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Original article

Potent naphthoquinones against antimony-sensitive and -resistant *Leishmania* parasites: Synthesis of novel α- and nor-α-lapachone-based 1,2,3-triazoles by copper-catalyzed azide–alkyne cycloaddition

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ABSTRACT

Continuing our screening program for novel anti-parasite compounds, we synthesized seven 1,4-naphthoquinones coupled to 1,2,3-triazoles, five nor-β-lapachone-based 1,2,3-triazoles and ten α-lapachone-based 1,2,3-triazoles. These and other naphthoquinonoid compounds were evaluated for their activity against promastigote forms of antimony-sensitive and -resistant strains of *Leishmania infantum* (syn. *Leishmania chagasi*) and *Leishmania amazonensis*. The toxicity of these compounds to mammalian cells was also examined. The substances were more potent than an antimonial drug, with IC_{50} values ranging from 1.0 to 50.7 μM. Nor-α-lapachone derivatives showed the highest antileishmanial activity, with selectivity indices in the range of 10–15. These compounds emerged as important leads for further investigation as antileishmanial agents. Additionally, one of these compounds exhibited cross-resistance in Sb-resistant *Leishmania* and could provide a molecular tool for investigating the multidrug resistance mechanisms in *Leishmania* parasites.

1. Introduction

Leishmaniasis, a neglected tropical disease [1], is a worldwide vector borne disease that affects developing countries. This disease is endemic in 88 countries, with an estimated 12 million cases worldwide [2]. Approximately 21 *Leishmania* species are capable of infecting vertebrate hosts. In humans, the syndrome is mostly associated with seven species of *Leishmania*: *Leishmania donovani*, *Leishmania infantum*, *Leishmania major*, *Leishmania tropica*, *Leishmania aethiopica*, *Leishmania braziliensis* and *Leishmania mexicana* [3,4]. Infection is caused by the bite of infected female sandflies of the genera *Phelobotomus* (Europe, Asia, Africa) and *Lutzomyia* (America) [5]. Metacyclic promastigotes, the infective stage, are phagocytized by macrophages and transformed into amastigotes. These multiply in an intracellular fashion, affecting different tissues. Leishmaniasis can manifest itself in three different clinical forms, depending on the *Leishmania* species: visceral, cutaneous and mucosal, which arise from parasite replication in the mononuclear phagocyte system, dermis and naso-oropharyngeal mucosa, respectively [3,6]. Pentavalent antimonials, miltefosine, paromomycin and amphotericin B are used to clinically treat leishmaniasis; due to their toxic side effects and the emergence of drug resistance, however, safer and more effective drugs are still needed [7,8].

Resistance to the first-line antimonial drugs has reached critical levels in some parts of the world, including Bihar State (India) [8]. The emergence of resistance is attributed to inappropriate drug exposure, resulting in a build-up of subtherapeutic blood levels of antimony and increasing the tolerance of the parasites to this treatment. Although antimonials are still the first-line drugs, they

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exhibit several limitations including severe side effects, the need for daily parenteral administration and drug resistance [9]. Increased levels of intracellular thiols and/or overexpression of ABC transporters are usually observed in metal-resistant *Leishmania* [10]. Other mechanisms such as diminished biological reduction of Sb(V) to Sb(III) [11], the loss of a single aquaglyceroporin (or its down regulation) [12] and hypoxic conditions [13] have been reported to cause increased resistance to pentavalent antimonials. Thus, to develop new drugs, it is important to ensure that cross-resistance with conventional drugs does not occur.

Efforts have been undertaken to find natural, bioactive compounds that can be used in the treatment of parasitic diseases [14]. Some natural naphthoquinones have emerged as promising subjects of medicinal chemistry research due to their structural properties. These compounds can generate reactive oxygen species (ROS), which lead to oxidative stress and subsequently to parasite death [15a]. Dubey and collaborators have described relevant quinonoid compounds with high leishmanicidal activity. The anticancer drugs, doxorubicin and mitomycin C, were reported as novel inhibitors of trypanothione reductase (TryR) in *Leishmania* and these compounds also showed significant effect on redox homeostasis of the parasite [15b]. Goulart and coworkers [16] described the leishmanicidal activity of lapachol and some derivatives, showing the potential utility of these substances against this parasite.

In the last few years, our research group has described quinonoid compounds with anticancer [17] and tuberculostatic activities [18], as well as compounds that are active against *Trypanosoma cruzi*, the etiologic agent of Chagas disease [19]. Guided by the concept of molecular hybridization and seeking compounds active against *T. cruzi* [20], we reported that triazole naphthoquinones are efficient trypanocidal agents. Treatment with these compounds causes impaired mitosis and increased ROS production leading to parasite death by an autophagic mechanism [21].

In the present study, triazole naphthoquinones were synthesized, and their leishmanicidal activities were evaluated against both Sb(III)-sensitive and -resistant *L. infantum* (syn. *Leishmania chagasi*) and *Leishmania amazonensis* promastigotes. Their toxicity to murine peritoneal macrophages was also examined.

2. Chemistry

2-Bromo-1,4-naphthoquinone (**1**) was acquired from Sigma–Aldrich (St. Louis, MO, USA). Lapachol (**10**) (2-hydroxy-3-(3′-methyl-2′-butenyl)-1,4-naphthoquinone) was extracted from the heartwood of *Tabebuia* sp. (Tecoma) and purified by a series of recrystallizations [22]. From this quinone, nor-lapachol (**24**) (2-hydroxy-3-(20-methyl-propenyl)-1,4-naphthoquinone) was obtained as a crystalline orange solid by Hooker oxidation [23,24].

The first group of 1,4-naphthoquinone coupled 1,2,3-triazoles (**3**–**9**) was obtained from 2-bromo-1,4-naphthoquinone (**1**) (Scheme 1). From compound **1**, 2-azido-1,4-naphthoquinone (**2**) was obtained by a simple reaction with sodium azide in dimethylformamide, as described previously [25]. Compound **2** was then submitted to copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction conditions [26].

The second group of compounds was obtained from lapachol (**10**) (Scheme 2) [27]. A four step synthesis afforded 3-azido-nor-β-lapachone (**11**), and nor-β-lapachone-based 1,2,3-triazoles **12**–**16** were prepared from this intermediate in high yields [27].

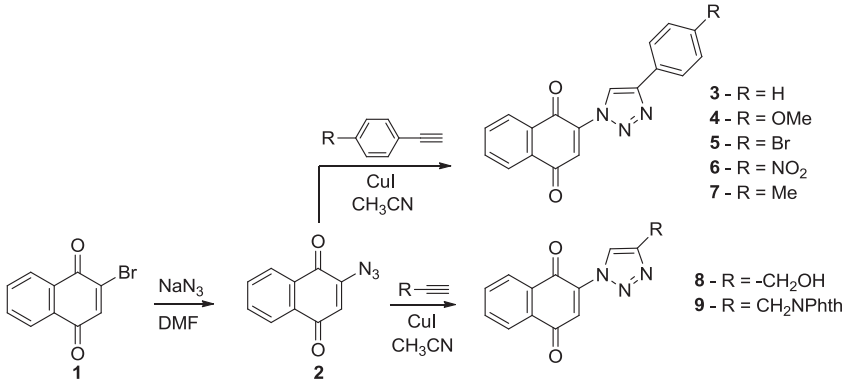
The third and fourth groups, the α- and nor-α-lapachone-based 1,2,3-triazoles, were synthesized for the first time as shown in Schemes 3 and 4. Lapachol (**10**) was used to prepare α-lapachone (**17**) by simple cyclization under acidic conditions. Next, the treatment of α-lapachone (**10**) with N-bromosuccinimide in the presence of benzoyl peroxide in carbon tetrachloride afforded 4-bromo-α-lapachone (**18**) was performed. Subsequent reaction with sodium azide gave the novel 4-azido-α-lapachone (**19**), the key intermediate used to obtain the triazole derivatives **20**–**23**. α-Lapachone-based 1,2,3-triazoles **20**–**23** were then obtained by Cu(I) catalysis following methodology described by Sharpless and coworkers [26] (Scheme 3).

The treatment of nor-lapachol (**24**) with HCl/AcOH provided nor-α-lapachone (**25**), which was transformed into 3-bromo-nor-α-lapachone (**26**). Reaction of **26** with sodium azide in dichloromethane gave the corresponding azide compound **27**, as described previously [28]. Azidonaphthoquinone **27** was used to synthesize the novel triazole derivatives **28**–**32** in moderate yields (Scheme 4).

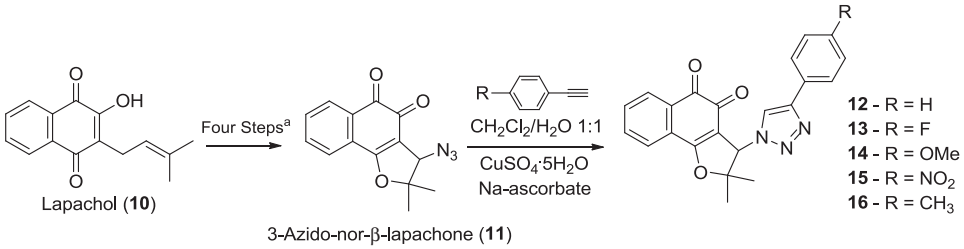
3. Results and discussion

We recently reported the synthesis of naphthoquinone coupled 1,2,3-triazoles **3**–**9** and nor-β-lapachone-based 1,2,3-triazoles **12**–**16** and their activity against bloodstream trypomastigotes, the infective forms of *T. cruzi* [27]. Their trypanocidal activity, with IC_{50}/24 h values in the range of 10.9–359.0 μM [27], led us to evaluate them further against *Leishmania*: *L. infantum* (syn. *L. chagasi*), the etiological agent for visceral leishmaniasis [29]. We also studied these compounds for activity against *L. amazonensis*, which is related to the cutaneous form of the disease in the New World [30]. These two species lead to different clinical manifestations, suggesting different metabolism and consequently distinct drug sensitivity. This observation highlights the importance of using different *Leishmania* species in drug screening [31], as well as strains with different susceptibilities to antimonials.

The first series of compounds, 1,4-naphthoquinone 1,2,3-triazole derivatives, was considered to be highly active against all



Scheme 1. Naphthoquinoidal compounds linked 1,2,3-triazoles **3**–**9**.



Scheme 2. Nor-β-lapachone-based 1,2,3-triazoles **12**–**16**. [a]Ref. [27].

Leishmania strains (with the exception of compound **6**, $IC_{50} > 100$ μM), with IC_{50} values in the range of 5.8–57.8 μM in the four cell lines studied (Table 1). However, these compounds were toxic to peritoneal macrophages with CC_{50} values in a similar range, from 5.4 to 50 μM (Table 1), leading to SI (selectivity index) values between 0.37 and 1.72 (Table 2). This index is obtained from the ratio between the CC_{50} for peritoneal macrophages and the IC_{50} in the parasites. Compounds **3**–**9** were approximately 2–10 times more active than the standard drug against the wild-type strains of both *L. infantum* (syn. *L. chagasi*) (2–10×) and *L. amazonensis* (2.2–6.6×).

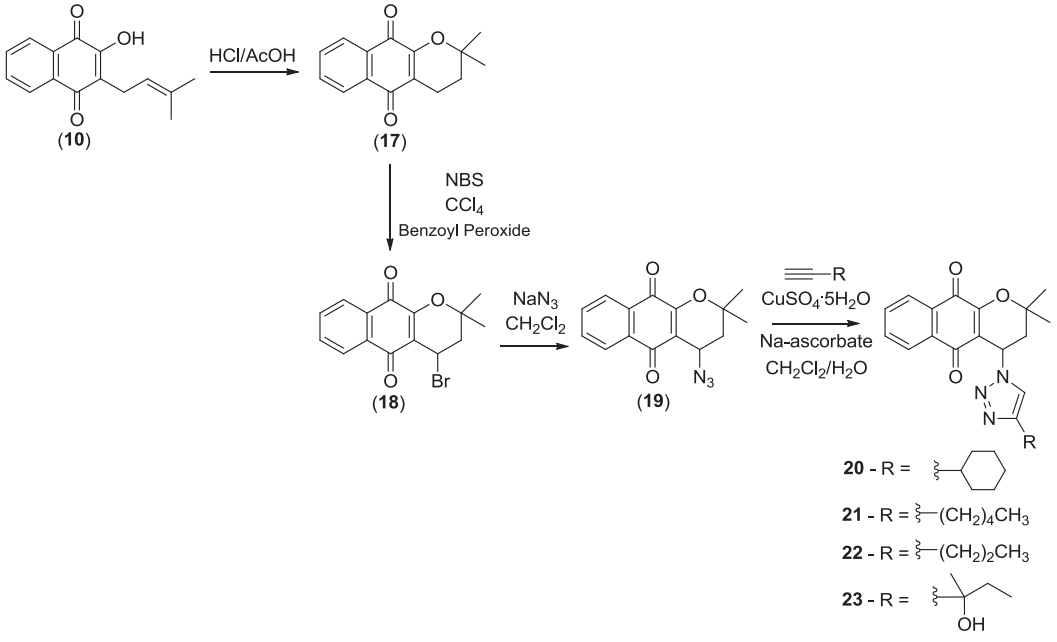
The second group of compounds, nor-β-lapachone-based 1,2,3-triazoles **12**–**16**, contained different electron donating and withdrawing groups linked to the phenyl group of the triazole ring. All compounds in this group showed similarly potent activity against *L. infantum* (syn. *L. chagasi*) and *L. amazonensis*. Activity was observed in both antimony-sensitive and resistant strains with IC_{50} values between 1.00 and 5.57 μM and CC_{50} values for murine peritoneal macrophages approximately 4 μM. Compounds **12**–**16** were also more effective than the naphthoquinoidal precursor lapachol (**10**) (IC_{50} values from 34.72 to 102.05 μM) but were less active than the *ortho*-quinones, β-lapachone and nor-β-lapachone (Table 1). Compounds **12**–**16** were approximately 20–90 times more active than potassium antimonyl tartrate against the wild-type strains of both *L. infantum* (syn. *L. chagasi*) (15.5–86×) and *L. amazonensis* (23–98×).

The novel α- and nor-α-lapachone-based 1,2,3-triazoles, compounds **20**–**23** and **28**–**33**, respectively, were synthesized and assessed against all of the *Leishmania* strains reported here. We observed that most of the nor-α-lapachone-based 1,2,3-triazoles were more active than α-lapachone coupled 1,2,3-triazoles (with the exception of compounds **23** and **31**), indicating that modification of the C-ring (pyran versus furan) is important for the leishmanicidal activity. On the other hand, nor-α-lapachone derivatives, except for compound **29**, were less cytotoxic towards macrophages than were α-lapachone compounds.

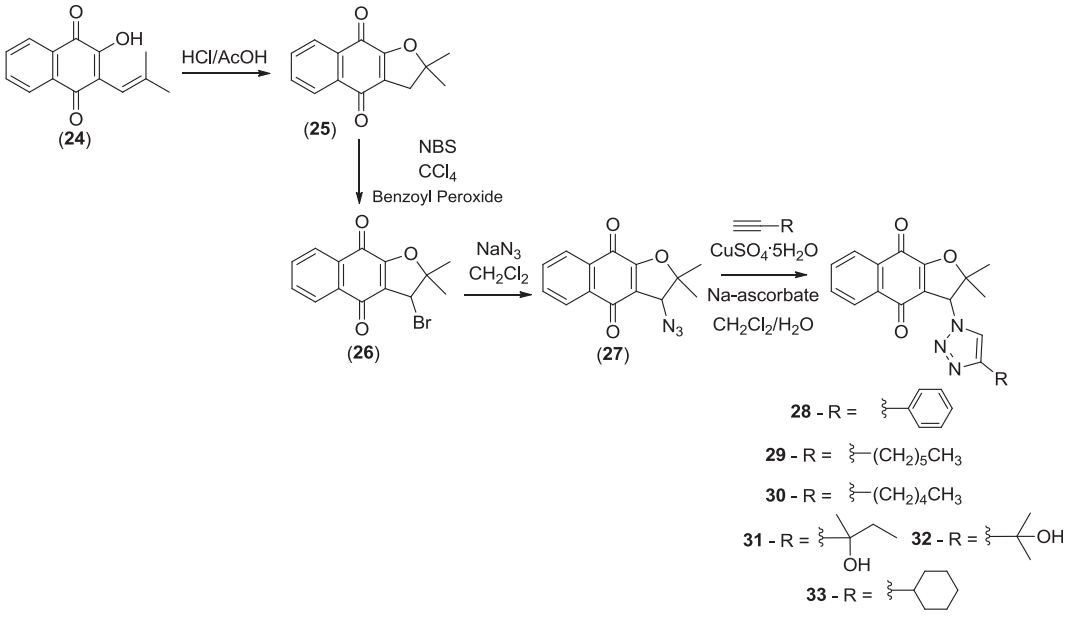
Most of the naphthoquinoidal compounds were derived from nor-α-lapachone, and compounds **28**, **30**, **32** and **33** in particular, exhibit high selectivity index and can be considered as the most promising drug candidates for use against both antimony-sensitive and -resistant leishmaniasis.

The need for a safe and highly effective drug against leishmaniasis is extremely relevant, especially in the cases of drug-resistant strains. One chemotherapeutic strategy entails the use of drug combinations to treat visceral leishmaniasis [32]. However, the long-term efficacy of this approach has been questioned in a recent study which shows the *in vitro* development of resistance to drug associations in *L. donovani* [33]. Improvements to the chemotherapeutic arsenal with new antileishmanial agents, starting from drug screening as described in this study are thus urgently required.

In comparison with the precursor quinones, lapachol (**10**), α-lapachone (**17**), nor-lapachol (**24**) and nor-α-lapachone (**25**) the



Scheme 3. α-Lapachone-based 1,2,3-triazoles **20**–**23**.



Scheme 4. Nor-α-lapachone-based 1,2,3-triazoles **28**–**33**.

strategy to insert the triazole ring was efficient since the novel compounds were, in general, more actives.

Of the twenty-seven substances tested herein, only compound **31** showed evidence of cross-resistance with respect to the trivalent antimonial potassium antimonyl tartrate. Interestingly, the only difference between compound **31** and **32** is the presence of an additional methyl substituent group in the triazole ring. As compound **32** is highly active against *Leishmania* and compound **31** presented cross-resistance in Sb-resistant parasites, this modification is most likely critical to recognize the drug molecule by the resistance mechanisms in the *Leishmania* parasite. These findings are relevant considering the importance of elucidating drug resistance mechanisms in *Leishmania* and the need to identify agents capable of reversing *Leishmania* drug resistance. Compound

Table 1
Cytotoxic effects of naphthoquinones on wild-type (WT) and antimony-resistant (2700R) promastigotes of *Leishmania* and murine peritoneal macrophages (MPM).

Compounds	*L. infantum* (*syn. L. chagasi*) WT	*L. infantum* (*syn. L. chagasi*) 2700R	*L. amazonensis* WT	*L. amazonensis* 2700R	MPM
	IC_{50} (μM)[a]				CC_{50} (μM)[b]
3	21.58	17.16	16.68	7.94	10.17
4	15.80	18.61	34.28	24.54	16.82
5	17.25	14.08	57.81	50.15	21.42
6	>100	>100	>100	>100	50.00
7	8.80	13.55	21.25	13.79	15.18
8	39.05	5.80	23.87	21.32	9.51
9	42.13	8.47	50.65	40.25	5.39
Lapachol (**10**)	34.72	99.88	102.05	63.28	59.30
12	~1.00	1.04	1.11	1.03	3.42
13	4.32	4.90	4.70	4.39	3.52
14	5.57	5.39	3.87	2.49	4.36
15	3.25	3.28	3.41	2.95	3.35
16	3.38	3.10	3.80	3.49	3.31
α-Lapachone (**17**)	13.88	5.85	16.08	15.15	3.80
20	2.26	2.24	9.23	1.9	8.59
21	2.04	2.20	6.00	1.95	6.89
22	5.90	3.43	4.37	8.04	14.79
23	17.73	3.30	35.19	21.55	9.63
Nor-lapachol (**24**)	>100	>100	>100	>100	14.58
Nor-α-lapachone (**25**)	1.54	1.28	2.72	1.54	0.77
28	1.00	1.01	2.14	1.2	8.01
29	1.12	1.12	11.00	4.23	2.77
30	1.05	1.14	2.19	1.14	15.96
31	1.45	25.36	18.00	27.04	17.92
32	1.55	1.32	14.24	7.31	19.81
33	1.83	1.78	3.50	1.39	18.72
Nor-β-lapachone	1.14	1.60	2.48	1.59	1.55
β-Lapachone	0.67	1.13	2.90	1.39	0.79
Potassium antimonyl tartrate – Sb(III)	86.1	>3000	109.7	>3000	62.89

[a] IC_{50}: drug concentration that inhibits *Leishmania* cell growth by 50%.
[b] CC_{50}: drug concentration that decreases the macrophage cell viability by 50%.

Table 2
Selectivity index (SI) of the triazole derivatives and the naphthoquinoidal compounds precursor.

Compounds	*L. infantum* (*syn. L. chagasi*) WT	*L. infantum* (*syn. L. chagasi*) 2700R	*L. amazonensis* WT	*L. amazonensis* 2700R
3	0.47	0.59	0.61	1.28
4	1.06	0.90	0.49	0.68
5	1.24	1.52	0.37	0.43
6	<0.50	<0.50	<0.50	<0.50
7	1.72	1.12	0.71	1.10
8	0.24	1.64	0.40	0.45
9	0.13	0.64	0.11	0.13
Lapachol (**10**)	1.71	0.59	0.58	0.94
12	~3.42	3.29	3.08	3.32
13	0.81	0.72	0.75	0.80
14	0.78	0.81	1.13	1.75
15	1.03	1.02	0.98	1.35
16	0.98	1.07	0.87	0.95
α-Lapachone (**17**)	0.27	0.65	0.24	0.25
20	3.80	3.83	0.93	4.52
21	3.38	3.13	1.15	3.53
22	2.50	4.31	3.38	1.83
23	0.54	2.92	0.27	0.45
Nor-lapachol (**24**)	<0.15	<0.15	<0.15	<0.15
Nor-α-lapachone (**25**)	0.50	0.60	0.28	0.50
28	8.01	7.93	3.74	6.67
29	2.47	2.47	0.25	0.65
30	15.02	14.0	7.29	14.0
31	12.36	0.70	0.99	0.66
32	12.78	15.01	1.39	2.71
33	10.23	10.51	5.35	13.47
Nor-β-lapachone	1.36	0.97	0.62	0.97
β-Lapachone	1.14	0.70	0.27	0.57
Potassium antimonyl tartrate – Sb(III)	0.73	<0.02	0.57	<0.02

SI = CC_{50}/IC_{50}; values below 1 mean that the compound is more toxic to mammalian cells than to the parasite.

31 should be investigated further as a molecular tool for the study and reversal of multidrug resistance mechanisms in *Leishmania* parasites.

The exposition of sensitive *Leishmania* to Sb(III) was previously shown to induce the efflux of thiols and the inhibition of TryR [34a], promoting redox homeostasis imbalance that leads to parasite death. Interestingly, naphthoquinones were also proposed to inhibit TryR and additionally to act as subversive substrates by converting the enzyme from an anti-oxidant to a pro-oxidant, resulting in an increase in ROS levels and consequently in cell death [15b,34b]. Thus, literature reveals some similarities in the mechanisms of action proposed for Sb(III) and naphthoquinones against *Leishmania* parasites. The cross-resistance observed for compound **31** may be attributed to a similar molecular target shared with Sb(III), however this feature cannot be generalized to the other naphthoquinones derivatives. Indeed, the subtle chemical difference between compounds **31** and **32** leading to completely different phenotypes was previously observed for other naphthoquinone-derived compounds in their mode of action and supports a multi-target mechanism model [34c,d].

4. Conclusions

In this study, we described a series of substances with potent antileishmanial activity. We evaluated twenty-seven compounds, and all naphthoquinones were found to be more active against promastigote forms of antimony-sensitive and resistant strains of *Leishmania* than the trivalent antimonial drug potassium antimonyl tartrate. Compounds **30** and **33**, with the largest selectivity indexes in the range of 10–15, are important drug candidates for further investigation. The behavior of these compounds was very similar across different species of *Leishmania*. Similar effects were also observed in both the Sb-resistant mutants and their respective parental sensitive WT strains. The results described here represent an important contribution to the discovery of novel leishmanicidal drugs.

5. Experimental

5.1. Chemistry

Melting points were obtained on a Thomas Hoover melting point apparatus and are uncorrected. Analytical grade solvents were used. Column chromatography was performed on silica-gel (Acros Organics, 0.035–0.070 mm, pore diameter ca. 6 nm). Infrared spectra were recorded on a Shimadzu IR Prestige-21 FTIR Spectrometer, ^{1}H and ^{13}C NMR were recorded at room temperature using a VNMRSYS-500, Varian MR-400 instrument, in the solvents indicated, with TMS as internal reference. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hertz. High resolution mass spectra (electrospray ionization) were obtained using a Shimadzu LCMS-IT-TOF. Chemical names for all compounds were generated using CS ChemDraw Ultra version 10.0.

5.2. General procedures for preparation of 4-bromo-α-lapachone (*19*)

A solution of 4-bromo-α-lapachone (**18**) (320 mg, 1 mmol) in dichloromethane (25 mL), was treated with excess sodium azide (78 mg, 1.2 mmol) at room temperature. The reaction mixture was stirred for 24 h and filtered. The crude material was purified by column chromatography over silica-gel, eluting with a gradient mixture of hexane:ethyl acetate (9:1 to 7:3, v/v) with increasing polarity. Compound **19** was obtained as an orange solid (141 mg, 0.50 mmol, 50% yield, m.p. 120–125 °C). IR (KBr) 1650 (C=O); 1683 (C=O); 2107 (N_3) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.88 (dd, J = 3.1, 5.6 Hz, 1H), 8.17–8.10 (2H, m), 7.76–7.69 (2H, m), 2.15 (dd, J = 2.9, 14.6 Hz, 1H), 2.02 (dd, J = 5.6, 14.8 Hz, 1H), 1.55 (3H, s), 1.5 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 183.7 (C=O), 179.6 (C=O), 154.7, 133.9, 132.7, 131.6, 130.8, 126.1, 125.9, 116.8, 78.4, 37.6, 25.2, 15.4, 49.5. Compound **27** was prepared as previously described [28].

5.3. General procedures for preparation of α- and nor-α-lapachone-based 1,2,3-triazoles

The azidoquinones were reacted with $CuSO_4 \cdot 5H_2O$ (9.3 mg, 0.04 mmol) and sodium ascorbate (22 mg, 0.11 mmol) and the desired alkyne in a mixture of CH_2Cl_2:H_2O (12 mL, 1:1, v/v). The mixture was stirred at room temperature until product formation was complete as determined by thin layer chromatography. The aqueous phase was extracted with dichloromethane, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with a gradient mixture of hexane:ethyl acetate with increasing polarity to 100% ethyl acetate.

5.3.1. 4-(4-Cyclohexyl-1H-1,2,3-triazol-1-yl)-2,2-dimethyl-3,4-dihyro-2H-benzo[g]chromene-5,10-dione **20**

The substituted alkyne (1 mmol) was reacted with 4-azido-α-lapachone (**19**), (283 mg, 1 mmol), $CuSO_4 \cdot 5H_2O$ (11.3 mg, 0.04 mmol) and sodium ascorbate (27 mg, 0.11 mmol) in CH_2Cl_2:H_2O (12 mL, 1:1, v/v). Compound **20** was obtained as a yellow solid (195 mg, 0.5 mmol, 50% yield, mp 130–135 °C); IR (KBr) 1682 (C═O), 1651 (C═O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.16–8.12 (m, 1H), 8.04–7.99 (m, 1H), 7.77–7.68 (m, 2H), 7.42 (s, 1H), 5.78 (t, J = 6.36 Hz, 1H), 2.80 (dd, J = 14.60, 6.41 Hz, 1H), 2.40–2.31 (m, 3H), 2.21–2.14 (m, 2H), 1.78–1.70 (m, 3H), 1.68–1.60 (m, 4H), 1.53 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 182.7 (C═O), 179.4 (C═O), 156.1, 149.0, 134.5, 133.4, 131.7, 131.0, 127.1, 126.6, 126.4, 125.1, 118.3, 115.2, 79.3, 49.5, 39.1, 26.6, 26.3, 25.2, 22.4, 22.1.

5.3.2. 2,2-Dimethyl-4-(4-pentyl-1H-1,2,3-triazol-1-yl)-3,4-dihydro-2H-benzo[g]chromene-5,10-dione **21**

The substituted alkyne (1 mmol) was reacted with 4-azido-α-lapachone (**19**), (283 mg, 1 mmol), $CuSO_4 \cdot 5H_2O$ (11.3 mg, 0.04 mmol) and sodium ascorbate (27 mg, 0.11 mmol) in CH_2Cl_2:H_2O (12 mL, 1:1, v/v). Compound **21** was obtained as a yellow solid (227 mg, 0.6 mmol, 60% yield, mp 176–178 °C); IR (KBr) 1653 (C═O), 1611 (C═O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: 8.16–8.12 (m, 1H), 8.04–8.01 (m, 1H), 7.76–7.69 (m, 2H), 7.29 (s, 1H), 5.78–5.75 (m, 1H), 2.85–2.80 (dd, J = 14.67, 6.35 Hz, 1H), 2.70–2.66 (m, 2H), 2.37–2.32 (dd, J = 14.67, 6.35 Hz, 1H), 1.67–1.60 (m, 4H), 1.59 (s, 3H), 1.33–1.29 (m, 2H), 1.30 (s, 3H) 0.87 (t, J = 7.00 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 182.7 (C═O), 179.4 (C═O), 156.1, 148.0, 134.5, 133.4, 131.7, 131.0, 126.6, 126.4, 120.6, 115.3, 79.3, 49.4, 39.1, 31.4, 29.0, 26.7, 26.5, 25.6, 22.3, 14.0; EI/HRMS (m/z) $[M + H]^+$ 380.1891. Calcd for $[C_{22}H_{25}N_3O_3H]^+$: 380.1974.

5.3.3. 2,2-Dimethyl-4-(4-propyl-1H-1,2,3-triazol-1-yl)-3,4-dihydro-2H-benzo[g]chromene-5,10-dione **22**

The substituted alkyne (1 mmol) was reacted with 4-azido-α-lapachone (**19**), (283 mg, 1 mmol), $CuSO_4 \cdot 5H_2O$ (11.3 mg, 0.04 mmol) and sodium ascorbate (27 mg, 0.11 mmol) in CH_2Cl_2:H_2O (12 mL, 1:1, v/v). Compound **22** was obtained as a yellow solid (175 mg, 0.5 mmol, 50% yield, mp 214.2–215.2 °C); IR (KBr) 1609 (C═O), 1651 (C═O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: 8.15–8.12 (m, 1H), 8.02–7.99 (m, 1H), 7.75–7.69 (m, 2H), 7.32 (s, 1H), 5.77 (t, J = 6.37 Hz, 1H), 2.83–2.76 (m, 1H), 2.66 (dt, J = 7.24, 1.44 Hz, 2H), 2.35 (dd, J = 14.59, 6.32 Hz, 1H), 1.71–1.62 (m, 2H), 1.53 (s, 3H), 1.31 (s, 3H), 0.93 (t, J = 7.37 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 182.7 (C═O), 179.4 (C═O), 156.1, 147.7, 134.5, 133.5, 131.7, 131.0, 126.6, 126.4, 120.8, 115.2, 79.3, 49.4, 39.1, 27.5, 26.7, 26.5, 22.5, 13.7; EI/HRMS (m/z) $[M + H]^+$ 352.1636. Calcd for $[C_{20}H_{21}N_3O_3]^+$: 352.1661.

5.3.4. 4-(4-(2-Hydroxybutan-2-yl)-1H-1,2,3-triazol-1-yl)-2,2-dimethyl-3,4-dihydro-2H-benzo[g]chromene-5,10-dione **23**

The substituted alkyne (1 mmol) was reacted with 4-azido-α-lapachone (**19**), (283 mg, 1 mmol), $CuSO_4 \cdot 5H_2O$ (11.3 mg, 0.04 mmol) and sodium ascorbate (27 mg, 0.11 mmol) in CH_2Cl_2:H_2O (12 mL, 1:1, v/v). Compound **23** was obtained as a yellow solid (304 mg, 0.8 mmol, 80% yield, mp 205–208 °C); IR (KBr) 1611 (C═O), 1650 (C═O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: 8.17–8.14 (m, 1H), 8.04–8.00 (m, 1H), 7.77–7.70 (m, 2H), 7.45 (d, J = 6.87 Hz, 1H), 5.80 (dt, J = 6.25, 3.33 Hz, 1H), 2.84 (dt, J = 14.41, 6.21 Hz, 1H), 2.41–2.33 (m, 2H), 1.95–1.82 (m, 2H), 1.57 (d, J = 6.89 Hz, 3H), 1.54 (d, J = 1.81 Hz, 3H), 1.29 (d, J = 1.81 Hz, 3H), 0.83 (q, J = 7.45 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 182.7 (C═O), 179.4 (C═O), 156.1, 154.1, 134.5, 133.5, 131.7, 131.0, 126.6, 126.4, 120.0, 115.0, 79.2, 71.3, 49.4, 39.1, 35.9, 28.0, 26.7, 26.3, 8.2; EI/HRMS (m/z) $[M + H]^+$ 382.1770. Calcd for $[C_{21}H_{23}N_3O_4H]^+$: 382.4329.

5.3.5. 2,2-Dimethyl-3-(4-phenyl-1H-1,2,3-triazol-1-yl)-2,3-dihydr onaphtho[2,3-b]furan-4,9-dione **28**

The substituted alkyne (1 mmol) was reacted with 3-azido-nor-α-lapachone (**27**), (269 mg, 1 mmol), $CuSO_4 \cdot 5H_2O$ (11.3 mg, 0.04 mmol) and sodium ascorbate (27 mg, 0.11 mmol) in CH_2Cl_2:H_2O (12 mL, 1:1, v/v). Compound **28** was obtained as a yellow solid (204 mg, 0.5 mmol, 55% yield, mp 227–230 °C); IR (KBr) 1649 (C═O), 1628 (C═O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.22–8.17 (m, 1H), 8.11–8.06 (m, 1H), 7.83–7.74 (m, 4H), 7.72–7.69 (m, 1H), 7.43–7.37 (m, 2H), 7.36–7.30 (m, 1H), 6.08 (s, 1H), 1.76 (s, 3H), 1.24 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 180.7 (C═O), 177.9 (C═O), 161.3, 147.8, 134.9, 133.5, 132.7, 131.6, 130.0, 128.8, 128.4, 126.8, 126.5, 125.7, 119.1, 118.4, 94.6, 67.5, 27.5, 21.0; EI/HRMS (m/z) $[M + H]^+$ 372.1213. Calcd for $[C_{22}H_{17}N_3O_3H]^+$: 372.13483.

5.3.6. 3-(4-Hexyl-1H-1,2,3-triazol-1-yl)-2,2-dimethyl-2,3-dihydr onaphtho[2,3-b]furan-4,9-dione **29**

The substituted alkyne (1 mmol) was reacted with 3-azido-nor-α-lapachone (**27**), (269 mg, 1 mmol), $CuSO_4 \cdot 5H_2O$ (11.3 mg, 0.04 mmol) and sodium ascorbate (27 mg, 0.11 mmol) in CH_2Cl_2:H_2O (12 mL, 1:1, v/v). Compound **29** was obtained as a yellow solid (155 mg, 0.4 mmol, 41% yield, mp 173–175 °C); IR (KBr) 1659 (C═O), 1637 (C═O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.18 (dd, J = 7.53, 1.39 Hz, 1H), 8.08 (dd, J = 6.99, 1.91 Hz, 1H), 7.81–7.73 (m, 2H), 7.20 (s, 1H), 6.00 (s, 1H), 2.68 (dt, J = 7.39, 2.83 Hz, 2H), 1.72 (s, 3H), 1.67–1.59 (m, 2H), 1.34–1.24 (m, 6H), 1.16 (s, 3H), 0.85 (t, J = 6.53 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.1 (C═O), 178.1 (C═O), 161.8, 147.6, 135.0, 134.0, 133.0, 132.3, 126.4, 126.0, 122.4, 118.7, 94.3, 66.6, 31.3, 29.1, 28.5, 27.0, 25.4, 22.4, 20.7, 14.3; EI/HRMS (m/z) $[M + H]^+$ 380.2038. Calcd for $[C_{22}H_{25}N_3O_3H]^+$: 380.46014.

5.3.7. 2,2-Dimethyl-3-(4-pentyl-1H-1,2,3-triazol-1-yl)-2,3-dihydr onaphtho[2,3-b]furan-4,9-dione **30**

The substituted alkyne (1 mmol) was reacted with 3-azido-nor-α-lapachone (**27**), (269 mg, 1 mmol), $CuSO_4 \cdot 5H_2O$ (11.3 mg, 0.04 mmol) and sodium ascorbate (27 mg, 0.11 mmol) in CH_2Cl_2:H_2O (12 mL, 1:1, v/v). Compound **30** was obtained as a yellow solid (109 mg, 0.3 mmol, 30% yield, mp 105–110 °C); IR (KBr) 1649 (C═O), 1632 (C═O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.17 (dd, J = 7.28, 1.53 Hz, 1H), 8.08 (dd, J = 7.03, 1.89 Hz, 1H), 7.83–7.72 (m, 2H), 7.20 (s, 1H), 5.99 (s, 1H), 2.68 (dt, J = 7.42, 3.06 Hz, 2H), 1.72 (s, 3H), 1.67–1.59 (m, 3H), 1.34–1.27 (m, 5H), 1.16 (s, 3H), 0.87 (t, J = 6.90 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.1 (C═O), 178.1 (C═O), 161.8, 147.6, 135.0, 134.0, 133.0, 132.3, 126.4, 126.0, 122.4, 118.7, 94.4, 66.5, 31.2, 28.9, 27.0, 25.4, 22.2, 20.7, 14.3; EI/HRMS (m/z) $[M + H]^+$ 366.1880. Calcd for $[C_{21}H_{23}N_3O_3H]^+$: 366.1817.

5.3.8. 3-(4-(2-Hydroxybutan-2-yl)-1H-1,2,3-triazol-1-yl)-2,2-dimethyl-2,3-dihydronaphtho[2,3-b]furan-4,9-dione **31**

The substituted alkyne (1 mmol) was reacted with 3-azido-nor-α-lapachone (**27**), (269 mg, 1 mmol), $CuSO_4 \cdot 5H_2O$ (11.3 mg, 0.04 mmol) and sodium ascorbate (27 mg, 0.11 mmol) in CH_2Cl_2:H_2O (12 mL, 1:1,

v/v). Compound **31** was obtained as a yellow solid (201 mg, 0.5 mmol, 55% yield, mp 154–156 °C); IR (KBr) 3312 (OH), 1657 (C=O), 1634 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.18 (dd, $J = 7.29$, 1.39 Hz, 1H), 8.07 (dd, $J = 7.44$, 1.22 Hz, 1H), 7.81–7.73 (m, 2H), 7.37 (d, $J = 1.31$ Hz, 1H), 6.01 (d, $J = 11.57$ Hz, 1H), 1.95–1.80 (m, 2H), 1.72 (s, 3H), 1.56 (d, $J = 10.10$ Hz, 3H), 1.15 (s, 3H), 0.80 (q, $J = 7.37$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.1 (C=O), 178.0 (C=O), 161.8, 155.2, 135.0, 133.9, 133.0, 132.3, 126.4, 126.0, 122.2, 118.8, 94.4, 70.1, 66.6, 35.9, 28.6, 27.1, 20.7, 8.7; EI/HRMS (m/z) $[M + H]^+$ 368.1667. Calcd for $[C_{20}H_{21}N_3O_4H]^+$: 368.1610.

5.3.9. 3-(4-(2-Hydroxypropan-2-yl)-1H-1,2,3-triazol-1-yl)-2,2-dimethyl-2,3-dihydronaphtho[2,3-b]furan-4,9-dione ***32***

The substituted alkyne (1 mmol) was reacted with 3-azido-nor-α-lapachone (**27**), (269 mg, 1 mmol), $CuSO_4 \cdot 5H_2O$ (11.3 mg, 0.04 mmol) and sodium ascorbate (27 mg, 0.11 mmol) in CH_2Cl_2:H_2O (12 mL, 1:1, v/v). Compound **32** was obtained as a yellow solid (141 mg, 0.4 mmol, 40% yield, mp 202–205 °C); IR (KBr) 3428 (OH), 1693 (C=O), 1645 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.18 (dd, $J = 7.05$, 1.65, 1H), 8.07 (dd, $J = 7.05$, 1.56, 1H), 7.82–7.73 (m, 2H), 7.39 (s, 1H), 6.00 (s, 1H), 1.73 (s, 3H), 1.61 (s, 3H), 1.60 (s, 3H), 1.15 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.2 (C=O), 178.1 (C=O), 161.9, 156.4, 135.0, 133.9, 133.0, 132.4, 126.4, 126.0, 121.3, 118.6, 94.5, 67.4, 66.6, 31.1, 31.0, 27.0, 20.7; EI/HRMS (m/z) $[M]^+$ 354.1501. Calcd for $[C_{19}H_{19}N_3O_4]^+$: 354.1454.

5.3.10. 3-(4-Cyclohexyl-1H-1,2,3-triazol-1-yl)-2,2-dimethyl-2,3-dihydronaphtho[2,3-b]furan-4,9-dione ***33***

The substituted alkyne (1 mmol) was reacted with 3-azido-nor-α-lapachone (**27**), (269 mg, 1 mmol), $CuSO_4 \cdot 5H_2O$ (11.3 mg, 0.04 mmol) and sodium ascorbate (27 mg, 0.11 mmol) in CH_2Cl_2:H_2O (12 mL, 1:1, v/v). Compound **33** was obtained as a yellow solid (150 mg, 0.4 mmol, 40% yield, mp 208–210 °C); IR (KBr) 1657 (C=O), 1630 (C=O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: 8.18 (dd, $J = 7.45$, 1.39 Hz, 1H), 8.08 (dd, $J = 7.44$, 1.35 Hz, 1H), 7.77 (m, 2H), 7.29 (s, 1H), 6.00 (s, 1H), 2.36–2.31 (m, 2H), 2.20–2.15 (m, 2H), 1.72 (s, 3H), 1.67–1.62 (m, 2H), 1.62–1.59 (m, 5H), 1.19 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 180.6 (C=O), 178.0 (C=O), 161.1, 149.5, 134.9, 133.5, 132.7, 131.6, 126.8, 126.7, 126.5, 125.7, 118.6, 117.6, 94.6, 67.3, 27.4, 26.2, 25.2, 22.3, 22.1, 20.8.

6. Antileishmanial activity

6.1. Parasite

Leishmania (*Leishmania*) *amazonensis* (strains MHOM/BR/1989/BA199, sensitive and resistant to Sb(III) at concentration up to 2700 μM) and *Leishmania* (*Leishmania*) *infantum* (syn. *L. chagasi*) (strains MCAN/BR/2002/BH400, sensitive and resistant to Sb(III) at concentration up to 2700 μM) promastigotes were maintained in minimum essential culture medium (α-MEM) (Gibco, Invitrogen NY, USA) supplemented with 10% (v/v) heat inactivated fetal calf serum (FBS, Cultilab, Campinas, SP, Brazil), 100 mg/mL kanamycin, 50 mg/mL ampicillin, 2 mmol L^{-1} L-glutamine, 5 mg/mL hemin, 5 mmol L^{-1} biopterin, (Sigma–Aldrich, St Louis, USA), at pH 7.0 and incubated at 25 °C. *L. amazonensis* and *L. infantum* (syn. *L. chagasi*) were selected for Sb(III) resistance as described previously [35,36]. The Sb(III)-resistant mutants *L. amazonensis* BA199 Sb(III) 2700.2 and *L. infantum* (syn. *L. chagasi*) BH400 Sb(III) 2700.2 were selected in 25 cm^2 flasks containing 5 mL of α-MEM in the presence of increasing Sb(III) concentrations up to 2700 μmol L^{-1}.

6.2. Antileishmanial activity assay

Compounds were evaluated *in vitro* for their activity against both Sb(III)-sensitive and -resistant *Leishmania* parasites, as described previously [37]. Briefly, log-phase *L. amazonensis* and *L. infantum* (syn. *L. chagasi*) promastigotes (1×10^6 parasites/mL) were seeded in 24-well cell culture plates with 1.5 mL of α-MEM. The cells were incubated under shaking at 25 °C for 72 h in the presence of seven different concentrations of the compound to be tested. Non-treated parasites were established for growth comparison. Stock solutions of the drugs were prepared in DMSO and diluted in α-MEM cell culture medium to obtain the range of tested concentrations. The final DMSO concentration did not exceed 0.2%, which is known to be nontoxic to *Leishmania* parasites [38,39]. For drug susceptibility assays, *Leishmania* growth curves were constructed by measuring absorbance at 600 nm. Antileishmanial activity is expressed as IC_{50}/72 h, which is the compound concentration that reduces cell growth by 50% compared to untreated control (relative growth). All experiments were performed in triplicate.

6.3. Cytotoxicity assay against peritoneal macrophages

The cytotoxicity of the compounds towards murine peritoneal macrophages was evaluated using the classical 3-(4,5-dimethyl thiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) method [40]. Briefly, macrophages were obtained by lavage of the peritoneal cavities of Swiss mice with 10 mL cold RPMI-1640 without FBS. After washing, the cell suspension (4.0×10^6/mL) was seeded (0.1 mL) in 96-well flat bottom plates. Macrophages were allowed to adhere for 1 h and non-adherent cells were removed by washing with RPMI. The compounds to be studied were then added to the wells at concentrations ranging from 1 to 20 μM and the cells were further cultured in RPMI supplemented with 10% FBS for 24 h at 38 °C in a humidified 5% CO_2 atmosphere. At that point, 10 μL of MTT solution (5 mg/mL) was added to each well, and the plates were incubated for an additional 4 h. Supernatants were aspirated, and the formazan crystals formed were dissolved in 100 μL of DMSO. After 15 min of incubation at room temperature, the absorbance of solubilized MTT formazan product was measured spectrophotometrically at 570 nm. Cell viability was calculated from the ratio of the absorbance of the well treated with the drug to that of the non-treated well. The concentration of drug that decreased cell viability by 50% (CC_{50}) was determined.

6.4. Statistical analysis

IC_{50} and CC_{50} values were calculated by nonlinear regression using the software GraphPad Prism 5.0. Significance was set to $p < 0.05$.

6.5. Selectivity index

The selectivity index (SI) of the compound was calculated as the ratio between the 50% cytotoxic concentration for murine peritoneal macrophages (CC_{50}) and the 50% inhibitory growth concentration in *Leishmania* promastigote (IC_{50}); $SI = CC_{50}/IC_{50}$. An SI value less than 1 means that a compound is more toxic to mammalian cells than it is effective against the parasite.

Conflict of interest

Authors declare no conflict of interest.

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