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Trypanocidal agents with low cytotoxicity to mammalian cell line: A comparison of the theoretical and biological features of lapachone derivatives

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Abstract—Starting from α- and β-lapachones, in this work we compared the biological and theoretical profile of several oxyran derivatives of lapachone as potential trypanocidal agents. Our biological results showed that the oxyrans tested act as trypanocidal agents against *Trypanosoma cruzi* with minimal cytotoxicity in the VERO cell line compared to naphthoquinones. The oxyran derivative of α-lapachone (**7a**) showed to be one of the most potent compounds. In our molecular modeling study, we analyzed the C-ring moiety and the redox center of β-lapachone molecule as the moieties responsible for the trypanocidal and cytotoxic effects on mammalian cell line. The computational methods used to delineate the structural requirements for the trypanocidal profile pointed out that the transposition of the C-ring moiety of β-lapachone, combined with its oxyran ring, introduced important molecular requirements for trypanocidal activity in the HOMO energy, HOMO orbital coefficient, LUMO density, electrostatic potential map, dipole moment vector, and calculated log *P* (clog *P*) parameter. This study could lead to the development of new antichagasic medicines based on α-lapachone analogs.

1. Introduction

American trypanosomiasis (Chagas' disease) is an important endemic disease caused by *Trypanosoma cruzi*, which affects more than 20 million people in Central and South America.[1,2] Currently, the treatment of this disease involves the use of the nitroheterocyclic benznidazole, which produces severe side effects.[3–5] In addition, the efficiency of the treatment depends on the susceptibility of different *T. cruzi* strains. Despite the progress made in the study of *T. cruzi* biochemistry and physiology, this parasitic infection remains part of a group of neglected diseases, for which chemotherapy needs to be developed.[6]

Quinones have been studied for antitumor,[7] molluscicidal,[8–10] antiparasitic,[11] anti-inflammatory,[12] antifungic,[13,14] antimicrobial,[15] and trypanocidal[16–18] activities. Literature points out that the biological profiles of these molecules are centered on their *ortho*- or *para*-quinonoid moiety. This group generally accepts one and/or two electrons (redox cycling) to form the corresponding radical anion or dianion species in situ.[19] Thus, the semi-quinone radicals accelerate intracellular hypoxic conditions by producing superoxide anion.[20–22] Due to this mechanism, quinones may present cytotoxicity in the mammalian cells, possibly by affecting enzymes such as topoisomerases, a group of enzymes that are critical for DNA replication in cells.[23]

Keywords: β-Lapachone; Trypanocidal agent; α-Lapachone; Cytotoxicity; Computational.
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β-Lapachone (**1**) is a 1,2-naphthoquinone (Fig. 1) isolated from the bark of the Lapacho tree (*Tabebuia avellanedae*).[23,24] As other quinones, **1** possesses a variety of pharmacological effects, including trypanocidal activity.[25–28] However, this molecule is also cytotoxic against several cell lines.[29,30]

Although the development of new effective compounds for the treatment of Chagas' disease is of interest, studies for designing new trypanocidal agents based on **1** were decelerated due to its high cytotoxicity against mammalian cells. On the other hand, the identification of **1** as a DNA topoisomerase I inhibitor increased the interest in developing itself as an anti-cancer agent.[23] More recently, searching for new compounds with reduced cytotoxicity while maintaining the trypanocidal profile of **1**, some derivatives were prepared modified at the redox center. Interestingly, Neves-Pinto et al. increased the trypanocidal activities of **1** by turning the *o*-quinone moiety into oxazoles (**2a**), imidazoles (**2b**),[31] and phenazine (**3**).[32] The electron density on the redox center of quinones structures is, indeed, affected by the addition of different substituents to the system.[33]

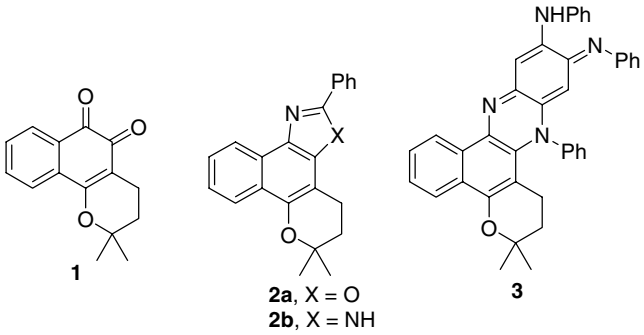
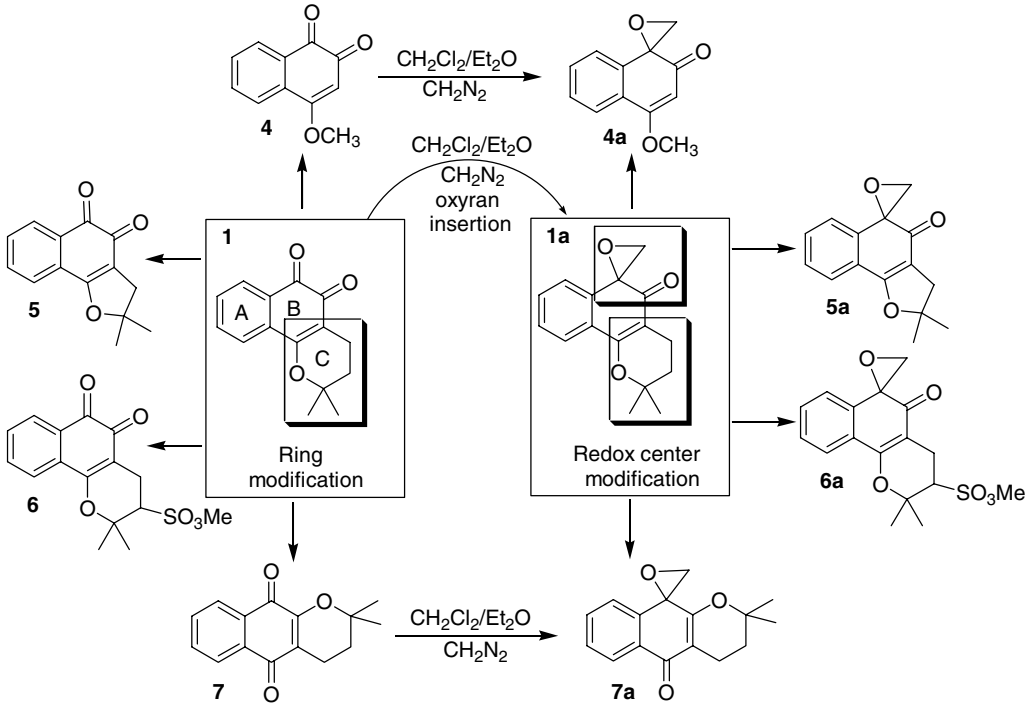


Figure 1. Some important derivatives of **1**.

In this work, we focused on the trypanocidal and cytotoxic effect on mammalian cell line of several oxyran derivatives of lapachones and naphthoquinones. Additionally, a theoretical comparison between these compounds regarding the redox center and C-ring moiety (pyran ring) was performed to determine the structural and stereoelectronic features that could lead to compounds presenting trypanocidal activity with low cytotoxicity against mammalian cells (Scheme 1). In order to analyze the role of the C-ring moiety of **1**, we used the following modifications: (a) the elimination of the C-ring with the maintenance of the oxygen atom as is the case of derivative **4** that possesses a methoxy group; (b) the contraction of the C-ring from 6 to 5 members (nor-β-lapachone) (**5**); (c) the replacement of the hydrogen closest to the dimethyl group in the C-ring by a methanesulfonate group, a more bulky and polar substituent (**6**); and (d) the transposition of the C-ring moiety (α-lapachone) (**7**) (Scheme 1). Considering the theoretical studies performed and the importance of the redox center for β-lapachone activity (**1**), we synthesized the oxyrans forms (**1a**, **4a**, **5a**, **6a**, and **7a**) from the corresponding naphthoquinones (**1**, **4**, **5**, **6**, and **7**) (Scheme 1). Theoretical parameters (HOMO's energy, HOMO orbital coefficients distribution, LUMO density, dipole moment, dipole moment vector and lipophilicity—c log *P*) for all derivatives were calculated by semi-empirical method, and correlated with the trypanocidal and cytotoxicity effects.



Scheme 1. General synthetic routes and structural features of compounds.

2. Chemistry

2.1. General procedures

Compound **1**, 4-methoxy-1,2-naphthoquinone (**4**), nor-β-lapachone (**5**), and α-lapachone (**7**) were prepared by standard procedures.[34] Reactions of the naphthoquinones with diazomethane leading to oxyrans had been previously described and their structures unequivocally established.[24,35,36] Briefly, the oxyrans (**1a**, **4a**, **5a**, **6a** and **7a**) were prepared from the corresponding naphthoquinones (**1**, **4**, **5**, **6** or **7**), by adding an ethereal solution of freshly prepared diazomethane (excess). The mixtures were kept at 5 °C for 48 h, and then the solvent was evaporated under reduced pressure. The resulting compounds were purified using a silica-gel chromatography column eluted with a gradient mixture of hexane and ethyl acetate. Spectroscopic data for compounds **1a**, **4a**, **5a**, and **7a** have been already reported elsewhere.[36,37]

2.1.1. 2-Methyl sulfonate-3-dihydro-2,2-dimethyl-spiro-[4*H*-1-oxafenanthrene-6,2′-oxyran]-5(6*H*)-one (6a). Obtained in 65% as slightly yellow oil. IR ν_{max} (cm^{-1}, film): 1652 (C=O), 1124 (SO_2), ^1H NMR (300 MHz, $CDCl_3$) δ 3.51 (dd, 1H, $J = 11.7$, 5.5), 3.15 (dd, 1H, $J = 17.7$, 5.5), 2.82 (dd, 1H, $J = 17.7$, 11.7), 7.12 (1H, ddd, $J = 7.4$, 2.1, 1.6), 7.43 (1H, ddd, $J = 12.9$, 7.4, 1.6), 7.38 (1H, ddd, $J = 12.9$, 7.2, 2.1), 7.80 (1H, ddd, $J = 7.2$, 2.1, 1.6), 3.43 (d, 1H, $J = 8.0$), 3.10 (d, 1H, $J = 8.0$), 3.96 (3H, s), 1.85 (3H, s), 1.56 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 78.3, 61.0, 19.5, 107.6, 190.1, 55.1, 128.3, 122.9, 130.6, 128.1, 123.7, 135.9, 160.5, 62.1, 55.8, 21.4, 28.1.

3. Biology

3.1. Trypanocidal assay

Stock solutions of all quinones (**1**, **4**–**7**) and oxyrans (**1a**, **4a**–**7a**) (5 mM) were prepared in dimethylsulfoxide (DMSO). Active *T. cruzi* Dm28c epimastigote forms from 1st and 3rd days cultures in BHI medium were counted in a Neubauer chamber using an optical microscopy (Olympus BX41). The parasites were incubated initially with the compounds at six different concentrations (50–0.3 μM), each one in quadruplicate, in order to determine the antiproliferative 50% inhibitory concentration against *T. cruzi* (IC_{50}). The final concentration of DMSO in the experiments never exceeded 1%. The cultures were evaluated after 72 h of incubation. Non-treated epimastigote forms control corresponded to 100% of living cells with no drug treatment. Results, calculated using the Probit statistical program, are presented in Table 1. Cultures containing non-treated epimastigote forms and 1.0% DMSO (v/v) were also included as negative controls, while the lead compound (**1**) was used as positive control. Crystal violet was also used as an additional molecule for comparison such as described elsewhere.[37,38]

3.2. Toxicity assay in mammalian cell

The cytotoxic effects of all compounds were evaluated in VERO cells (ATCC, CRL-1586™), a mammalian cell line from the kidney of African green monkeys (*Cercopithecus aethiops*), based on modifications over Margis and Borojevic methods.[39] Briefly, cells were maintained at 37 °C in Dulbecco's modified minimum essential medium (Sigma Chemical Co., St. Louis, MO), supplemented with 10% fetal bovine serum (Cultilab, Campinas, SP, Brazil) and Hepes buffer (Sigma) in 24-well plates (Nuclon) (10^5 cells/well) during 12 h, for adhesion. Then the compounds diluted in 100% DMSO solution were added to the cells at six different concentrations, each one in quadruplicate, during 72 h. Controls containing non-treated cells and 1.0% DMSO (v/v) were also included. After 72 h-incubation, treated and untreated cells were washed twice with PBS and fixed with 0.2% formaldehyde in PBS for 15 min. Cells were stained for 1 h at room temperature with 0.2% CBBR-250 solution and then the stain was eluted from the cells with 1.0 ml of 1% sodium dodecyl sulfate during another hour.[30] Quantification was performed using the correlation (r) between cell number and the CBBR-250 absorbance. Compounds' toxicity on VERO cells is shown in Table 1, expressed as the concentration that induces 50% of VERO Cell death (CC_{50}), calculated using the Probit statistical program. Non-treated cultures and 1.0% DMSO (v/v) were also included as

Table 1. Comparison of trypanocidal activity (IC_{50}), cytotoxicity against VERO cells (CC_{50}), and molecular electronic properties (E_{HOMO}, dipole, clog P) of naphthoquinones and oxyrans derivatives

Compound	Experimental		Theoretical		
	IC_{50}[a] (μM)	CC_{50}[b] (μM)	E_{HOMO} (eV)	Dipole (Debye)	clog P
1	0.9	<3.1	−9.22	5.56	1.66
4	11	22	−9.60	5.59	1.04
5	>50	<3.1	−9.23	5.24	1.24
6	>50	>50	−9.51	6.31	0.61
7	>50	>50	−9.61	0.55	0.90
1a	12	>50	−9.02	4.15	1.58
4a	4.0	32	−9.37	4.30	0.96
5a	3.5	25	−9.03	3.93	1.17
6a	43	>50	−9.31	5.54	0.54
7a	1.3	>50	−9.35	2.33	0.83

[a] IC_{50} represents the antiproliferative 50% inhibitory concentration against *T. cruzi* Dm28c epimastigote forms.
[b] Compounds' cytotoxicity against VERO cells was expressed as the cytotoxic concentration that leads to 50% of VERO cells death (CC_{50}).

negative controls, while the lead compound (**1**) was used as positive control. Crystal violet was also used as an additional molecule for comparison.

4. Molecular modeling and theoretical comparison

All computations were performed using SPARTAN'04 (wavefunction Inc., Irvine, CA, 2000). Structures were minimized and the equilibrium geometry was obtained in vacuum using a semi-empirical AM1 module. In order to evaluate the electronic properties of the AM1 minimal energy conformations, they were submitted to a single-point ab initio calculation with a 6-31-G* basis set of the SPARTAN'04 package. The three-dimensional isosurfaces of the molecular electrostatic potential maps (MEPs) at the van der Waals contact surface represented electrostatic potentials superimposed onto a surface of constant electron density (0.002 e/au^3). They were generated in a range from −65 to +23 kcal/mol. These color-coded isosurface values provide an indication of the overall molecular size and location of negative (red) or positive (blue) electrostatic potentials. The electronic properties (HOMO's energy, HOMO orbital coefficients distribution, LUMO density, dipole moment, dipole moment vector, and lipophilicity—c log *P*) were calculated for all compounds. Theoretical log *P* (c log *P*) was calculated at AM1 semi-empirical level using the Villar method, included in Spartan.

5. Results and discussion

5.1. Rationale

The search for new compounds with activities against *T. cruzi*, with low toxicity to mammalian cells, and with increased efficacies during the chronic phase of Chagas' disease continues. The C-ring moiety of α- and β-lapachones is an important structural feature for its biological activities. For instance, the natural product Rhicanthone, isolated from *Rhinacanthus nasusts* and used in folk medicine for the treatment of cancer, hepatitis, and skin diseases, is an isomer of **1** with different mode of action.[40,41]

In order to identify other structural feature involved in the trypanocidal activity of **1** than the redox center, we tested a set of related compounds presenting a modified C-ring moiety (**1**, **4**–**7**, **1a**, and **4a**–**7a**) against *T. cruzi*. Since the literature had showed the strong influence of the redox center on biological activity, we decided also to prepare a set of closely related compounds to determine a new lead structure. It was interesting to assess the influence of the redox center by replacing one carbonyl by an oxyran ring. Many authors have pointed out that oxyran derivatives are trypanocidal agents, which target some specific proteinases.[42] Natural E-64,[43] for example, and several others reported synthetic derivatives[44] show potent trypanocidal activity. This transformation was carried out from the readily available naphthoquinones (**1**, **4**, **5**, **6**, and **7**), which were straightforwardly treated with an ethereal solution of diazomethane to produce the oxyran derivatives (**4a**, **5a**, **6a**, and **7a**).

5.2. Drug screening

All compounds were prepared and tested in vitro against epimastigote forms of *T. cruzi*. Our naphthoquinones' results showed only **4** (IC_{50} = 11 μM) with a significant trypanocidal activity (IC_{50} < 50 μM) compared to the lead compound **1** (IC_{50} = 0.9 μM) (Table 1) and crystal violet (IC_{50} < 3.1 μM). These data pointed out the importance of C-ring moiety since modifications in this region interfered directly with the trypanocidal activity in all naphthoquinones tested, including **4** that presented a ~10-fold lower activity compared to **1**.

The evaluation of the influence of the redox center in **1a**–**7a** derivatives' biological profile showed that all oxyrans were still able to inhibit the parasite growth with IC_{50} ranging from 1.3 to 43 μM. These data are in agreement with the literature that describes oxyrans as pharmacologically active compounds.[45] Although in a significant lower level, the derivative **1a** still presented a trypanocidal effect (IC_{50} = 12 μM) when compared to **1** (IC_{50} = 0.9 μM). This result may reinforce the importance of C-ring moiety's structural feature that may be sustaining the biological activity of **1a**. Interestingly, α-lapachone (**7**) is described as a non-producing free radical molecule,[46] and generated **7a** (IC_{50} = 1.3 μM), the most potent compound among the oxyrans tested in this study.

The cytotoxicity (CC_{50}) against mammalian cells (Vero cells) was investigated and revealed all naphthoquinones, except for the derivative **5**, presenting a lower cytotoxicity than **1** (CC_{50} < 3.1 μM) and crystal violet (CC_{50} = 8.9 mM). Despite showing no trypanocidal effect, derivative **5** presented a significant degree of cytotoxicity against mammalian cells (CC_{50} < 3.1 μM) (Table 1). This result suggests different structural features to rule each **5** derivative's biological profile (trypanocidal activity and cytotoxicity).

All oxyrans tested presented lower cytotoxicity values (CC_{50} = 25 to >50 μM) when compared to the corresponding naphthoquinones (Table 1). In agreement with the literature, these data implied the direct correlation of the redox center with the cytotoxic level in the naphthoquinones, as its replacement by an oxyran ring led to compounds less toxic to the mammalian cells. Interestingly the most potent compound (**7a**) presented a lower cytotoxicity profile, which leads to a high selectivity (CC_{50}/IC_{50}) against parasites compared to **1**. Thus, our biological results pointed **7a** as a lead compound for design of new trypanocidal agents with low cytotoxicity against mammalian cells.

5.3. Molecular modeling and theoretical studies

5.3.1. Naphthoquinones analysis. Considering the computational studies we evaluated structural and electronic properties of the current compounds to gain insight on their role in modulating the trypanocidal

and cytotoxicity profiles. The overall analysis showed that the HOMO energy and lipophilicity (clog *P*) data decreased for most of the naphthoquinones (except for **5**) as much as the trypanocidal effect did, different from that observed for some naphthoquinones described by Molfetta et al. (Table 1).[47] In contrast, both dipole moments (0.55–6.31 D), and dipole moment vector that was oriented at the same direction for all compounds (except for **7**), presented no clear or direct correlation with trypanocidal activity.

The molecular electrostatic potential maps (MEP), HOMO orbital coefficient, and LUMO density showed an overall similarity for all naphthoquinone derivatives, except for **7** (Fig. 2). In a more specific analysis of the naphthoquinones group, the elimination of the C-ring of **1** with the maintenance of an oxygen atom as in **4** revealed no significant changes in the electronic density in the MEP in comparison to **1** (Fig. 2). This result reinforced the suggestion that the structural requirement represented by C-ring is important to the trypanocidal activity.

Compound **5,** which presents the contraction of C-ring from six to five members, demonstrated the influence of the restricted flexibility and its impact in the trypanocidal profile. Interestingly, derivative **5** showed no significant variation in its electronic properties, which is comparable to **1** (Fig. 2). However, **5** was not active against *T. cruzi* and curiously the cytotoxicity had increased. Thus, this result led us to infer that the six-membered C-ring arrangement of **1** may define an oxygen-specific orientation, which is probably significant to the interaction with the target in *T. cruzi*. This region is more restricted in **5** and differently oriented in **4**, due to the conformational freedom of the methoxy group, which apparently compromised the antiparasite activity (Fig. 2 and Table 1).

Due to the presence of the electron-withdrawing group, derivative **6** showed a decrease of the electronic density represented by the intense blue color in the ring systems as noted in the molecular modeling studies (Fig. 2). The replacement of the hydrogen closest to the dimethyl groups in the C-ring by the substituent methanesulfonate, a more polar and voluminous substituent, raised the possibility of steric hindrance as this group clearly occupied the largest area in this compound (Fig. 2 and Table 1). Thus, both **6** electronic effect and steric hindrance may be deleterious to trypanocidal activity. Similar to compounds **4** and **5**, the dipole moment vector of **6** is directed to the oxygen atom in C-ring (not shown).

The last naphthoquinone studied, derivative **7**, showed the electrophilic center distributed in both carbonyls (Fig. 2). The C-ring (pyran) moiety transposition in **7** also induced a significant decrease of the trypanocidal activity (Table 1). This reinforces the importance of the right orientation of the pyran ring to an ideal interaction with the target present in the parasite. The absence of activity in **7** is also in agreement with the experimental pharmacological data described in the literature.[43] We also analyzed the three-dimensional coefficient isosurface of both orbitals of **7** to determine the atomic contribution on the frontier HOMO orbitals. Our analysis revealed the highest HOMO contribution for this compound in the unsaturated bond in the B- and C-ring junction closest to the oxygen atom of C-ring (Fig. 2). This result is different from that observed for other derivatives, where these contributions were distributed among the ring systems (Fig. 2). The analysis of **7** LUMO contributions showed no significant difference from those of other naphthoquinones of this series (data not shown).

5.3.2. Oxyrans analysis. In order to assess structural features and electronic properties that could affect the

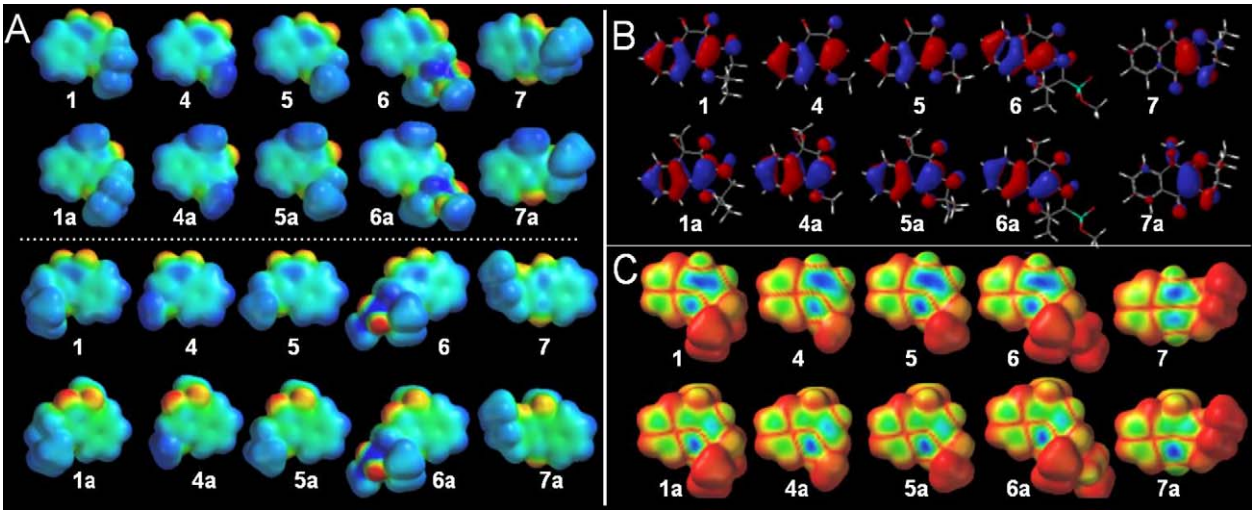


Figure 2. Comparison of the naphthoquinones (**1** and **4**–**7**) and the respective compounds with oxyran ring (**1a** and **4a**–**7a**). (A) Front (up) and back view (down) of molecular electrostatic potential energy isosurfaces (MEP) superimposed onto total electron density of 0.002 e/a.u.^{3} The color code is in the range of −65.0 (deepest red) to −23.0 (deepest blue) kcal/mol. (B) HOMO orbital coefficient and (C) LUMO encoded onto a surface of constant electron density (0.002 e/a.u.^{3}) (LUMO density).

trypanocidal activity, we performed the computational studies by analyzing the addition of the oxyran ring in the quinone moiety. The overall analysis of theoretical parameters showed that the dipole moment, except for **6a**, and the lipophilicity (clog *P*) decreased for most of the oxyrans but with no clear correlation with trypanocidal activity.

The three-dimensional molecular electrostatic potential maps (MEP) and LUMO density provide a measure of charge distribution from the point of view of an approaching molecule.[48] In general oxyrans, MEP and LUMO density significantly differed from the corresponding naphthoquinones (Fig. 2). The MEPs revealed a more negative electrostatic potential extending at the ring systems' surface in all oxyrans when compared with naphthoquinone derivatives (Fig. 2). The LUMO delineated electron deficient areas, which may be susceptible to interaction with the electron density regions of the target of the parasite. In Figure 2, regions where the absolute value of the LUMO is greatest are shown in blue, while regions where it is least are shown in red. The LUMO electron deficient (blue) area of naphthoquinones concentrated in both carbon carbonyls and in the carbon atom bond to the oxygen pyran ring. In oxyrans, this area was restricted to the carbon atom closest to the pyran oxygen atom (Fig. 2). In contrast, HOMO's orbital coefficients and the dipole moment vector showed no apparent modifications when compared to the original quinone derivatives (Fig. 2B).

The individual analysis of oxyran **1a** showed its lower but consistent trypanocidal effect, suggesting that the quinone system is not essential to this activity. Despite of the variation in some structural parameters such as dipole moment and clog *P*, no direct correlation could be strictly determined to **1a** when compared to other derivatives. Interestingly, **1a** MEP showed the orientation of the methylene bridge, which could impose an important steric hindrance and limit the perfect match with the target (Fig. 2).

The trypanocidal activity of oxyrans **4a**, **5a**, and **6a** also reinforced the importance of other structural features than the quinone moiety for presenting this biological profile. Unlike the naphthoquinone derivatives **4** and **5**, the addition of the oxyran group simultaneously to the ring modulation did not abolish the trypanocidal activity in **4a** and **5a**. The derivative **6a** still presented a slight level of antiparasite activity, although the voluminous substituent may interfere with most of the favorable interactions with the target (Fig. 2).

Among the planned drugs, derivative **7a** was the most potent compound with at least 40-fold activity improvement over **7** and at a similar level to **1** (Fig. 3 and Table 1). In addition, this oxyran was also shown to be less toxic, even at a high concentration (50 μM), pointing it as the best candidate for a more specific study about its mechanism of action. The molecular modeling analysis pointed out the variation of all electronic and molecular properties, compared to **1**, which led to the highest trypanocidal activity of this series of compounds without a significant cytotoxicity against mammalian cells (Fig. 3). Apparently, the presence of the oxyran group decreased the lipophilicity (clog $P = 0.83$) and the dipole moment (2.33 D) of the compound and, interestingly enough, also the cytotoxicity of these compounds ($CC_{50} > 50$ μM) (Table 1).

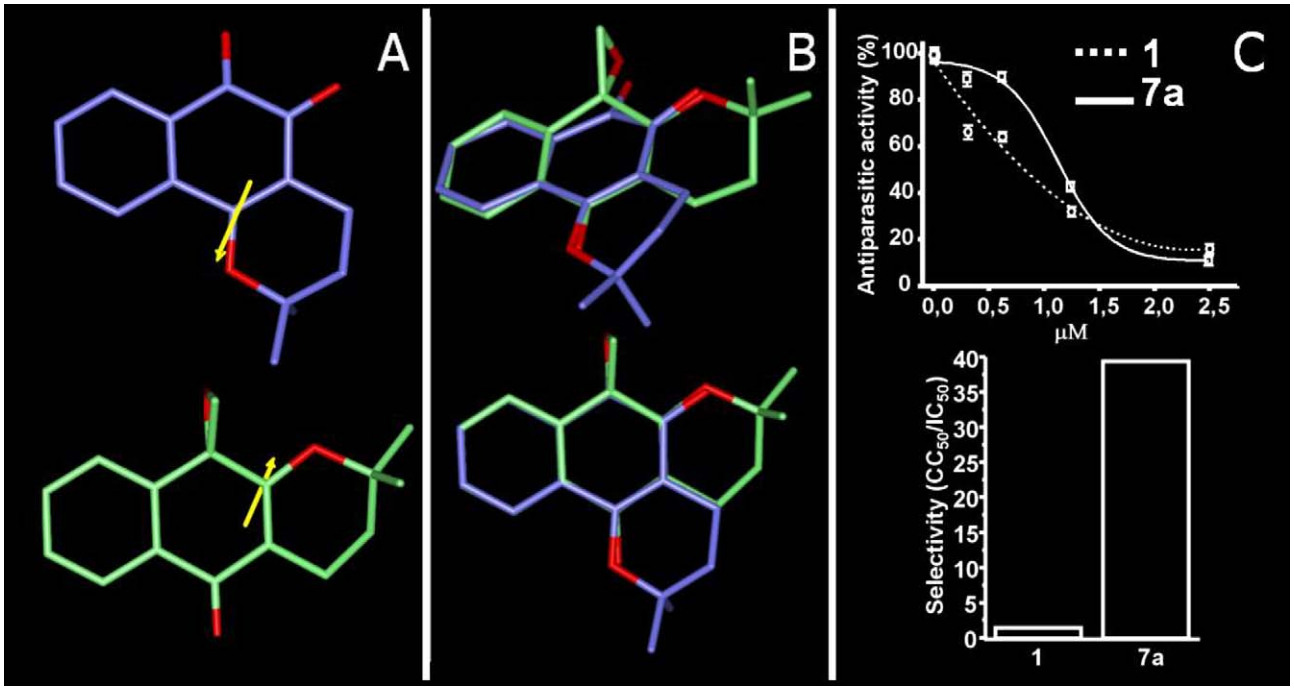


Figure 3. Comparison of the structural and biological features of **1** and **7a**. (A) Dipole moment vector orientation, (B) stereo diagram of the superimposition of both structures from two different views, and (C) antiparasitic profile (up) and the predicted selectivity (CC_{50}/IC_{50}) against parasites (down).

The dipole moment and the molecule vector also compose a relevant parameter for evaluating ligands approaching the binding site. Although compounds **1a**, **4a**, **5a**, and **6a** showed lower dipole moments and also lower cytotoxicity against mammalian cells values than its precursors, its vectors pointed to the same region, showing that the dipole region is similar for all derivatives (not shown). The only exception was **7a**, which showed a higher dipole moment than its quinone precursor, while its vector was in an opposite direction (Fig. 3). This vector orientation showed that the polarity of the molecule is different when compared to that of the lead compound (**1**) (Fig. 3). This feature might be extremely relevant for maximizing the interaction with the target in the *T. cruzi,* revealing **7a** as the most potent compound of the oxyran series, and the closest to the biological profile of **1**. This may also be important for the selectivity degree (CC_{50}/IC_{50}) of **7a** to parasites against mammalian cells, which is at least 10-fold higher than **1** (Fig. 3).

In brief, all the molecular modeling calculations suggested that these compounds might act by a different mechanism against the *T. cruzi*. In addition, the cytotoxicity data also suggest that the new oxyran derivatives are active in the parasite without critically affecting the mammalian cells, in contrast to the naphthoquinones. The superimposition of **1** and **7a** showed that the six-membered ring occupies different regions, which might be extremely important to bind adequately with the target in the parasites (Fig. 3).

6. Conclusion

The presence of the redox center of the quinones is described in the literature as important to maintain the antiproliferative activity against *T. cruzi*. In this work, we designed and synthesized a set of related compounds with modifications in the C-ring moiety and on the quinone moiety to correlate them with antiparasite, cytotoxicity, and molecular properties. This study pointed out the naphthoquinones with an oxyran ring as a new class of trypanocidal compounds with low cytotoxicity against mammalian cells. Despite presenting totally different electronic properties, **1** and **7a** showed a similar activity profile against the parasites. These features may account for its different levels of reactivity, and this, on the other hand, could lead to different pharmacological mechanisms. In agreement with this hypothesis, compound **7a** showed a lower cytotoxicity in the VERO cell line, which shows it as a potential lead compound for the future design of drugs to be used in the treatment of Chagas' disease.

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References and notes

1. Williams-Blangero, S.; VandeBerg, J. L.; Blangero, J.; Correa-Oliveira, R. *Front. Biosci.* **2003**, *8*, e337.
2. Dias, J. C.; Silveira, A. C.; Schofield, C. J. *Mem. Inst. Oswaldo Cruz* **2002**, *97*, 603.
3. Brener, Z. *Pharmacol. Ther.* **1979**, *7*, 71.
4. Schmuñis, G. A.; Szarfman, A.; Coarasa, L.; Guilleron, C.; Peralta, J. M. *Am. Soc. Trop. Med. Hyg.* **1980**, *29*, 170.
5. Cançado, J. R.; Brener, Z. In *Trypanosoma cruzi e Doença de Chagas*; Brener, Z., Andrade, Z., Eds.; Guanabara Koogan: Rio de Janeiro, 1979; p 362.
6. Hotez, P. J.; Remme, J. H.; Buss, P.; Alleyne, G.; Morel, C.; Breman, J. G. *Clin. Infect. Dis.* **2004**, *38*, 871.
7. Subramanian, S.; Ferreira, M. M. C.; Trsic, M. *Struct. Chem.* **1998**, *9*, 47.
8. dos Santos, A. F.; Ferraz, P. A. L.; de Abreu, F. C.; Chiari, E.; Goulart, M. D. F.; Sant'Ana, A. E. G. *Planta Med.* **2001**, *67*, 92.
9. Barbosa, T. P.; Camara, C. A.; Silva, T. M. S.; Martins, R. M.; Pinto, A. C.; Vargas, M. D. *Bioorg. Med. Chem.* **2005**, *13*, 6464.
10. Silva, T. M. S.; Camara, C. A.; Barbosa, T. P.; Soares, A. Z.; da Cunha, L. C.; Pinto, A. C.; Vargas, M. D. *Bioorg. Med. Chem.* **2005**, *13*, 193.
11. Teixeira, M. J.; Almeida, Y. M.; Viana, J. R.; Holanda Filha, J. G.; Rodrigues, T. P.; Prata, J. R. C., Jr.; Coelho, I. C. B.; Rao, V. S.; Pompeu, M. M. L. *Phytother. Res.* **2001**, *15*, 44.
12. Almeida, E. R. *J. Ethnopharmacol.* **1990**, *29*, 239.
13. Marr, J. J.; Docampo, R. *Rev. Infect. Dis.* **1986**, *8*, 884.
14. Garnier, S.; Wolfender, J. L.; Nianga, M.; Stoeckli-Evans, H.; Hostettmann, K. *Phytochemistry* **1996**, *42*, 1315.
15. Abreu, F. C.; Ferreira, D. C. M.; Wadhawan, J.; Amatore, C.; Ferreira, V. F.; da Silva, M. N.; de Souza, M. C. B. V.; Gomes, T. S.; Ximenes, E. A.; Goulart, M. O. F. *Electrochem. Commun.* **2005**, *7*, 767.
16. Ferreira, V. F.; Pinto, A. V.; Silva, J. S.; Gilbert, B. *Mem. Inst. Oswaldo Cruz.* **1992**, *87*, 345.
17. Pinto, C. N.; Dantas, A. P.; De Moura, K. C. G.; Emery, F. S.; Polequevitch, P. F.; Pinto, M. C. F. R.; De Castro, S. L.; Pinto, A. V. *Arzneim. Forsch./Drug Res.* **2000**, *50*, 1120.
18. Menna-Barreto, R. F. S.; Henrique-Pons, A.; Pinto, A. V.; Morgado-Diaz, J. A.; Soares, M. J.; De Castro, S. L. *J. Antimicrob. Chemother.* **2005**, *56*, 1034.
19. Babior, B. M. *Braz. J. Med. Biol. Res.* **1997**, *30*, 141.
20. Santos, E. V. M.; Carneiro, J. W. M.; Ferreira, V. F. *Bioorg. Med. Chem.* **2004**, *12*, 87.
21. Silva, M. N.; Ferreira, V. F.; de Souza, M. C. B. V. *Quim. Nova.* **2003**, *26*, 407.
22. Monks, T. J.; Hanzlik, R. P.; Cohen, G. M.; Ross, D.; Graham, D. G. *Toxicol. App. Pharmacol.* **1992**, *112*, 2.
23. Pardee, A. B.; Li, Y. Z.; Li, C. J. *Curr. Cancer Drug Targets* **2002**, *2*, 227.
24. Pinto, A. V.; Pinto, M. C. R.; Gilbert, B.; Pellegrino, J.; Mello, R. T. *Trans. Roy. Soc. Trop. Med. Hyg.* **1977**, *71*, 133–135.
25. Ravelo, A. G.; Estévez-Braun, A.; Chávez-Orellana, H.; Pérez-Sacau, E.; Mesa-Siverio, D. *Curr. Top. Chem.* **2004**, *4*, 241–244.
26. Chiang, J.; Averboukh, L.; Pardee, A. B. *J. Biol. Chem.* **1993**, *268*, 22463–22468.
27. Frydman, B.; Marton, L. J.; Sun, S. J.; Neder, K.; Witiak, T. D.; Liu, A. A.; Wang, H. M.; Mao, Y.; Wu, H. Y.; Sanders, M. M.; Liu, L. F. *Cancer Res.* **1997**, *57*, 620–627.

28. Dubin, M.; Fernadez Villamil, S. H.; Stoppani, A. O. *Medicina* **2001**, *61*, 343–350.
29. Frydman, B.; Marton, L. J.; Sun, J. S. *Cancer Res.* **1995**, *57*, 620–627.
30. Pinto, A. V.; Pinto, M. C. R.; Gilbert, B.; Pellegrino, J.; Mello, R. T. *Trans. Roy. Soc. Trop. Med. Hyg.* **1977**, *71*, 133.
31. De Moura, K. C. G.; Salomão, K.; Menna-Barreto, R. F. S.; Emery, F. S.; Pinto, M. C. F. R.; Pinto, A. V.; Castro, S. L. *Eur. J. Med. Chem.* **2004**, *39*, 639.
32. Neves-Pinto, C.; Malta, V. R. S.; Pinto, M. C. F. R.; Santos, R. H. A.; de Castro, S. L.; Pinto, A. V. *J. Med. Chem.* **2002**, *45*, 2112.
33. Abreu, F. C.; Ferraz, P. A. L.; Goulart, M. O. F. *J. Braz. Chem. Soc.* **2002**, *13*, 19.
34. Hooker, S. C. *J. Chem. Soc.* **1892**, 611.
35. Ferreira, V. F.; Pinto, A. V.; Pinto, M. C. R. F.; da Silva, M. N.; Skakle, J. M. S.; de Souza, M. C. B. V.; Wardell, S. M. S. V. *Acta. Crystallogr.* **2002**, *C58*, 560.
36. Silva, M. N.; de Souza, M. C. B. V.; Ferreira, V. F.; Pinto, A. V.; Pinto, M. C. R. F.; Wardell, S. M. S. V.; Wardell, J. L. *Arkivoc* **2003**, *10*, 156.
37. Gadelha, F. R.; Moreno, S. N.; De Souza, W.; Cruz, F. S.; Docampo, R. *Mol. Biochem. Parasitol.* **1989**, *34*, 117.
38. de Souza, A. O.; Hemerly, F. P.; Gomes-Cardoso, L.; Santa-Rita, R. M.; Leon, L. L.; de Castro, S. L.; Duran, N. *J. Chemother.* **2004**, *16*, 530.
39. Margis, R.; Borojevic, R. *Anal. Biochem.* **1989**, *181*, 209.
40. Kongkathip, N.; Luangkamin, S.; Kongkathip, B.; Sangma, C.; Grigg, R.; Kongsaeree, P.; Prabpai, S.; Pradidphol, N.; Piyaviriyagul, S.; Siripong, P. *J. Med. Chem.* **2004**, *47*, 4427.
41. Kongkathip, N.; Kongkathip, B.; Siripong, P.; Sangma, C.; Luangkamin, S.; Niyomdecha, M.; Pattanapa, S.; Piyaviriyagul, S.; Kongsaeree, P. *Bioorg. Med. Chem.* **2003**, *11*, 3179.
42. Cazzulo, J. J. *Curr. Top. Med. Chem.* **2002**, *2*, 1257.
43. Lacobuzio-Donahue, C. A.; Shuja, S.; Cai, J.; Peng, P.; Murname, M. J. *J. Biol. Chem.* **1997**, *272*, 29190.
44. Roush, W. R.; González, F. V.; McKerrow, J. H.; Hansell, E. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2809.
45. Marco-Contelles, J.; Molina, M. T.; Anjum, S. *Chem. Rev.* **2004**, *104*, 2857.
46. Krishnan, P.; Bastow, K. F. *Biochem. Pharmacol.* **2000**, *60*, 1367.
47. Molfetta, F. A.; Bruni, A. T.; Honório, K. M.; da Silva, A. B. F. *Eur. J. Med. Chem.* **2005**, *40*, 329.
48. Bhattacharjee, A. K.; Majundar, D.; Guha, S. J. *J. Chem. Soc., Perkin Trans. 2* **1992**, 805.