1.00		
MSA	0.48	−0.05	−0.39	0.60	−0.21	0.00	0.78	0.57	−0.19	−0.28	1.00	
PSA	0.17	0.07	−0.83	−0.29	−0.03	−0.63	0.52	0.94	0.38	−0.52	0.38	1.00

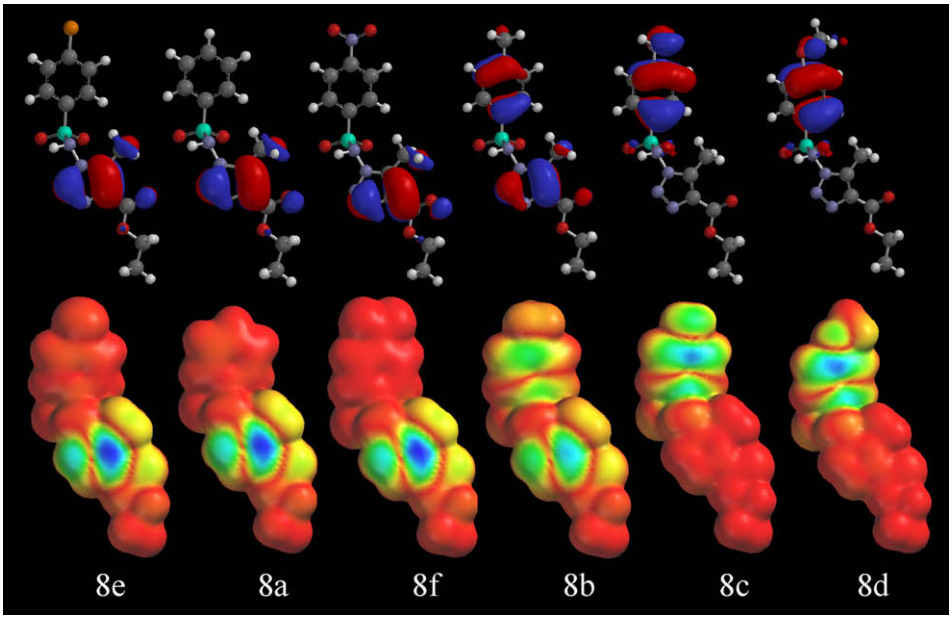


Figure 3. Coefficient distribution (up) and density maps (down) of the Highest Occupied Molecular Orbital (HOMO) of the compounds aligned according to the hemolysis IC_{50} potency (30–83 μM). Blue and red areas in the density map indicate higher and lower HOMO energy values, respectively.

Silica Gel 60 (HF-254, E. Merck) to a thickness of 0.25 mm. The developed chromatograms were viewed under ultraviolet light at 254 nm. E. Merck silica gel (60–200 mesh) was used for column chromatography. Microanalyses were performed using a Perkin–Elmer Model 2400 instrument and all values were within ±0.4 of the calculated compositions.

4.1. General procedure for the preparation of the 4-carbethoxy-triazole derivatives 8a–f

To the sulfonylhydrazide solution (**10a–f**, 1 mmol) in MeOH/acetic acid (5:1) (10 mL) was added ethyl 2-diazoacetoacetate (0.156 g, 1 mmol). Stirring was kept, at room temperature, for

24 h, and the resulting mixture was concentrated under reduced pressure. The residue was purified by column chromatography using silica gel and ethyl acetate:hexane (3:7) as eluent to give the pure triazoles **8a–f**.

4.1.1. 5-Methyl-1-(phenylsulfonylamino)-1*H*-[1,2,3]-triazole-4-carboxylic acid ethyl ester 8a

Obtained in 60% yield as a yellow solid; mp 151–152 °C; IR (KBr) ν_{max} (cm^{-1}) 3099 (N–H); 1694 (C=O), 1292 (C–O); ^{1}H NMR (300.00 MHz, $CDCl_3$) δ: 1.40 (t, 3H, J = 7.1, OCH_2CH_3), 2.61 (s, 3H, CH_3), 4.42 (q, 2H, J = 7.1, OCH_2CH_3), 7.45–7.50 (m, 2H, H-3′ and H-5′), 7.64 (tt, 1H, J = 7.5 and 1.2, H-4′), 7.69–7.73 (m, 2H, H-2′ and H-6′), 9.38 (br s, 1H, N–*H*) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 9.1 (CH_3), 14.3 (OCH_2CH_3), 61.3 (OCH_2CH_3), 128.5 (C-2′ and C-6′), 129.1 (C-3′ and C-5′), 134.2 (C-4′), 134.6 (C-4 or C-5), 136.4 (C-1′), 140.8 (C-4 or C-5), 160.8 (C=O) ppm. Anal. Calcd for $C_{12}H_{14}N_4O_4S$: C, 46.44; H, 4.55; N, 18.05. Found: C, 46.48; H, 4.39; N, 17.09. HRMS (ESI) $[M+Na]^+$ calcd for $C_{12}H_{14}N_4O_4SNa$ 333.0627. Found: 333.0622.

4.1.2. 5-Methyl-1-(4′-methylphenylsulfonylamino)-1*H*-[1,2,3]-triazole-4-carboxylic acid ethyl ester 8b

Obtained in 61% yield as a yellow solid; mp 140–141 °C; IR (KBr) ν_{max} (cm^{-1}) 3106 (N–H); 1697 (C=O); 1292 (C–O); ^{1}H NMR (300.00 MHz, $CDCl_3$) δ: 1.44 (t, 3H, J = 7.3, OCH_2CH_3), 2.43 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 4.42 (q, 2H, J = 7.1, OCH_2CH_3), 7.29 (d, 2H, J = 8.1, H-3′ and H-5′), 7.58 (d, 2H, J = 8.1, H-2′ and H-6′), 9.02 (br s, 1H, N–*H*) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 9.2 (CH_3), 14.2 (OCH_2CH_3), 21.7, (CH_3), 61.3 (OCH_2CH_3), 128.6 (C-2′ and C-6′), 130.0 (C-3′ and C-5′), 132.9 (C-1′), 135.0 (C-4 or C-5), 140.8 (C-4 or C-5), 145.9 (C-4′), 160.9 (C=O) ppm. Anal. Calcd for $C_{13}H_{16}N_4O_4S$: C, 48.14; H, 4.97; N, 17.27. Found: C, 48.03; H, 4.35; N, 16.63. HRMS (ESI) $[M+Na]^+$ calcd for $C_{13}H_{16}N_4O_4SNa$ 347.0784. Found: 347.0785.

4.1.3. 1-(4′-Aminophenylsulfonylamino)-5-methyl-1*H*-[1,2,3]-triazole-4-carboxylic acid ethyl ester 8c

Obtained in 55% yield as a yellow solid; mp 200–203 °C; IR (KBr) ν_{max} (cm^{-1}) 2997 (N–H); 1699 (C=O), 1264 (C–O); ^{1}H NMR (300.00 MHz, $CDCl_3$) δ: 1.27 (t, 3H, J = 7.2, OCH_2CH_3), 2.53 (s, 3H, CH_3), 4.42 (q, 2H, J = 7.2, OCH_2CH_3), 7.29 (d, 2H, J = 8.7, H-2′ and H-6′), 7.61 (d, 2H, J = 9.0, H-3′ and H-5′), 12.1 (br s, 2H, NH_2), 6.40 (br s, 1H, N–*H*) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 9.0 (CH_3), 9.6 (OCH_2CH_3), 60.6 (OCH_2CH_3), 112.7 (C-3′ and C-5′), 120.3 (C-1′), 130.2 (C-2′ and C-6′), 134.1 (C-4 or C-5), 140.1 (C-4 or C-5), 154.3 (C-4′), 160.5 (C=O) ppm. Anal. Calcd for $C_{12}H_{15}N_5O_4S$: C, 44.30; H, 4.65; N, 21.53. Found: C, 44.68; H, 5.07; N, 20.15. HRMS (ESI) $[M+Na]^+$ calcd for $C_{12}H_{15}N_5O_4SNa$ 348.0736. Found: 348.0738.

4.1.4. 5-Methyl-1-(4′-methoxiphenylsulfonylamino)-1*H*-[1,2,3]-triazole-4-carboxylic acid ethyl ester 8d

Obtained in 60% yield as a yellow solid; mp 173–175 °C; IR (KBr) ν_{max} (cm^{-1}) 3109 (N–H); 1699 (C=O), 1264 (C–O); ^{1}H NMR (300.00 MHz, $CDCl_3$) δ: 1.40 (t, 3H, J = 7.2, OCH_2CH_3), 2.65 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 4.41 (q, 2H, J = 7.2, OCH_2CH_3), 6.93 (d, 2H, J = 8.9, H-3′ and H-5′), 7.64 (d, 2H, J = 8.9, H-2′ and H-6′), 9.70 (br s, 1H, N–*H*) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 9.2 (CH_3), 14.2 (OCH_2CH_3), 55.7 (OCH_3), 61.3 (OCH_2CH_3), 114.5 (C-3′ and C-5′), 127.3 (C-1′), 130.9 (C-2′ and C-6′), 135.0 (C-4 or C-5), 140.8 (C-4 or C-5), 160.9 (C=O), 164.3 (C-4′) ppm. Anal. Calcd for $C_{13}H_{16}N_4O_5S$: C, 45.88; H, 4.74; N, 16.46. Found: C, 45.71; H, 4.96; N, 17.29. HRMS (ESI) $[M+Na]^+$ calcd for $C_{13}H_{16}N_4O_5SNa$ 363.0733. Found: 363.0724.

4.1.5. 1-(4′-Chlorophenylsulfonylamino)-5-methyl-1*H*-[1,2,3]-triazole-4-carboxylic acid ethyl ester 8e

Obtained in 60% yield as a yellow solid; mp 155–157 °C; IR (KBr) ν_{max} (cm^{-1}) 3095 (N–H); 1700 (C=O), 1289 (C–O); ^{1}H NMR (300.00 MHz, $CDCl_3$) δ: 1.40 (t, 3H, J = 7.3, OCH_2CH_3), 2.61 (s, 3H, CH_3), 4.41 (q, 2H, J = 7.3, OCH_2CH_3), 7.41 (d, 2H, J = 8.8, H-2′ and H-6′), 7.65 (d, 2H, J = 8.8, H-3′ and H-5′), 9.92 (br s, 1H, N–*H*) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 9.1 (CH_3), 14.2 (OCH_2CH_3), 61.5 (OCH_2CH_3), 129.4 (C-2′ and C-6′), 130.0 (C-3′ and C-5′), 134.7 (C-4 or C-5), 135.0 (C-1′), 140.9 (C-4 or C-5), 141.0 (C-4′), 160.9 (C=O) ppm. Anal. Calcd for $C_{12}H_{13}ClN_4O_4S$: C, 41.80; H, 3.80; N, 16.25. Found: C, 42.04; H, 4.09; N, 16.41. HRMS (ESI) $[M+Na]^+$ calcd for $C_{12}H_{13}ClN_4O_4SNa$ 367.0238. Found: 367.0236.

4.1.6. 5-Methyl-1-(4′-nitrophenylsulfonylamino)-1*H*-[1,2,3]-triazole-4-carboxylic acid ethyl ester 8f

Obtained in 63% yield as a yellow solid; mp 195–197 °C; IR (KBr) ν_{max} (cm^{-1}) 3114 (N–H); 1719 (C=O), 1288 (C–O); ^{1}H NMR (300.00 MHz, $CDCl_3$) δ: 1.27 (t, 3H, J = 7.2, OCH_2CH_3), 2.53 (s, 3H, CH_3), 4.42 (q, 2H, J = 7.2, OCH_2CH_3), 8.0 (d, 2H, J = 8.9, H-2′ and H-6′), 8.40 (d, 2H, J = 8.9, H-3′ and H-5′), 11.56 (br s, 1H, N–*H*) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 9.0 (CH_3), 14.0 (OCH_2CH_3), 60.6 (OCH_2CH_3), 124.6 (C-3′ and C-5′), 129.5 (C-2′ and C-6′), 134.3 (C-4 or C-5), 140.0 (C-4 or C-5), 143.2 (C-1′), 150.6 (C-4′), 160.4 (C=O) ppm. Anal. Calcd for $C_{12}H_{13}N_5O_6S$: C, 40.56; H, 3.69; N, 19.71. Found: C, 40.63; H, 3.81; N, 19.63. HRMS (ESI) $[M+Na]^+$ calcd for $C_{12}H_{13}N_5O_6SNa$ 378.0478. Found: 378.0492.

4.2. Antiophidic assays

4.2.1. Snake venom and antiserum

L. muta snake venom and anti-lachesis serum were provided from Fundação Ezequiel Dias (FUNED), Belo Horizonte, MG, Brazil.

4.3. Antihemolytic activity

The hemolytic activity of *L. muta* venom was determined by the indirect hemolytic test using rabbit erythrocytes and hen's egg yolk emulsion as substrate.[31] The activity was performed in a two-step reaction including (1) incubation of *L. muta* crude venom with egg yolk emulsion and (2) measurement of the hemolytic capacity of released lysolecithin by monitoring the hemoglobin at *A*578 nm. The compounds were pre-incubated with *L. muta* crude venom for 30 min at room temperature and then hemolytic activity was evaluated. The Inhibitory Concentration (IC_{50}) was determined as the concentration of compound (μM) able to inhibit 50% of hemolysis caused by snake venom.

4.4. Statistical analysis

Results are expressed as means ± SD obtained with the indicated number of antihemolytic assays performed. The statistical significance of differences among experimental groups was evaluated using ANOVA test. P value of <0.05 was considered statistically significant.

4.5. Molecular modeling

4.5.1. Structure–activity relationship (SAR) evaluation

The non-substituted derivative 5-methyl-1-(phenylsulfonylamino)-1*H*-[1,2,3]-triazole-4-carboxylic acid ethyl ester **8a** was submitted to the default systematic conformational analysis procedure, available in the SPARTAN'06 software package (Wavefunction Inc. Irvine, CA, 2000), using the MMFF94 force field,[32] and the

most stable conformer was used to construct the other derivatives **8b–f**. In order to evaluate the stereoelectronic properties, all structures were submitted to a full geometry optimization process, using the Recife Model 1 (RM1) semi-empirical Hamiltonian,[33] and, subsequently, to a single-point energy ab initio calculation, using Hartree–fock method at 6–311+G** level[34] available in SPARTAN'06. Then, some electronic properties, such as HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) energy and orbital coefficients distribution, molecular dipole moment (μ), and molecular electrostatic potential (MEP) maps were calculated.[35] In addition, descriptors, such as molecular volume, molecular surface area were calculated using SPARTAN'06, whereas molecular mass (MM), *c*log *P* (octanol/water partition coefficient) and *c*log *S* (water solubility) were calculated using the Osiris Property Explorer on-line system (available at http://www.organic-chemistry.org/prog/peo/).

Since the compounds are considered for oral delivery, they were also submitted to the analysis of 'Lipinski Rule of Five' using Molinspiration program (http://www.molinspiration.com/cgi-bin/properties).

Acknowledgments

The authors are grateful to FAPERJ, CAPES, CNPq, and the International Foundation for Science (IFS-Grant F/4571-1) for financial support and fellowships and would like to thank Universidade Federal do Rio de Janeiro, Departamento de Química Orgânica, LABEM—Laboratório Multiusuário de Espectrometria de Massas, for obtaining the mass spectra data.

References and notes

1. Fuly, A. L.; Machado, A. L.; Castro, P.; Abrahão, A.; Redner, P.; Lopes, U. G.; Guimarães, J. A.; Koatz, V. L. G. *Toxicon* **2007**, *50*, 400.
2. Fuly, A. L.; Calil-Elias, S.; Zingali, R. B.; Guimarães, J. A.; Melo, P. A. *Toxicon* **2000**, *38*, 961.
3. Colombini, M.; Fernandes, I.; Cardoso, D. F.; Moura-da-Silva, A. M. *Toxicon* **2001**, *39*, 711.
4. Stephano, M. A.; Guidolin, R.; Higashi, H. G.; Tambourgi, D. V.; Sant'Anna, O. A. *Toxicon* **2005**, *45*, 467.
5. Ferreira, T.; Camargo, E. A.; Ribela, M. T. C. P.; Damico, D. C.; Marangoni, S.; Antunes, E.; De Nucci, G.; Landucci, E. C. T. *Toxicon* **2009**, *53*, 69.
6. Oliveira, C. Z.; Menaldo, D. L.; Marcussi, S.; Santos-Filho, N. A.; Silveira, L. B.; Boldrini-França, J.; Rodrigues, V. M.; Soares, A. M. *Biochimie* **2008**, *90*, 1506.
7. Basappa; Kumar, M. S.; Swamy, S. N.; Mahendra, M.; Prasad, J. S.; Viswanath, B. S.; Rangappa, K. S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3679.
8. Pungerčar, J.; Križaj, I. *Toxicon* **2007**, *50*, 871.
9. Damico, D. C. S.; Nascimento, J. M.; Lomonte, B.; Ponce-Soto, L. A.; Joazeiro, P. P.; Novello, J. C.; Marangoni, S.; Collares-Buzato, C. B. *Toxicon* **2007**, *49*, 678.
10. White, J. *Toxicon* **2005**, *45*, 951.
11. Cardoso, J. L. C.; Fan, H. W.; França, F. O. S.; Jorge, M. T.; Leite, R. P.; Nishioka, S. A.; Avila, A.; Sano-Martins, I. S.; Tomy, S. C.; Santoro, M. L.; Chudzinski, A. M.; Castro, S. C. B.; Kamiguti, A. S.; Kelen, E. M. A.; Hirata, M. H.; Mirandola, R. M. S.; Theakston, R. D. G.; Warrell, D. A. *Quart. J. Med.* **1993**, *86*, 315.
12. da Silva, A. J. M.; Coelho, A. L.; Simas, A. B. C.; Moraes, R. A. M.; Pinheiro, D. A.; Fernandes, F. F. A.; Arruda, E. Z.; Costa, P. R. R.; Melo, P. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 431.
13. Mors, W. B.; Nascimento, M. C.; Pereira, B. M. R.; Pereira, N. A. *Phytochemistry* **2000**, *55*, 627.
14. Sánchez, E. E.; Rodríguez-Acosta, A. *Immunopharmacol. Immunotoxicol.* **2008**, *30*, 647.
15. Souza, A. D. L.; Rodrigues-Filho, E.; Souza, A. Q. L.; Pereira, J. O.; Calgarotto, A. K.; Maso, V.; Marangoni, S.; Da Silva, S. L. *Toxicon* **2008**, *51*, 240.
16. Ticli, F. K.; Hage, L. I. S.; Cambraia, R. S.; Pereira, P. S.; Magro, A. J.; Fontes, M. R. M.; Stábeli, R. G.; Giglio, J. R.; França, S. C.; Soares, A. M.; Sampaio, S. V. *Toxicon* **2005**, *46*, 318.
17. Murakami, M. T.; Arruda, E. Z.; Melo, P. A.; Martinez, A. B.; Calil-Elias, S.; Tomaz, M. A.; Lomonte, B.; Gutierrez, J. M.; Ami, R. K. *J. Mol. Biol.* **2005**, *350*, 416.
18. Fernandes, R. S.; Assafim, M.; Arruda, E. Z.; Melo, P. A.; Zingali, R. B.; Monteiro, R. Q. *Toxicon* **2007**, *49*, 931.
19. Murakami, M. T.; Gava, L. M.; Zela, S. P.; Arruda, E. Z.; Melo, P. A.; Gutierrez, J. M.; Arni, R. K. *Biochim. Biophys. Acta* **2004**, *1703*, 83.
20. Khanum, S. A.; Murari, S. K.; Vishwanth, B. S.; Shashikanth, S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4100.
21. Cunha, A. C.; Figueiredo, J. M.; Tributino, J. L. M.; Miranda, A. L. P.; Castro, H. C.; Zingali, R. B.; Fraga, C. A. M.; De Souza, M. C. B. V.; Ferreira, V. F.; Barreiro, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 2051.
22. Menegatti, R.; Cunha, A. C.; Ferreira, V. F.; Perreira, E. F. R.; El-Nabawi, A.; Eldefrawi, A. T.; Albuquerque, E. X.; Neves, G.; Rates, S. M. K.; Fraga, C. A. M.; Barreiro, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 4807.
23. Da Silva, E. N., Jr.; Menna-Barreto, R. F. S.; Pinto, M. C. F. R.; Silva, R. S. F.; Teixeira, D. V.; de Souza, M. C. B. V.; De Simone, C. A.; De Castro, S. L.; Ferreira, V. F.; Pinto, A. V. *Eur. J. Med. Chem.* **2008**, *43*, 1774.
24. Gallardo, H.; Conte, G.; Bryk, F.; Lourenço, M. C. S.; Costa, M. S.; Ferreira, V. F. *J. Braz. Chem. Soc.* **2007**, *18*, 1285.
25. Ferreira, S. B.; Costa, M. S.; Boechat, N.; Bezerra, R. J. S.; Genestra, M. S.; Canto-Cavalheiro, M. M.; Kover, W. B.; Ferreira, V. F. *Eur. J. Med. Chem.* **2007**, *42*, 1388.
26. Jordão, A. K.; Ferreira, V. F.; Lima, E. S.; De Souza, M. C. B. V.; Carlos, E. C. L.; Castro, H. C.; Geraldo, R. B.; Rodrigues, C. R.; Almeida, M. C. B.; Cunha, A. C. *Bioorg. Med. Chem.* **2009**, *17*, 3713.
27. Danheiser, R. L.; Miller, R. F.; Brisbois, R. G.; Park, S. Z. *J. Org. Chem.* **1990**, *55*, 1959.
28. Audrieth, L. F.; vox Brhuchitsch, M. *J. Org. Chem.* **1956**, *21*, 426.
29. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
30. Fuly, A. L.; Francischetti, I. M.; Zingali, R. B.; Carlini, C. R. *Braz. J. Med. Biol Res.* **1993**, *26*, 459.
31. Fuly, A. L.; de Miranda, A. L. P.; Zingali, R. B.; Guimarães, J. A. *Biochem. Pharmacol.* **2002**, *63*, 1589.
32. Halgren, T. A. *J. Comput. Chem.* **1999**, *20*, 720.
33. Rocha, G. B.; Freire, R. O.; Simas, A. M.; Stewart, J. J. P. *J. Comput. Chem.* **2006**, *27*, 1101.
34. Davidson, E. R.; Feller, D. *Chem. Rev.* **1988**, *86*, 661.
35. Da Silva, F. C.; De Souza, M. C. B. V.; Frugulhetti, I. I. P.; Castro, H. C.; De Souza, S. L. O.; Souza, T. M. L.; Rodrigues, D. Q.; Souza, A. M. T.; Abreu, P. A.; Passamani, F.; Rodrigues, C. R.; Ferreira, V. F. *Eur. J. Med. Chem.* **2009**, *44*, 373.