



Strategies towards potent trypanocidal drugs: Application of Rh-catalyzed [2 + 2 + 2] cycloadditions, sulfonyl phthalide annulation and nitroalkene reactions for the synthesis of substituted quinones and their evaluation against *Trypanosoma cruzi*

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ABSTRACT

Rhodium-catalyzed [2 + 2 + 2] cycloadditions, sulfonyl phthalide annulations and nitroalkene reactions have been employed for the synthesis of 56 quinone-based compounds. These were evaluated against *Trypanosoma cruzi*, the parasite that causes Chagas disease. The reactions described here are part of a program that aims to utilize modern, versatile and efficient synthetic methods for the one or two step preparation of trypanocidal compounds. We have identified 9 compounds with potent activity against the parasite; 3 of these were 30-fold more potent than benznidazole (Bz), a drug used for the treatment of Chagas disease. This article provides a comprehensive outline of reactions involving over 120 compounds aimed at the discovery of new quinone-based frameworks with activity against *T. cruzi*.

1. Introduction

Chagas disease, caused by the hemoflagellate protozoan *Trypanosoma cruzi*, is a neglected tropical disease endemic in Latin America, affecting approximately 6 million individuals and leading to almost 10,000 deaths every year.¹ Typically, this disease is related to poverty, and low-income rural populations have been most severely affected.^{2–3} The intense migratory flux of infected people to well-developed countries (USA, Spain, Japan, Australia, among others) led to the occurrence of Chagas disease in non-endemic areas.^{4,5} Due to successful control programmes, transfusional and vectorial (triatomines) transmissions have progressively declined, and oral and congenital routes have been associated with new cases.^{6,7} Clinically, Chagas disease is characterised by acute and chronic phases. In the acute phase, which is frequently asymptomatic, a high number of bloodstream parasites are detected. On the other hand, the chronic

phase shows an important reduction in parasitemia, but different clinical manifestations can be observed: indeterminate (asymptomatic), cardiac (30% of the individuals) and digestive forms (megasyndromes). More rarely, polyneuropathy is observed.^{8–10} Up to now, only two trypanocidal drugs have been clinically employed, the 2-nitroimidazole benznidazole (Bz) and the 5-nitrofuran nifurtimox; both are very effective for acute cases, but their activity decreases with disease progression.^{11,12} Side-effects coupled with debatable efficacy in chronic cases, justifies the search for alternative drugs/combinations for Chagas disease.¹³

In the present manuscript, we describe our efforts to identify new lead compounds with trypanocidal activity. Here, we outline the synthesis and trypanocidal evaluation of 56 compounds. The synthesis of these compounds involved the preparation of more than 120 substances; this work lays the foundations for the discovery of a new and versatile family of trypanocidal compounds.

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2. Results and discussion

2.1. Chemistry

In this work we have prepared and evaluated the trypanocidal activity of three families of quinones. The first group of compounds was generated via a [2 + 2 + 2] cycloaddition-oxidation process using a Rh-catalyst. Next, we have explored a second family of compounds accessed by the reaction of sulfonyl phthalide and 2-nitrobenzofurans in an annulation type reaction. A third set of derivatives has been prepared via reactions of lawsone and 2-aminonaphthoquinone with α -bromonitroalkenes and nitroallylic acetates. Finally, three compounds were obtained via Hauser-Kraus annulation of sulfonyl phthalide with Rauhut-Currier adducts of nitroalkenes.

2.1.1. Rh-catalyzed [2 + 2 + 2] cycloaddition-oxidation process towards A-ring substituted naphthoquinones

A series of 6,7-fused tricyclic naphthoquinones were prepared utilizing a Rh-catalyzed [2 + 2 + 2] cycloaddition-oxidation protocol reported recently by our group.¹⁴ This methodology enabled efficient preparation of naphthoquinones with various A-ring substitution patterns from simple 1,6-diyne and benzoquinones. Various metal catalysts have been evaluated for this process, and these studies highlighted the efficiency of a rhodium-based system.¹⁴ Dipropargyl malonate **2**, which can be prepared in one step by alkylation of dimethyl malonate (**1**),¹⁵ underwent Rh-catalyzed cycloaddition with two equivalents of benzoquinone (**3**) to afford indane-type naphthoquinone **4** in 70% yield (Scheme 1). For the purpose of clarity, the numbering system shown in Scheme 1 is used during the following discussion.

Dipropargyl malonate **2** also underwent cycloaddition with a range of easily accessible 2-mono- or 2,3-di-substituted benzoquinones to generate B-ring substituted naphthoquinones, from which some trends in reactivity could be observed. Benzoquinones with electron-donating substituents are generally more effective substrates; 2-methoxy- and 2,3-dimethyl substituted systems provided naphthoquinones **14** and **15** in 78% and 91% yield, respectively (Scheme 2). Meanwhile, halogen substituents on the benzoquinone ring were tolerated to varying degrees. For example, chloride **16** was obtained in 60% yield, while bromide **17** and iodide **18** were obtained in diminished yields of 32% and 22%, respectively. An aniline-substituted substrate **20** was prepared by treatment of *N*-methylaniline (**19**) with excess benzoquinone **3**.¹⁶ Cycloaddition of this substrate with diyne **2** afforded aniline-substituted naphthoquinone **21** in 58% yield. Benzoquinones with electron-withdrawing substituents were investigated as substrates for this Rh-catalyzed cycloaddition methodology, but were found to undergo decomposition.

Aryl-substituted benzoquinone substrates were readily prepared from boronic acids using a procedure developed by Fujiwara *et al.*¹⁷ The benzoquinones **26–29** thus obtained underwent efficient Rh-mediated cycloaddition with diyne **2**, and here it is noteworthy that a less-activated aryl bromide **28** was well tolerated, delivering naphthoquinone **32** in 56% yield (Scheme 3). The Rh-catalyzed cycloaddition conditions could be extended to the synthesis of anthraquinones; cycloaddition of 1,4-naphthoquinone (**34**) with diyne **2** afforded **35** in 62% yield (Scheme 4).

As we were interested in evaluating the trypanocidal activity of naphthoquinones with varying A-ring substituents, different diyne substrates were subjected to the Rh-catalyzed cycloaddition with 1,4-benzoquinone (**3**). A range of 1,6-diyne tethers were found to be compatible with the cycloaddition methodology; for example, ether- and sulfonamide-linked diynes furnished **39** and **43** in 46% and 66% yield, respectively (Scheme 5). Internal diynes featuring alkyl substituents were also well-tolerated, however, efficient reaction of butyl-substituted diyne **42** required higher temperatures and excess benzoquinone **3** to proceed. Nevertheless, preparation of naphthoquinone **44** was achieved in 69% yield, and it is noteworthy that this product possesses a fully-decorated A-ring.

Dimethyl malonate (**1**) provided a versatile starting point for the preparation of differently substituted diynes (Scheme 6). Diynes **45** and **46**, each possessing one internal alkyne, underwent cycloaddition with benzoquinone **3** at the same reaction temperature used for terminal diyne substrates. Silyloxymethyl-substituted naphthoquinone **48** was produced in good yield (82%) and provides a synthetic handle for further functionalization.

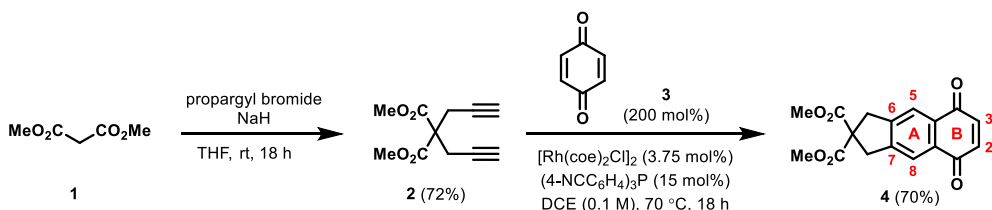
Sonogashira reaction of diyne **2** with aryl iodides enabled expedient access to aryl-substituted naphthoquinones **54–56** (Scheme 7). Electron-rich aryl substituted diynes are more competent substrates in the rhodium-catalyzed cycloaddition, a trend which is evidenced by the yields obtained for naphthoquinones **54–56**.

Indole and benzofuran-fused naphthoquinones **60** and **64** were accessed from ynamide **59** and ethynyl phenyl ether **63** (Scheme 8). These *N*- and *O*-tethered alkynes were in turn obtained from their respective aniline and phenol precursors by addition to dichloroacetylene, generated in situ from trichloroethylene.¹⁸ Halogen-lithium exchange, then elimination of the dichlorovinyl adducts provided the moderately unstable alkynes **59** and **63**, which were used in the Rh-catalyzed cycloaddition reaction immediately after isolation.

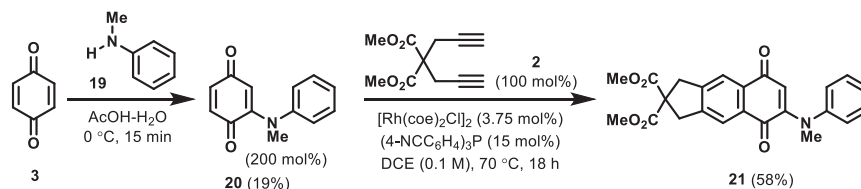
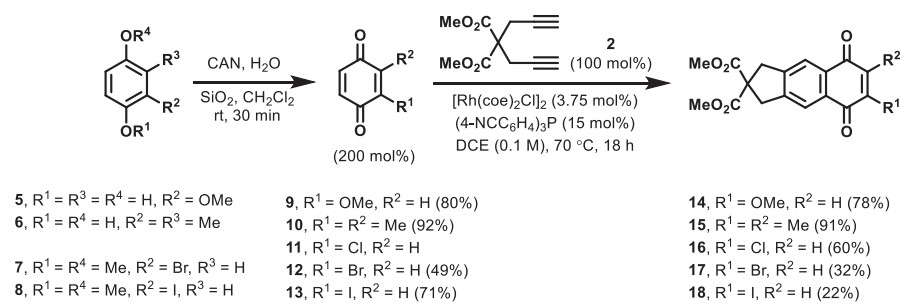
The anti-leishmanial natural product justicidone (**68**)¹⁹ was prepared in 42% yield from diyne **67** and benzoquinone **9** (Scheme 9). Diyne **67** was prepared in high yield over two steps by Sonogashira reaction of iodide **65** and propargyl alcohol, and then Steglich esterification of alcohol **66** with propiolic acid. Interestingly, the penta-substituted benzenoid core of justicidone (**68**) was afforded as a single regioisomer, despite diyne **67** and benzoquinone **9** both being non-symmetrical substrates.

1,6-Diynes with heteroatom or electron-withdrawing substituents at C-1 or C-7 were not competent substrates in the [2 + 2 + 2] cycloaddition reaction; however, the cycloadducts described above could be modified to provide a broader range of C5 and C8-substituted naphthoquinones. Silyl ether **48** underwent desilylation and oxidation to give carboxylic acid **69** when treated with Jones reagent (Scheme 10). Recently developed conditions for carbonyl-directed Ru-catalyzed C–H oxidation²⁰ enabled regioselective transformation of naphthoquinone **14** to its C-5-hydroxylated product **70** in 81% yield. 5-Hydroxynaphthoquinone **70** is activated towards further oxidation by PIFA,²¹ providing 5,8-dihydroxynaphthoquinone **71** in 75% yield under metal-free conditions.

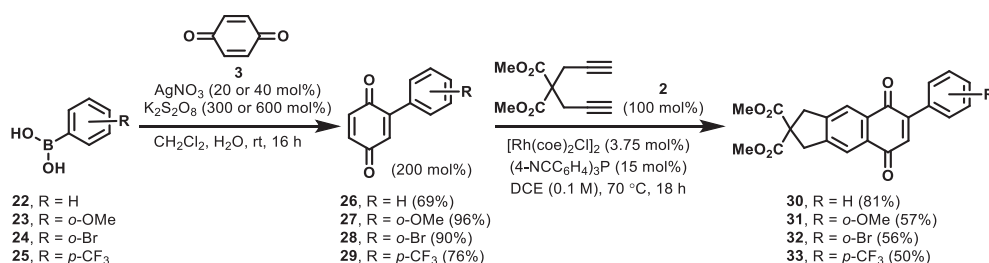
The mechanism of the [2 + 2 + 2] cycloaddition presumably commences with oxidative coupling of diyne to provide rhodacyclopentadiene. From here, cycloaddition with benzoquinone occurs to provide



Scheme 1. Synthesis of naphthoquinone **4**.



Scheme 2. Synthesis of B-ring substituted naphthoquinones 14–18 and 21.



Scheme 3. Synthesis of aryl-substituted naphthoquinones 30–33.

intermediate **1'**; this process could occur via either a migratory insertion/reductive elimination sequence or a Diels-Alder pathway.²² Oxidation of **1'** to the respective 1,4-naphthoquinone occurs in situ and is enabled by reduction of a sacrificial equivalent of benzoquinone (Scheme 11).

2.1.2. Annulation of sulfonyl phthalide with 2-nitrobenzofurans towards naphthoquinones

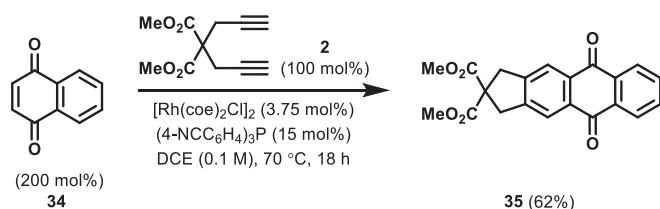
The synthesis of the second family of compounds started with the preparation of 3-sulfonyl phthalide **75** in three steps following the procedure described by Ramström et al.²³ Commercially available 2-carboxybenzaldehyde **72** was reacted with methyl iodide in acetone to generate methyl 2-formylbenzoate **73**, which on treatment with thiophenol in the presence of triethylamine in chloroform afforded **74**. Compound **74** was then oxidized with *m*-CPBA to provide 3-sulfonyl phthalide **75** in good yield (Scheme 12A). Subsequently, 2-nitrobenzofurans were prepared on the basis of a protocol reported in the literature.²⁴ 2-Hydroxy- β -nitrostyrenes **82–87** were obtained from commercially available salicylaldehydes **76–81** by their reaction with nitromethane in the presence of NH₄OAc/AcOH at 90 °C. Then, the formation of 2-nitrobenzofurans **88–93** was performed in 2 steps. Conjugate reduction using NaBH₄ in CHCl₃-*i*PrOH at 0 °C was followed

by intramolecular cyclization and oxidation using PIDA and TBAI in the presence of triethylamine and acetonitrile as solvent (Scheme 12B).

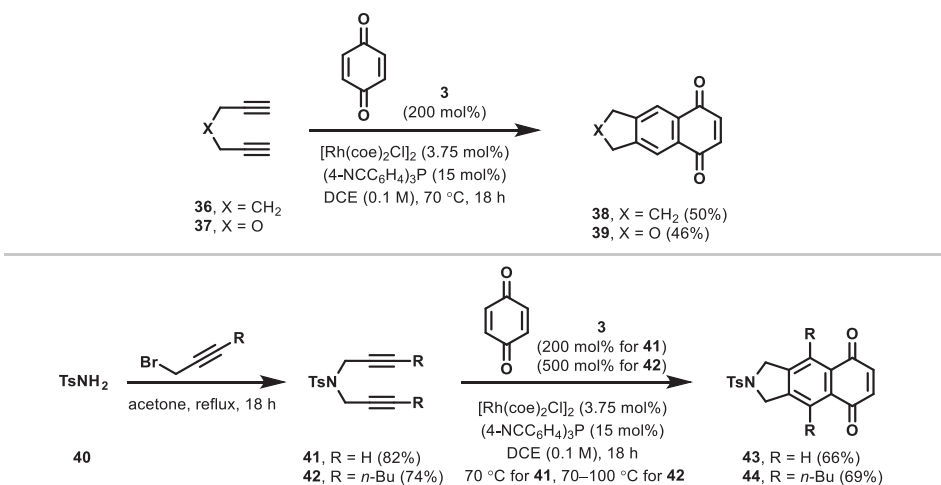
With 2-nitrobenzofurans in hand, we decided to optimize the annulation reaction of 3-sulfonyl phthalide **75** and 2-nitrobenzofuran **88** to enable subsequent studies (Table 1). Initially, we evaluated the use of Cs₂CO₃ in different solvents, such as DCM, MeCN, toluene and THF (entries 1–4). The use of Cs₂CO₃ as base and THF as solvent allowed us to prepare the desired product in 81% yield. Subsequent studies with different bases offered no further improvements (entries 5–8).

With the optimized reaction conditions in hand, the scope of different 2-nitrobenzofurans **88–93** was explored. Nitroalkenes with electron-neutral and electron-rich substituents were treated with 3-sulfonyl phthalide **75** to afford the benzofuran fused naphthoquinone derivatives **94–99** as summarized below (Scheme 13). The reaction with parent 2-nitrobenzofuran **88** under the optimized reaction conditions delivered the desired product **94** in 81% yield. 2-Nitrobenzofurans **89–91** containing a strongly electron donating alkoxy groups underwent smooth reaction with phthalide **75** to afford the benzofuran fused naphthoquinones **95–97** in excellent yields (84–87%). Notably, annulations with nitroalkenes bearing one or two methyl groups, such as **92–93**, provided the corresponding benzofuran fused naphthoquinones **98–99** in 70–72% yields. Compounds **94–99** are described here for the first time and have been characterized by various analytical and spectroscopic techniques. In addition, compound **95** was analysed by X-ray crystallography - the ORTEP-3 structure is shown in Fig. 1.

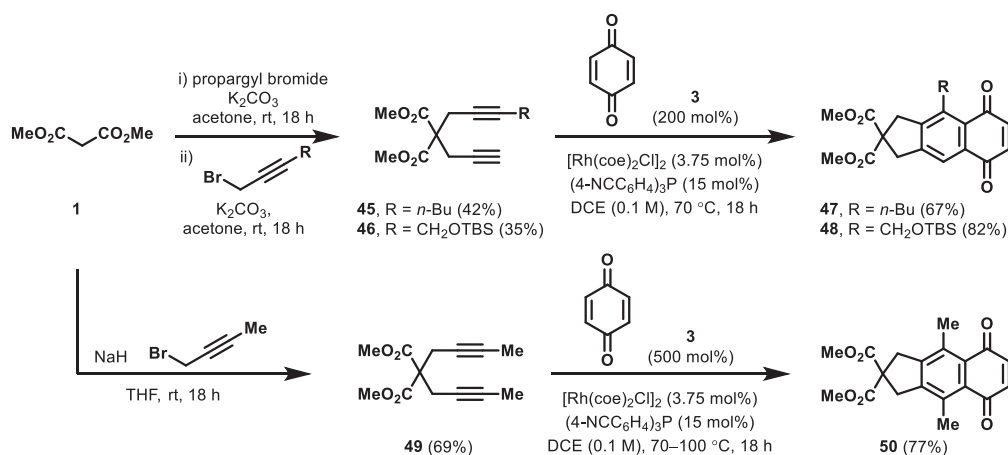
The proposed mechanism of the annulation reaction is based on relevant literature, including our recent work.²⁵ 3-Sulfonyl phthalide **75** functions as a 1,4-dipolar synthon (donor and acceptor) in the Hauser-Kraus annulation. First, base-mediated deprotonation of phthalide **75** generates stabilized carbanion **I**, which participates in Michael addition with 2-nitrobenzofuran **88**, resulting in the formation of Michael adduct **II** (Scheme 14). This undergoes Thorpe-Ingold



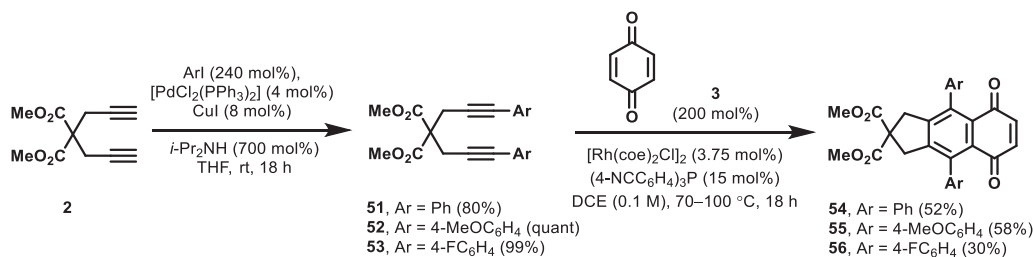
Scheme 4. Synthesis of anthraquinone 35.



Scheme 5. Synthesis of naphthoquinones 38, 39, 43 and 44.



Scheme 6. Synthesis of 5- and 8-substituted naphthoquinones 47, 48 and 50.



Scheme 7. Synthesis of 5,8-diarylnaphthoquinones 54–56.

facilitated Dieckmann cyclization with the lactone resulting in the formation of **III**. Intermediate **III** undergoes elimination of a sulfonyl anion as well as the nitro group, which results in the formation of benzofuran-fused naphthoquinone **94**.

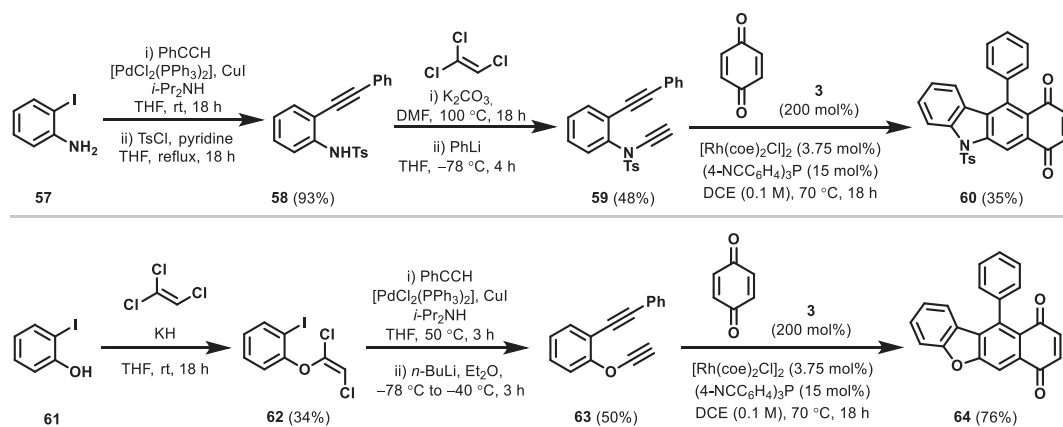
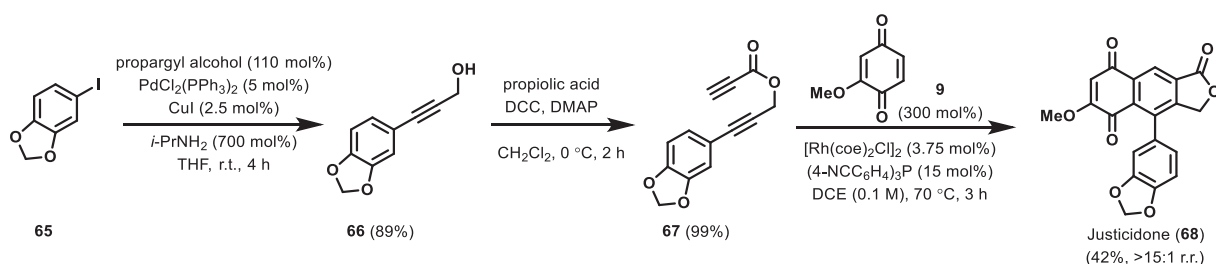
2.1.3. Naphthoquinones via reactions of lawsone and 2-aminonaphthoquinone with α -bromonitroalkenes

Using a protocol reported recently by our group, a series of pyrrolonaphthoquinones and furanonaphthoquinones were prepared by reacting lawsone and 2-aminonaphthoquinone with α -bromonitroalkenes.²⁶ Initially, we accomplished the reaction between substituted α -bromonitroalkenes and various *N*-arylated aminonaphthoquinones to prepare compounds 100–112 in good to moderate yields (Scheme 15A). In Scheme 15B we demonstrate the preparation of furanonaphthoquinones 114–119. The methodology used here was also

based on our previous report²⁶ and the method described by Zhang and co-workers.²⁷ We used lawsone (**113**) for reactions with α -bromonitroalkenes to obtain compounds 114–119 in good to excellent yields (56–87%).

2.1.4. Compounds prepared via Hauser-Kraus annulation of sulfonyl phthalide with Rauhut-Currier adducts of nitroalkenes

The last series of compounds described in this manuscript was prepared using the methodology recently described by the Namboothiri group.²⁸ Here, Hauser-Kraus annulation of phthalide **75** with α,β -disubstituted nitroalkenes led to the formation of unsymmetrically substituted naphthoquinones 120–122 in good to high yield (64–75%) (Scheme 16).

Scheme 8. Synthesis of indole and benzofuran-fused naphthoquinones **60** and **64**.Scheme 9. Synthesis of justicidone (**68**).

2.2. Biological studies

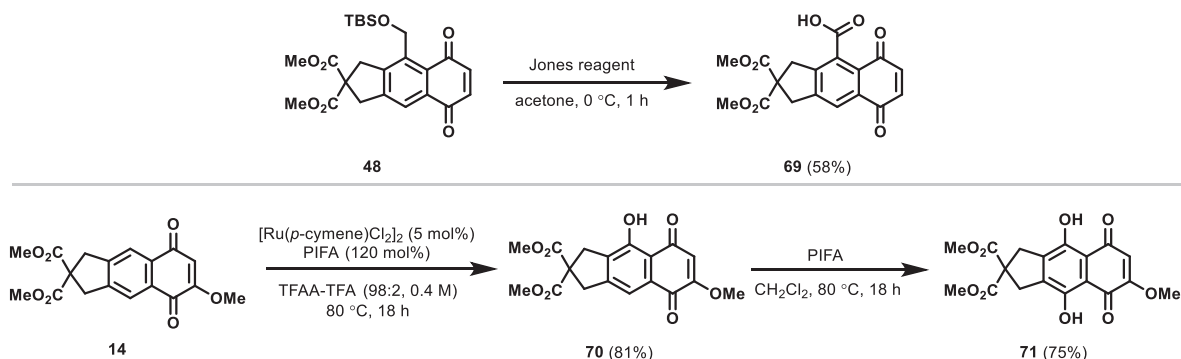
In the past decade, the da Silva Júnior, de Castro, and Mennabarreto groups have evaluated the trypanocidal potential of quinones and heterocycles as part of a Medicinal Chemistry program for the evaluation of compounds against the parasite *Trypanosoma cruzi*.²⁹ Our mission is to identify synthetic strategies for the discovery of compounds with powerful trypanocidal activity and low cytotoxicity. This could enable the establishment of a research base for the synthesis of efficient prototypes to combat the parasite.

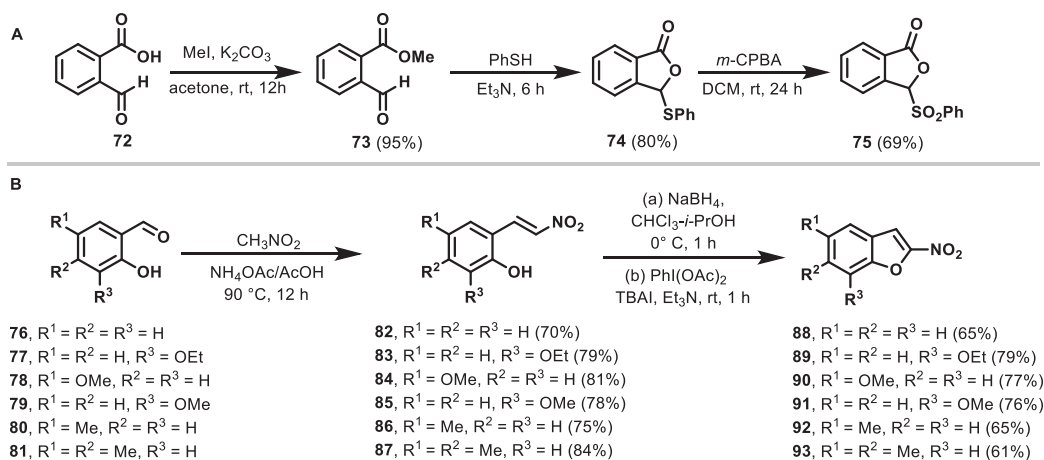
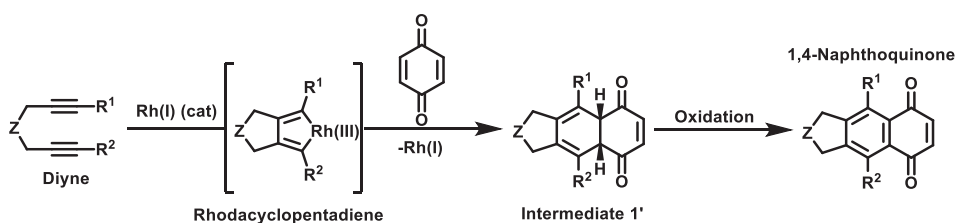
The search for novel compounds with features suitable for eventual progression to the clinic is well known to be challenging.³⁰ We are aware of the need to approach the synthesis of trypanocidal substances via versatile and efficient synthetic routes. This kind of approach can provide important details on structure-activity relationships.

The quinoidal framework presents dual reactivity, possessing both nucleophilic or electrophilic behavior; the relative propensity of these characteristics depends on the reaction medium and specific conditions used.³¹ Aware of these aspects, our research group developed

transition-metal catalyzed reactions that allow the direct modification of the A-ring of naphthoquinones.³² Controlling reactions to modify A-ring quinonoid systems allowed us to synthesize several series of compounds and evaluate their trypanocidal activity. In this context, we assayed quinoidal molecules containing oxygen,²⁰ selenium,³³ sulfur,³⁴ iodine and aryl,³⁵ triazole³⁶ and alkene³⁷ groups on the A-ring. These studies clearly indicated that the substitution of this ring intensifies trypanocidal activity - the substitution pattern is closely related to the ability of the compound to kill the parasite and also to its cytotoxicity (Scheme 17).

The first family of compounds described here was prepared via a direct and modern strategy using [2 + 2 + 2] cycloaddition reactions to prepare A-ring modified quinones. Our strategy has proved efficient, as we have identified eight compounds with trypanocidal activity greater than benznidazole, a drug used against *T. cruzi* in the clinic. Compound **4**, prepared in only two synthetic steps, showed $IC_{50}/24\text{ h} = 51.5\ \mu\text{M}$ and is twofold more potent than benznidazole (Table 2). Similarly, derivatives **16**, **18** and **32** were prepared from commercially available reactants in two steps and present outstanding

Scheme 10. Derivatization of naphthoquinones **48** and **14**.



trypanocidal activity with $IC_{50}/24\text{ h}$ values = 56.6, 51.3 and 51.5 μM , respectively. The synthesis of compound **38** was performed in only one step and has an $IC_{50}/24\text{ h}$ = 49.2 μM , which is around two times more active than benzimidazole. Easy access to this substance allows the planning of structural modifications to increase its antiparasitic potential. Compound **47** was also prepared in two steps and presented $IC_{50}/24\text{ h}$ = 64.4 μM . This derivative was also more active than Bz. The most potent compounds described here are derivatives **54**, **55** and **56** with IC_{50} values lower than 3.5 μM . We also observed that the addition of aryl groups to the A-ring increased trypanocidal activity. This effect was most pronounced for electron rich aromatic units, as in the case of compound **55**. Arenes with electron withdrawing groups, such as compound **56**, showed significant but slightly attenuated trypanocidal activity (Scheme 18).

Table 1
Selected optimization results.^a

Entry	Base	Solvent	Yield (%) ^b
1	Cs_2CO_3	DCM	50
2	Cs_2CO_3	MeCN	71
3	Cs_2CO_3	Toluene	46
4	Cs_2CO_3	THF	81
5	KO^tBu	THF	20
6	LiO^tBu	THF	40
7	DBU	THF	12
8	K_2CO_3	THF	50

^a Reaction conditions: 3-sulfonyl phthalide (**75**, 1 equiv), 2-nitrobenzofuran (**88**, 1 equiv), base (1 equiv) in 5–10 mL of solvent stirred at rt under N_2 atmosphere.

^b Yield after purification by column chromatography.

Unfortunately, we did not observe significant trypanocidal activity for compounds prepared by annulation reactions with 3-sulfonyl phthalide and 2-nitrobenzofurans, or for those prepared in Section 2.1.3. Compounds **94–112** and **114–119** had $IC_{50}/24\text{ h}$ > 500 μM and so were not active against *T. cruzi*.

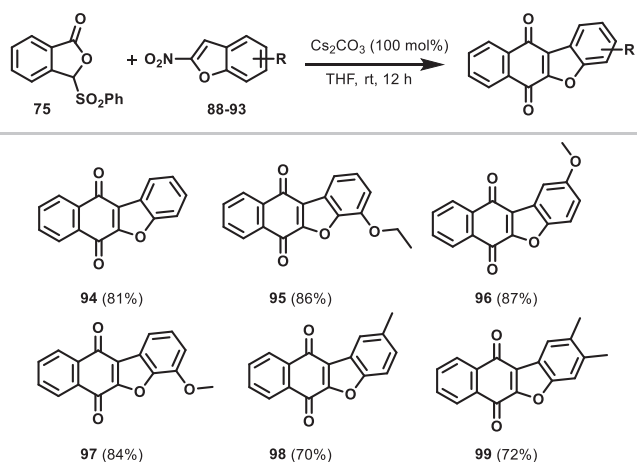
The last series of compounds **120–122** was also evaluated against *T. cruzi* and we identified two compounds with similar activity to benzimidazole (Table 2). The cytotoxicity of the compounds was also evaluated (Table 3). Compounds **120** and **121** with SI = 1.38 and 1.74, could be also considered important prototypes for subsequent studies.

We also evaluated the cytotoxicity of 11 compounds that showed outstanding trypanocidal activity (Table 3). In general, the compounds showed cytotoxicity against mammalian cells, but still had a selectivity index near 1, as in the case of compounds **55** and **56**. These results are promising and define these structures as special prototypes for the preparation of new bioactive compounds that are efficient for combating parasites, such as *T. cruzi*.

Since the 1990s, Pinto and de Castro's groups have established the foundations for trypanocidal studies involving quinones and heterocyclic compounds.³⁸ The trypanocidal assays have been standardized as: (a) 24 h of incubation; (b) 4 °C (blood bank temperature); and (c) presence of 5% mouse blood (due to the inactivation by serum components).³⁹ Trypanocidal activity is expressed as an IC_{50} value corresponding to the concentration that lyses 50% of the parasites. It is well-known that low temperatures can lead to low biological activity and so the values of $IC_{50}/24\text{ h}$ may be higher than values obtained when the analyses are performed at 37 °C (host temperature). Accordingly, we decided to evaluate the trypanocidal activity of the three most active compounds **54–56** at 37 °C in the absence of blood (Table 4). As expected, the activity of **54**, **55** and **56** increased at least 5x, with IC_{50} values in the nanomolar range. Consequently, the SI values increased and were 3.7, 13.9 and 9.3 for compounds **54**, **55** and **56**, respectively.

2.3. Structure activity relationships: some general aspects

To consider further the potential of the quinones described here as



Scheme 13. Synthesis of quinones 94–99 from 3-sulfonyl phthalide 75.

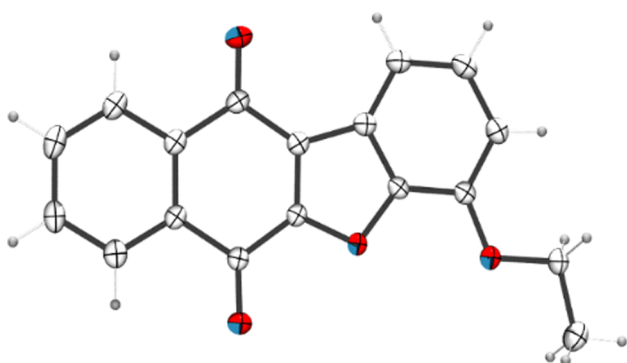
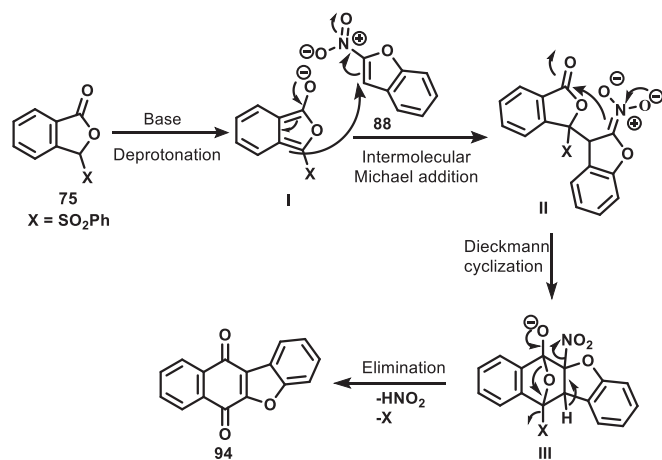
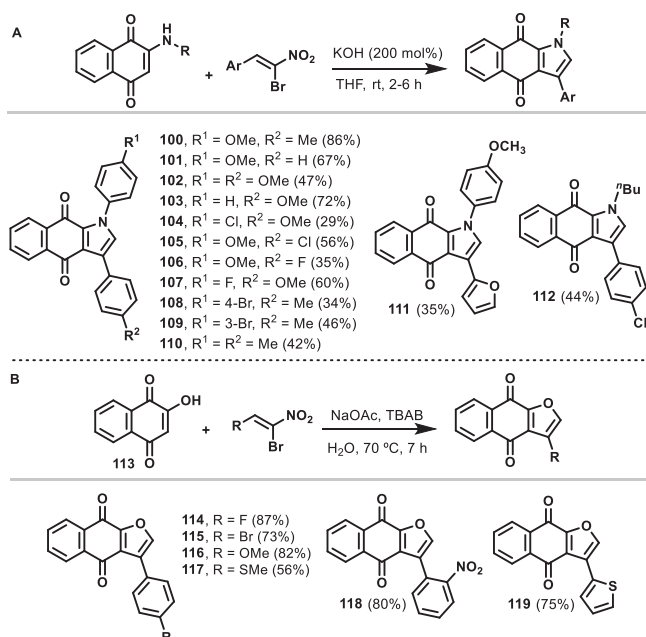


Fig. 1. ORTEP-3 projection of the compound 95 with 50% probability displacement ellipsoids.

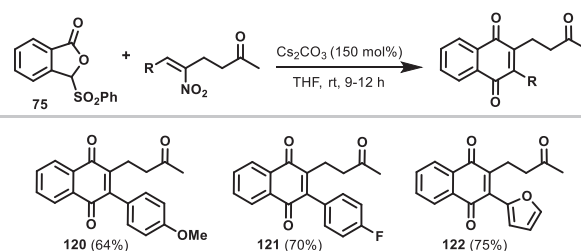


Scheme 14. Proposed mechanism for the formation of compounds 94–99.

trypanocidal compounds, it is important to establish details that enable the understanding of the relationship between structure and activity. In total 56 compounds were evaluated against *T. cruzi* in this work. Although the compounds have in common the quinonoid nucleus, the library is diverse and can be broadly divided into 5 subgroups, according to the substitution in the A- and B-rings in comparison with 1,4-naphthoquinone (34). We categorize the molecules into: (1) A-ring functionalized quinones; (2) A- and B-ring functionalized quinones; (3) B-ring modified quinones with oxygenated heterocycles; (4) B-ring modified quinones with nitrogenated heterocycles and; (5) B-ring functionalized quinones.



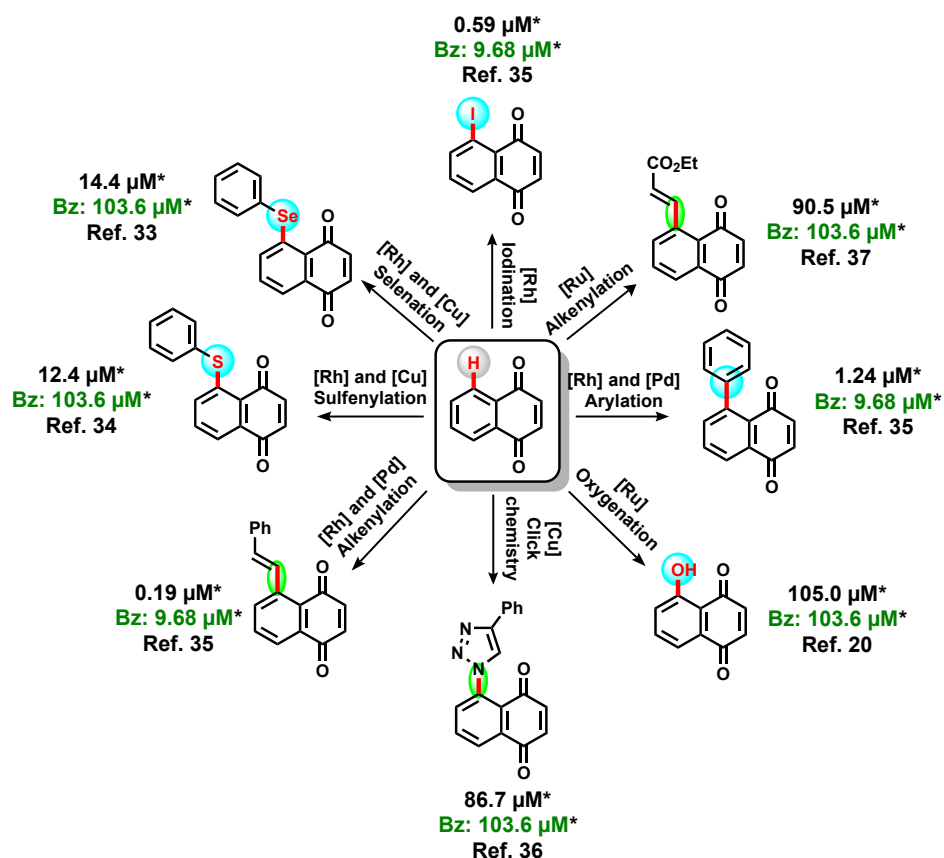
Scheme 15. Synthesis of pyrrolonaphthoquinones 100–112 and furanonaphthoquinones 114–119.



Scheme 16. Synthesis of compounds 120–122 prepared via Hauser-Kraus annulation of 3-sulfonyl phthalide (75).

1. *A-ring functionalized quinones*: The strategy for obtaining new trypanocidal compounds was based on the structural modification of the basic nucleus of 1,4-naphthoquinone (34). Here it is important to note that we are discussing the design of new bioactive molecules and not synthetic routes for the preparation of new compounds, which can be prepared from 1,2-benzoquinones or other quinonoid derivatives. The structure of 34 is composed of two rings, which we define as the A- and B-rings. Studies previously published by our group have described that the simple modification of these rings can cause either an increase or decrease in trypanocidal activity.^{33–37} We observed that A-ring modified compounds containing a fused cyclopentane were highly active against *T. cruzi*. The appendage of substituted aryl groups also enhances the trypanocidal activity. On the other hand, when the A-ring was substituted by *O*- or *N*-heterocyclic compounds we observed that trypanocidal activity was only moderate. The substitution of the A-ring with electron donating groups, for instance methyl groups, delivers compounds that are inactive against *T. cruzi* (see Table 5 for more details).

2. *A- and B-ring functionalized quinones*: The compound library included 13 A- and B-ring modified quinones. Three molecules from this group were highly active against *T. cruzi* with IC₅₀ in the range of 51.3 and 56.6 μM. In general, we observed that the presence of chlorine and iodine on the B-ring did not cause a significant change in the trypanocidal activity of the respective compounds 16 and 18, and did not decrease the ability of the compounds to kill the parasite. On the contrary, the presence of bromine on the B-ring decreased activity against the parasite. Aryl bromides on the B-ring did not cause a change in



Scheme 17. Trypanocidal A-ring substituted quinones. *IC₅₀/24 h values for the lytic activity on bloodstream trypomastigotes.

Table 2
IC₅₀/24 h (μM) of quinones against the trypomastigote form of *T. cruzi*.^a

Compounds	IC ₅₀ /24 h	Compounds	IC ₅₀ /24 h
4	51.5 (± 13.2)	94	> 500.0
14	> 500.0	95	> 500.0
15	> 500.0	96	> 500.0
16	56.6 (± 7.6)	97	> 500.0
17	149.5 (± 15.3)	98	> 500.0
18	51.3 (± 3.3)	99	> 500.0
21	> 500.0	100	> 500.0
30	368.7 (± 32.4)	101	> 500.0
31	102.0 (± 9.7)	102	> 500.0
32	51.5 (± 5.2)	103	> 500.0
33	361.6 (± 45.5)	104	> 500.0
35	> 500.0	105	> 500.0
38	49.2 (± 4.4)	106	> 500.0
39	192.9 (± 22.3)	107	> 500.0
43	> 500.0	108	> 500.0
44	> 500.0	109	> 500.0
47	64.4 (± 1.8)	110	> 500.0
48	> 500.0	111	> 500.0
50	> 500.0	112	> 500.0
54	2.5 (± 0.1)	114	> 500.0
55	2.3 (± 0.2)	115	> 500.0
56	3.3 (± 0.1)	116	> 500.0
60	122.4 (± 4.9)	117	> 500.0
64	247.3 (± 4.7)	118	> 500.0
68	> 500.0	119	> 500.0
69	> 500.0	120	109.2 (± 10.9)
70	> 500.0	121	113.9 (± 11.7)
71	> 500.0	122	> 500

^a Mean ± SD of at least three independent experiments, 5% of blood at 4 °C. IC₅₀/24 h for benzimidazole = 103.6 (± 0.6)⁴⁹.

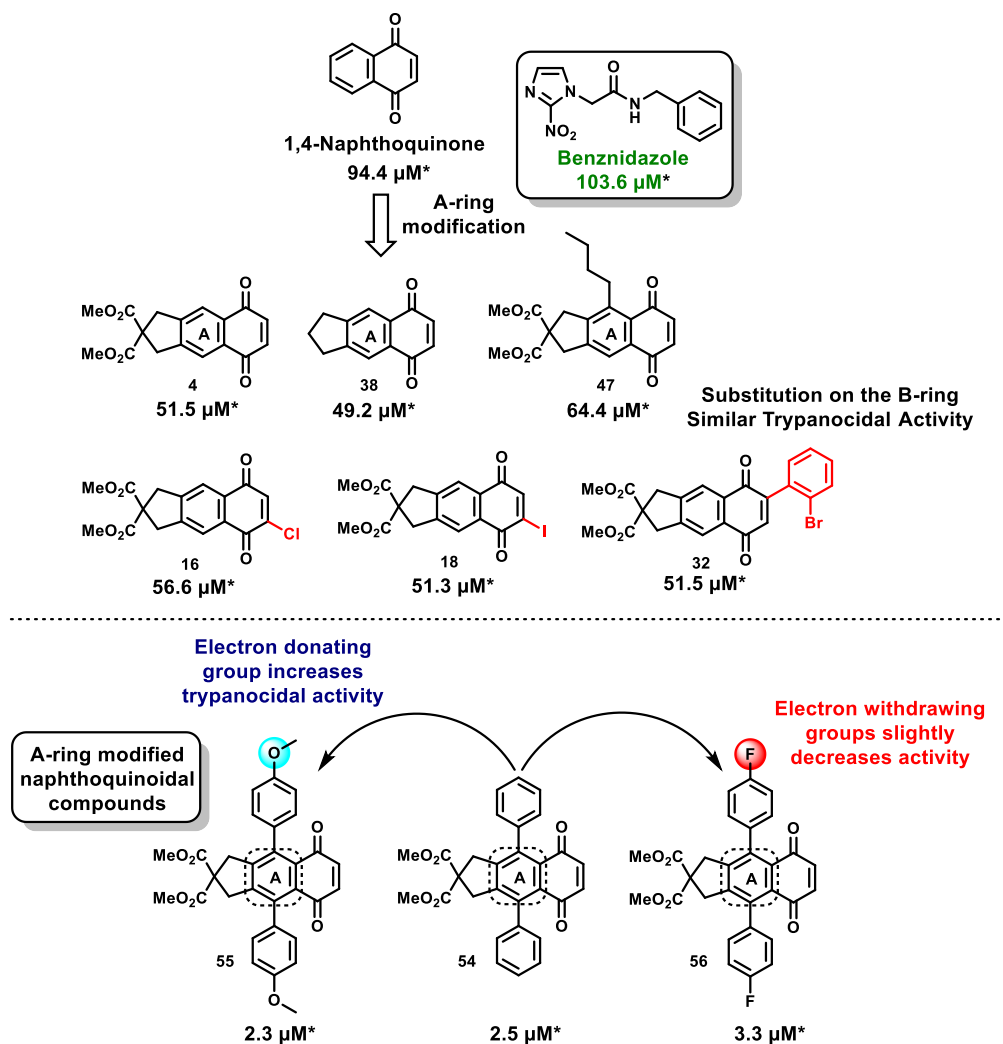
trypanocidal activity, but aryl groups without substitution or with other substitution, such as methoxy or trifluoromethyl groups, decreased trypanocidal activity. An important observation is that electron donating substituents on B-ring, such as methoxy, methyl and arylamino groups, deactivate the compound's ability to eliminate the parasite (for more details, see Table 6).

3. *B*-ring modified quinones with oxygenated heterocycles and 4. *B*-ring modified quinones with nitrogenated heterocycles: Unfortunately, all compounds with these modifications were inactive against *T. cruzi* (compounds 94–112 and 114–119). To gain a better understanding of the biological aspects, studies with derivatives of this series of these compounds are in progress in our laboratories and will be published in due course.

5. *B*-ring functionalized quinones: Compounds 120–122 with substitution only on the B-ring were also prepared and evaluated against *T. cruzi*. Quinones 120 and 121 showed moderate activity against the parasite, showing that different patterns of substitution on the A-ring are important for trypanocidal activity. In general, the trypanocidal activity of quinones is intrinsically related to the generation of ROS. The mechanism of pharmacological action of the compounds described here has not yet been established, but studies previously published in the literature demonstrate that functionalization of benzenoid or quinoid rings can cause changes in the redox system with direct consequences on the parasitic activity observed.²⁹

3. Conclusions

Effective methods for the synthesis of quinones have been described. The strategies discussed here allowed the preparation of bioactive molecules in a fast, simple and direct manner. This synthetic efficiency underpinned the evaluation of 56 compounds and the



Scheme 18. Trypanocidal A-ring modified quinones. *IC₅₀/24 h values for the lytic activity on bloodstream trypomastigotes.

Table 3
IC₅₀/24 h, LD₅₀/24 h (μM) and Selectivity Index (SI) of most active quinones.

Compounds	IC ₅₀ /24 h ^a	LD ₅₀ /24 h	SI
4	51.5 (± 13.2)	1.6 (± 0.7)	0.03
16	56.6 (± 7.6)	5.0 (± 0.8)	0.09
18	51.3 (± 3.3)	1.6 (± 0.3)	0.03
32	51.5 (± 5.2)	3.8 (± 1.5)	0.07
38	49.2 (± 4.4)	1.7 (± 0.3)	0.03
47	64.4 (± 1.8)	4.2 (± 1.7)	0.07
54	2.5 (± 0.1)	1.6 (± 0.5)	0.60
55	2.3 (± 0.2)	1.9 (± 0.3)	0.81
56	3.3 (± 0.1)	3.0 (± 1.2)	0.89
120	109.2 (± 10.9)	79.0 (± 4.7)	1.38
121	113.9 (± 11.7)	65.4 (± 6.5)	1.74

^a 5% of blood at 4 °C.

Table 4
IC₅₀/24 h, LD₅₀/24 h (μM) and Selectivity Index (SI) of most quinones 54–56.

Compounds	IC ₅₀ /24 h ^a	LD ₅₀ /24 h	SI
54	0.44 (± 0.04)	1.6 (± 0.5)	3.7
55	0.14 (± 0.04)	1.9 (± 0.3)	13.9
56	0.32 (± 0.04)	3.0 (± 1.2)	9.3

^a 0% of blood at 37 °C. IC₅₀/24 h for benznidazole = 8.8 (± 1.1) μM , LD₅₀/24 h > 1000 μM .

identification of various trypanocidal derivatives more active than benznidazole, a drug used in the treatment of Chagas disease. For instance, we identified 3 compounds that are around 30-fold more active than Bz. After evaluating the cytotoxicity of these compounds in mammalian cells, the selectivity index for these substances was close to 1. In general terms, we describe here a set of substances with potent trypanocidal activity that offer high potential for further optimization. Our ongoing aim is to target more active compounds with lower cytotoxicity. Additionally, studies into the mechanism of pharmacological action of the more active compounds are ongoing in our laboratories and will be published in due course.

4. Experimental section

4.1. Chemistry

4.1.1. General experimental details

Starting materials sourced from commercial suppliers (Acros, Aldrich, Alfa Aesar, Fluorochem, TCI) were used as received unless otherwise stated. Anhydrous 1,2-dichloroethane was sourced from Aldrich and used as received. Other dry solvents, where necessary, were obtained by distillation using standard procedures or by passage through a column of anhydrous alumina using equipment from Anhydrous Engineering. Petrol refers to the fraction of petroleum ether boiling in the range of 40–60 °C. The removal of solvents in vacuo was achieved using both a Büchi rotary evaporator (bath temperatures up to

Table 5
A-ring functionalized quinones.

A-ring functionalized quinones						
Highly active compounds						
(4) 51.5 μM^* 	(38) 49.2 μM^* 	(47) 64.4 μM^* 	(54) 2.5 μM^* 	(55) 2.3 μM^* 	(56) 3.3 μM^* 	
- A-ring modification can increase trypanocidal activity - Addition of aryl groups onto the A-ring increases trypanocidal activity strongly - Electron donating aryl groups are slightly more active against <i>T. cruzi</i>						
Moderately active compounds			Inactive compounds			
(39) 129.9 μM^* 	(60) 122.4 μM^* 	(64) 247.3 μM^* 	>500 μM^* 	(48) >500 μM^* 	(50) >500 μM^* 	(69) >500 μM^*
- Addition of heterocycles onto the A-ring decreases trypanocidal activity - Electron donating groups, for instance <i>n</i> -Bu and Me groups, makes the compounds inactive against the <i>T. cruzi</i>						

*IC₅₀/24 h values for the lytic activity on bloodstream trypomastigotes.

45 °C) at a pressure of either 4 mbar (diaphragm pump) or 1 mbar (oil pump), as appropriate, and a high vacuum line at room temperature. Reactions requiring anhydrous conditions were run under an atmosphere of dry nitrogen or argon; glassware was either flame dried immediately prior to use or placed in an oven (200 °C) for at least 2 h and allowed to cool either in a desiccator or under an atmosphere of nitrogen or argon; liquid reagents, solutions or solvents were added via syringe through rubber septa; solid reagents were added via Schlenk type adapters. Commercially available Merck Kieselgel 60F254 aluminium backed plates were used for TLC analysis. Visualisation was achieved by either UV fluorescence, basic KMnO₄ solution and heat. Flash column chromatography was performed using silica gel (Aldrich 40–63 μm , 230–400 mesh). The crude material was applied to the

column as a solution in CH₂Cl₂ or by pre-adsorption onto silica, as appropriate. Melting points were determined using a Reichert melting point table and temperature controller and are uncorrected. Infrared spectra were recorded in the range 4000–600 cm⁻¹ on a Perkin Elmer Spectrum either as neat films or solids compressed onto a diamond window. NMR spectra were recorded using either a Varian 400-MR, or Bruker Nano 400. Chemical shifts (δ) are quoted in parts per million (ppm), coupling constants (*J*) are given in Hz to the nearest 0.5 Hz. ¹H and ¹³C NMR spectra were referenced to the appropriate residual solvent peak. Mass spectra were determined using a Shimadzu GCMS QP2010+ (EI + mode), Bruker Daltonics FT-ICRMS Apex 4e 7.0T FT-MS (ESI + mode), Thermo Scientific Orbitrap Elite (APCI mode).

Table 6
A- and B-ring functionalized quinones.

A- and B-ring functionalized quinones				
Highly active compounds			Moderately active compounds	
(16) 56.6 μM^* 	(18) 51.3 μM^* 	(32) 51.5 μM^* 	(17) 149.5 μM^* 	
- Chlorine and iodine substituents on the B-ring did not cause a significant change in trypanocidal activity - A bromine substituent on the B-ring decreased activity against the parasite - Aryl bromide substituents on the B-ring did not cause a change in trypanocidal activity - Aryl groups without substitution, or with OMe or CF ₃ groups decreased trypanocidal activity				
Inactive compounds				
(14) >500 μM^* 	(15) >500 μM^* 	(21) >500 μM^* 	(35) >500 μM^* 	
- The compounds were inactive against <i>T. cruzi</i> when methoxy, methyl or arylamino groups are inserted on B-ring - The presence of an aromatic C-ring also caused the loss of trypanocidal activity				

*IC₅₀/24 h values for the lytic activity on bloodstream trypomastigotes.

4.1.2. Rh-Catalyzed [2 + 2 + 2] cycloaddition-oxidation process towards A-ring substituted naphthoquinones

General procedure for the [2 + 2 + 2] cycloaddition reactions was based on our previous report.¹⁴ A flame-dried reaction tube, fitted with a magnetic stirrer, was charged with [Rh(coe)₂Cl]₂ (3.75 μmol, 2.69 mg, 3.75 mol%), (4-NCC₆H₄)₃P (15 μmol, 5.06 mg, 15 mol%) and the appropriate quinone substrate (0.4 mmol, 200 mol% or 1.0 mmol, 500 mol%, as specified). The tube was flushed with argon, then fitted with a rubber septum, placed under a balloon of argon, and DCE (0.25 mL) was added via syringe. The reaction tube was placed in a preheated heating block at 70 °C, then the appropriate diyne substrate (0.1 mmol, 100 mol%) in DCE (0.75 mL) was added dropwise by syringe pump over 2 h. After the addition of diyne was complete, the reaction mixture was stirred at 70 °C or 100 °C (as specified) for 16 h, then cooled to room temperature and concentrated *in vacuo*. The crude reaction mixture was purified by flash column chromatography on silica gel using either a petroleum ether/ethyl acetate or acetone/toluene eluent system to yield the target naphthoquinones.

4.1.3. Annulation of sulfonyl phthalide with 2-nitrobenzofurans towards naphthoquinones

General procedure for the synthesis of annulated quinones. To a stirred solution of 3-sulfonyl phthalide **75** (0.2 mmol, 54 mg, 1.0 equiv) and 2-nitrobenzofurans **88–93** (0.2 mmol, 1.0 equiv) in THF (5.0 mL) was added Cs₂CO₃ (0.2 mmol, 66 mg, 1.0 equiv) at room temperature until completion of the reaction (overnight, 12 h, monitored by TLC). The solvent was evaporated *in vacuo*. The residue was then subjected to silica gel column chromatography by gradient elution with ethyl acetate/hexane (2:98) affording the respective quinones **94–99**.

Naphtho [2,3-*b*] benzofuran-6,11-dione (94). Yellow solid; Yield 40 mg, 81%, mp 231–233 °C; IR (neat, cm⁻¹) 3077 (vw), 2915 (vw), 2847 (vw), 1660 (br vs), 1563 (m), 1486 (w), 1223 (m), 1179 (m), 987 (m), 747 (s), 713 (s); ¹H NMR (500 MHz, CDCl₃) δ 7.50 (t, *J* = 7.8 Hz, 1H), 7.59 (t, *J* = 7.8 Hz, 1H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.75–7.84 (m, 2H), 8.20–8.28 (m, 2H), 8.31 (d, *J* = 7.8 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 113.1, 122.9, 124.2, 126.3, 127.0, 127.1, 129.8, 132.5, 133.5, 134.1, 134.4, 153.7, 156.6, 175.7, 181.6; HRMS (ES +) calcd for C₁₆H₉O₃ (MH⁺) 249.0544, found 249.0546.

4-Ethoxynaphtho [2,3-*b*] benzofuran-6,11-dione (95). Orange solid; Yield 50 mg, 86%, mp 224–226 °C; IR (neat, cm⁻¹) 3080 (w), 2974 (m), 2920 (m), 1673 (br vs), 1284 (m), 1241 (m), 780 (vs), 710 (vs); ¹H NMR (500 MHz, CDCl₃) δ 1.54 (t, *J* = 7.0 Hz, 3H), 4.30 (q, *J* = 7.0 Hz, 2H), 7.03 (d, *J* = 8.0 Hz, 1H), 7.38 (t, *J* = 8.0 Hz, 1H), 7.75–7.79 (m, 2H), 7.84 (d, *J* = 8.0 Hz, 1H), 8.21–8.25 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 14.8, 65.0, 112.1, 115.3, 124.4, 124.5, 126.8, 126.9, 127.0, 132.4, 133.3, 133.8, 134.1, 145.6, 146.4, 153.4, 175.2, 181.5; HRMS (ES +) calcd for C₁₈H₁₂O₄Na (MNa⁺) 315.0628, found 315.0628.

2-Methoxynaphtho [2,3-*b*] benzofuran-6,11-dione (96). Yellow solid; Yield 48 mg, 87%, mp 211–213 °C; IR (neat, cm⁻¹) 2937 (vw), 1670 (vs), 1559 (w), 1490 (m), 1454 (w), 1357 (w), 1277 (m), 1266 (m), 1245 (s), 1204 (m), 1026 (m), 710 (m); ¹H NMR (500 MHz, CDCl₃) δ 3.91 (s, 3H), 7.14 (dd, *J* = 9.1, 2.5 Hz, 1H), 7.45 (d, *J* = 9.1 Hz, 1H), 7.63 (d, *J* = 2.5 Hz, 1H), 7.73–7.79 (m, 2H), 8.17–8.23 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 56.2, 104.1, 113.8, 120.3, 123.6, 124.3, 126.9, 127.1, 132.6, 133.4, 134.0, 134.3, 151.7, 154.1, 158.6, 175.4, 181.7; HRMS (ES +) calcd for C₁₇H₁₁O₄ (MH⁺) 279.0652, found 279.0657.

4-Methoxynaphtho [2,3-*b*] benzofuran-6,11-dione (97). Yellow solid; Yield 41 mg, 84%, mp 227–229 °C; IR (neat, cm⁻¹) 2950 (w), 1665 (br vs), 1596 (vs), 1502 (m), 1376 (s), 1331 (s), 1278 (s), 1246 (s), 1075 (m), 943 (s), 778 (m), 711 (m); ¹H NMR (500 MHz, CDCl₃) δ 4.06 (s, 3H), 7.04 (d, *J* = 7.8 Hz, 1H), 7.40 (t, *J* = 7.8 Hz, 1H), 7.70–7.80 (m, 2H), 7.85 (d, *J* = 7.8 Hz, 1H), 8.21–8.25 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 56.5, 111.3, 115.6, 124.5, 124.6, 127.0, 127.1,

127.2, 132.6, 133.5, 134.0, 134.3, 146.3, 146.4, 153.7, 175.3, 181.6; HRMS (ES +) calcd for C₁₇H₁₁O₄ (MH⁺) 279.0652, found 279.0651.

2-Methylnaphtho [2,3-*b*] benzofuran-6,11-dione (98). Yellow solid; Yield 37 mg, 70%, mp 251–253 °C; IR (neat, cm⁻¹) 3107 (w), 2915 (m), 1643 (br s), 1598 (vs), 1422 (m), 1351 (m), 1263 (s), 1045 (m), 966 (m), 821 (m), 736 (vs); ¹H NMR (500 MHz, CDCl₃) δ 2.52 (s, 3H), 7.39 (d, *J* = 8.8 Hz, 1H), 7.57 (d, *J* = 8.8 Hz, 1H), 7.75–7.81 (m, 2H), 8.09 (s, 1H), 8.21–8.26 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 21.6, 112.5, 123.0, 123.6, 124.2, 127.0, 127.1, 131.4, 132.6, 133.5, 134.0, 134.3, 136.4, 153.8, 155.2, 175.7, 181.8; HRMS (ES +) calcd for C₁₇H₁₁O₃ (MH⁺) 263.0703, found 263.0702.

2,3-Dimethylnaphtho [2,3-*b*] benzofuran-6,11-dione (99). Yellow solid; Yield 39 mg, 72%, mp 225–227 °C; IR (neat, cm⁻¹) 3055 (w), 2922 (m), 1672 (vs), 1659 (vs), 1591 (m), 1574 (s), 1560 (s), 1456 (m), 1251 (s), 1189 (vs), 987 (s), 712 (s); ¹H NMR (500 MHz, CDCl₃) δ 2.41 (s, 3H), 2.43 (s, 3H), 7.45 (s, 1H), 7.74–7.80 (m, 2H), 8.04 (s, 1H), 8.20–8.26 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 20.3, 21.3, 113.1, 120.7, 123.6, 124.4, 126.9, 127.0, 132.7, 133.5, 134.0, 134.2, 135.8, 140.3, 153.2, 155.9, 175.6, 181.9; HRMS (ES +) calcd for C₁₈H₁₃O₃ (MH⁺) 277.0859, found 277.0858.

4.1.4. Naphthoquinones via reactions of lawsone and 2-aminonaphthoquinone with α-bromonitroalkenes

General procedure for the synthesis of pyrrolonaphthoquinones 100–112 was based on our previous report.²⁶ To a stirred solution of 2-aminonaphthoquinone (0.5 mmol, 1.0 equiv) in THF (4 mL), KOH (56 mg, 1.0 mmol, 2 equiv) was added and the reaction mixture was stirred at room temperature under N₂ atmosphere. After 5 min, α-bromonitroalkene (0.75 mmol, 1.5 equiv) in THF (2 mL) was added dropwise to the reaction mixture and continued stirring at room temperature. After completion of the reaction (as evidenced by TLC), the solvent was removed *in vacuo* and water (4 mL) was added to the crude residue. The aqueous phase was extracted with ethyl acetate (3 × 10 mL) and the combined organic layers were washed with brine (10 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue on purification by column chromatography (neutral alumina) using ethyl acetate/petroleum ether (5:95 to 10:90) yielded the respective pyrrolonaphthoquinones. **General procedure for the synthesis of furanonaphthoquinones 114–119** was based on our previous report.²⁶ To a stirred solution of lawsone 113 (26 mg, 0.15 mmol) and α-bromonitroalkene (0.3 mmol, 2 equiv) in water (1 mL) were added NaOAc (15 mg, 0.18 mmol, 1.2 equiv) and tetrabutylammonium bromide (TBAB, 10 mg, 0.03 mmol, 20 mol%). The reaction mixture was heated at 70 °C for 7 h. After completion of the reaction, the crude product was isolated by filtration and washed with water. The product was further purified by recrystallization from EtOAc.

4.1.5. Compounds prepared via Hauser-Kraus annulation of sulfonyl phthalide with Rauhut-Currier adducts of nitroalkenes

General procedure for the synthesis of compounds 120–122 was based on our previous report.²⁸ To a stirred solution of 3-sulfonyl phthalide **75** (90 mg, 0.33 mmol, 1.1 equiv) in THF (4 mL), Cs₂CO₃ (146 mg, 0.45 mmol, 1.5 equiv) was added. After 5 min, the RC adduct of nitroalkene (0.3 mmol, 1 equiv) was added and the reaction mixture was stirred until the completion of reaction. The solvent was removed *in vacuo* and the crude residue was directly subjected to silica gel column chromatography and the product was isolated by gradient elution with ethyl acetate/petroleum ether (10:90 to 18:82).

4.2. Crystallographic data

X-ray diffraction data collection for three compounds was performed on an Enraf-Nonius Kappa-CCD diffractometer (95 mm CCD camera on κ-goniostat) using graphite monochromated MoK radiation (0.71073 Å), at room temperature. Data collection was carried out

using the COLLECT software⁴⁰ up to 50° in 2θ. Integration and scaling of the reflections, correction for Lorentz and polarization effects were performed with the HKL DENZO-SCALEPACK system of programs.⁴¹ The structure of the compounds was solved by direct methods with SHELXS-97.⁴² The models were refined by full-matrix least squares on F² using the SHELXL-97.⁴³ The program ORTEP-3⁴⁴ was used for graphic representation and the program WINGX⁴⁵ to prepare materials for publication. All H atoms were located by geometric considerations (C–H = 0.93–0.97; O–H = 0.82 Å) and refined as riding with Uiso (H) = 1.5Ueq(C-methyl) or 1.2Ueq(other). Crystallographic data for the structures were deposited in the Cambridge Crystallographic Data Centre, with numbers CCDC 1985681.

4.3. Animals

Albino Swiss mice were employed for the trypanocidal and cytotoxicity assays, in accordance to the guidelines of the Colégio Brasileiro de Experimentação Animal (COBEA), and these were performed under biosafety conditions. All animal experimentation procedures were approved by the Comissão de Ética em Experimentação Animal (CEUA/Fiocruz), license L-005/2017.

4.4. Trypanocidal assay

The experiments were performed with the Y strain of *T. cruzi*.⁴⁶ Stock solutions of the compounds were prepared in dimethylsulfoxide (DMSO), with the final concentration of the latter in the experiments never exceeding 0.1%. Preliminary experiments showed that at concentrations of up to 0.5%, DMSO has no deleterious effect on the parasites.⁴⁷ Bloodstream trypomastigotes were obtained from infected Albino Swiss mice at the peak of parasitemia by differential centrifugation. The parasites were resuspended to a concentration of 10 × 10⁶ parasites/mL in DMES medium. This suspension (100 µL) was added to the same volume of each of the compounds, which had been previously prepared at twice the desired final concentrations. The incubation was performed in 96-well microplates (Nunc Inc., Rochester, USA) at 4 °C for 24 h. Benznidazole (Lafepe, Brazil), the standard drug for treatment of chagasic patients, was used as control. Cell counts were performed in a Neubauer chamber, and the activity of the compounds corresponding to the concentration that led to 50% lysis of the parasites was expressed as the IC₅₀/24 h.

4.5. Cytotoxicity to mammalian cells

The cytotoxicity assays were performed using primary cultures of peritoneal macrophages obtained from Albino Swiss mice. For the experiments, 2.5 × 10⁴ cells in 200 µL of RPMI-1640 medium (pH 7.2 plus 10% foetal bovine serum and 2 mM glutamine) were added to each well of a 96-well microtiter plate and incubated for 24 h at 37 °C. The treatment of the cultures was performed in fresh supplemented medium (200 µL/well) for 24 h at 37 °C. After this period, 110 µL of the medium was discarded and 10 µL of PrestoBlue (Invitrogen) was added to complete the final volume of 100 µL. Thus, the plate was incubated for 2 h and the measurement was performed at 560 and 590 nm, as recommended by the manufacturer. The results were expressed as the difference in the percentage of reduction between treated and untreated cells being the LC₅₀/24 h value, corresponding to the concentration that leads to damage of 50% of the mammalian cells.⁴⁸

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bmc.2020.115565>.

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