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Research paper

Palladium-catalysed synthesis of arylnaphthoquinones as antiprotozoal and antimycobacterial agents

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ABSTRACT

Malaria and tuberculosis are still among the leading causes of death in low-income countries. The 1,4-naphthoquinone (NQ) scaffold can be found in a variety of anti-infective agents. Herein, we report an optimised, high yield process for the preparation of various 2-arylnaphthoquinones by a palladium-catalysed Suzuki reaction. All synthesised compounds were evaluated for their *in-vitro* antiprotozoal and antimycobacterial activity. Antiprotozoal activity was assessed against *Plasmodium falciparum* (*P.f.*) NF54 and *Trypanosoma brucei rhodesiense (T.b.r.)* STIB900, and antimycobacterial activity against *Mycobacterium smegmatis (M.s.)* mc^2 155. Substitution with pyridine and pyrimidine rings significantly increased antiplasmodial potency of our compounds. The 2-aryl-NQs exhibited trypanocidal activity in the nM range with a very favourable selectivity profile. (Pseudo)halogenated aryl-NQs were found to have a pronounced effect indicating inhibition of mycobacterial efflux pumps. Cytotoxicity of all compounds towards L6 cells was evaluated and the respective selectivity indices (SI) were calculated. In addition, the physicochemical parameters of the synthesised compounds were discussed.

1. Introduction

Infectious diseases (IDs) are caused by a diverse group of pathogenic microorganisms, such as bacteria, viruses, fungi and parasites. At least 25% of about 60 million deaths occurring worldwide annually are estimated to be induced by IDs [1]. Especially mortality caused by lower respiratory tract infections has risen sharply over the last decade, which is mainly attributed to the spread of drug-resistance.

Vector-borne parasitic diseases like malaria, human African trypanosomiasis (HAT, also known as sleeping sickness), leishmaniasis and Chagas disease are still among the leading causes of morbidity and mortality in countries of the developing world without access to adequate health care systems [2]. Even though huge efforts are put forth in eradicating these maladies, they are still affecting more than 1 billion people, or about 15% of the world population [3,4]. With no vaccines available, vector control and drug therapy remain the cornerstones of disease control [5–8]. Caused by the apicomplexan genus *Plasmodium* (*P. falciparum, P. vivax, P. malariae, P. ovale and P. knowlesi*), malaria is the most common and clinically relevant vector-borne parasitic disease. Transmitted by infected *Anopheles* mosquitos, this ID is claiming the lives of more than 435 000 people each year [5].

Efforts in malaria control often show poor outcome due to the highly resilient and adaptive nature of the *Plasmodium* parasite and the consequential emergence of drug-resistance. In some areas the number of drug-resistant strains is continuously rising, which limits the efficacy of currently available therapies [5,6].

Another pathogen posing an enormous threat to human life is *Mycobacterium tuberculosis*, a gram-positive mycobacterium that causes tuberculosis (TB). This ID which mainly affects the lungs has been declared a global health emergency by the WHO due to its latent occurrence in nearly one quarter of the global population [9].

With the emergence of drug-resistant strains, TB control efforts have been further complicated in recent decades. Multidrug-

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resistant (MDR), extensively drug-resistant (XDR) and totally drug-resistant (TDR) strains are a great reason of concern [10]. Extrusion of antibiotics from the bacterial cells by efflux pumps, i.e. bacterial transporters which are able to confer resistance to groups of chemically diverse compounds, is a frequently occurring mechanism of resistance [11]. In this study, selected compounds were tested for potential antimycobacterial effects using *Mycobacterium smegmatis*, a fast growing mycobacterial strain, in a microbroth dilution assay. In addition, subinhibitory concentrations of the test compounds were evaluated for their potency to lower the minimal inhibitory concentration (MIC) of the efflux pump substrate ethidium bromide (EtBr). Reduction of the MIC of EtBr in combination with a test compound could be indicative for a potential inhibitor of bacterial efflux, and could be useful in combination with existing antituberculotics in MDR TB [12].

Despite the effective use of chemotherapeutic approaches to treat and prevent parasitic infections, satisfactory medications are often still lacking. Many of the currently used drugs suffer from major drawbacks such as significant toxicity, variable efficacy, or lack of oral bioavailability [13,14].

Special efforts have to be placed on the development of new trypanocidal drugs, as these therapeutic agents must be able to cross the blood brain barrier (BBB) in order to effectively combat late-stage *T. b. rhodesiense* infections [7,15].

The naphthoquinone (NQ) scaffold can be found in a variety of secondary plant metabolites, e.g. shikonin, with a broad spectrum of biological activities [16–19]. Some mechanisms of action have been proposed for these substances, mainly related to excessive generation of reactive oxygen species (ROS) [20] and inhibition of DNA gyrase [21]. Previous studies demonstrated that NQs represent an interesting pharmacophore for further drug development due to their activity against several apicomplexan parasites and their antimicrobial properties in general [22,23]. For example, the 2-hydroxynaphthoquinones lapachol and atovaquone (ATV) act as potent antimalarial redox-active agents. ATV has been used for chemoprophylaxis against malaria infections since its introduction to market, as it acts both on liver and blood stages of the parasite. Used in a fixed-dose combination with proguanil, ATV inhibits the mitochondrial electron transport chain (mETC) at the level of the cytochrome bc_1 complex, which results in a loss of mitochondrial function [24]. The emergence of ATV-resistant *P. falciparum* strains highlights limitations to its clinical efficacy in areas with endemic malaria and provides motivation for the synthesis of second-generation bc_1-targeted inhibitors. Fortunately, many of these research compounds used as leads (including ATV analogues) demonstrate little cross resistance with atovaquone-resistant parasite strains [22,25].

In the present study, we modified the alkyl side chain of naturally occurring shikonin to create naphthoquinone derivatives that bear various aryl moieties instead. In contrast to atovaquone, shikonin has no hydroxyl group adjacent to its lipophilic attachment (see Fig. 1). Moreover, it shows numerous beneficial pharmacological properties [18].

Herein, we report a highly efficient method for the introduction of aromatic substituents in position 2 of the 1,4-naphthoquinone scaffold and our findings on the antiprotozoal and antimycobacterial activity of 18 naphthoquinone derivatives.

2. Results and discussion

2.1. Synthetic chemistry

2-Arylnaphthoquinones have been obtained only through a few general methods that depend on the substitution pattern and only in rare cases by direct arylation [26,27]. The palladium-catalysed cross-coupling of aryl halides with arylboronic acids (Suzuki-Miyaura cross-coupling) has become a reliable technique in organic synthesis for the introduction of aryl groups due to its versatility and efficiency under mild reaction conditions [28]. Recent developments focused especially on the reduction of the amount of transition metal needed by introduction of highly specialised directing ligands, as well as the use of non-organic solvents [29,30]. However, for the arylation of haloquinones, these methods have received limited attention because of the poor stability of bromo- and iodoquinones and the limited susceptibility of chloronaphthoquinones to Suzuki couplings.

Nevertheless, we were encouraged by published protocols [31] to attempt such couplings on 2-chloro-1,4-naphthoquinones, but the preparation of the chlorinated precursor was tedious and the yields of the following coupling reaction were unsatisfactory.

Therefore, we focused on the utilisation of bromonaphthoquinone **2** as reagent for the Suzuki reaction. Oxidative bromination of 1-naphthol (**1**) using *N*-bromosuccinimide (NBS) in acetic acid [32] delivered **2** in excellent yield. Unfortunately, our attempts to couple intermediate **2** with phenylboronic acid via a published Pd-catalysed Suzuki coupling [33] gave only moderate yields, which was perhaps due to the fact that the reaction conditions in the original paper were not specified in detail. Therefore, we evaluated the effects of different Pd catalysts, bases and solvents that are frequently used for this type of reactions with **2** and phenylboronic acid as model compounds (see Supplementary Table S1 for details). Optimal conversion of the starting material was achieved using $Pd(PPh_3)_4$ (2.5 mol %), aromatic boronic acid (1.5 equiv.) and Cs_2CO_3 (1.5 equiv.) in THF-water (20:3) as a solvent. A further decrease in the equivalents of boronic acids and Pd catalyst reduced the respective yields. Optimal solvent composition not only led to high yields, but also to a dramatic reduction of reaction time. Under these improved conditions, **3a** was obtained in 94% yield (the highest described for this type of Suzuki coupling so far) in 1.5 h without the usage of specially designed ligands and minimised Pd load. Use of the corresponding boronic acid pinacol ester is also possible, resulting in a similar overall yield.

Next, arylnaphthoquinones **3a-r** were synthesised from **2** with commercially available aromatic boronic acids under optimised

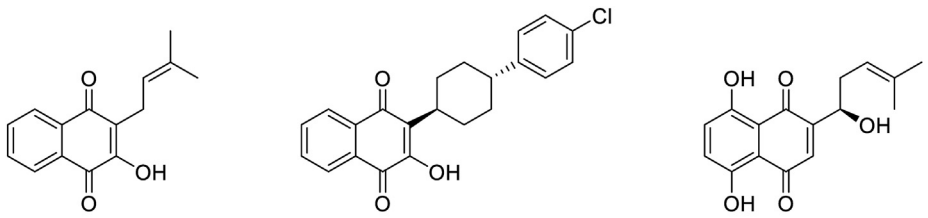


Fig. 1. Naphthoquinones with remarkable biological activity.

conditions in good to excellent yields with the exception of the furyl (**3g, 3h**) and the quinolyl (**3i**) derivatives (Scheme 1). Fortunately, the starting material is completely consumed even in the low yield examples **3g-i** and thus simplifying subsequent chromatographical purification. The preparation of **3s** and **3t** failed, which suggests that the described method is, as of yet, limited to the use of aromatic boronic acids.

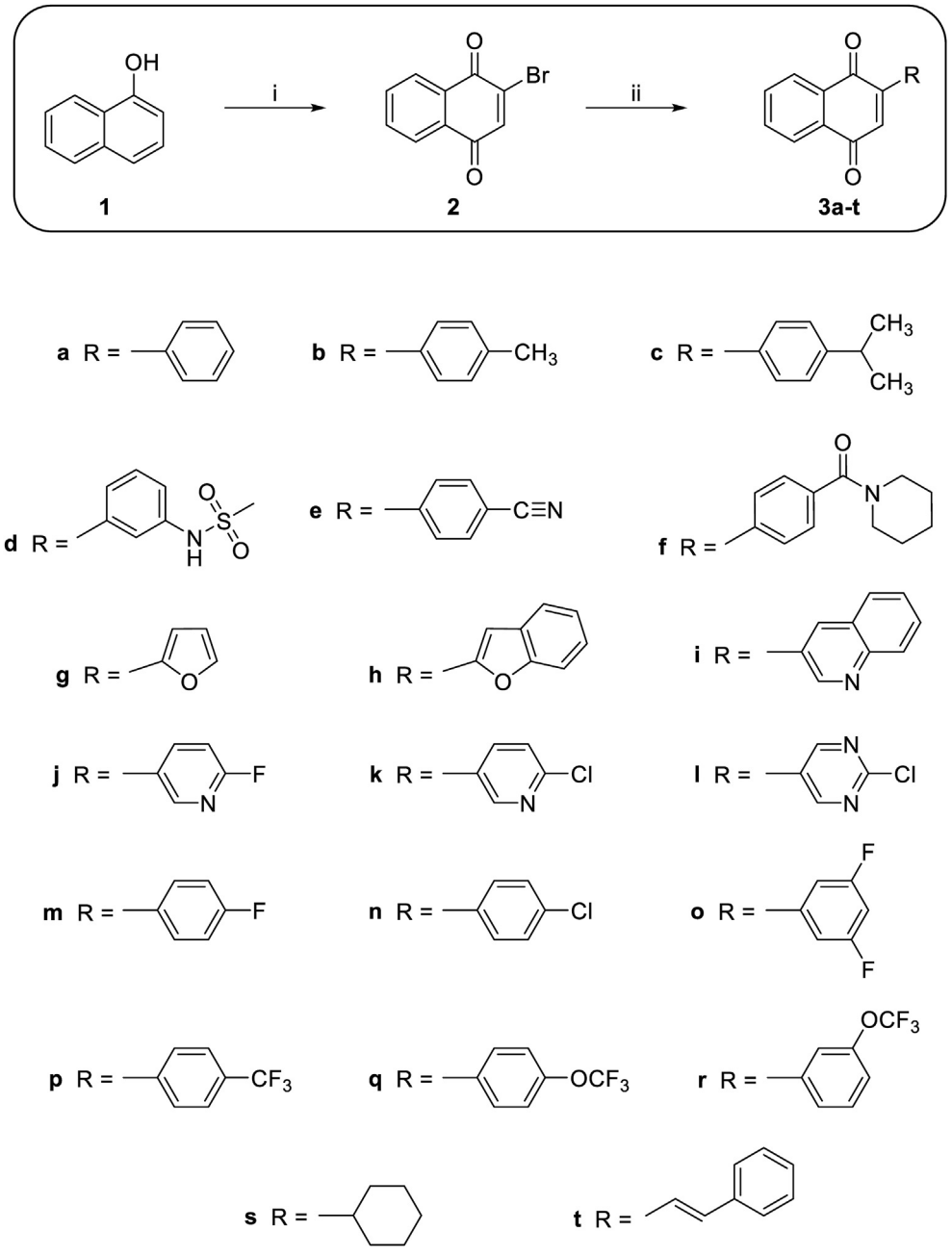
2.2. Biological evaluation

2.2.1. Antiprotozoal activity

The synthesised 2-arylnaphthoquinones (**3a-r**) were evaluated *in vitro* for their antiprotozoal activity against *P. falciparum* (NF54) and *T. brucei rhodesiense* (STIB900). Cytotoxicity was measured using L6 rat skeletal myoblasts to calculate a selectivity index for each parasite (SI = $IC_{50(L6)}/IC_{50(parasite)}$).

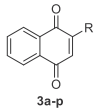
According to TDR (Special Program for Research and Training in Tropical Diseases, WHO) criteria [34], all derivatives showed moderate (IC_{50} = 1–10 μM) or high (IC_{50} < 1 μM) antiplasmodial activity towards NF54 (Table 1), except the inactive phenylnaphthoquinone **3a**, and the benzofuranyl derivative **3h**, with IC_{50} values > 10 μM. Strongest activity in the NF54 assay with excellent selectivity was exhibited by the 2-chloro-5-pyridyl naphthoquinone **3k** (IC_{50} = 0.07 μM, SI = 462.6). Except for **3k**, the selectivity indices ranged between 1.8 (**3a**) and 26.1 (**3r**) and have to be improved to target the *Plasmodium* parasites effectively.

High trypanocidal activity could also be observed. According to the criteria set above, most of the tested derivatives showed high



Scheme 1. Reagents and conditions: (i) NBS, AcOH; H_2O, 45 °C, 1 h (94%); (ii) boronic acid, $Pd(PPh_3)_4$, Cs_2CO_3, THF/H_2O (20:3), 65 °C, 1.5–20 h (**3a**: 94%, **3b**: 83%, **3c**: 81%, **3d**: 92%, **3e**: 82%, **3f**: 88%, **3g**: 22%, **3h**: 12%, **3i**: 12%, **3j**: 75%, **3k**: 82%, **3l**: 56%, **3m**: 84%, **3n**: 74%, **3o**: 68%, **3p**: 95%, **3q**: 91%, **3r**: 98%, **3s**: 0%, **3t**: 0%).

Table 1
In vitro antiparasitic activity, host toxicity and lipophilic ligand efficiency of the tested compounds.



3a-p

compd	R	*P.f.*[a]	SI[b]	*T.b.r.*[c]	SI[b]	Cyt. L6[d]	*P.f.*[a]	T.b.r.[c]	*P.f.*	T.b.r.
		IC_{50} μM		IC_{50} μM		IC_{50} μM	pIC_{50}[e]	pIC_{50}[e]	LLE[f]	LLE[f]
Chl.		0.003					8.52			
Mel.				0.006				8.22		
Pod.						0.024				
3a	phenyl	10.95	1.8	0.06	322.8	19.37	4.96	7.26	2.27	4.56
3b	4-methylphenyl	3.86	1.9	0.08	90.8	7.26	5.41	7.12	2.37	4.08
3c	4-isopropylphenyl	2.96	6.9	1.45	14.1	20.40	5.53	5.84	1.65	1.96
3d	3-(methylsulfonylamino)phenyl	2.56	3.6	0.20	46.3	9.25	5.59	6.70	3.85	4.95
3e	4-cyanophenyl	1.11	6.5	0.28	25.8	7.21	5.96	6.56	3.43	4.03
3f	4-(piperidin-1-ylcarbonyl)phenyl	2.37	4.1	0.75	12.9	9.71	5.63	6.13	2.09	2.59
3g	2-furyl	2.07	3.6	0.13	57.2	7.44	5.68	6.89	3.80	5.01
3h	2-benzo[b]furanyl	24.96	8.4	1.36	153.9	209.37	4.60	5.87	1.40	2.67
3i	3-quinolyl	0.45	12.5	0.22	25.6	5.64	6.35	6.65	3.34	3.64
3j	6-fluoro-3-pyridyl	0.11	19.1	0.14	15.0	2.10	6.94	6.86	4.80	4.71
3k	6-chloro-3-pyridyl	0.07	462.6	2.20	14.7	32.38	7.18	5.66	4.78	3.26
3l	2-chloro-5-pyrimidinyl	0.47	2.2	0.13	7.8	1.02	6.33	6.89	4.35	4.91
3m	4-fluorophenyl	1.81	5.0	0.22	41.2	9.06	5.74	6.66	2.95	3.87
3n	4-chlorophenyl	2.64	3.4	0.22	40.7	8.96	5.58	6.65	2.28	3.35
3o	3,5-difluorophenyl	0.59	16.4	0.24	40.3	9.67	6.23	6.62	3.33	3.72
3p	4-(trifluoromethyl)phenyl	1.08	14.0	0.08	189.0	15.12	5.97	7.12	2.42	3.58
3q	4-(trifluoromethoxy)phenyl	3.87	4.3	2.36	7.0	16.62	5.41	5.63	1.62	1.83
3r	3-(trifluoromethoxy)phenyl	0.89	26.1	1.80	12.9	23.23	6.05	5.75	2.26	1.95

[a] *P. falciparum*, strain NF54, erythrocytic stages.
[b] SI is defined as the ratio: IC_{50} in L6 cells/IC_{50} in each parasite.
[c] *T. brucei rhodesiense*, strain STIB900 trypomastigote forms.
[d] cytotoxicity L6 cells rat skeletal myoblasts.
[e] $pIC_{50} = -\log_{10} IC_{50}$ (M).
[f] LLE was calculated using the OSIRIS Datawarrior 5.0.0 (http://www.openmolecules.org/). Reference drugs: chloroquine (chl.), melarsoprol (mel.), podophyllotoxin (pod.). The IC_{50} value of each reference drug is the mean from multiple measurements in parallel with the compounds of interest. IC_{50} values of the tested compounds are means of 2–3 measurements. The SD was <5%.

activity against *T.b. rhodesiense* with IC_{50} values as low as 0.06 μM and favourable selectivity, especially in the case of **3a**, **3b**, **3g**, **3p** (SI = 57.2–322.8). Good selectivity was also observed for the benzofuranyl derivative **3h** (SI = 153.9), but biological activity was among the weakest overall.

2.2.2. Antimycobacterial and resistance-modulatory activity

The synthesised compounds were also tested against the strain *Mycobacterium smegmatis* mc^2 155 (ATCC 700084) to further gain insight into the antimycobacterial potential of these substances. Each compound was screened for their minimal inhibitory concentration (MIC) and the modulation factor (MF) tested against ethidium bromide (EtBr). The MIC value indicates the concentration (in μg/mL) required to inhibit visible growth of the bacteria. The MF shows the x-fold reduction of the MIC of ethidium bromide when incubated using a constant subinhibitory (½ MIC) concentration of modulator (**3a-r**). An MF of 8 states that an 8 times lower amount is needed to exert the same antibiotic combined effect. In contrast, an MF of 1 indicates no effect of the modulator on the MIC of the tested substance.

The most active compound according to the obtained MIC is **3l** with an MIC of 4 μg/mL (see Table 2). Similarly pronounced growth inhibitory effects were seen for **3a** and **3i** with MIC of 8 μg/mL and **3j**, **3n** and **3q** with MIC of 16 μg/mL, respectively. However, all MIC values were above the respective IC_{50} values of cytotoxicity in L6 cells.

An MF of 4 can be considered as cut-off for a promising MF. Nearly half (8 of 18) of the synthesised arylnaphthoquinones showed an MF of at least 128. Highly promising candidates with an MF of >128 are compounds **3e**, **3j**, **3m** and **3n**.

Table 2
Antimycobacterial and resistance-modulatory activity of the tested compounds against *Mycobacterium smegmatis* mc^2 155.

compd	MIC[a] μg/mL	MF[b] (EtBr)
INH	4–8	–
EtBr	8	–
3a	8	4
3b	32	2
3c	64	4
3d	>128	128
3e	64	>128
3f	32	8
3g	128	2
3h	>128	2
3i	8	8
3j	16	>128
3k	32	128
3l	4	2
3m	128	>128
3n	16	>128
3o	>128	4
3p	128	4
3q	16	128
3r	64	128

[a] Minimal inhibitory concentration for the *Mycobacterium smegmatis* mc^2 155 strain.
[b] modulation factor tested against ethidium bromide. Reference drugs: isonicotinic acid hydrazide (INH), ethidium bromide (EtBr).

2.3. Physicochemical parameters

To find the ideal balance of potency and physicochemical properties is of utmost relevance in the drug development process [35,36]. For this reason, an assessment of drug-likeness was made for all tested compounds, and various physicochemical properties were calculated (see Suppl. Material).

All compounds had relatively low molecular weights within the range of 224–345 g mol^{-1}. Except the methylsulfonylamino derivative **3d**, all compounds were predicted to possess good central nervous system (CNS) penetration [37,38], a key feature for the treatment of second stage HAT. All synthesised arylnaphthoquinones fulfil the Lipinsky rule of five [39], Veber's rule [40] and the drug-likeness classifier defined by Ghose et al. [41].

Ligand efficiency, especially lipophilic ligand efficiency (LLE) has gained traction in recent years as a helpful metric in lead discovery and optimisation [42–45]. LLE normalises a molecule's biological potency in relation to its lipophilicity. The use of LLE as a metric can greatly simplify multi-parameter optimisation in drug development by focussing on the design of potent compounds with preferably low lipophilicity i.e. high LLE [46].

An LLE above 3 indicates favourable compounds for further development. Several of the newly developed compounds were found well above this threshold. For the *P. falciparum* parasite, the heteroaryls **3j**, **3k** and **3l** showed drug-like behaviour in this regard (LLE = 4.35–4.80). For *T.b. rhodesiense*, good LLE values were observed for compounds **3a**, **3b**, **3d**, **3g**, **3j** and **3l** (LLE = 4.08–5.01).

2.4. Structure-activity relationships (SAR) of the antiparasitic activity

By taking a closer look at the LLE and pIC_{50} values of the arylnaphthoquinone derivatives, we observed a clear clustering of compounds with similar structural elements and trypanocidal activities (Fig. 2). Sterically hindered or strongly polarised compounds (**3c**, **3f**, **3h**, **3q**, **3r**; pIC_{50} = 5.63–6.13) turned out to be inactive. (Pseudo)-halogenated compounds (**3e**, **3m**, **3n**, **3o**; pIC_{50} = 6.56–6.66) also showed a distinct clustering with an increase of 0.5–1 log unit for this set. Small heteroaryls (**3g**, **3j**, **3l**; pIC_{50} = 6.86–6.89) represent the third group with a further increase in activity of around 0.3 log units. Small lipophilic compounds (**3a**, **3b**, **3p**) were the most potent (pIC_{50} = 7.12–7.26) with SI values in the range of 100–300. Only the 3-(methylsulfonylamino)phenyl derivative **3d**, the quinolylnaphthoquinone **3i**, and the 2-chloro-4-pyridyl naphthoquinone **3k**, do not seem to follow any clear trend from an SAR point of view.

A relationship between trypanocidal potency and SI can also be found in this dataset (Fig. 3). This hints at target-specific effects of the tested arylnaphthoquinones, opposed to non-target specificity often exhibited by the quinone structure class. Compounds with SI > ~100 and pIC_{50} values greater than 6.7 (corresponding to activity < 0.2 μM) combine high activity with acceptable selectivity over an L6 cell line and deemed to be promising drug candidates for a further study based on this assessment [34].

Regarding antiplasmodial activity, the pyridyl and pyrimidinyl derivatives **3j-l** showed the strongest biological activity. In particular, the 2-chloro-5-pyridyl-1,4-naphthoquinone **3k** (IC_{50} = 0.07 μM; SI = 462.6) was the most active compound with excellent selectivity tested in the *P.f.* NF54. Compared to the fluoro analogon **3j** (IC_{50} = 0.11 μM; SI = 19.1), the introduced chlorine group significantly reduces the cytotoxicity while simultaneously increasing its antiplasmodial potency.

Still, in comparison to their trypanocidal effects, the antiplasmodial activities of the tested arylnaphthoquinones were rather sparse.

Interestingly, cytotoxicity and antiplasmodial activity are strongly correlated (R^2 = 0.909). The benzofuranyl derivative **3h** showed the generally lowest cytotoxicity, but it is still strongly correlated with its antiplasmodial activity. Remarkably, a strong influence of the position of the trifluoromethoxy group of compounds **3q** and **3r** was observed: the *meta*-position is more favourable, as antiplasmodial activity is raised by one log unit and SI increases fivefold.

In view of antimycobacterial activity, compound **3j**, **3n** and **3q** appear to be the most promising, since they combine high activity in both the MIC (16 μg/mL) and MF assay (≥128). Chlorinated compounds often showed considerably higher activity in the MIC assay (**3l**, **3n**) compared to derivatives with fluorine substitution (**3m**, **3o**) (Fig. 4). The pyrimidine moiety (**3l**) resulted in the lowest observed MIC of all tested compounds at 4 μg/mL, but this modification leads to nearly a complete loss of efflux pump modulatory activity.

Another general observation is that the *para* (pseudo)-halogenation of aryl derivatives is crucial for high efflux inhibitory effects.

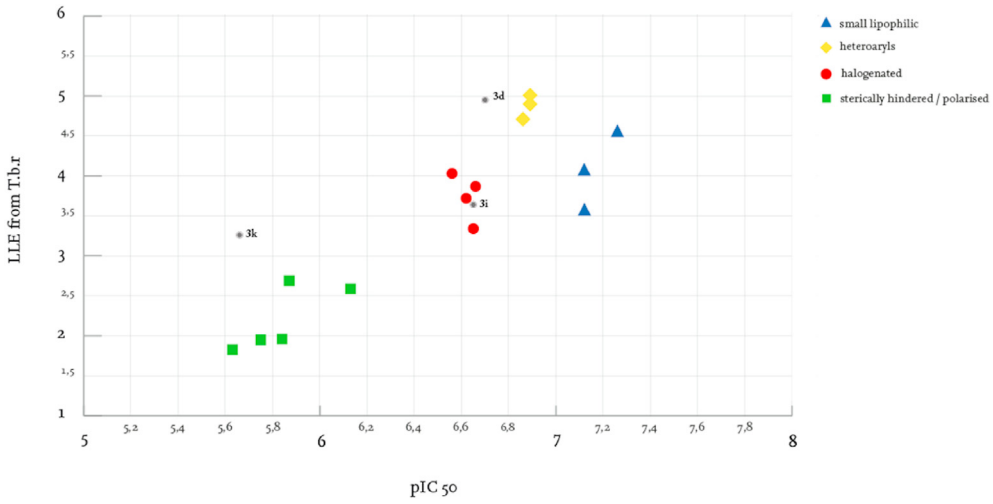


Fig. 2. Plot of pIC_{50} (*T.b. rhodesiense*) data obtained in this study (x) vs. calculated lipophilic ligand efficiency (y). Substances are labelled in the following way: green (sterically hindered or strongly polarised: **3c**, **3f**, **3h**, **3q**, **3r**), red (halogenated: **3e**, **3m**, **3n**, **3o**), yellow (heteroaryls: **3g**, **3j**, **3l**), blue (small lipophilic residues: **3a**, **3b**, **3p**). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

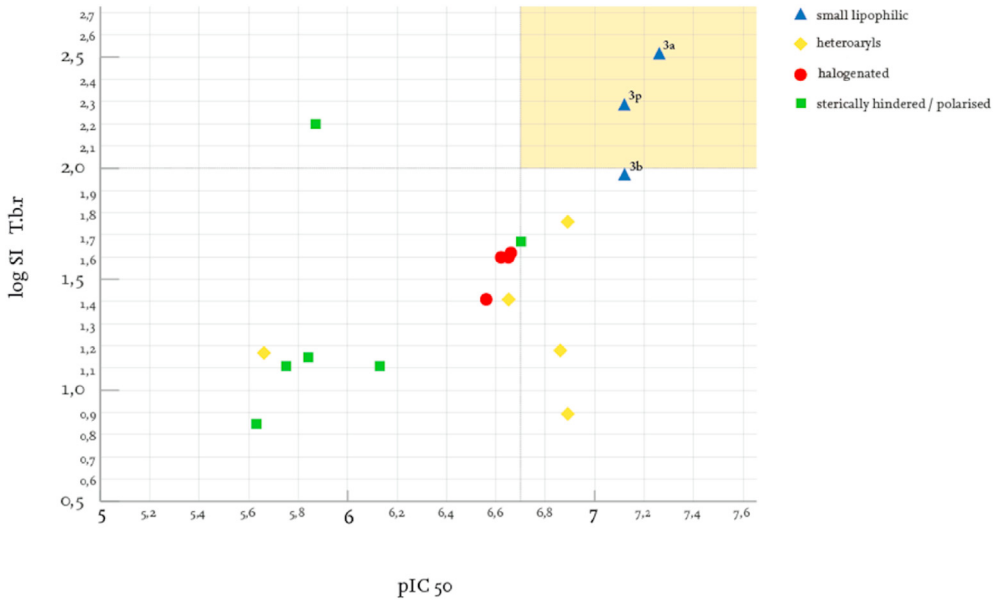
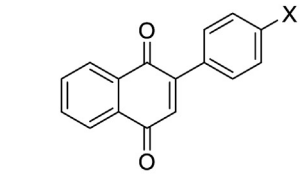
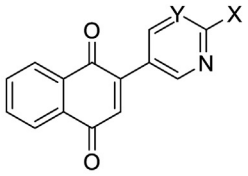


Fig. 3. Plot of pIC_{50} (*T.b. rhodesiense*) data obtained in this study (x) vs. calculated log SI (*T.b. rhodesiense*) (y). Substances appearing in the yellow shaded area combined high activity with acceptable selectivity over an L6 cell line. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



	MIC	MF
X = CN (**3e**)	64	> 128
X = F (**3m**)	128	> 128
X = Cl (**3n**)	16	> 128



	MIC	MF
X = F, Y = C (**3j**)	16	> 128
X = Cl, Y = C (**3k**)	32	128
X = Cl, Y = N (**3l**)	4	2

Fig. 4. Comparison between the (pseudo)-halogenated phenyl and pyridyl NQs with regards to their antimycobacterial properties.

With exception of the pyrimidine derivative **3l**, all tested compounds with this structural feature (**3e**, **3j**, **3k**, **3m**, **3n, 3q**) showed an MF $\geq$ 128.

3. Conclusions

We present an efficient, high-yield synthetic procedure for the preparation of 2-arylnaphthoquinones by a Suzuki type cross-coupling, which gave good to excellent yields for a variety of aromatic boronic acids. Many of the new synthesised compounds showed encouraging biological activities against *P. falciparum, T.b. rhodesiense* and *M. smegmatis* (Fig. 5), as well as favourable physicochemical properties. Trypanocidal effects were extraordinary and remarkable selectivity could be achieved, especially in the case of **3a** (IC_{50} = 0.06 μM; SI = 322.8) and **3p** (IC_{50} = 0.08 μM; SI = 189.0). The pyridyl derivative **3k** exhibits the lowest IC_{50} of all prepared compounds against the *P. falciparum* NF54 strain at 0.07 μM with very good selectivity (SI = 462.6), demonstrating that these heteroaromatics represent promising chemical leads for further derivatisation.

Remarkable antimycobacterial properties have also been observed: the most active compound against *M. smegmatis* proved to be **3l** with an MIC of 4 μg/mL. In addition, efflux pump modulation of *M. smegmatis* was outstanding, with **3e**, **3j**, **3m** and **3n** showing an MF > 128 in the ethidium bromide efflux assay. The findings presented in this work demonstrate the potential for the 2-aryl-1,4-naphthoquinones in the development of new anti-infective drugs.

4. Experimental section

4.1. General

All reagents and solvents were purchased from Sigma-Aldrich and Fluorochem Ltd. The moisture-sensitive reactions were performed under an inert atmosphere of argon. Each reaction was monitored by TLC on Merck TLC plates (silica gel 60 F_{254} 0.2 mm, 200 × 200 mm) and detected at 254 nm. All the compounds were purified by flash column chromatography using silica gel 60 (Merck, 70–230 mesh, pore-diameter 60 Å), unless otherwise specified. Purity and homogeneity of the final compounds were assessed by TLC and high-resolution mass spectrometry. The melting points were determined with a digital melting point apparatus (Electrothermal IA 9200). The structure elucidation was

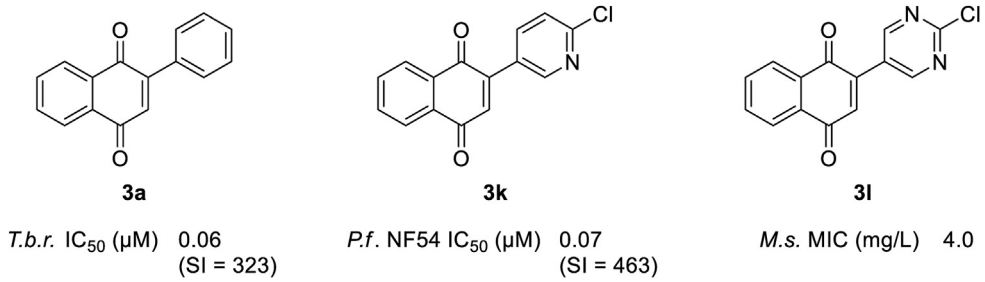


Fig. 5. Overview of the most active compounds in their respective assays.

confirmed by ^{1}H and ^{13}C NMR (1D and 2D) on a Varian Unity Inova 400 MHz instrument (at 298 K) using 5 mm tubes. The chemical shifts are expressed in δ (ppm) using tetramethylsilane (TMS) as internal standard or the ^{13}C signal of the solvent ($CDCl_3$ δ 77.04), coupling constants (J) were reported in Hertz (Hz). ^{1}H and ^{13}C resonances are numbered as given in the formulae, the signals marked with an asterisk are interchangeable. High-resolution EI mass spectra (70 eV, source temperature 220 °C) were recorded on an orthogonal TOF spectrometer (Waters GCT Premier) equipped with a direct insertion (DI) probe. Typically, 0.2 μl of a solution of the sample (c = 0.1 mg/mL) were placed in the glass cup used for DI, dried under atmospheric pressure, and transferred into the vacuum. Mass spectra (50–800 Da; 1 spectrum/s; resolution appr. 7500 FWHM) were continuously acquired while the sample was evaporated rapidly. ESI mass spectra were acquired on an Exactive Orbitrap mass spectrometer equipped with a heated ESI II source.

4.2. Synthesis of 2-bromo-1,4-naphthoquinone (**2**)

To a vigorously stirred solution of NBS (5.34 g, 30 mmol) in AcOH (15 mL) and H_2O (30 mL), a mixture of 1-naphthol (1.08 g, 7.5 mmol) in AcOH (15 mL) was added dropwise at 45 °C within 20 min. The clear solution slowly turned orange and a yellow precipitate was formed. Stirring at 45 °C was continued until TLC showed complete consumption of the starting material (1 h). Then, additional 40 mL of H_2O were added and the reaction mixture was cooled to 0 °C. The yellow precipitate was filtered, washed two times with ice-cold H_2O and dried under reduced pressure to obtain 1.67 g (94%) of **2** as a bright yellow solid. R_f = 0.37 ($CHCl_3$:CH = 2:1), mp 127–128 °C (lit [32]. mp 132–133 °C). The spectroscopic data were in accordance with those described in Ref. [32].

4.3. General synthetic procedures for arylnaphthoquinones ***3a-r***

Under exclusion of air or humidity, bromonaphthoquinone **2** (237 mg, 1 mmol) and Cs_2CO_3 (489 mg, 1.5 mmol) were added successively to a stirred solution of $Pd(PPh_3)_4$ (29 mg, 0.025 mmol) in anhydrous THF (4 mL). Then, 0.6 mL of H_2O and 1.5 mmol of the corresponding boronic acid were added and the reaction mixture was heated to 65 °C until TLC showed complete consumption of the starting material (1.5–20 h). After cooling to ambient temperature, the mixture was diluted with water (15 mL) and extracted several times with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to give a residue, which was purified by flash chromatography.

4.3.1. 2-Phenyl-1,4-naphthoquinone (***3a***)

Compound **3a** was obtained after heating for 1.5 h and purified by flash chromatography using toluene. Yellow solid; yield: 94%; R_f = 0.32 (toluene); mp: 107–108 °C (lit [47]. mp 106–108 °C). The spectroscopic data were found to be identical to the ones described in Ref. [47,48]. Although the structure of **3a** is already known, to the best of our knowledge, a complete assignment of the NMR signals has never been published: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23–8.17 (m, 1H, H-8), 8.17–8.09 (m, 1H, H-5), 7.85–7.77 (m, 2H, H-6, H-7), 7.74 (d, J = 8.3 Hz, 2H, H-3′, H-5′), 7.11 (s, 1H, H-3), 7.69 (d, 2H, H-2′, H-6′) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.1 (C-4), 184.3 (C-1), 148.1 (C-2), 135.2 (C-3), 133.9 (C-7), 133.8 (C-6), 133.4 (C-1′), 132.4 (C-8a), 132.1 (C-4a), 130.0 (C-4′), 129.4 (C-2′, C-6′), 128.5 (C-3′, C-5′), 127.0 (C-8), 126.0 (C-5) ppm.

4.3.2. 2-(4-Methylphenyl)-1,4-naphthoquinone (***3b***)

Compound **3b** was obtained after heating for 4 h and purified by flash chromatography using toluene. Yellow solid; yield: 83%; R_f = 0.44 (toluene); mp: 109–110 °C (lit [47]. mp 100–104 °C). Although the structure of **3b** is already known [47], to the best of our knowledge, a complete assignment of the NMR signals has never been published: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21–8.16 (m, 1H, H-8), 8.14–8.08 (m, 1H, H-5), 7.80–7.74 (m, 2H, H-6, H-7), 7.49 (d, J = 7.9 Hz, 2H, H-2′, H-6′), 7.28 (d, J = 7.9 Hz, 2H, H-3′, H-5′), 7.07 (s, 1H, H-3), 2.42 (s, 3H, C**H**$_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.2 (C-4), 184.6 (C-1), 148.0 (C-2), 140.4 (C-4′), 134.6 (C-3), 133.8 (C-6, C-7), 132.5 (C-8a), 132.1 (C-4a), 130.5 (C-1′), 129.4 (C-2′, C-6′), 129.2 (C-3′, C-5′), 127.0 (C-8), 125.9 (C-5), 21.4 (**C**H$_3$) ppm; HRMS (EI) calcd. for $C_{17}H_{12}O_2$ $[M]^+$ = 248.0837; found: 248.0837.

4.3.3. 2-(4-Isopropylphenyl)-1,4-naphthoquinone (***3c***)

Compound **3c** was obtained after heating for 4 h and purified by flash chromatography using toluene. Yellow crystallising oil; yield: 81%; R_f = 0.31 (toluene); mp: 85–86 °C. Although the structure of **3c** is already known [49], to the best of our knowledge, a complete assignment of the NMR signals has never been published: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21–8.16 (m, 1H, H-8), 8.15–8.09 (m, 1H, H-5), 7.81–7.73 (m, 2H, H-6, H-7), 7.53 (d, J = 8.0 Hz, 2H, H-2′, H-6′), 7.34 (d, J = 8.0 Hz, 2H, H-3′, H-5′), 7.07 (s, 1H, H-3), 2.97 (hept, J = 7.0 Hz, 1H, C**H**$(CH_3)_2$), 1.29 (d, J = 7.0 Hz, 6H, (C**H**$_3)_2$–CH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.2 (C-4), 184.6 (C-1), 151.3 (C-4′), 148.0 (C-2), 134.6 (C-3), 133.8 (C-6, C-7), 132.5 (C-8a), 132.1 (C-4a), 130.8 (C-1′), 129.5 (C-2′, C-6′), 127.0 (C-8), 126.7 (C-3′, C-5′), 125.9 (C-5), 34.1 (**C**H-$(CH_3)_2$), 23.8 ((**C**H$_3)_2$–CH) ppm; HRMS (EI) calcd. for $C_{19}H_{16}O_2$ $[M]^+$ = 276.1150; found: 276.1151.

4.3.4. 2-(3-(Methylsulfonylamino)phenyl)-1,4-naphthoquinone (***3d***)

Compound **3d** was obtained after heating for 20 h and purified by flash chromatography using toluene/EtOAc (5:1). Orange oil; yield: 92%; R_f = 0.22 (toluene:EtOAc = 5:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20–8.17 (m, 1H, H-8), 8.15–8.12 (m, 1H, H-5), 7.83–7.77 (m, 2H, H-6, H-7), 7.48 (t, J = 8.0 Hz, 1H, H-5′), 7.46–7.44 (m, 1H, H-2′), 7.41–7.36 (m, 1H, H-6′), 7.38–7.34 (m, 1H, H-4′), 7.10 (s, 1H, H-3), 6.61 (s, 1H, NH), 3.10 (s, 3H, C**H**$_3$-(SO_2)N) ppm; ^{13}C NMR (100 MHz,

$CDCl_3$) δ 184.9 (C-4), 184.1 (C-1), 147.0 (C-2), 136.8 (C-3′), 135.7 (C-3), 135.0 (C-1′), 134.1 (C-6, C-7), 132.3 (C-8a), 132.0 (C-4a), 129.9 (C-5′), 127.1 (C-8), 126.2 (C-6′), 126.1 (C-5), 121.9 (C-4′), 121.5 (C-2′), 39.7 (**C**H_3-(SO_2)N) ppm; HRMS (EI) calcd. for $C_{17}H_{13}NO_4S$ $[M]^+$ = 327.0565; found: 327.0557.

4.3.5. 2-(4-Cyanophenyl)-1,4-naphthoquinone (**3e**)

Compound **3e** was obtained after heating for 20 h and purified by flash chromatography using toluene/EtOAc (5:1). Yellow solid; yield: 82%; R_f = 0.62 (toluene:EtOAc = 5:1); mp: 191–192 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22–8.17 (m, 1H, H-8), 8.17–8.12 (m, 1H, H-5), 7.85–7.80 (m, 2H, H-6, H-7), 7.78 (d, J = 8.3 Hz, 2H, H-3′, H-5′), 7.71–7.66 (m, 2H, H-2′, H-6′), 7.10 (s, 1H, H-3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.5 (C-4), 183.6 (C-1), 146.3 (C-2), 137. 8 (C-1′), 136.3 (C-3), 134.2 (C-6, C-7), 132.1 (C-3′C-5′), 132.1 (C-8a), 132.0 (C-4a), 130.1 (C-2′, C-6′), 127.2 (C-8), 126.2 (C-5), 118.3 (CN), 113.6 (C-4′) ppm; HRMS (EI) calcd. for $C_{17}H_{19}NO_2$ $[M]^+$ = 259.0633; found: 259.0635.

4.3.6. 2-(4-(Piperidin-1-ylcarbonyl)phenyl)-1,4-naphthoquinone (**3f**)

Compound **3f** was obtained after heating for 3 h and purified by flash chromatography using toluene/EtOAc (5:1). Brownish oil; yield: 88%; R_f = 0.14 (toluene:EtOAc = 5:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (dd, J = 6.4, 2.8 Hz, 1H, H-8), 8.14 (dd, J = 5.8, 3.3 Hz, 1H, H-5), 7.84–7.77 (m, 2H, H-6, H-7), 7.62 (d, J = 7.9 Hz, 2H, H-2′, H-6′), 7.50 (d, J = 7.9 Hz, 2H, H-3′, H-5′), 7.10 (s, 1H, H-3), 3.74 (s, 2H, H-6″), 3.39 (s, 2H, H-2″), 1.73–1.69 (m, 2H, H-5″), 1.72–1.68 (m, 2H, H-4″), 1.57–1.53 (m, 2H, H-3″) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.0 (C-4), 184.2 (C-1), 169.5 ((**C**O)–N), 147.4 (C-2), 138.0 (C-4′), 135.6 (C-3), 134.3 (C-1′), 134.0 (C-6, C-7), 132.3 (C-8a), 132.1 (C-4a), 129.6 (C-2′, C-6′), 127.1 (C-8), 126.9 (C-3′, C-5′), 126.1 (C-5), 48.8 (C-2″), 43.2 (C-6″), 26.6 (C-3″), 25.6 (C-4″), 24.6 (C-5″) ppm; HRMS (EI) calcd. for $C_{22}H_{19}NO_3$ $[M]^+$ = 345.1365; found: 345.1359.

4.3.7. 2-(2-Furyl)-1,4-naphthoquinone (**3g**)

Compound **3g** was obtained after heating for 4 h and purified by flash chromatography using toluene. Red solid; yield: 22%; R_f = 0.35 (toluene); mp: 162–163 °C (lit [50]. mp 158–160 °C). Although the structure of **3g** is already known [50,51], to the best of our knowledge, a complete assignment of the NMR signals has never been published: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (dd, J = 5.9, 3.1 Hz, 1H, H-8), 8.09 (dd, J = 5.9, 3.2 Hz, 1H, H-5), 7.78–7.71 (m, 2H, H-6, H-7), 7.63 (d, J = 3.6 Hz, 1H, H-3′), 7.61 (d, J = 1.7 Hz, 1H, H-5′), 7.31 (s, 1H, H-3), 6.61 (dd, J = 3.6, 1.8 Hz, 1H, H-4′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.9 (C-4), 183.0 (C-1), 146.7 (C-2′), 145.4 (C-5′), 135.4 (C-2), 133.9* (C-6), 133.6* (C-7), 132.3 (C-8a), 132.1 (C-4a), 128.2 (C-3), 126.7 (C-8), 126.0 (C-5), 118.9 (C-3′), 113.4 (C-4′) ppm; HRMS (EI) calcd. for $C_{14}H_8O_3$ $[M]^+$ = 224.0473; found: 224.0470.

4.3.8. 2-(1-Benzofuran-2-yl)-1,4-naphthoquinone (**3h**)

Compound **3h** was obtained after heating for 8 h and purified by flash chromatography using toluene. Red solid; yield: 12%; R_f = 0.21 (toluene); mp: 181–182 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (dd, J = 5.7, 3.4 Hz, 1H, H-8), 8.12 (dd, J = 5.7, 3.3 Hz, 1H, H-5), 8.02 (s, 1H, H-3′), 7.78 (dd, J = 5.8, 3.3 Hz, 2H, H-6, H-7), 7.69 (d, J = 7.5 Hz, 1H, H-4′), 7.56 (s, 1H, H-3), 7.53 (d, J = 8.3 Hz, 1H, H-7′), 7.46–7.37 (m, 1H, H-6′), 7.28 (t, J = 7.5 Hz, 1H, H-5′) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.7 (C-4), 182.9 (C-1), 155.0 (C-7'a), 148.0 (C-2′), 135.7 (C-2), 134.0* (C-6), 133.8* (C-7), 132.5 (C-8a), 132.1 (C-4a), 130.5 (C-3), 129.0 (C-3'a), 127.2 (C-6′), 126.9 (C-8), 126.1 (C-5), 123.5 (C-5′), 122.5 (C-4′), 115.2 (C-3′), 111.4 (C-7′) ppm; HRMS (EI) calcd. for $C_{18}H_{10}O_3$ $[M]^+$ = 274.0630; found: 274.0625.

4.3.9. 2-(3-Quinolyl)-1,4-naphthoquinone (**3i**)

Compound **3i** was obtained after heating for 4.5 h and purified by flash chromatography using CH/EtOAc (1:1). Yellow solid; yield: 12%; R_f = 0.35 (CH:EtOAc = 1:1); mp: 197–198 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (d, J = 2.2 Hz, 1H, H-2′), 8.49 (d, J = 2.2 Hz, 1H, H-4′), 8.24 (dd, J = 5.9, 3.2 Hz, 1H, H-8), 8.19–8.13 (m, 2H, H-5, H-8′), 7.96–7.91 (m, 1H, H-5′), 7.85–7.79 (m, 2H, H-6, H-7), 7.82–7.78 (m, 1H, H-7′), 7.63 (t, J = 7.5 Hz, 1H, H-6′), 7.27 (d, J = 1.5 Hz, 1H, H-3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.6 (C-4), 184.1 (C-1), 149.7 (C-2′), 148.3 (C-8'a), 145.1 (C-2), 137.6 (C-4′), 135.8 (C-3), 134.2* (C-6), 134.1* (C-7), 132.2 (C-8a), 132.1 (C-4a), 130.9 (C-7′), 129.3 (C-8′), 128.6 (C-5′), 127.4 (C-6′), 127.2 (C-8, C-4′a), 126.3 (C-3′), 126.2 (C-5) ppm; HRMS (EI) calcd. for $C_{19}H_{11}NO_2$ $[M]^+$ = 285.0790; found: 285.0802.

4.3.10. 2-(6-Fluoro-3-pyridyl)-1,4-naphthoquinone (**3j**)

Compound **3j** was obtained after heating for 3 h and purified by flash chromatography using toluene/EtOAc (5:1). Yellow solid; yield: 75%; R_f = 0.54 (toluene:EtOAc = 5:1); mp: 170–171 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, J = 2.5 Hz, 1H, H-2′), 8.23–8.18 (m, 1H, H-8), 8.17–8.12 (m, 1H, H-5), 8.07 (td, J = 8.4, 2.5 Hz, 1H, H-4′), 7.85–7.79 (m, 2H, H-6, H-7), 7.13 (s, 1H, H-3), 7.07 (dd, J = 8.5, 3.0 Hz, 1H, H-5′) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.5 (C-4), 183.8 (C-1), 164.3 (d, $^1J_{C,F}$ = 243.6 Hz, C-6′), 148.1 (d, $^3J_{C,F}$ = 15.6 Hz, C-2′), 144.0 (C-2), 142.4 (d, $^3J_{C,F}$ = 8.4 Hz, C-4′), 135.6 (C-3), 134.3* (C-6), 134.2* (C-7), 132.0 (C-4a, C-8a), 127.3 (d, $^4J_{C,F}$ = 4.7 Hz, C-3′), 127.2 (C-8), 126.3 (C-5), 109.4 (d, $^2J_{C,F}$ = 37.3 Hz, C-5′) ppm; HRMS (EI) calcd. for $C_{15}H_8FNO_2$ $[M]^+$ = 253.0539; found: 253.0532.

4.3.11. 2-(6-Chloro-3-pyridyl)-1,4-naphthoquinone (**3k**)

Compound **3k** was obtained after heating for 2 h and purified by flash chromatography using toluene/EtOAc (3:1). Orange solid; yield: 82%; R_f = 0.63 (toluene:EtOAc = 3:1); mp: 205–206 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 2.5 Hz, 1H, H-2′), 8.23–8.17 (m, 1H, H-8), 8.17–8.11 (m, 1H, H-5), 7.92 (dd, J = 8.3, 2.5 Hz, 1H, H-4′), 7.85–7.79 (m, 2H, H-6, H-7), 7.46 (d, J = 8.3 Hz, 1H, H-5′), 7.13 (s, 1H, H-3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.4 (C-4), 183.6 (C-1), 153.0 (C-6′), 149.5 (C-2′), 144.0 (C-2), 139.6 (C-4′), 135.8 (C-3), 134.3* (C-6), 134.2* (C-7), 132.0 (C-4a, C-8a), 128.2 (C-3′), 127.2 (C-8), 126.3 (C-5), 124.0 (C-5′) ppm; HRMS (EI) calcd. for $C_{15}H_8ClNO_2$ $[M]^+$ = 269.0244; found: 269.0240.

4.3.12. 2-(2-Chloro-5-pyrimidyl)-1,4-naphthoquinone (**3l**)

Compound **3l** was obtained after heating for 5 h and purified by flash chromatography using toluene/EtOAc (5:1). Orange solid; yield: 56%; R_f = 0.50 (toluene:EtOAc = 5:1); mp: 237–238 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 2H, H-4′, H-6′), 8.24–8.19 (m, 1H, H-8), 8.19–8.13 (m, 1H, H-5), 7.85 (dd, J = 5.8, 3.3 Hz, 2H, H-6, H-7), 7.19 (s, 1H, H-3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.8 (C-4), 183.0 (C-1), 162.4 (C-2′), 159.3 (C-4′, C-6′), 141.2 (C-2), 136.2 (C-3), 134.7* (C-6), 134.5* (C-7), 132.0 (C-4a), 131.7 (C-8a), 127.3 (C-8), 126.5 (C-5), 126.0 (C-5′) ppm; HRMS (EI) calcd. for $C_{14}H_7ClN_2O_2$ $[M]^+$ = 270.0196; found: 270.0199.

4.3.13. 2-(4-Fluorophenyl)-1,4-naphthoquinone (**3m**)

Compound **3m** was obtained after heating for 3 h and purified by flash chromatography using toluene. Yellow solid; yield: 84%; R_f = 0.25 (toluene); mp: 139–140 °C (lit [51]. mp 140–142 °C). Although the structure of **3m** is already known [51], to the best of our knowledge, a complete assignment of the NMR signals has never been published: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (dd, J = 5.9, 3.1 Hz, 1H, H-8), 8.12 (dd, J = 5.9, 3.1 Hz, 1H, H-5), 7.82–7.75 (m, 2H, H-6, H-7), 7.62–7.55 (m, 2H, H-2′, H-6′), 7.17 (t, J = 8.6 Hz, 2H, H-3′, H-5′), 7.06 (s, 1H, H-3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.0 (C-4), 184.3 (C-1), 163.9 (d, $^1J_{C,F}$ = 251.4 Hz, C-4′), 146.9 (C-2), 135.0 (C-

3), 134.0* (C-6), 133.9* (C-7), 132.3 (C-8a), 132.0 (C-4a), 131.5 (d, $^{3}J_{C,F}$ = 8.5 Hz, C-2′, C-6′), 129.4 (d, $^{4}J_{C,F}$ = 3.4 Hz, C-1′), 127.1 (C-8), 126.0 (C-5), 115.7 (d, $^{2}J_{C,F}$ = 21.7 Hz, C-3′, C-5′) ppm; HRMS (EI) calcd. for $C_{16}H_9FO_2$ $[M]^+$ = 252.0587; found: 252.0582.

4.3.14. 2-(4-Chlorophenyl)-1,4-naphthoquinone (**3n**)

Compound **3n** was obtained after heating for 2 h and purified by flash chromatography using toluene. Yellow solid; yield: 74%; R_f = 0.50 (toluene); mp: 152–153 °C (lit [50]. mp 170–178 °C). Although the structure of **3n** is already known [50], to the best of our knowledge, a complete assignment of the NMR signals has never been published: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21–8.16 (m, 1H, H-8), 8.14–8.10 (m, 1H, H-5), 7.82–7.77 (m, 2H, H-6, H-7), 7.56–7.51 (m, 2H, H-2′, H-6′), 7.48–7.43 (m, 2H, H-3′, H-5′), 7.07 (s, 1H, H-3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.9 (C-4), 184.1 (C-1), 146.9 (C-2), 136.4 (C-4′), 135.2 (C-3), 134.0 (C-6, C-7), 132.3 (C-8a), 132.0 (C-4a), 131.7 (C-1′), 130.7 (C-2′, C-6′), 128.8 (C-3′, C-5′), 127.1 (C-8), 126.0 (C-5) ppm; HRMS (EI) calcd. for $C_{16}H_9ClO_2$ $[M]^+$ = 268.0291; found: 268.0288.

4.3.15. 2-(3,5-Difluorophenyl)-1,4-naphthoquinone (**3o**)

Compound **3o** was obtained after heating for 2 h and purified by flash chromatography using toluene. Yellow solid; yield: 68%; R_f = 0.44 (toluene); mp: 178–179 °C (lit [51]. mp 194–196 °C). Although the structure of **3o** is already known [51], to the best of our knowledge, a complete assignment of the NMR signals has never been published: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22–8.16 (m, 1H, H-8), 8.15–8.10 (m, 1H, H-5), 7.84–7.77 (m, 2H, H-6, H-7), 7.13 (dt, J = 6.5, 2.1 Hz, 2H, H-2′, H-6′), 7.07 (s, 1H, H-3), 6.94 (tt, $^{3}J_{H,F}$ = 8.9, J = 2.4 Hz, 1H, H-4′) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.6 (C-4), 183.5 (C-1), 162.8* (d, $^{1}J_{C,F}$ = 249.3 Hz, C-3′), 162.7* (d, $^{1}J_{C,F}$ = 249.3 Hz, C-5′), 145.8 (C-2), 136.2 (d, $^{3}J_{C,F}$ = 10.0 Hz, C-1′), 136.0 (C-3), 134.2* (C-6), 134.1* (C-7), 132.1 (C-8a), 131.9 (C-4a), 127.2 (C-8), 126.1 (C-5), 112.6 (dd, $^{2}J_{C,F}$ = 26.4, $^{4}J_{C,F}$ = 7.5 Hz, C-2′, C-6′), 105.3 (t, $^{2}J_{C,F}$ = 25.3 Hz, C-4′) ppm; HRMS (EI) calcd. for $C_{16}H_{18}F_2O_2$ $[M]^+$ = 270.0492; found: 270.0495.

4.3.16. 2-(4-(Trifluoromethyl)phenyl)-1,4-naphthoquinone (**3p**)

Compound **3p** was obtained after heating for 4 h and purified by flash chromatography using toluene. Yellow solid; yield: 95%; R_f = 0.33 (toluene); mp: 126–127 °C (lit [51]. mp 122–124 °C). Although the structure of **3p** is already known [51], to the best of our knowledge, a complete assignment of the NMR signals has never been published: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23–8.17 (m, 1H, H-8), 8.17–8.09 (m, 1H, H-5), 7.85–7.77 (m, 2H, H-6, H-7), 7.74 (d, J = 8.3 Hz, 2H, H-3′, H-5′), 7.69 (d, J = 8.3 Hz, 2H, H-2′, H-6′), 7.11 (s, 1H, H-3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.8 (C-4), 183.8 (C-1), 146.8 (C-2), 136.8 (C-1′), 136.1 (C-3), 134.1 (C-6, C-7), 132.2 (C-8a), 132.0 (C-4a), 131.8 (q, $^{2}J_{C,F}$ = 32.7 Hz, C-4′), 129.8 (C-2′, C-6′), 127.1 (C-8), 126.1 (C-5), 125.4 (q, $^{3}J_{C,F}$ = 3.8 Hz, C-3′, C-5′), 123.8 (q, $^{1}J_{C,F}$ = 272.4 Hz, **C**F_3) ppm; HRMS (EI) calcd. for $C_{17}H_9F_3O_2$ $[M]^+$ = 302.0555; found: 302.0556.

4.3.17. 2-(4-(Trifluoromethoxy)phenyl)-1,4-naphthoquinone (**3q**)

Compound **3q** was obtained after heating for 3 h and purified by flash chromatography using toluene. Orange solid; yield: 91%; R_f = 0.44 (toluene); mp: 90–91 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22–8.17 (m, 1H, H-8), 8.15–8.11 (m, 1H, H-5), 7.83–7.77 (m, 2H, H-6, H-7), 7.66–7.61 (m, 2H, H-2′, H-6′), 7.36–7.30 (m, 2H, H-3′, H-5′), 7.08 (s, 1H, H-3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.9 (C-4), 184.1 (C-1), 150.5 (q, $^{3}J_{C,F}$ = 1.8 Hz, C-4′), 135.5 (C-3), 134.0 (C-6, C-7), 132.3 (C-8a), 132.0 (C-4a), 131.8 (C-1′), 131.1 (C-2′, C-6′), 127.1 (C-8), 126.1 (C-5), 120.7 (C-3′, C-5′), 120.4 (q, $^{1}J_{C,F}$ = 258.2 Hz, O**C**F_3) ppm; HRMS (EI) calcd. for $C_{17}H_9F_3O_3$ $[M]^+$ = 318.0504; found: 318.0495.

4.3.18. 2-(3-(Trifluoromethoxy)phenyl)-1,4-naphthoquinone (**3r**)

Compound **3r** was obtained after heating for 4 h and purified by flash chromatography using toluene. Yellow solid; yield: 98%; R_f = 0.40 (toluene); mp: 86–87 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22–8.18 (m, 1H, H-8), 8.16–8.12 (m, 1H, H-5), 7.82–7.79 (m, 2H, H-6, H-7), 7.53–7.50 (m, 2H, H-4′, H-6′), 7.47 (s, 1H, H-2′), 7.38–7.31 (m, 1H, H-5′), 7.10 (s, 1H, H-3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.8 (C-4), 183.9 (C-1), 149.1 (q, $^{3}J_{C,F}$ = 1.8 Hz, C-3′), 146.5 (C-2), 135.9 (C-3), 135.2 (C-1′), 134.1 (C-6, C-7), 132.2 (C-8a), 132.0 (C-4a), 129.9* (C-4′), 127.7* (C-6′), 127.1 (C-8), 126.1 (C-5), 122.4 (C-5′), 122.2 (C-2′), 120.4 (q, $^{1}J_{C,F}$ = 257.5 Hz, O**C**F_3) ppm; HRMS (EI) calcd. for $C_{17}H_9F_3O_3$ $[M]^+$ = 318.0504; found: 318.0507.

4.4. Assay for in vitro antimalarial activity

In brief, *in vitro* activity against erythrocytic stages of *P. falciparum* was determined by a modified [^{3}H]-hypoxanthine incorporation assay [52] using the drug-sensitive NF54 strain and the standard drug. For details, we refer to the supplementary material.

4.5. Assay for in vitro trypanocidal activity

The assay for trypanocidal activity was carried out as Alamar blue assay previously described in Ref. [53,54], it is detailed out in the supplementary material.

4.6. Assay for cytotoxicity

Cytotoxicity tests were performed using L6 cells with podophyllotoxin as positive control, further details are listed in the supplementary material.

4.7. Assay for in vitro antimycobacterial and resistance modulating activity

The antimycobacterial activity of all arylnaphthoquinones was evaluated against the strain *Mycobacterium smegmatis* mc^2 155 (ATCC 700084). Test concentration was obtained by serial dilution with isonicotinic acid hydrazide (INH) as control. After incubation, visual analysis was carried out using MTT (thiazolyl blue tetrazolium bromide) colorimetric test. Further experimental details are given in the supplementary material.

Author contributions

Conceptualisation, M.-M.K. A.P.; Data curation; M.-M.K, A.P. F.B. W.S. R.S. S.O. J.S. M.K.; Formal analysis, A.P. and R.S.; Investigation, M.-M.K, S.O. J.S. W.S.; Methodology, M.-M.K, A.P. F.B. W.S. R.S. M.K; Project administration, A.P.; Resources, A.P.; Supervision, A.P.; Validation, A.P. F.B. W.S. R.S. M.-M.K.; Writing-original draft, A.P, M.-M.K.; Writing-review & editing, A.P. M.-M.K, F.B. W.S. R.S. M.K.

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Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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