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RESEARCH ARTICLE

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Synthesis, activity, and molecular modeling studies of 1,2,3-triazole derivatives from natural phenylpropanoids as new trypanocidal agents

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Abstract

The search for compounds with new structural scaffolds is an important tool to the discovery of new drugs against Chagas disease. We report herein the synthesis of 1,2,3-triazoles obtained from eugenol and di-hydroeugenol and their in vitro and in vivo trypanocidal activity. These derivatives were obtained by a three-step objective route and were suitably characterized by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry. Two compounds (**9** and **10**) showed activity against epimastigote forms of *Trypanosoma cruzi* (Y strain) in the range 42.8–88.4 μM and were weakly toxic to cardiomyoblast cells (H9c2 cells). The triazole **10** was the most active derivative and could reduce more than 50% of parasitemia after a 100-mg/kg oral treatment of mice infected with *T. cruzi*. Molecular docking studies suggested this compound could act as a trypanocidal agent by inhibiting cruzain, an essential enzyme for *T. cruzi* metabolism, usually inhibited by triazole compounds.

KEYWORDS

1,2,3-triazoles, cruzain inhibitors, di-hydroeugenol, eugenol, trypanocidal activity

1 | INTRODUCTION

Eugenol (4-allyl-2-methoxyphenol) is a natural phenylpropanoid present in essential oils of some plants as India's clove (*Syzygium aromaticum*) and cinnamon-brava (*Croton zenhtneri*). Several authors have reported different biological activities for eugenol, analogues, and derivatives, such as the antimicrobial (Dai et al., 2013; Yadav, Chae, Im, Chung, & Song, 2015), antitumoral (Manikandan, Senthil, Priyadarsini, Vinothini, & Nagini, 2010), anti-inflammatory (Daniel et al., 2009), anti-parasitic (Machado et al., 2011), and antioxidant (Gülçin, 2011) actions.

In view of this great interest, our research group has been working on the chemical manipulation of this allylphenol and its analogues, as isoeugenol and di-hydroeugenol, in order to identify new active derivatives from them. Recently, we have discovered some derivatives of eugenol with antibacterial (Cazelli et al., 2017; Souza et al., 2015) and antifungal (Abrão et al., 2015; Souza et al., 2016, 2014) actions (Figure 1).

Following our efforts in obtaining optimized derivatives of eugenol, we have designed a new structural pattern containing the eugenol or di-hydroeugenol nucleus attached to a 1,2,3-triazole ring. This structural pattern was designed considering the biological versatility of eugenol or di-hydroeugenol combined with different reports of di-substituted 1,2,3-triazole derivatives active against *T. cruzi* (Andrade et al., 2015; Silva et al., 2008; Porta et al., 2017). This heterocycle is isostere of 1,2,4-triazole and imidazole rings present in different drugs as fluconazole, ketoconazole, benznidazole, and metronidazole. Then, we hypothesized that the triazole core could help to improve the antiparasitc properties of phenylpropanoids. Thus, we synthesized these derivatives and evaluated them as trypanocidal agents by in vitro and in vivo activity tests. Further, we performed molecular docking studies to check its ability to interact with cruzain as a possible target in *T. cruzi*.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The 1,2,3-triazole derivatives from eugenol and di-hydroeugenol were synthesized in a short and objective synthetic route as shown in Scheme 1.

Firstly, eugenol (**1**) and di-hydroeugenol (**2**) were converted to the epoxides **3** and **4**, respectively, by reactions with epychlorohydrine (Jin et al., 2004), which in sequence afforded the alkylazides **5** and **6** after ring opening with sodium azide (Carvalho et al., 2010). The reaction of alkyl azides with different alkynes by a click reaction afforded the triazoles **7–14** in good yields after purification by column chromatography, following the procedure described by Souza et al. (2015). The click reaction is a 1,3-dipolar cycloaddition catalyzed by Cu(I) and has been largely employed for the synthesis of biologically actives 1,2,3-triazoles (Deobald et al., 2011; Freitas et al., 2011). In the ^{1}H nuclear magnetic resonance spectra of compounds **7–14**, it was possible to observe a signal corresponding to the triazole proton near 8 ppm, besides the diastereotopic methylene protons as a double of doublets between 3.5 and 4.5 ppm.

2.2 | In vitro assays

The cytotoxicity of the compounds was evaluated against cardiac cells obtained from neonatal rat cardiomyoblasts (H9c2 cells). Following, the selectivity indexes (SI) of the synthesized compounds could be determined. All derivatives and the two phenylpropanoids were evaluated against epimastigote forms of *Trypanosoma cruzi* by the resazurin microtiter assay (Table 1). The triazole **10**, obtained from di-hydroeugenol, showed inhibition at 42.8 μM against this form of the parasite, similarly to that presented by the control drug benznidazole. Although this derivative had a lower SI than benznidazole, it can be considered an innovative structural core for optimization and design of new tripanocidal agents. Moreover, the triazole **10** was twice as active as the corresponding eugenol derivative **9** (IC_{50}

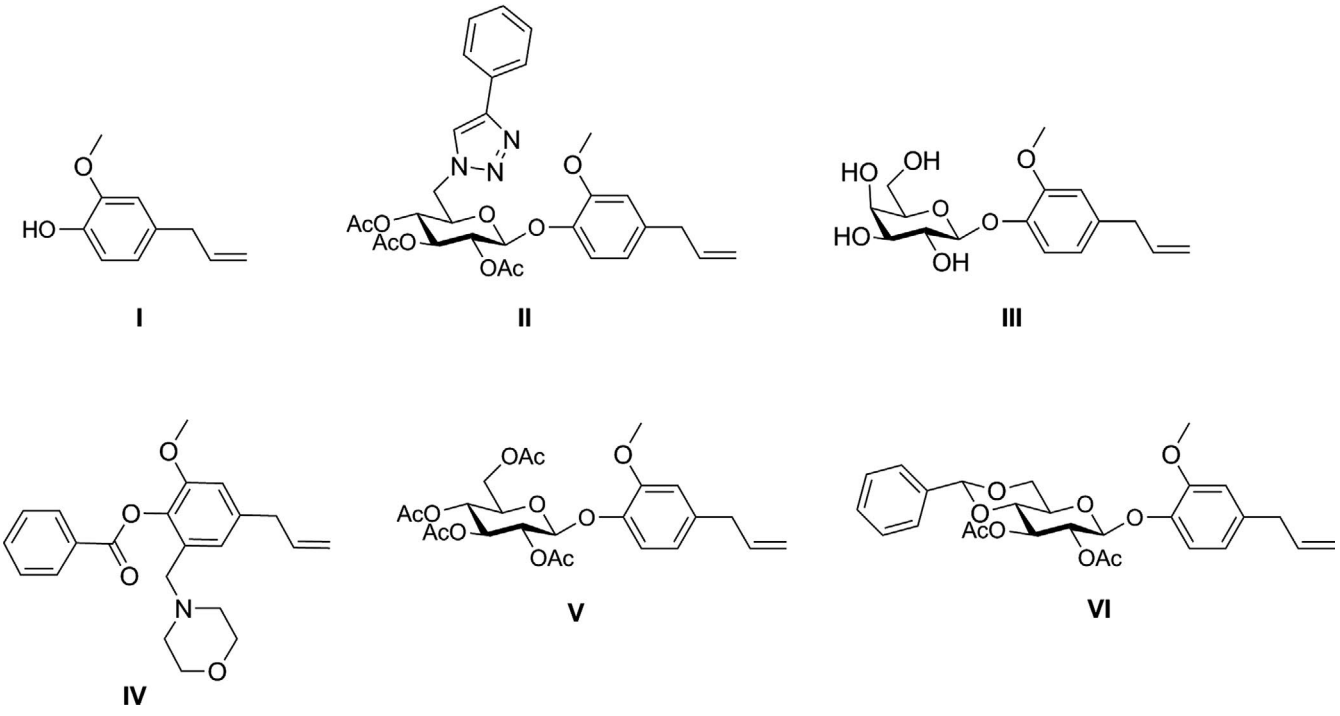


FIGURE 1 Eugenol (**I**) and derivatives with antibacterial (**II** and **III**) and antifungal (**IV**, **V,** and **VI**) activities

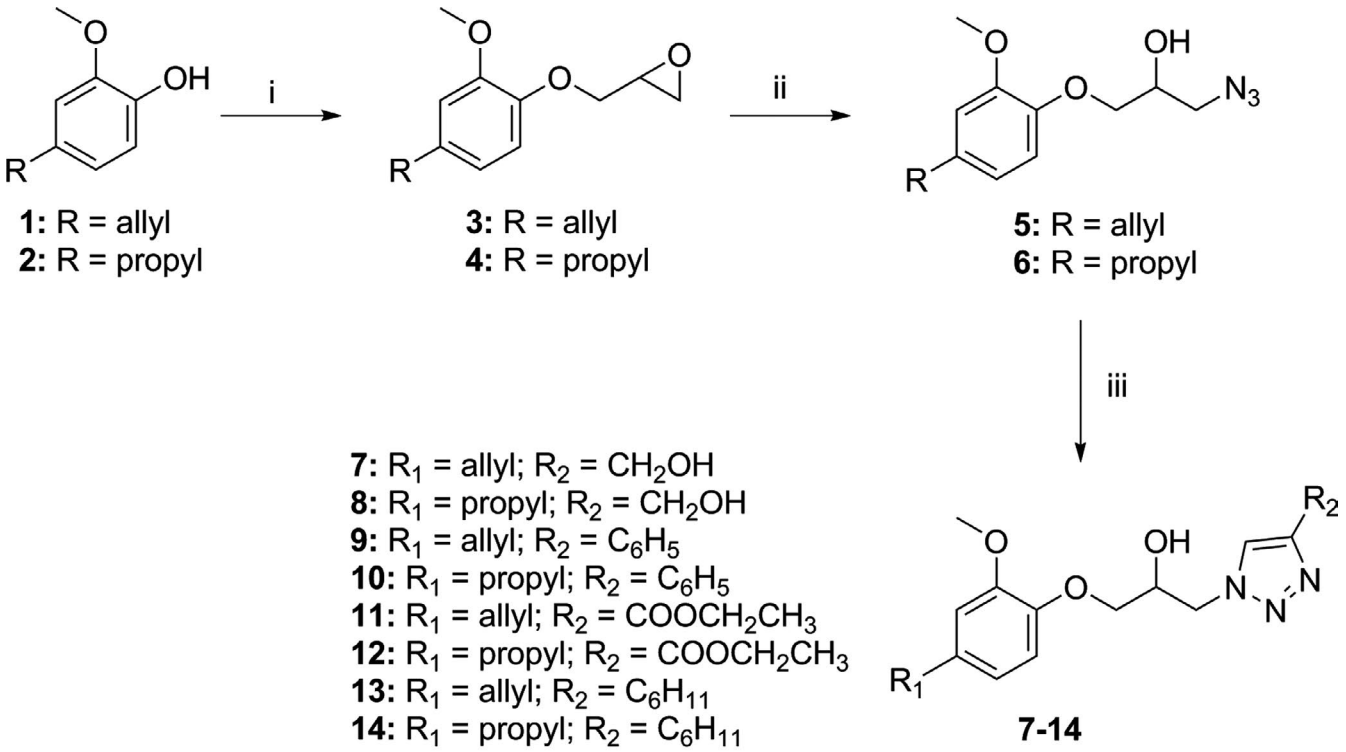
88.4 μM) pointing the importance of the *n*-propyl side chain for this activity. It is possible to note among these 1,2,3-triazoles that the phenyl group (present in derivatives **9** and **10**) was the best substituent at the triazole core, because derivatives with hydroxymethyl (derivatives **7** and **8**), acetyl (derivatives **11** and **12**) or cyclohexyl (derivatives **13** and **14**) groups showed lower or no trypanocidal activity.

2.3 | Docking studies

Some azoles are known as compounds that show anti-trypanosomal activity and this action may come from cruzain inhibition (Brak et al., 2010). The compounds synthesized in this work present a di-substituted 1,2,3-triazole ring, so they could act as such possibly by inhibiting this enzyme, which is crucial in trypanosomatides survival. In the current study, a crystallographic complex formed by this enzyme and a di-substituted 1,2,3-triazole inhibitor (1,2,3-triazole-tetrafluorophenoxymethyl ketone) was selected as the model (Brak et al., 2010). Docking studies were carried using the conformers of minimal energy from compounds **9** and **10** with chemical structures generated by Spartan software (Shao et al., 2006), and the results from the main interactions with cruzain are shown in Tables 2 and 3. According to these studies, it was possible to indicate the individual poses relative to a selective binding to the cruzain active site.

The empirical scoring function of iGemdock is characterized by the sum of Van der Waals, H-bonding, and electrostatic energies. At this point, the best poses for ligands **9** and **10** showed affinity energies of −102.25 and −102.05 kcal/mol, respectively. Calculated values for both molecules were similar to each other but the compound **9** showed lower Van der Waal and higher H-bonding values in comparison to compound **10**. No electrostatic interactions were observed for these compounds and the amino acid residues in cruzain active site.

The data of docking experiments indicate that the main interactions between the triazoles **9** and **10** and cruzain active site involve the residues GLY 23, CYS 25, TRP 26, SER 64, GLY 65, GLY 66, LEU 67, MET 68, LEU 160, HIS 162, and GLY 163. Additionally, the active interactions between the compounds **9** and **10** and cruzain active site are shown in Figure 2. After applying the postscreening analysis GLY 65 was detected as the main residue evolved in this



i: Epichlorohydrin, KOH, EtOH, r.t.; ii: NaN_3, H_2O, CH_3CN, 80°C; iii: corresponding alkine, sodium ascorbate, copper acetate, THF/H_2O, r.t.

SCHEME 1 Synthesis of 1,2,3-triazole derivatives from eugenol and di-hydroeugenol

ligand–receptor binding (with *Z*-score −1.09 and WPharma 1.00). Some of these residues (CYS 25, GLY 65, GLY 66, and LEU 67) are the same observed in the docking study performed by Brak et al. (2010) with tetrafluorophenoxymethyl ketone and the active site of cruzain (figure available in "Supporting Information").

According to the results generated by these docking studies, 1,2,3-triazoles **9** and **10** interact in the same region at the cruzain active site as observed in the model generated with the 1,2,3-triazole inhibitor (Brak et al., 2010). The main intermolecular interactions are those observed between GLY 23 and the triazole ring/N3 (2.74 Å), CYS 25 thiol and the N-2 from the same ring (2.20 Å). Other important H-bonding interaction observed in this model occurs between the secondary alcohol and the GLY 163 nitrogen (2.5 Å). These results show a good predicted binding pose between derivatives **9** and **10** and suggest they may be candidates to structural optimization to follow development as trypanocidal agents acting probably as cruzain inhibitors, a special target used in anti-trypanosomatide drug design.

TABLE 1 In vitro activity (IC_{50}) against epimastigote forms of *T. cruzi*, cytotoxicity (CC_{50}) against H9c2 cells and SI for eugenol (**1**), di-hydroeugenol (**2**) and derivatives **7–14**

Compound	*T. cruzi* IC_{50} (μM)	*H9c2 cells* CC_{50} (μM)	Selectivity Index
1	383.3	>1,000	>3.2
2	658.6	>1,000	>1.8
7	—[a]	144.4	—
8	293.9	319.4	1.1
9	88.4	141.4	1.6
10	42.8	155.3	3.6
11	—	>500	—
12	—	>500	—
13	108.2	363.6	3.3
14	178.9	375.0	2.1
Bzn	35.8	655.1	18.3

Abbreviation: —[a]: no significant activity; Bzn: Benznidazole; SI: CC_{50}/IC_{50}.

2.4 | In vivo assay

The two most potent derivatives against *T. cruzi* epimastigote forms (compounds **9** and **10**) were evaluated in mice infected with *T. cruzi* (Y strain) trypomastigotes. These compounds were given orally for seven consecutive days as suspensions at 100 mg/kg weight, and benznidazole was used as the control drug (Table 4).

TABLE 2 Docking results and Van der Waals (VDW), H-bond, and electrostatic interactions (in kcal/mol) for derivatives **9** and **10** in *Trypanosoma cruzain* active site (PDB code 3IUT), using IGemdock 2.1 software

Compounds	Affinity energy (Kcal/mol)	VDW (Kcal/mol)	H-bond (Kcal/mol)	Electrostatic (Kcal/mol)
9	−102.25	−82.88	−19.37	0.0
10	−102.05	−87.37	−14.68	0.0

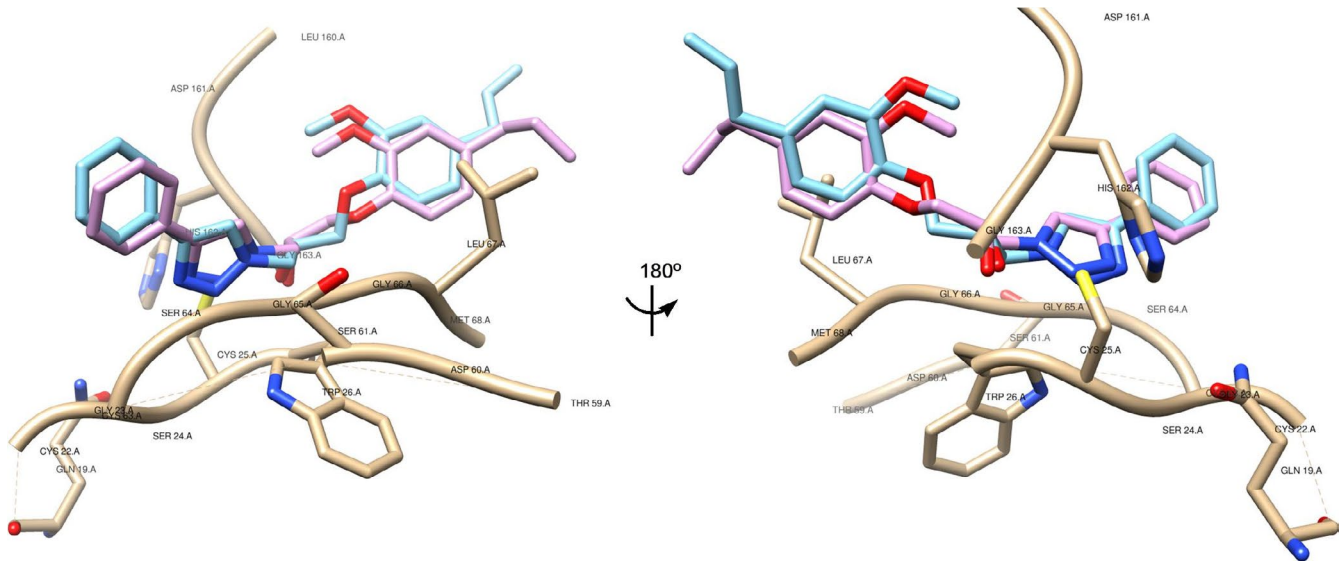


FIGURE 2 Binding poses of triazoles **9** (blue) and **10** (purple) in the *Trypanosoma cruzi* cruzain active site (3IUT) calculated by Igemdock 2.1 and visualized using Chimera software (v. 1.10.1)

TABLE 3 Central Van der Waals and H-bond interactions (in kcal/mol) of compounds **9** and **10** with the *Trypanosoma cruzi* cruzain active site (PDB code 3IUT) applying the Residues Consensus Analysis using Igemdock 2.1 software

Compounds interactions[a]	GLY 23	CYS 25	TRP 26	SER 64	GLY 65	GLY 66	LEU 67	MET 68	LEU 160	HIS 162	GLY 163
9 Van der Waals[a]	−5.9	−4.0	−5.1	−6.3	−7.3	−5.4	−4.6	−5.5	−4.7	−5.4	0.0
H-Bond[a]	−5.8	−6.5	−3.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	−3.5
10 Van der Waals[a]	−6.1	−4.2	−7.6	−6.3	−7.8	−7.1	−7.2	−6.7	−2.5	−6.3	0.0
H-Bond[a]	−2.6	−5.1	−3.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	−3.5

[a]Total interaction energy in kcal/mol.

TABLE 4 In vivo evaluation of compounds **9** and **10** in the reduction of parasitaemia

Compound	Number of parasites/0.1 ml blood
UA	1,425.000
Bzn	18.333
9	1,101.000
10	625.000

Note: The values represent the means of parasitemia obtained in peripheral blood samples from mice infected with 5,000 of *T. cruzi* (Y strain) trypomastigotes in the maximum peak of parasitemia.
Abbreviation: UA, untreated animals.

Untreated infected animals showed maximal parasitemia (1.425×10^6 parasites/ml blood) as expected. The dihydroeugenol derivative **10** reduced more than 50% of the parasitemia when compared to the untreated animals. The compound **9** showed no significant ability to reduce the infection. Although compound **10** was not as good as benznidazole in reducing parasitaemia, it is important to note that it has an innovative structural core. As such it can be seen as an alternative in acting against benznidazole-resistant parasites.

3 | CONCLUSION

Eight new 1,2,3-triazoles were synthesized from eugenol and di-hydroeugenol and were assayed as trypanocidal agents. Some important structure–activity relationships could be noted for best trypanocidal activity and higher selectivity index, as a preference for a propyl instead of an allyl side chain in the phenylpropanoid residue and for a phenyl group as the substituent attached to the triazole ring. Docking studies showed that the triazoles **9** and **10** interact with the active site of cruzain similarly to a well-known 1,2,3-triazole inhibitor of this enzyme. In vivo studies showed that the triazole **10** could reduce parasitaemia in infected mice and, as such, can be seen as a good prototype for the development of new anti-trypanosomal agents.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

All experimental protocols were conducted in accordance with the guidelines issued by the Brazilian College of Animal Experimentation (COBEA) and approved by the Ethics Committee in Animal Research at UNIFAL-MG (number 59/2017).

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as all data have been made available in the "Supplementary Material" section.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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