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Synthesis, characterization and biological activities of 3-aryl-1,4-naphthoquinones – Green palladium-catalysed Suzuki cross couplings

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Quinones are important scaffolds that are present in a variety of natural occurrings or synthetic bioactive molecules. Arylation is an important strategy for accomplishing structural modifications, leading to new potential candidates to drugs. In the present work, palladium-catalysed, ligandless and phosphine-free Suzuki couplings between 2-hydroxy-3-iodo-1,4-naphthoquinone and boronic acids were employed to prepare several 2-hydroxy-3-aryl-1,4-naphthoquinones in aqueous conditions using microwave irradiation or conventional heating. Because the biological activities of quinones, which are related to their ability to accept electrons to form semiquinones and hydroquinones, the electrochemical behaviour of the synthesized molecules was investigated. The Osiris and Molinspiration Cheminformatic programs, utilized *in silico* analyses, imply that these naphthoquinones are candidates inclined to drug which was reinforced by the *in vitro* antifungal and trypanocidal activities tests outcomes. Our *in vitro* data indicated a MIC value of 8 µg/mL against *Candida albicans* ATCC 24433 strains, and an EC_{50} of 0.67 µm with respect to trypanocidal activity against *Trypanosoma cruzi* epimastigote strains (Y).

Introduction

Quinones occur widely in nature and play important roles in the life cycle of cells. Quinone-based compounds display a variety of pharmacological activities, such as anticancer, antiviral and anti-neurodegenerative activities, among others.[1]

Atovaquone (Figure 1) is a potent anti-malarial drug that inhibits the mitochondrial electron transport chain (cytochrome bc1 complex) of parasites. The naphthoquinone moiety in atovaquone interacts with highly conserved aminoacids residues of cytochrome b by multiple non-polar interactions. Currently, atovaquone is used for the treatment and prophylaxis of malaria in association with proguanil hydrochloride (Malarone®– GlaxoSmithKline, UK).

Other biologically active molecules were synthesized based on **1** as the lead compound using rational drug design.[2]

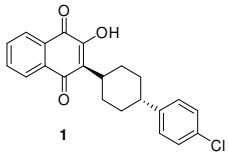

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Figure 1 – Atovaquone

Fungi are eukaryotic organisms that are found in the commensal microbiota of healthy humans. They may colonize mucosal surfaces such as the skin, gastrointestinal tract and oral cavity. These organisms may present different forms, including a rounded form, a hyphae form or a dimorphic form.[3]

Candida spp are fungi of clinical importance that undergo phenotypic switching from yeast to hyphae forms, becoming infectious and causing candidiasis. There are several predisposing factors for the development of candidiasis, such as AIDS, diabetes, cytotoxic therapy and prolonged antibiotic therapy.[4]

Over the last few years, *Candida* strains have been recognized as a major cause of opportunistic fungal infections that occur around the world. They represent a great threat, especially to immune compromised patients, and cause significant morbidity and mortality.[5] In this regard, *Candida albicans* is the most common aetiological agent of candidiasis, but non-albicans *Candida* species, such as *C. glabrata, C. krusei, C. parapsilosis* and *C. tropicalis,* are also important in causing this infection.

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Currently, there are a few classes of antifungal agents that are available for the treatment of candidiasis. They include polyenes, azoles and echinocandins, but none of them are fully effective due to their lack of efficacy or their toxicity. This scenario worsens because of the emergence and/or increase in fungi resistance against the antifungals that are clinically in use. Widespread and non-rational use of these antifungal agents has led to a strong selection of resistant strains.[6]

The emergence of antifungal resistance and the side effects of current antifungal therapy make it difficult to the treatment of the fungi-caused infections. Therefore, new treatment options ought to be developed by researching new antifungal prototypes with effective activities against *Candida* strains and with low toxicity.

A similar scenario is observed in a protozoan infection that affects millions of people and kills 12,000 per year. Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi*. The transmission of such a disease can occur in different ways (e.g., via the faeces of bloodsucking bugs known as 'kissing bugs'). Within urban areas, it is not only caused by transfusion of contaminated blood, but also by food contaminated with the parasite, as well as by urine or anal secretions from infected marsupials, to name a few. Additionally, mothers can transmit *T. cruzi* to their foetus during pregnancy.[7]

In Latin America, Chagas disease affects mainly poor communities, where it sets in endemically. However, this neglected disease has been scattered to other continents, and more than 6 million people are estimated to be infected.[8]

The main drugs used in the treatment of Chagas disease are Nifurtimox **2** and Benznidazole **3** (Figure 2). These drugs are very effective if applied in the acute phase, but not in the chronic phase, when the effectiveness decreases. In addition, treatments with these drugs are of long duration and have severe side effects.[9] Similar to the Candidiasis scenario, it is also necessary to discover new trypanocidal agents to replace the few that are currently available for treating Chagas disease.

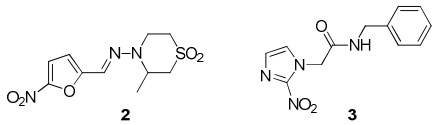

Figure 2 – Trypanocidal agents

Several reports in the literature describe the potential of naphthoquinones as antifungals and trypanocidal agents. Tran and co-workers demonstrated the antifungal activity of several halo-1,4-naphthoquinones,[10] Yoshizaki and co-workers reported on the antifungal activity of naphthoquinone derivatives constituting *Lithospermum erythrorhizon*[11] and terpenyl-1,4-naphthoquinones were considered active *in vitro* against pathogenic yeasts and filamentous fungi.[12] In our research group and in others,[13,14] several naphthoquinones were prepared that displayed promising chemotherapeutic activity against *T. cruzi*.

T. cruzi shows some similarities to fungi in terms of the sterol lipid biosynthesis, being both susceptible to the inhibitors of the sterol biosynthesis. Antifungals such as posaconazole or ketoconazole can inhibit the growth of the *T. cruzi* strains.[15]

Arylation is an interesting approach that can accomplish structural modifications of quinones and naphthoquinones with the goal of synthesizing new substances with biological activities.[16] In this regard, palladium-catalysed Suzuki coupling between boronic acids and electrophiles is an important C-C bond-forming tool. Additionally, greener protocols, including reactions in aqueous medium, have been developed to accomplish the Suzuki couplings.[17]

3-Aryl-2-hydroxy-1,4-naphthoquinones can be considered analogues of atovaquone, but with a simpler structure. This is very interesting because although atovaquone is a highly active and safe drug against protozoan infections its production costs have precluded its use in the developing countries.

Arylations of naphthoquinones have already been reported in the literature by using C-H activation procedures,[18] diazonium salts, Lewis acid-catalyzed arylations with aromatic aldehydes, arenes or hydrazines, Stille and Suzuki cross-coupling reactions.[18] Many of the procedures reported fail when it comes in to introducing electron-poor groups at the C-3 position of the naphthoquinones. Additionally, there are few reports regarding the arylation of unprotected 2-hydroxy-1,4-naphthoquinones. Spyroudis and co-workers[19] reported the Suzuki cross-coupling of phenyliodonium ylides of hydroxyquinones with arylboronic acids (0.5 mmol of iodonium, 1.1 mmol boronic acid, 4% $Pd(OAc)_2$, LiOH, DME:H_2O, R_3P, 5-8 h). Yields were found to be dependent on the phosphine employed, with better yields when P(*t*-Bu)$_3$ was used, which was attributed to the minimization of transylidation reaction with formation of the corresponding phosphonium ylides. Iodobenzene was formed as side product and coupled with the excess of the boronic acids, resulting in the formation of Ph-Ar. 3-Aryl-2-hydroxy-1,4-naphthoquinones bearing electron-withdrawing groups (F, CHO or CN) in the phenyl ring were not synthesized using the procedure reported by Spyroudis. 2-Hydroxy-3-iodo-1,4-naphthoquinone or 2-amino-3-iodo-1,4-naphthoquinone were not employed in the Suzuki cross-couplings, phosphine-free couplings were not reported for these substrates, as well as the effect of microwave irradiation in these reactions was not investigated. Furthermore, the 3-aryl-2-hydroxy-1,4-napthoquinones were not evaluated concerning their antifungal or trypanocidal activities.

We prepared different 3-aryl-2-hydroxy-1,4-naphthoquinone analogues of atovaquone under aqueous conditions, employing conventional heating or microwave irradiation using inexpensive phosphine-free sources of palladium and inexpensive bases. These molecules were tested for *in vitro* antifungal and trypanocidal activities, and an *in silico* study was performed to evaluate their potential as pharmaceuticals, as well as their pharmacokinetic properties and toxicity.

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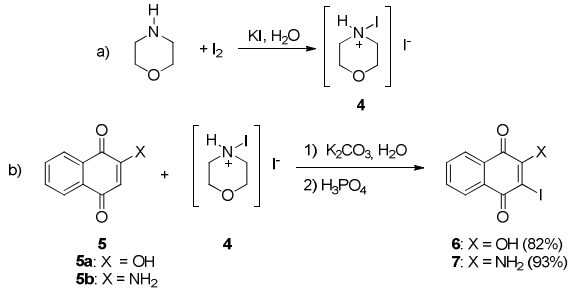
Results and discussion

Different 3-arylnaphthoquinones were prepared via palladium-catalysed Suzuki couplings between arylboronic acids and 3-iodo-naphthoquinones in aqueous conditions, using conventional heating or microwave irradiation.

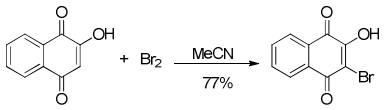
Synthesis and characterization of 3-halo-1,4-naphthoquinones

Initially, a morpholine-iodine complex **4** was prepared by reacting morpholine and iodine[20] in an aqueous solution (Scheme 1). This complex was, then, used in the iodination of 2-amino-1,4-naphthoquinone and 2-hydroxy-1,4-naphthoquinone under basic conditions. The 3-iodonaphthoquinones **6** and **7** were obtained in excellent yields (82 and 93%, respectively) after acidification with a concentrated phosphoric acid solution.[21] Iodination at position C-3 was confirmed by the absence of a signal at 6.20 ppm (DMSO-d_6) in an ^{1}H-NMR spectrum that corresponds to the vinylic CH of lawsone **5a** (3-hydroxy-1,4-naphthoquinone). In lawsone, the C-3 signal in the ^{13}C-NMR spectrum is located at 110.9 ppm, whereas halogenation of this position produces a shielding effect, and the C-3 of 2-hydroxy-3-iodo-1,4-naphthoquinone resonates at 92.7 ppm. The upfield trend in the chemical shifts caused by bromination or iodination of a carbon is attributed to an increase in the diamagnetic shielding, which is due to the introduction of a large number of electrons by the bromine or iodine atoms. The "shielding effect" on the α-carbon is known as "the heavy atom effect". Bromo-1,4-naphthoquinone can also decompose, but this process seems to be slower than the iodine loss. Halogenation of 2-amino-1,4-napthoquinone **5b** was confirmed in a similar manner: a signal for the vinylic CH of the reagent (5.8 ppm) was absent in the ^{1}H-NMR spectrum of the product, and the iodination of C-3 produces a shielding effect: from 102.3 ppm in 2-amino-1,4-naphthoquinone to 82.2 ppm in 2-amino-3-iodo-1,4-naphthoquinone (DMSO-d_6).



Scheme 1 – Preparation of iodo-1,4-naphthoquinones **6** and **7**

Lawsone was also brominated at the C-3 position using bromine in acetonitrile (Scheme 2), and 3-bromo-2-hydroxy-1,4-naphthoquinone[22] **8** was obtained in 77% yield. Although bromine can, in principle, also cause an upfield effect in the chemical shifts of the Cα signal, this "heavy atom effect" due to bromination wasn't observed at the C-3 position of the brominated lawsone, because this carbon resonates at 111.11 ppm (lawsone C-3 = 110.9 ppm).



5a 8

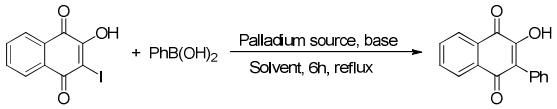
Scheme 2 –Preparation of 3-bromo-lawsone

3-Iodo-1,4-naphthoquinones must be stored protected from light to prevent the loss of iodine. 2-Amino-3-iodo-1,4-naphthoquinone **7** appear to be less prone to iodine loss, as it can be stored for a longer period of time without losing iodine. This compound is resistant to GC/MS (EI) analysis conditions and the molecular ion (m/z = 299) can be recognized as being the most prominent signal in the spectrum, followed by a peak that corresponds to the loss of iodine (m/z = 172). 2-Hydroxy-3-iodo-1,4-naphthoquinone **6**, however, is more prone to the deiodination process and therefore must be stored carefully. This compound does not survive the conditions under which GC/MS analysis is performed, and the molecular ion cannot be observed. Iodo-lawsone **6** appears to lose iodine in the chromatographic step.

Substitution of hydrogen in the C-3 position by halogen atoms resulted in the appearance of an additional band at approximately 414 nm in the UV electromagnetic spectrum, indicating halogenation at this position.[23]

Synthesis and characterization of 3-aryl-1,4-naphthoquinones

3-Halo-1,4-naphthoquinones were subject to Suzuki couplings with boronic acids. Perez and co-workers[24] employed $Pd(OAc)_2/K_2CO_3/H_2O$ to couple 3-iodolawsone with electron-deficient olefins in a Heck coupling procedure and obtained coupled naphthoquinones in high yields. Therefore, we used the conditions described by Perez as the initial conditions for the Suzuki couplings in the present work, using the reaction between 2-hydroxy-3-iodo-1,4-naphthoquinone **6** and phenylboronic acid **9** as a model system. Palladium was tested from different sources, bases and solvents (Table 1, Scheme 3).



6 9 10a

Scheme 3 – Suzuki coupling with phenylboronic acid

Under basic conditions for the Suzuki couplings, 2-hydroxy-3-iodo-1,4-naphthoquinone **6** (yellow) is soluble in water, as it forms their lawsonate (red), as well as the reaction product is in the form of a salt. After acidification of the aqueous reaction medium, the crude product precipitates from water (crude yields are shown in Table 1). We analysed the aqueous phase and observed that the majority of the product precipitates.

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Therefore, extraction of the aqueous phase was not necessary to improve the recovery of the product.

Table 1 – Suzuki coupling with phenylboronic acid

Conditions	Catalyst	Base	Solvent	Recovery (%)
1	$Pd(OAc)_2$	K_2CO_3	H_2O	85 (53)*
2	$Pd(OAc)_2$	K_2CO_3	EtOH	66
3	$Pd(OAc)_2$	Na_2CO_3	H_2O	69
4	$PdCl_2$	K_2CO_3	H_2O	44
5	Pd_2dba_3	K_2CO_3	H_2O	84 (54)*
6	$PdCl_2(PPh_3)_2$	K_2CO_3	H_2O	99
7	Pd/C	K_2CO_3	H_2O	86
8	Pd(II)/$BaSO_4$	K_2CO_3	H_2O	75
9	Pd(0)/$CaCO_3$	K_2CO_3	H_2O	81

Reaction conditions: 0.5 mmol of 2-hydroxy-3-iodo-1,4-naphthoquinone, 0.75 mmol of boronic acid, 5 mol% of the palladium source, 2.5 mmol K_2CO_3 and 5 mL of solvent. The reactions were monitored by TLC analysis, and after 6 h, virtually no more consumption of **6** occurred, however by-products began to form. Reactions were performed in water and crude products were analysed by ^{1}H-NMR. *Yield after column chromatography.

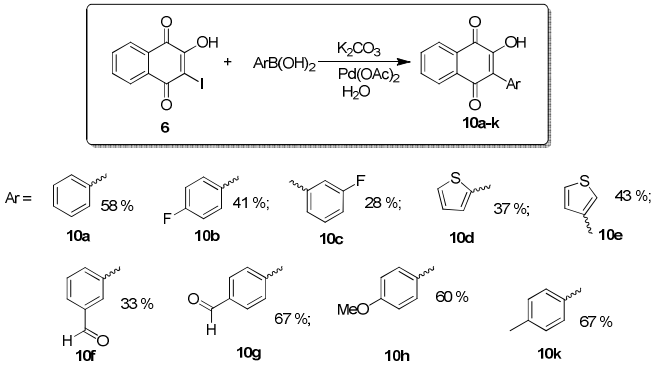
Products were obtained with low crude yields when alcohol was employed as the solvent (entry 2) and ^{1}H-NMR analysis indicated a 4% yield of lawsone (product of deiodination). This is likely due to the insolubility of carbonate in ethanol, thus producing heterogeneous conditions; whereas both the base (carbonate) and the substrate are soluble under aqueous basic conditions. No lawsone was observed when $Pd(OAc)_2/K_2CO_3/H_2O$ (entry 1) was employed, whereas 9% lawsone was obtained using $Pd(OAc)_2/Na_2CO_3/H_2O$ (entry 3). Water was considered the best solvent, and potassium carbonate the best base. Several catalysts were tested using H_2O/K_2CO_3.

During homogeneous catalysis, palladium dichloride exhibited the worst performance. TLC indicated a high content of **6**, which was confirmed by NMR analysis. Although a high recovery of the product from acidified water was accomplished when Pd_2dba_3 and $PdCl_2(PPh_3)_2$ (entries 5 and 6) were employed, TLC analysis of the crude products revealed a significant formation of lawsone, and in the case of Pd_2dba_3, a significant amount of iodo-lawsone **6** remained. NMR analysis identified numerous impurities in the aromatic region.

Supported catalysts were also evaluated (entries 7-9). All of the catalysts that were tested were successful in the Suzuki coupling reactions, but the reactions appeared to be slower with $Pd/CaCO_3$ and $Pd/BaSO_4$ (entries 8 and 9), because after 6h, a significant amount of iodo-lawsone remained. Alternatively, when Pd/C was used, 6% lawsone was obtained, and some impurities were detected in the resonance spectra.

When the reactions were heated for more than 6-8h, by-products began to form, and iodo-lawsone was not fully consumed.

The conditions described in Table 1, entry 1 (5% $Pd(OAc)_2$, K_2CO_3, H_2O) were considered the best of those that were evaluated and were applied to the synthesis of other arylnaphthoquinones for use the coupling of 3-halo-1,4-naphthoquinones with different arylboronic acids (Scheme 4). Crude products were recovered easily (60-99 %) from the acidified solutions. However, low to moderate yields were obtained, after flash chromatography.



Scheme 4 – Suzuki coupling of **6** with different boronic acids

Microwave irradiation can improve yields and accelerate many organic reactions. Beneficial effects of the use of microwave irradiation in cross-coupling reactions have already been reported in the literature.[25] Therefore, we employed the conditions that were determined to be optimal for coupling with conventional heating (5% $Pd(OAc)_2$, K_2CO_3, H_2O). Intensive deiodination occurred when 5% $Pd(OAc)_2$ was employed, using microwave heating (P = 300 W, T = 120° C, 10 min). Increasing temperatures (150, 200° C) also resulted in deiodination of 2-hydroxy-3-iodo-1,4-naphthoquinone **6**. At 80° C, a large amount of unreacted iodo compounds were recovered. The best reaction conditions were: 1% $Pd(OAc)_2$/100°C/H_2O. Therefore, using microwave irradiation, excellent results were obtained using a lower loading of palladium (1% irradiation versus 5% conventional) and shorter reaction times (10 min versus 6 h). Using these conditions, different boronic acids were employed in cross-coupling reactions with 3-halo-1,4-naphthoquinones (Table 2). The products that were obtained were higher in purity than those obtained using the conventional conditions, and better yields were obtained using flash chromatography (yields are an average of duplicates). Moderate to good yields were obtained, except for that of the reaction with 2-thiopheneboronic acid (entry 4). Several conditions were evaluated to improve the yield (increasing temperatures, catalyst loading, time or different solvents), but in all cases a low conversion of the iodinated precursor was obtained.

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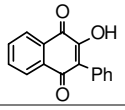
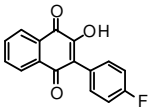
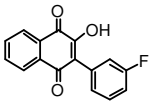
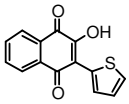
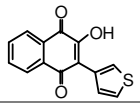
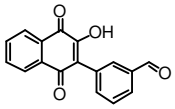
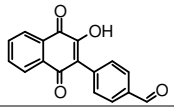
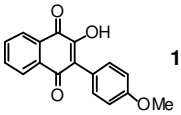
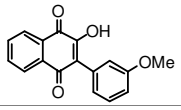
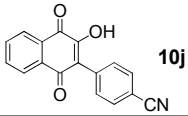
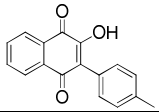
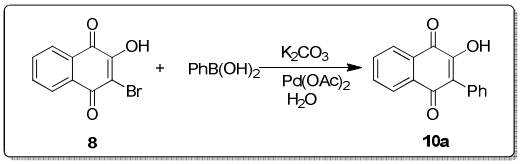
Table 2 – Suzuki reactions under microwave irradiation

	Compounds		Yield (%)*
1		**10a**	71
2		**10b**	64
3		**10c**	78
4		**10d**	4
5		**10e**	72
6		**10f**	60 (83)**
7		**10g**	43 (65)**
8		**10h**	75
9		**10i**	66
10		**10j**	43
11		**10k**	77

Reaction conditions: (0.5 mmol of **6**), 0.75 mmol of boronic acid, 1.0 mol % of the $Pd(OAc)_2$, 2.5 mmol K_2CO_3, and 5 mL of H_2O. *Isolated yields (flash column chromatography). **Pd/C (1%).

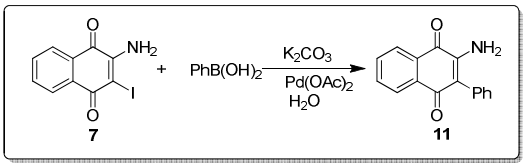
Pd/C is a low cost, interesting, heterogeneous pre-catalyst. Therefore, in this work, we also tested this catalyst with microwave heating for the reactions of **6** with formylbenzeneboronic acids to obtain compounds **10f** and **10g** (entries 6 and 7). The moderate isolated yields that were obtained with formylbenzeneboronic acids were due to the small retention factor (rf) difference between the products (**10f** and **10g**) and the boronic acids (3-formylphenylboronic acid and 4-formylphenylboronic acid, respectively). This small difference made the purification by column chromatography difficult. The difference between **10f** and 3-formylphenylboronic acid, however, was slightly larger than the difference between **10g** and 4-formylphenylboronic acid. Hence, the isolated yields were higher in all the reactions with 3-formylphenylboronic acid, despite of reaction conditions (microwave *versus* conventional heating and $Pd(OAc)_2$ *versus* Pd/C).

Because of the apparent greater stability of 3-bromo-2-hydroxy-1,4-naphthoquinone compared with 2-hydroxy-3-iodo-1,4-naphthoquinone, couplings with the former were also studied (Scheme 5). Under conventional heating and employing the best conditions that were employed in couplings with 2-hydroxy-3-iodo-1,4-naphthoquinone ($Pd(OAc)_2/K_2CO_3/H_2O$/6h), a low conversion was observed. Increasing the time of the reaction to 24 hours did not improve the conversion rate. Taking into consideration the observation that microwave irradiation was beneficial to the couplings with 2-hydroxy-3-iodo-1,4-naphthoquinone, we evaluated different conditions using microwave heating. The best conditions that led to the greatest conversion of 3-bromo-2-hydroxy-1,4-naphthoquinone were: 5% $Pd(OAc)_2/H_2O$/25 min/ **8**: $PhB(OH)_2$ (2:1) (Y = 40%).



Scheme 5 – Coupling reactions with **8**

The low solubility of 2-amino-3-iodo-1,4-naphthoquinone affected its conversion to product. Therefore, a mixture 4:1 water/EtOH was employed in the couplings with this naphthoquinone. The coupling product **11** is partially soluble in water and could not be recovered in higher yields by filtration (47% the crude product). The pure product was obtained at 26% yield by extraction of the aqueous acidified phase. Microwave conditions were also tested in the coupling of 2-amino-3-iodo-1,4-naphthoquinone with phenylboronic acid. The best condition obtained using microwave irradiation was: 5% $Pd(OAc)_2/H_2O$:EtOH(1:1)/25 min, using 100% excess of $PhB(OH)_2$, instead of 50% that was used with **6** (Yield = 54%, column chromatography).



Scheme 6 – Coupling reactions with the naphthoquinone **7**

***In silico* analysis of 3-aryl-1,4-naphthoquinones**

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Recently, several research groups have exploited the potential of cheminformatic tools to conduct a rational approach to drug design. Currently, these tools are used in partnership with experimental tests.[26] In view of the interest in the synthesis of new antifungal and trypanocidal drug candidates and the potential of naphthoquinones, compounds **10a-10k** were evaluated *in silico* using the Osiris Property Explorer[27] and Molinspiration Cheminformatic programs[28]. The toxicological risks, bioavailability properties and biological activity scores for six receptor classes (GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors and enzyme inhibitors) were calculated for all of the compounds and were compared with Fluconazole and Benznidazole, which are antifungal and trypanocidal drugs, respectively.

Table 3 – Comparison of the *in silico* results of the 3-aryl-1,4-naphthoquinones series

	cLogP	Sol	M	T	R_{Ef}	I	DL	DS	L. R. viol.	GCPRL	ICM	KI	NRL	PI	EI
10a	**2.19**	**-3.16**	**-**	**-**	**-**	**-**	**-1,12**	**0.53**	**0**	**-0.26**	**-0.28**	**0.15**	**-0.09**	**-0.40**	**0.27**
10b	**2.3**	**-4.04**	**-**	**-**	**-**	**-**	**-1.79**	**0.46**	**0**	**-0.20**	**-0.29**	**0.22**	**-0.01**	**-0.37**	**0.25**
10c	2.3	-4.04	-	-	-	-	-4.76	0.41	0	-0.17	-0.27	0.25	0.04	-0.34	0.26
10d	**2.06**	**-3.74**	**-**	**-**	**-**	**-**	**0.29**	**0.66**	**0**	**-0.40**	**-0.40**	**-0.01**	**-0.23**	**-0.57**	**0.10**
10e	**1.98**	**-3.63**	**-**	**-**	**-**	**-**	**-1.63**	**0.5**	**0**	**-0.32**	**-0.28**	**0.23**	**-0.15**	**-0.47**	**0.26**
10f	2.13	-4.05	+	-	-	+	-5.89	0.26	0	-0.24	-0.29	0.18	0.12	-0.43	0.23
10g	2.13	-4.05	-	-	-	+	-8.8	0.24	0	-0.27	-0.31	0.14	0.07	-0.46	0.20
10h	2.12	-3.75	-	-	+	-	-1.29	0.30	0	-0.21	-0.34	0.16	-0.02	-0.34	0.20
10i	2.12	-3.75	-	-	-	-	-2.07	0.46	0	-0.22	-0.34	0.19	-0.01	-0.36	0.21
10j	2.03	-4.50	-	-	-	-	-10.0	0.38	0	-0.14	-0.27	0.31	0.12	-0.25	0.30
10k	2.54	-4.07	-	-	-	-	-2.59	0.43	0	-0.26	-0.37	0.12	-0.07	-0.41	0.19
Benz	-0.25	-1.62	-	-	+	-	-1.66	0.33	0	-0.33	-0.39	-0.49	-0.71	-0.05	-0.02
Flu	-0.31	-1.55	-	-	-	-	4.84	0.95	0	0.04	0.01	-0.09	-0.23	-0.09	0.03

Properties calculated by the Osiris Explorer program: clogP, Sol = solubility, M = mutagenic, T = tumourigenic, Ref = reproductive effect, I = irritant, DL = druglikeness and DS = drug-score. Properties estimated by the Molinspiration Program: L.R.viol. = number of violations of Lipinski's rule. Theoretical inhibitory profile against GPCRL = GPCR ligand, ICM= ion channel modulator, KI = kinase inhibitor, NRL= nuclear receptor ligand, PI = protease inhibitor, EI = enzyme inhibitor. Flu = Fluconazole, Benz = Benznidazole

According to our *in silico* analysis, most of the 3-aryl-1,4-naphthoquinones presented a low toxic risk profile regarding mutagenic, tumourigenic, irritant and reproductive effects. This low toxicity theoretical profile was as low as, or even lower than the antifungal positive control (Fluconazole) or the trypanocidal positive control (Benznidazole) (Table 3).

Water solubility is an important parameter to be evaluated for the drug candidates because it is related to their distribution in the body. Interestingly, suitable values for logs > -4 were observed for the naphthoquinones that were synthesized herein. Analogously, lipophilicity and hydrophilicity are important properties that affect the absorption of a drug in the body. On this subject, the Lipinsk's rule of five[29] is used to predict oral bioavailability. According to our analysis, none of the compounds studied violated Lipinski's rule of five, which indicates a good theoretical biovailability.

Our *in silico* analyses revealed negative values for druglikeness parameter of these naphthoquinones, which suggested fragments different from those present in commercial pharmaceuticals. The drug-score, which is a global parameter that is related to the potential of a molecule to be a drug candidate, indicates that the 3-aryl-1,4-naphthoquiones are good drug candidates. Analysis of the predictions of bioactivities, according to the Molinspiration tool, indicated the inhibition of kinase and other enzymes as latent activities of the compounds under study. In agreement, most of the drugs that interact with enzymes in the body show a clogP between 2 and 5 and all of the naphthoquinones that were evaluated herein presented clogP values in this range.

Based on the *in silico* analyses that suggested the potential biological profiles for this new series, we performed *in vitro* antifungal and antiparasitic tests to search for biological

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activities associated with the arylnaphthoquinones. Among all of the compounds that were tested, **10a**, **10b**, **10d** and **10e** were found to inhibit the growth of *Candida* strains, whereas compound **10e** exhibited a high trypanocidal activity, which was superior to that observed with Benznidazole (Tables 4 and 5 and Figures 3-5). These data are in agreement with those of the active compounds detected *in silico* that presented drug-scores higher than 0.4 except for compound **10d**, and scores for kinase inhibitor and enzyme inhibition greater than 0.2. These experimental observations reinforced our *in silico* predictions, as the *in vitro* data are in good agreement with them. The low theoretical toxicity value was also confirmed by the *in vitro* viability studies in mice primary cells that were exposed to the arylnaphthoquinones. These results supported additional studies on the spectrum and level of their biological activities, which we present in the *in vitro* assays that follow.

Toxicity assay in mouse cell lines

Naphthoquinone-induced injuries to J774.G8 mouse macrophage cells derived from a sarcoma (Figure 3), or mouse primary peritoneal macrophages (Figure 4), were measured in terms of the ability to reduce resazurin. Cell viability decreased significantly when J774.G8 cells were exposed to naphthoquinone **10g** at a concentration of 100 µM for 24 h (Fig. 3). However, the concentration used in the testes is 10-100 times greater than the concentration that was applied in the subsequent biological assays. None of naphthoquinones displayed toxicity when tested with wild type primary cells.

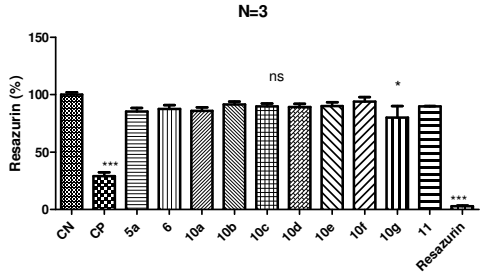

Figure 3 – Naphthoquinone-induced injury. J774.G8 cells treated with naphthoquinones (100 µM) for 24 h. Cytotoxicity was measured using resazurin reduction to resorufin as an index of proliferation. Data shown are the mean ± SD of four independent experiments. Comparisons: *** or *, treated vs. non-treated. n.s. – no significant difference

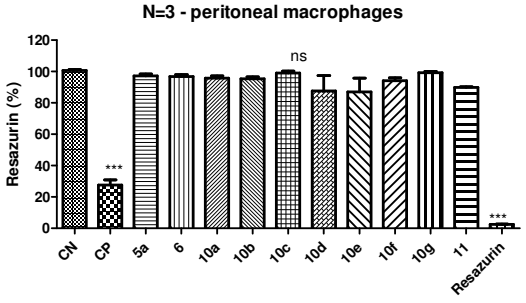

Figure 4 – Naphtoquinones-induced injury. Mouse peritoneal macrophages were treated with naphtoquinones (100 µM) for 24 h. Cytotoxicity was measured using resazurin reduction to resorufin as an index of proliferation. Data shown are the mean ± SD of four independent experiments. Comparisons: ***, treated vs. non-treated. n.s. – no significant difference

Disk diffusion susceptibility test (DDST)

Among the naphthoquinones that were tested (**6**, **8**, **10a-k)**, four exhibited antifungal activity. Compound **10b** presented the highest inhibition growth zone against *C. albicans* ATCC 24433, as well as the widest spectrum of action of all compounds that were tested. Compounds **10a**, **10b** and **10d** showed a great spectrum of action, being able to inhibit the growth of three strains. None of the compounds were active against the strain *C. parapsilosis,* ATCC 90028 (data not shown).

Table 4 - Comparison of the inhibitory effects (halo = mm) of the active naphthoquinones (**10a**, **10b**, **10d** and **10e**) in the DDST against *Candida* strains of clinical importance

MO strain	Inhibition growth zone (halo = mm)				
	Compounds				Control
	10a	10b	10d	10e	Fluconazole
C. tropicalis ATCC 750	0	11	11	15	14
C. glabrata ATCC 90030	09	15	0	0	12
C. krusei ATCC 34135	13	11	11	0	14
C. albicans ATCC 24433	13	18	11	0	13

Determination of the minimum inhibitory concentrations (DMIC)

The four compounds that were active in the DDST were submitted to the MIC test to determine the minimum inhibitory concentration. Compound **10d** presented the lowest MIC against *C. albicans* ATCC 2433 MIC = 8 µg/mL) (Table 5). Most of the compounds had MIC values that were at the same level of the antifungals most used in clinical practice (0.125 to 32 µg/mL) (listed in: CLINICAL LABORATORY STANDARDS INSTITUTE - Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard - Second Edition. M27-A2. CLSI, Wayne, PA.2013). This level of activity suggested their use as food molecules for further exploration against multiresistant strains.

Table 5 – Comparison of the minimum inhibitory concentration (MIC) values for the derivatives that presented antifungal activities in the *DDST*

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MO strain	MIC µg/mL				
	10a	10b	10d	10e	Fluconazole
C. albicans ATCC 24433	16	16	08	-	0.75
C. krusei ATCC 34135	>512	128	256	-	64
C. tropicalis ATCC 750	-	>512	32	32	03
C.glabrata ATCC 90030	16	32	-	-	03

***In vitro* antiparasitic activity**

The effect of 3-aryl-1,4-naphthoquinones on *in vitro* cultures of epimastigotes of *T. cruzi* was evaluated in comparison with Benznidazole. Initially, we treated the cells with a fixed dose of 10 µM naphthoquinones (see the Experimental section and Figure 5) and compared the results with those obtained with Benznidazole treatment. Treatment with 100 µM Benznidazole was considered our positive control (approximately 0-30% survive), whereas untreated cells were the negative control (approximately 90-100% survive). The results are shown in Figure 5 as % of trypanocidal activity. The derivative that reduced the trypanosome viability below 75% (compared with the positive control) was selected for additional assays and MTT was used to perform the initial screening of activity.

According to our data the analogues **5a**, **6**, **10a**, **10b**, **10c**, **10d**, **10e**, **10f, 10g** and **11** did not exhibit a response comparable to Benznidazole after 24 h (Figure 5). After 72 h of continuous exposure, only the derivative **10e** exhibited trypanocidal activity (Figure 6). Therefore, this naphthoquinone analogue was tested against epimastigote *T. cruzi* strains (Y) using increasing concentrations ranging from 1 nM – 1 mM for 72 h (Figure 7). Interestingly, the EC_{50} value for **10e** to cause trypanocidal activity (0,67 µM) was more potent than that described for Benznidazole (EC_{50} = 40 µM).[30] Thus, although the **10e** derivative effects were observed only after 72h, the potency that was measured was approximately 59 times greater than that with Benznidazole, indicating that the development of new derivatives of this class would be desirable.

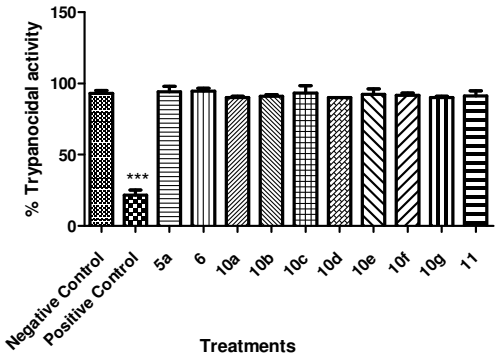

Figure 5 - Trypanocidal activity in 24 h (10 µM naphthoquinones, n=3, Y strain). This graph is representative of triplicate treatments performed on 4 distinct days

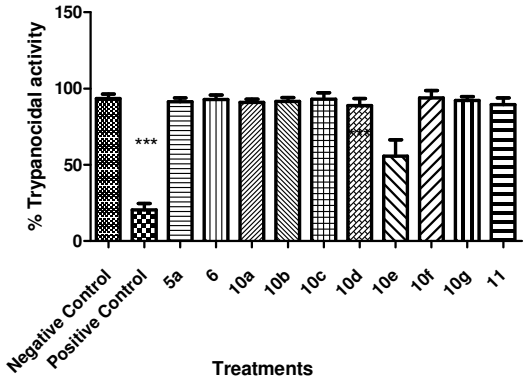

Figure 6- Trypanocidal activity in 72 h (10µM naphthoquinones, n=3, Y strain). This graph is representative of triplicate treatments performed on 4 distinct days

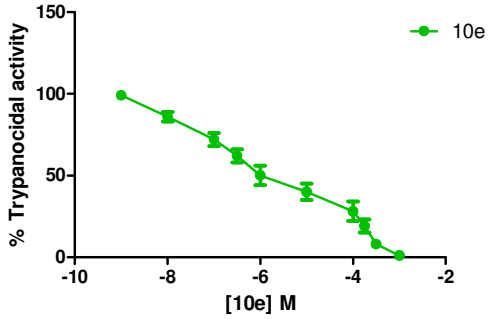

Figure 7– Analysis of the trypanocidal effect of **10e** after 72 h using increasing concentrations (1nM – 1mM) against Y strains. This graph is representative of triplicate treatments performed on 3 distinct days

Electroanalytical studies

Naphthoquinones are known for their biological activities that are often related to their ability to accept electrons (in

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general one or two), forming semiquinones and hydroquinones.[31,32,33] Such interesting electrochemical behaviour encouraged us to conduct electroanalytical studies. Thus, cyclic voltammetry[33] was chosen as exploratory technique. For the assessment of the electroanalytical behaviour of the synthesized naphthoquinones, voltammetric measurements were performed using an IVIUM CompactSTAT potentiostat (IviumTechnologies). The synthesized naphtoquinones (from **5a**, **6**, **10a-k**) were added to a 10 mL electrochemical cell filled with a supporting electrolyte, and in which a three electrode arrangement was assembled. The electrodes consisted of a platinum wire as a counter electrode, $Ag|AgCl_{(sat.)}$ as a pseudo reference electrode, and a commercially available glassy carbon as a working electrode (ϕ = 0.0519 cm^2, Metrohm). Initially, solutions prepared by direct solubilization of lithium perchlorate or tetrabutylammonium perchlorate (0.1 mol/L) in acetonitrile were used as supporting electrolytes; however, because noisy and non-reproductive electroanalytical responses were obtained using the former, all subsequent studies were performed in tetrabutylammonium perchlorate solutions. Analytes were assessed by direct dissolution of suitable amounts in the supporting electrolytes, reaching a final concentration of 1 mmol/L just before the electrochemical measurements were conducted. Cyclic voltammograms were recorded from -2.00 to +1.25 V, at different scan rates (from 50 to 200 mV/s), and were assessed against respective blanks. From these experiments, some issues were raised. It could be noted that the arylnaphthoquinones exhibited one reduction peak (from -1.33 to -1.24 V) and one oxidation peak (from -1.16 to -1.28 V).[32] Complimentary experiments were performed by inverting the sweep rate, from +1.25 to -2.00 V, which indicated an independence between the observed peaks, which was then confirmed by narrowing the potential window (assessing this time from 0.00 to +2.00 V). Within this new potential window it was possible to note that the peaks, which appear at more positive potentials, are independent of the others (Figure 8).

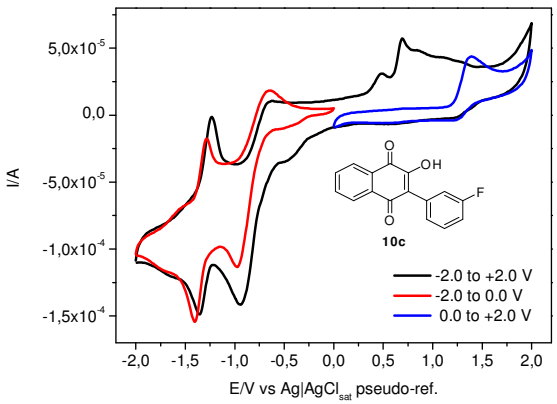

Figure 8 - Cyclic voltammogram of **10c**: 200 mV/s

Thus the electrochemical studies demonstrated that the assessed naphthoquinones have quasi-reversible redox peaks. A diffusion-controlled mass transport may be considered limiting in this case, because a linear relationship was obtained between I_p (μA) and $v^{1/2}$ (mV/s). From the series of synthesized compounds, three were exceptional, because during voltammetric measurements, solutions containing compounds **5a**, **10a** and **10d** exhibited colour changes from colourless to red (Figure 9) in the surroundings of the working and counter electrodes; such changes were monitored by coupling elecroanalytical (this time using an ITO transparent electrode and fixing the potential into suitable values) and spectrophotometric measurements (Red Tide Fiber Optic Spectrometer USB-650-UV-VIS). When UV-VIS spectrum was obtained by applying a potential when a colour change occurred on the working electrode, it detected a new band in the spectrum. According to Singh,[23] this is due to a broad local excitation band (L. E.) in the 400-500 nm region which is attributable to a n→π* transition of the quinone carbonyls.

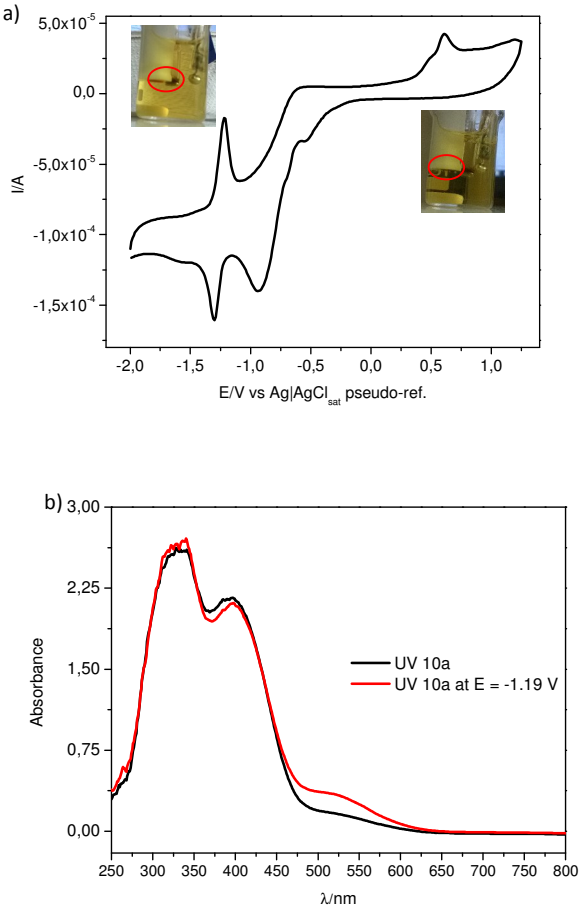

Figure 9: a) Cyclic voltammogram of **10a**, b) UV-VIS of **10a** at potential of -1.19 V

Although naphthoquinone activities can, in many cases, be related to their redox properties, in the present work, it was not possible to correlate the electrochemical behaviour of the synthesized arylnaphthoquinones with their biological

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activities, as both of bioactive and the inactive compounds exhibited the same reduction and oxidation values. Chemical activity, in this case, may involve another mechanism of action.

Conclusions

Arylnaphthoquinones were obtained in moderate to good yields by conducting palladium-catalysed Suzuki reactions using conventional heating and microwave irradiation. Suzuki couplings were performed under aqueous conditions using inexpensive palladium phosphine-free pre-catalysts. Microwave irradiation resulted in an improvement of the isolated yields, decreasing reaction times and decreasing catalyst load (1% with microwave vs. 5% with conventional heating). Five of the aryl-naphthoquinones synthesized herein are new. Arylnaphthoquinones were evaluated *in silico* with respect to their ADME/Tox and druglikeness properties. The *in silico* analysis indicated good biological and toxicological profiles for the synthesized naphthoquinones, and this was reinforced by the biological assays. None of the arylnaphthoquinones displayed toxicity to wild type mouse primary peritoneal macrophages at a concentration of 100 µM (24 h). Four of the arylnaphthoquinones that were prepared (**10a**, **10b**, **10d** and **10e**) exhibited antifungal activities against strains of clinical importance (**10d**, MIC = 8 µg/mL, against *C. albicans* ATCC 24433). Compound **10d** exhibited an antiparasitic effect against the epimastigote strain (Y) of *T. cruzi*, with an EC_{50} of 0.67 µM. Although the trypanocidal effects were observed only after 72h, this EC_{50} value is 59 times higher than the reported EC_{50} value for Benznidazole, a trypanocidal agent that is used clinically. These results indicate that arylnaphthoquinones are promising hit compounds whose structures deserve to be optimized.

Experimental

Materials and measurement

Boronic acids were purchased from Sigma Aldrich Brazil LTDA and Combi-Blocks. They were used without further purification. 2-Hydroxy-1,4-naphthoquinone and other reagents and solvents were purchased from Sigma Aldrich Brazil LTDA. Column flash chromatography was performed using silica gel 60 (35-70 µm). Analytical thin-layer chromatography was conducted on silica gel plates (Silicycle Ultrapure Silica Gels, F254) and the spots were visualized using UV light or iodine vapours. Yields refer to isolated yields after flash chromatography. Melting points were recorded on a Fisatom 413D apparatus. Infrared spectra were measured using KBr pellets on a Perkin-Elmer model 1420 FT-IR Spectrophotometer. NMR spectra were recorded on a Varian VNMRS (300 MHz or 500 MHz) instrument in DMSO-d_6 or $CDCl_3$. The chemical shift data are reported in units of δ (ppm) downfield from tetramethylsilane, which was used as an internal standard. Coupling constants (*J*) are reported in Hertz units and refer to apparent peak multiplicities. The gas chromatography/mass spectrometry (GS/MS) analysis was conducted on an Agilent® 6890 chromatograph coupled to the Agilent® 5973 mass spectrometer. The fragmentation values and molecular ions are reported in *m/z* units. An Agilent® 122-5532 DB-5MS column was used for GC analysis. High-resolution mass spectra (HRMS) were recorded on a Mass spectrometer MICROTOF Bruker Daltonics mass spectrometer. Samples were dissolved in CH_3Cl and diluted in MeOH and could not be ionized in the positive mode.

Biological assays

Five fungal strains of different species of the genus *Candida* were obtained from America Type Culture Collection and were used to evaluate the antifungal activity of the naphthoquinones: *C. parapsilosis* ATCC 90028, *C. albicans* ATCC 24433, *C. krusei* ATCC 34135, *C. glabrata* ATCC 90030 and *C. tropicallis* ATCC 750.

Disk diffusion susceptibility test (DDST)

To determine the susceptibility of each of the five *Candida* ATCC strains to the compounds used, a suspension of each strain in 5 mL of saline solution was prepared. Then, an aliquot of 500 µL was inoculated onto a Sabouraud-Dextrose Agar plate, and a 6 mm filter paper disk impregnated with the naphthoquinones diluted in dimethyl sulfoxide (DMSO) (5 mg/mL) was placed on the agar plate. The plate was then incubated for 24-48 h at a temperature of 35°C. Fungal growth inhibition zones were measured when the compounds displayed activity against the *Candida* strains tested. DMSO was used as a negative control, and Fluconazole (5 mg/mL) as a positive control.

Determination of minimum inhibitory concentrations (*DMIC*)

The compounds that exhibited antifungal activity in the *DDST* were evaluated further to define the lowest concentrations of the compounds that inhibited visible growth of the *Candida* strains (MIC). Serial dilutions of the compounds were prepared using Sabouraud-Dextrose Broth medium in a 96-well microtiter plate (0.5 µg/mL to 512µg/mL) and 100 µL inoculums of the *Candida* strains. The microtiter plate was incubated for 24-48 h at a temperature of 35°C, and the minimum inhibitory concentration (MIC) was defined as the lowest concentration that was capable of inhibiting visible fungal growth.

In silico analysis of the toxicity

The compounds **10a**-**10k** were submitted to toxicological analysis *in silico* using the Osiris Property Explorer[27] and Molinspiration programs.[28] The Osiris program presents a predictive algorithm that evaluates the potential toxicological risks of the compounds tested by analysis of the molecular structure using a database of 3300 drugs. This program predicts whether the compounds are mutagenic, tumourigenic, irritants, or have reproductive effects. Other properties, including solubility, lipophilicity, druglikeness, TPSA (permeability of the molecule in the plasma membrane) and molecular weights are also estimated. The Osiris program indicates the potential toxicological risks using a colour table in which a green colour represents a low potential toxic risk, a

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yellow colour represents a medium potential toxic risk, and a red colour represents a high potential toxic risk.

Violations of the Lipinski's rule of five[29] were determined using Molinspiration web based software. Important pharmacokinetic properties in humans (absorption, distribution, metabolism, excretion and toxicity – ADME/Tox) are described by this rule. The activity score for GPCR ligands, ion channel modulators, nuclear receptor ligands, kinase inhibitors, protease inhibitors and enzyme inhibitors were calculated using Molinspiration. The results obtained were compared with the values obtained with standard drugs. Molecules with positive scores are considered active. Scores between -5.0 and 0.0 indicate moderate activities and scores bellow -5.0, are considered inactive.

Trypanosomatid cultures

T. cruzi epimastigote Y strains were cultured in LIT medium (Tryptose liver infusion) supplemented with 10% foetal calf serum at a temperature of 28°C, with strong agitation (100 rpm) as reported previously, in the absence or presence of either naphthoquinone derivatives or Benznidazole. Live parasites were counted daily using a Neubauer chamber. The initial parasite concentration was 10^6 parasites/mL. Either the drug or vehicle (DMSO) was added after 24h and 72h for each condition. At least 3 independent experiments were performed for each drug and dose, and the 50% effective concentration (EC_{50}) was determined using Prisma GraphPad 5.0.

Trypomastigote viability assay

Viability assays were performed using the formazan formation method, also referred to as the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as previously described.[33] Briefly, $1x10^7$ trypomastigotes were incubated in RPMI 1640 culture medium at 37 °C for 24 h with and without addition of the naphthoquinones at a final concentration of 10 µM and then incubated for 24 or 72h. Naphthoquinones with trypanocidal activities were tested at concentrations range from 1nM – 1mM. Formation of formazan was monitored at 570 nm in a multi-well reader (Spectramax M5, Molecular Devices).

Cell cultures

Macrophages were obtained from the peritoneal cavities of 4-week-old Swiss Webster mice. After centrifugation, the cells were counted using a Neubauer chamber, maintained in serum-free medium for macrophages (Gibco, USA) and cultured at 37 °C in an atmosphere of 5% CO_2 for 24 hours. Our protocols adhered to the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation and were approved by the Fiocruz Research Ethics Committee (number L-041/08).

The murine macrophage line J774.G8 was used in all experiments. This strain is derived from the original J774.A1 cell line from the American Type Cell Collection (ATCC, Rockville, MD). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM)(Sigma, St. Louis, MO) containing 10% foetal bovine serum (FBS) (Cultilab - Campinas, Brazil) at 37 °C in a humidified atmosphere with 5% CO_2. On reaching 80-90% confluence, the cells were detached using a solution of 0.025% trypsin and 0.4% EDTA (ethylene diamine tetraacetic acid) for 1 minute and then used in flow cytometry experiments.

Resazurin (Alamar Blue) proliferation assay

Reduction of the dye resazurin to resorufin was used to measure proliferation of the cell in cultures. Cells were seeded at 1 x 10^5 trypan blue excluding cells/mL in 96-well microtiter plates.[34] After incubating for 24 h, the DMEM medium was removed and replaced with fresh medium containing 40 µM resazurin, followed by an incubation period of 24 h, after which reduction of resazurin to resorufin was determined by fluorescence (excitation 530 nm; emission 590 nm) using a M5 microplate fluorometer (Molecular Devices, Florida, U.S.A.). Appropriate cell free controls were included in the test to account for potential interactions between the derivatives and resazurin.

Synthesis of 3-halo-1,4-naphthoquinones

Iodination of 1,4-naphthoquinones 5a and 5b

The morpholine-iodine complex was synthesized using the procedure described by Frota and co-workers and then stored protected from light.[21]

Morpholine-iodine complex (12.8 g, 37.5 mmol) was added to a 500 mL Erlenmeyer flask containing 1,4-naphthoquinone (lawsone **5a** or 2-amine-1,4-naphthoquinone **5b** 30 mmol), K_2CO_3 (12.4g, 90 mmol) and distilled water (300 mL) in small portions every 15 minutes over a period of two hours. The reaction mixture was then stirred for at least 30 minutes. Subsequently, the mixture was cooled using an ice bath and acidified with concentrated H_3PO_4. The solution remained under agitation at room temperature for at least 24 hours, after which the mixture was filtered using a vacuum, and the solid obtained was washed with ice-cold water. The solid was dried at room temperature protected from light and then stored protected from light and under refrigeration.[21]

2-Hydroxy-3-iodonaphthalene-1,4-dione (6) - The reaction produced the compound **6** (7.38 g, 82%) as an orange solid, mp 172-173°C (lit. 177°C).[21] IR (KBr) υ_{max}/cm^{-1} 1620 (C=O), 1670 (C=O), 3161 (OH). δH (500 MHz; DMSO-d_6; TMS) 8.06-7.98 (2H, m), 7.85-7.78 (2H, m). δC (125 MHz; DMSO-d_6) 179.5, 177.2, 162.2, 134.4, 133.3, 130.7, 129.6, 126.6, 126.3, 92.7 ppm.

2-Amino-3-iodonaphthalene-1,4-dione. The reaction produced the compound **7** (0.592 g, 91%) as a red-brick solid mp 188-190°C (lit. 201-202°C).[21] IR (KBr) υ_{max}/cm^{-1} 1271 (C-N), 1579 (N-H), 1621 (C=O), 1671 (C=O), 3346 and 3447 (NH_2). δH (500 MHz; DMSO-d^6; J in Hz) 8.12-8.10 (2H, m), 7.91 (1H, td, *J* = 1.5, 7.5 Hz), 7.88-7.85 (1H, m). δC (125 MHz; DMSO-d^6) 177.3, 176.5, 152.5, 134.5, 132.5, 131.5, 129.4, 126.4, 126.3, 82.2 ppm.

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2-Bromo-3-hydroxynaphthalene-1,4-dione (8) – Lawsone **5a** (1.16 g, 6.7 mmol) and acetonitrile (100 mL) were added to a 250 mL flask equipped with a magnetic stirrer. Bromine (0.5 mL) was added after solubilization of **5a** and the resulting solution was refluxed for 3 hours and then 1.2 mL of cyclohexene was added. Solvents were removed using a rotary evaporator. The residue was washed with hexane, solubilized in ether and treated with charcoal. Subsequently, the solution was filtered, and the ether was evaporated. The solid that was obtained was dried at room temperature protected from light and was then stored protected from light and under refrigeration. This reaction produced compound **8** (1.306 g, 77%) as a yellow solid, mp 203-205°C (lit. 201-203°C).[22, 35] IR (KBr) υ_{max}/cm^{-1} 3176 (O-H), 1672 (C=O), 1580 and 1454 (C=C), 3161 (OH). δH (500 MHz; DMSO-d^6; J in Hz) 8.03-8.00 (2H, m); 7.85-7.80 (2H, m). δC (125 MHz; DMSO-d^6) 179.2, 178.6, 158.7, 135.1, 134.0, 131.9, 130.2, 126.9, 126.8, 111.1 ppm.

General procedure for preparing 3-arylnaphthoquinones under conventional heating 10a-k

2-Hydroxy-3-iodo-1,4-naphthoquinone (0.150 g, 0.5 mmol), K_2CO_3 (0.346 g, 2.5 mmol), arylboronic acid (0.75 mmol) and $Pd(OAc)_2$ (0.005 g, 5 mol%) were added to a 25 mL round-bottom flask equipped with a reflux condenser and a magnetic stirrer. Then, 5.0 mL of distilled water was added. The reaction mixture was refluxed for 6h. The reaction mixture was acidified with concentrated H_3PO_4 (pH ≅ 2). The solid that formed was vacuum filtered, dried at room temperature and purified by flash chromatography using silica gel and was eluted with an increasing polarity gradient mixture of hexane and dichloromethane - 4:1 to 1:1).

General procedure for preparing 3-arylnaphthoquinones under microwave irradiation 10a-k

A 10 mL Pyrex tube equipped with a magnetic stirrer was charged with 3-halo-1,4-naphthoquinone (0.5 mmol), K_2CO_3 (0.346 g, 2.5 mmol), arylboronic acid (0.75 mmol) and $Pd(OAc)_2$ (0.001 g, 1 mol%). The mixture was irradiated for 10 min at 100°C. Then, the reaction media were acidified with H_3PO_4 (pH ≅ 2). The solid that was obtained was purified by flash column chromatography on silica gel and eluted with an increasing polarity gradient mixture of hexane and dichloromethane.

2-Hydroxi-3-phenylnaphthalene-1,4-dione (10a) – Product **10a** was obtained as a yellow solid (0.089 g, 0.356 mmol, 71%), mp 145-146°C (lit. 146°C).[36] IR (KBr) υ_{max}/cm^{-1} 1649 (C=O), 1659 (C=O), 3346 (OH). δH (500 MHz; DMSO-d^6; J in Hz) 8.19 (1H, dd, *J* = 1.0, 7.5 Hz); 8.15 (1H, dd, *J* = 1.0, 7.5 Hz); 8.02-7.99 (1H, m), 7.95 (1H, td, *J* = 1.5, 7.5 Hz); 7,55-7.45 (5H, m, Ph). δC (125 MHz; $CDCl_3$) 183.7, 181.8, 152.2, 135.3, 133.1, 132.8, 130.6, 130.0, 129.3, 128.6, 127.9, 127.3, 126.1, 122.2 ppm. MS (EI^+) *m/z* (%): 250 (M^+, 100), 221 (19) 194 (21), 165 (47).

2-(4-Fluorophenyl)-3-phenylnaphthalene-1,4-dione (10b) - The reaction produced the compound **10b** as a yellow solid (0.086 g, 0.321 mmol, 64%), mp 185-188°C. IR (KBr) υ_{max}/cm^{-1} 1643 (C=O), 1665 (C=O), 3330 (OH). δH (500 MHz; DMSO-d^6; J in Hz) 8.07 (1H, dd, *J* = 1.0, 7.5 Hz); 8.03 (1H, dd, *J* = 1.5, 7.5 Hz); 7.88 (1H, td, *J* = 1.5, 7.5 Hz); 7.84 (1H, td, *J* = 1.5, 7.5 Hz); 7.45-7.41 (2H, m); 7.27-7.22 (2H, m). δC (125 MHz; $CDCl_3$) 183.3, 181.3, 163.0, 159.8, 154.9, 134.6, 133.2, 132.8, 132.0, 129.9, 127.6, 126.0, 125.5, 121.1, 114.4 ppm. MS (EI^+) *m/z* (%): 268 (M^+, 100), 239 (18) 240 (19), 183 (57).

2-(3-Fluorophenyl)-3-phenylnaphthalene-1,4-dione (10c) - The reaction produced the compound **10c** as an orange solid (0.105 g, 0.391 mmol, 78%), mp 163-165°C. IR (KBr) υ_{max}/cm^{-1} 1648 (C=O), 1672 (C=O), 3246 (OH). δH (500 MHz; DMSO-d^6; J in Hz) 8.07 (1H, dd, J = 1.5, 7.5 Hz); 8.04 (1H, dd, J = 1.0, 7.5 Hz); 7.89 (1H, td, J = 1.5, 7.5 Hz); 7.84 (1H, td, J = 1.5, 7.5 Hz); 7.48-7.43 (1H, m); 7.23 (1H, dd, J = 1.0, 7.5 Hz); 7.20-7.17 (2H, m).δC (125 MHz; DMSO-d^6) 183.4, 181.5, 162.6, 160.7, 155.3, 135.0, 133.9, 133.5, 132.1, 130.1, 129.5, 127.1, 126.3, 125.8, 121.0, 117.6, 114.6 ppm. HRMS (ESI) *m/z* calc for $C_{16}H_9FO_3$ $[M-H]^-$ 267.0457, found 267.0462.

2-Hydroxy-3-(thiophen-2-yl)naphthalene-1,4-dione (10d) - The reaction produced the compound **10d** as a purple solid (0.046 g, 0.179 mmol, 36%), mp 146-147°C (lit. 134-137°C).[16d] IR (KBr) υ_{max}/cm^{-1} 1652 (C=O), 3344 (OH). δH (500 MHz; DMSO-d^6; J in Hz) 8.27 (1H, dd, *J* = 1.0, 4.0 Hz); 8.18 (1H, dd, *J* = 1.0, 7.5 Hz); 8.13 (1H, dd, *J* = 1.0, 7.5 Hz); 7.98 (1H, td, *J* = 1.0, 7.5 Hz); 7.94-7,91 (1H, m); 7.85 (1H, dd, *J* = 1.0, 5.5 Hz); 7.32-7.30 (1H, m). δC (125 MHz; DMSO-d^6) 183.5, 180.5, 153.4, 134.7, 133.4, 132.0, 131.9, 130.9, 129.8, 129.7, 126.5, 126.4, 125.5, 115.4 ppm. MS (EI^+) *m/z* (%): 256 (M^+, 100), 228 (33), 172 (27), 171 (64), 76 (17).

2-Hydroxy-3-(thiophen-3-yl)naphthalene-1,4-dione (10e) - The reaction produced the compound **10e** as a red-brick solid (0.092 g, 0.359 mmol, 72%), mp 132-134°C (lit. 125-127°C).[16] IR (KBr) υ_{max}/cm^{-1} 1649 (C=O), 3325 (OH). δH (500 MHz; DMSO-d^6; J in Hz) 8.06-8.03 (2H, m); 7.94-7.93 (1H, m); 7.87 (1H, td, *J* = 1.5, 7.5 Hz); 7.82 (1H, td, *J* = 1.5, 7.5 Hz); 7.56-7.53 (2H, m). δC (125 MHz; DMSO-d^6) 183.5, 181.2, 154.4, 134.6, 133.2, 132.2, 130.8, 129.9, 129.8, 127.9, 126.2, 125.4, 123.7, 117.0 ppm. MS (EI^+) *m/z* (%): 256 (M^+, 100), 228 (63), 172 (34), 171 (95), 76 (28).

3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzaldehyde (10f) - The reaction produced the compound **10f** as a yellow solid (0.083 g, 0.300 mmol, 60%), mp 221-223°C. IR (KBr) υ_{max}/cm^{-1} 1667 (C=O), 1691 (C=O), 3137 (OH). δH (500 MHz; DMSO-d^6; J in Hz) 10.05 (1H, s, CHO); 8.09–8.04 (2H, m); 7.93 (1H, s); 7.91–7.88 (2H, m); 7.86–7.83 (1H, m); 7.73 (1H, dt, *J* = 1.5; 7.5 Hz); 7.66 (1H, t, *J* = 7.5 Hz). δC (125 MHz; DMSO-d^6) 193.0, 183.3, 181.3, 155.4, 136.8, 135.6, 134.7, 133.3, 132.5, 132.0, 131.6, 130.0, 128.8, 128.3, 126.1, 125.6, 120.8 ppm. HRMS (ESI) *m/z* calc for $C_{17}H_{10}O_4$ $[M-H]^-$ 277.0501,found 277.0510.

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4-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzaldehyde (10g) - The reaction produced the compound **10g** as a yellow solid (0.058 g, 0.208 mmol, 42%), mp 217-218°C. IR (KBr) υ_{max}/cm^{-1} 1671 (C=O), 1689 (C=O), 3261 (OH). δH (500 MHz; DMSO-d^6; J in Hz) 10. 05 (1H, s, CHO); 8.07 (1H, dd, *J* = 1.0, 7.,5 Hz); 8.04 (1H, dd, *J* = 1.0, 7.5 Hz); 7.95 (2H, d, *J* = 8,0 Hz); 7.89 (1H, td, *J* = 1.0, 7.5 Hz); 7.84 (1H, td, *J* = 1.0, 7.5 Hz); 7.62 (2H, d, *J* = 8.0 Hz). δC (125 MHz; DMSO-d^6) 192.6, 183.0, 181.2, 155.4, 137.9, 135.0, 134.7, 133.2, 131.9, 131.4, 129.9, 128.4, 126.0, 125.6, 120.9 ppm. HRMS (ESI) *m/z* calc for $C_{17}H_{10}O_4$ $[M-H]^-$ 277.0501, found 277.0509.

2-hydroxy-3-(4-methoxyphenyl)naphthalene-1,4-dione (10h) -The reaction produced the compound **10h** as a red darkness solid (0.106 g, 0.378 mmol, 75%), mp 180-182°C (lit. 172-173°C).[18f] IR (KBr) υ_{max}/cm^{-1} 1609 (C=O), 1635 (C=O), 3366 (OH). δH (500 MHz; DMSO-d^6; J in Hz) 8.07-8.01 (2H, m), 7.90-7.79 (2H, m), 7.37-7.32 (2H, m), 7.00-6.95 (2H, m), 3.80 (3H, s, CH_3). δC (125 MHz; DMSO-d^6) 184.4, 181.8, 159.2, 154.9, 135.2, 133.8, 132.5, 132.4, 130.3, 126.6, 126.0, 123.7, 122.5, 113.5, 55.6 ppm.

2-Hydroxy-3-(3-methoxyphenyl)naphthalene-1,4-dione (10i) - The reaction produced the compound **10i** as a yellow solid (0.092 g, 0.328 mmol, 66%), mp 126-127°C. IR (KBr) υ_{max}/cm^{-1} 1652 (C=O), 1661 (C=O), 3359 (OH). δH (500 MHz; DMSO-d^6; J in Hz) 8.06 (1H,dd, *J* = 0.5, 7.5 Hz), 8.03 (1H,dd, *J* = 1.0, 7.5 Hz); 7.88 (1H, td, *J* = 1.5, 7.5 Hz); 7.83 (1H, td, *J* = 1.0, 7.5 Hz); 7.34-7.31 (1H, m); 6.94-6.92 (3H, m); 3.76 (3H, s, CH_3). δC (125 MHz; DMSO-d^6) 183.4, 181.4, 158.4, 154.8, 134.6, 133.1, 132.7, 132.0, 129.9, 128.3, 126.0, 125.5, 123.0, 122.1, 116.4, 113.0, 55.0 ppm. HRMS (ESI) *m/z* calc for $C_{17}H_{12}O_4$ $[M-H]^-$ 279.0657, found 279.0658.

4-(3-Hydroxy-14-dioxo-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzonitrile (10j) -The reaction produced the compound **10j** as a yellow solid (0.059 g, 0.214 mmol, 43%), mp 253-255°C. IR (KBr) υ_{max}/cm^{-1} 1653 (C=O), 1674 (C=O), 2239 (C≡N), 3211 (OH). δH (500 MHz; DMSO-d^6; J in Hz) 8.07 (1H, dd, *J* = 1.5, 7.5 Hz), 8.05-8.03 (1H, m), 7.91-7.83 (4H, m), 7.59 (2H, d, *J* = 8,0 Hz). δC (125 MHz; DMSO-d^6) 182.9, 181.1, 155.5, 136.8, 134.8, 133.3, 131.9, 131.7, 131.2, 130.0, 126.0, 125.6, 120.4, 118.8, 110.0 ppm. HRMS (ESI) *m/z* calc for $C_{17}H_9NO_3$ $[M-H]^-$ 274.0504, found 274.0520.

2-Hydroxy-3-(*p*-tolyl)naphthalene-1,4-dione (10k) -The reaction produced the compound **10k** as an orange solid (0.102 g, 0.386 mmol, 77%), mp 169-171°C (lit. 168°C).[36] IR (KBr) υ_{max}/cm^{-1} 1645 (C=O), 3361 (OH). δH (500 MHz; DMSO-d^6; J in Hz) 8.06-8.05 (1H, m), 8.03-8.02 (1H, m), 7.89-7.86 (1H, m), 7.83 (1H, td, *J* = 1.0, 7.5 Hz), 7.27 (2H,d, *J* = 8.5 Hz), 7.22 (2H,d, *J* = 8.0 Hz), 2.35 (3H, s). δC (125 MHz; DMSO-d^6) 183.5, 181.3, 154.5, 136.7, 134.5, 133.0, 131.9, 130.4, 129.8, 128.2, 127.8, 125.9, 125.4, 122.0, 20.8 ppm.

Synthesis of 2-amino-3-phenylnaphthalene-1,4-dione using conventional heating

2-amino-3-iodo-1,4-naphthoquinone (0.149 g, 0.5 mmol), K_2CO_3 (0.346 g, 2.5 mmol), arylboronic acid (0.75 mmol) and $Pd(OAc)_2$ (0.005 g, 5 mol%) were added to a 25 mL round-bottom flask equipped with a reflux condenser and a magnetic stirrer. Then, 4.0 mL of distilled water and 1.0 mL of ethanol were added. The reaction mixture was refluxed for 6h. The reaction mixture was then acidified with concentrated H_3PO_4 (pH ≅ 2). The solid that formed was filtered, dried at room temperature and purified by extraction with ethyl acetate.

2-Amino-3-phenylnaphthalene-1,4-dione (11) - The reaction produced the compound **11** in 24% as a red darkness solid, m.p. 177-178°C (lit. 178-179°C).[37] IR (KBr) υ_{max}/cm^{-1} 1603 (C=O), 1675 (C=O), 3343 and 3416 (NH_2). δH (500 MHz; DMSO-d^6; J in Hz) 8.13 (1H,d, *J* = 7.5 Hz); 8.10 (1H,d, *J* = 7,0 Hz); 7.95 (1H,td, *J* = 1.0, 7.5 Hz); 7.86 (1H,td, *J* = 1.0, 7.5 Hz); 7,57 (2H, t, *J* = 7.5 Hz); 7.47 (1H, t, *J* = 7.5 Hz); 7.41-7.39 (2H, m). δC (125 MHz; DMSO-d^6) 181.7, 180.2, 146.1, 134.7, 130.4, 128.3, 127.3, 125.7, 125.5, 114.8 ppm.

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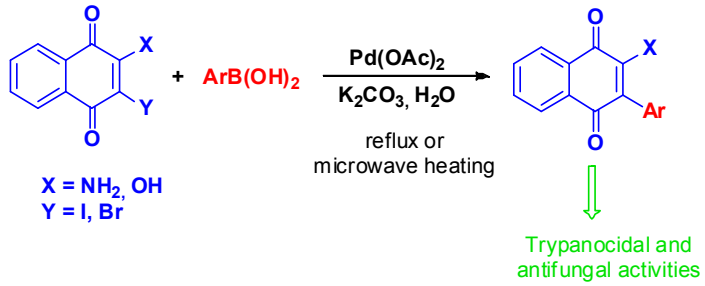

Antifungal and trypanocidal aryl-1,4-naphthoquinones were prepared through aqueous Suzuki protocol with reflux or microwave irradiation.