RSC Medicinal Chemistry

ROYAL SOCIETY OF CHEMISTRY

RESEARCH ARTICLE

View Article Online
View Journal

Cite this: DOI: 10.1039/d0md00072h

Synthesis of quinone imine and sulphur-containing compounds with antitumor and trypanocidal activities: redox and biological implications†

Renata G. Almeida, ‡[a] Wagner O. Valença, ‡[ab] Luísa G. Rosa, [a] Carlos A. de Simone,[c] Solange L. de Castro, [d] Juliana M. C. Barbosa, [d] Daniel P. Pinheiro, [e] Carlos R. K. Paier, [e] Guilherme G. C. de Carvalho,[e] Claudia Pessoa, [e] Marilia O. F. Goulart, [f] Ammar Kharma [ag] and Eufrânio N. da Silva Júnior *[a]

Received 3rd March 2020,
Accepted 8th June 2020

DOI: 10.1039/d0md00072h

rsc.li/medchem

Ortho-Quinones represent a special class of redox active compounds associated with a spectrum of pronounced biological activities, including selective cytotoxicity and antimicrobial actions. The modification of the quinone ring by simple nitrogen and sulphur substitutions leads to several new classes of compounds with their own, distinct redox behaviour and equally distinct activities against cancer cell lines and *Trypanosoma cruzi*. Some of the compounds investigated show activity against *T. cruzi* at concentrations of 24.3 and 65.6 μM with a selectivity index of around 1. These results demonstrate that simple chemical modifications on the *ortho*-quinone ring system, in particular, by heteroatoms such as nitrogen and sulphur, transform these simple redox molecules into powerful cytotoxic agents with considerable "potential", not only in synthesis and electrochemistry, but also, in a broader sense, in health sciences.

1. Introduction

The rapid expansion and simultaneous ageing of populations around the globe represent a demographical change and a challenge that, among many other issues, such as shortage of nutrition and increase of pollution, is also associated with higher incidence of new cases of cancer and related deaths. The projection for the year 2030 estimates 27 million new cases of cancer with 17 million cancer-related deaths worldwide.[1] At the same time, an increased global population is also more susceptible to the outbreak and spread of infectious diseases, such as Chagas disease. This disease is caused by the protozoan *Trypanosoma cruzi*, which is considered by the World Health Organization (WHO) as one of the twenty neglected tropical diseases, affecting more than 5 million people worldwide. Its current chemotherapy is still restricted to the nitroderivatives benznidazole and nifurtimox, available for half a century, which present limited activity and severe adverse effects.[2–8] These demographical developments and new ways of transmission, combined with the emergence of drug resistance, require new and effective drugs for the treatment of such diseases, *i.e.* agents which may be effective as (cyto)toxins and also selective for their targets.

Within this context, naphthoquinoidal compounds have been studied widely.[9–13] The derivatization of naphthoquinones has been a subject of considerable interest among medicinal chemists and a wide variety of natural and synthetic naphthoquinones have already been reported as potent trypanocidal and anticancer agents.[14–17] Mechanistically, the quinone core may undergo one-electron reduction under aerobic conditions to form a semiquinone radical which will redox cycle to release reactive oxygen species (ROS).[10,18–20] Within the context of cancer, the cytotoxicity of quinones is therefore mainly associated with the catalytic generation of ROS and the alkylation of crucial proteins and nucleic acids, both

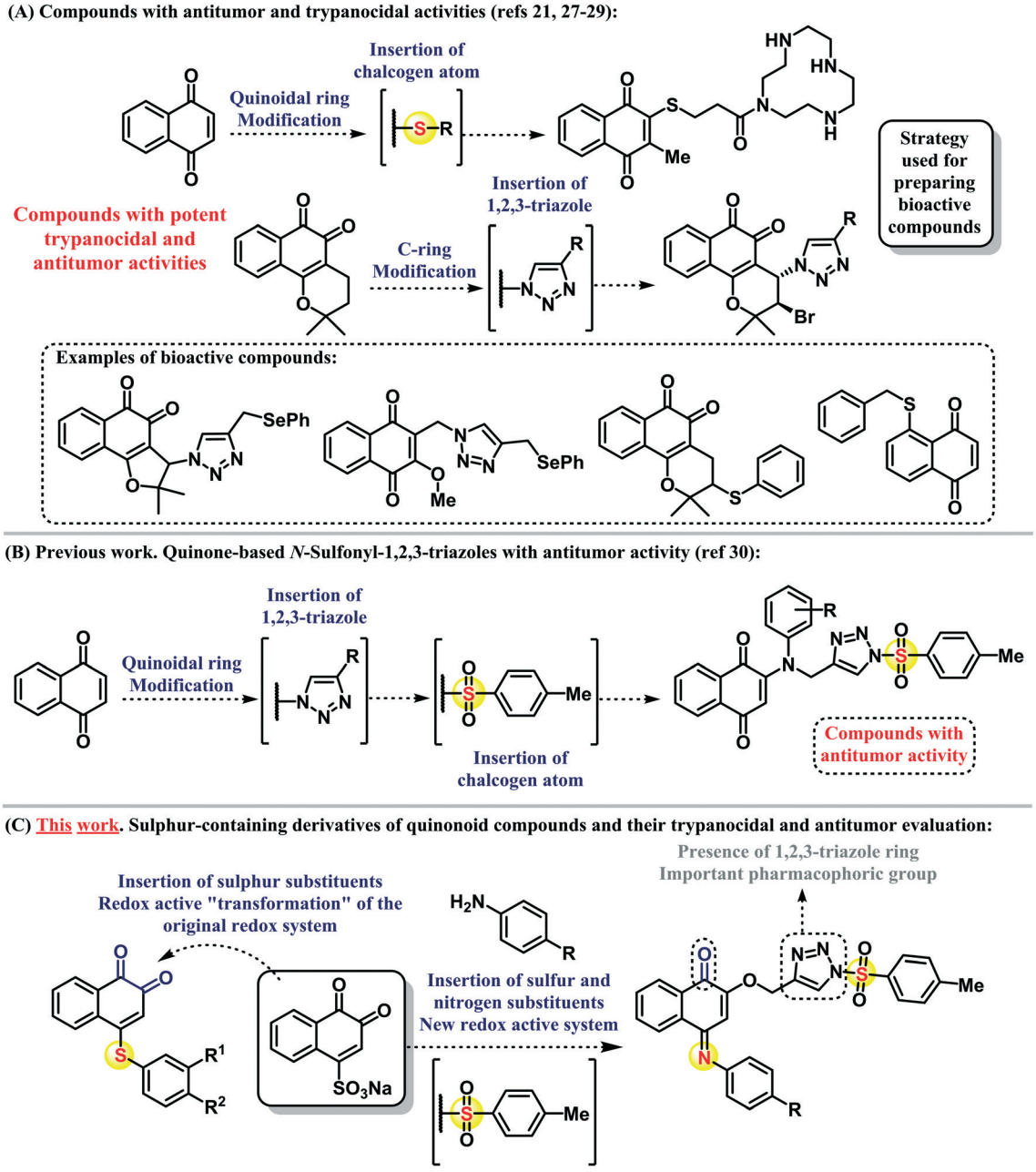
[a] *Institute of Exact Sciences, Department of Chemistry, Federal University of Minas Gerais, Belo Horizonte, 31270-901, MG, Brazil. E-mail: eufranio@ufmg.br*
[b] *Center for the Development of Chemical Technologies, State University of Mato Grosso do Sul, Naviraí, 79950-000, MS, Brazil*
[c] *Department of Physics and Informatics, Institute of Physics, University of São Paulo, São Carlos, 13560-160, SP, Brazil*
[d] *Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, 21045-900, RJ, Brazil*
[e] *Department of Physiology and Pharmacology, Federal University of Ceará, Fortaleza, CE, 60430-270, Brazil*
[f] *Institute of Chemistry and Biotechnology, Federal University of Alagoas, CEP 57072-970, Maceió, AL, Brazil*
[g] *Division of Bioorganic Chemistry, School of Pharmacy, University of Saarland, D-66123 Saarbruecken, Germany*

† Electronic supplementary information (ESI) available. CCDC 1928261, 1928296, 1928326, 1928361, 1928382 and 1928407. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/d0md00072h

‡ These authors contributed equally to this work.

processes, which provoke cell damage.[14–16] Similar considerations also apply to pathogenic microorganisms, some of which are affected considerably by – similar – redox modulating agents thanks to their weak(er) cellular antioxidant defence systems. Indeed, the concept of catalytic "sensor/effector" agents, *i.e.* "smart" redox molecules responding to specific intracellular redox signatures and peculiarities, and thereby combining considerable activity with selectivity, has been evaluated for over a decade now, often with amazing results.[21–23]

For almost twenty years, our groups have been involved in the synthesis and biological evaluation of naphthoquinones.[24–26] Whilst initial studies on such catalytic agents have employed comparably simple redox agents, subsequent ones have developed hybrid molecules with two or more redox sites integrated into one molecule. The hybridization of a triazole nucleus with β-lapachone, for instance, has already resulted in a derivative with high antitumor activity ($IC_{50} < 2$ μM) for different cancer lineages and, more recently, we have also reported the antitumor activity of selenium-containing quinone-based triazoles, which were inspired by earlier quinone–chalcogen structures tested more than a decade ago (Scheme 1A).[21,27–29] Quinone-based *N*-sulfonyl-1,2,3-triazoles were also prepared by our group using click chemistry reactions (Scheme 1B).[30] In this



Scheme 1 A schematic overview of the strategies employed in the preparation of bioactive compounds and transformation of *ortho*-quinones into *para*-quinone imines and sulphur containing compounds.

work, we employed as a strategy the insertion of the 1,2,3-triazolic nucleus associated with a chalcogen atom, allowing the formation of a redox active system. The compounds were subsequently evaluated against eight types of cancer cell lines and some derivatives exhibited potent antitumor activity.[30] Interestingly, the application of such "smart" catalytic agents is not limited to cancer research, and a pronounced – and selective – cytotoxicity also affects protozoan pathogens, such as *T. cruzi*, with some molecules exhibiting trypanocidal activity about two or six times higher than that of the standard drug benznidazole.[31–35]

Interestingly, the *ortho*-quinone redox centre found in β-lapachone is prone to chemical modifications which impact considerably the redox behaviour of these agents.[10] Here, we have investigated modifications with nitrogen and sulphur substituents, as both heteroatoms are redox active and, besides electronic effects, promise a major "transformation" of the original redox system into considerably more active species (Scheme 1C). We are now able to report the synthesis, electrochemistry, anticancer and trypanocidal evaluation of quinoidal derivatives containing alkynes and *N*-sulfonyl triazoles and propose a distinct relationship between the chemical structure and redox system, and their electrochemical potentials and biological activities.

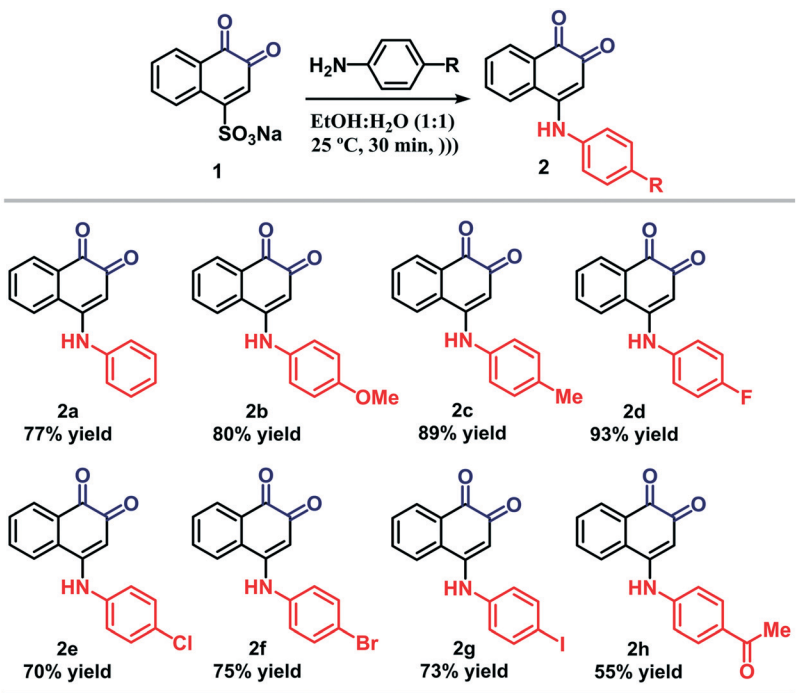
2. Results and discussion

2.1. Chemistry

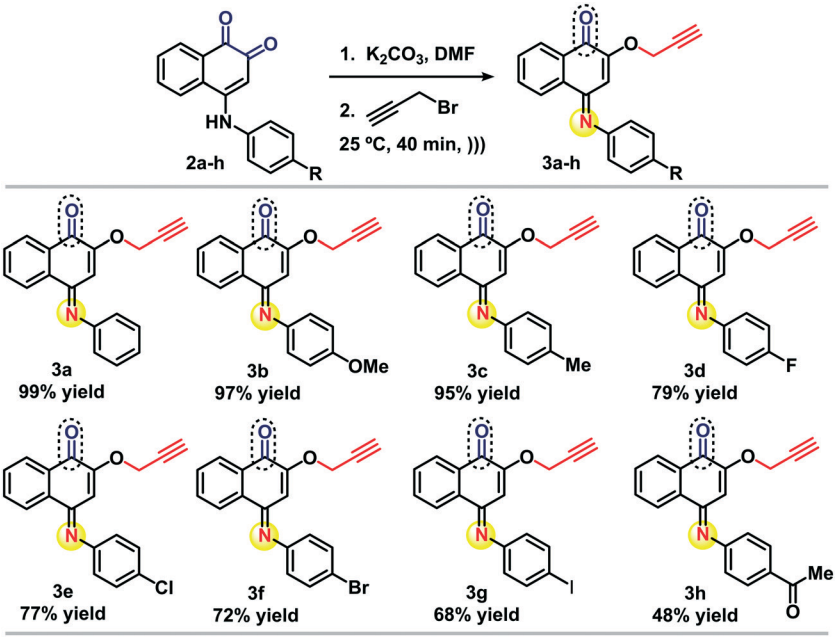
The first class of compounds with a nitrogen substituent at the ring was prepared from commercially available 1,2-naphthoquinone-4-sulfonic acid sodium salt (**1**). Initially, we prepared arylamino naphthoquinones (**2a–h**) by the reaction of **1** and the respective anilines following a procedure published previously in the literature with minor modifications.[36–38] In general, the desired products were obtained in moderate to excellent yields (Scheme 2). It should be noted from the outset that these compounds now possess more complicated redox cores spanning the original *ortho*-quinone and the amine functionality in the *para*-position.

With the quinoidal compounds **2a–h** available, the changed "redox core" was modified further, this time by alkylation of one of the oxygen atoms. Notably, this kind of alkylation not only simply "blocks" one of the oxygen atoms of the *ortho*-quinone, it also transforms the electronic structure of the entire ring system by engaging the nitrogen in the *para*-position in the form of a distinctive *para*-quinone imine redox core (Scheme 3).[39,40] The consequences of this apparently innocent *O*-alkylation for the redox and biological activity will be discussed later. Eight alkyne derivatives **3a–h** were synthesized in moderate to excellent yields ranging from 48% to 99% according to Scheme 3 and in the presence of DMF as a solvent, excess K_2CO_3 as a base, and propargyl bromide.

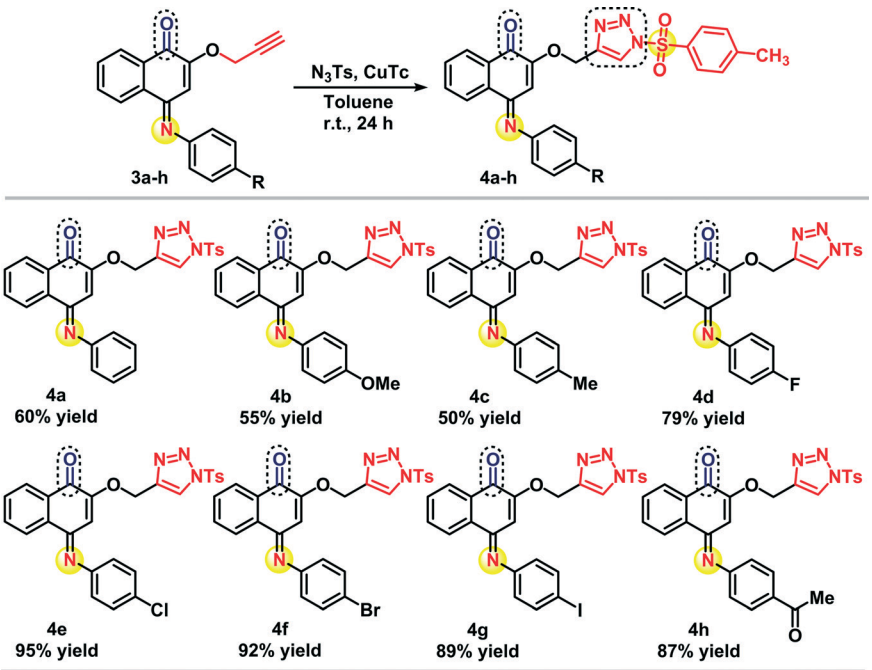
Alkynes derived from *ortho*-naphthoquinones represent important intermediates for the synthesis of 1,2,3-triazole derivatives, and such molecules can be synthesized easily by means of a 1,3-dipolar cycloaddition reaction between an azide and alkyne using copper(I) as a catalyst, in a process known as a click chemistry reaction (Scheme 4). This type of reaction is considered an important tool to synthesize hybrid molecules with two or more redox centres and pronounced



Scheme 2 Synthesis of arylamino *ortho*-naphthoquinones **2a–h**.



Scheme 3 Synthesis of alkynes derived from arylamino *ortho*-naphthoquinones **3a–h**.



Scheme 4 Synthesis of *N*-sulfonyl-1,2,3-triazoles **4a–h**.

biological activities.[41] In this context, the synthesis of the *N*-sulfonyl-1,2,3-triazoles was accomplished according to the methodology described by Fokin *et al.*, employing copper(I)-thiophene-2-carboxylate (CuTC) as a catalyst, as this catalyst promises high yields and selective formation of the 1,4-regioisomer of the product.[42] The reaction was carried employing the corresponding alkynes (**3a–h**) (Scheme 3) with an excess of tosyl azide in toluene, as a solvent, and at room temperature according to Scheme 4. In general, novel compounds were obtained in moderate to excellent (50–95%) yields.

Nitrogen is not the only nucleophile able to replace the sulfonate group in compound **1**. Sulphur has a similar nucleophilic character and is also redox active. Indeed, recent studies have shown that naphthoquinone compounds substituted with chalcogen atoms often exhibit considerable

biological activity.[17,43] Whilst these studies have focused primarily on *para*-quinones, compound **1** provides the basis for a series of similar *ortho*-quinones. For this synthesis, a methodology adapted from the literature[36,37] yielded compounds **5a–5d** in low to moderate yields (30–40%) (Scheme 5).

The structures of the novel compounds were determined initially by ^{1}H and ^{13}C NMR, and were corroborated further using electrospray ionization mass spectra. In the case of compounds **3a**, **3d**, **3f**, **3g**, **3h** and **5d**, X-ray crystallographic analysis with suitable crystals obtained by the slow evaporation method was performed. Here, the bond lengths and angles are in good agreement with the expected values reported in the literature.[44] The atoms of the naphthoquinonic ring (C1–C10) of all structures are coplanar and the largest deviation from the least-squares plane for each one is: 0.077(3) Å for atom C1 in **3a**; 0.044(3) Å for atom C5 in **3d**; 0.035(2) Å for atom C1 in **3f**; 0.039(2) Å for atom C5 in **3g**; 0.039(2) Å for atom C8 in **3h** and 0.017(4) Å for atom C5 in **5d**. The dihedral angles between the planes of the rings (C1–C10) and (C11–C12) are: 48.5(3)° for **3a**; 57.4(2)° for **3d**; 86.9(3)° for **3g**; 57.1(4)° for **3h**; 61.01° for **3f** and 86.9° for **5d**. ORTEP-3 diagrams of each molecule are shown in Fig. 1.

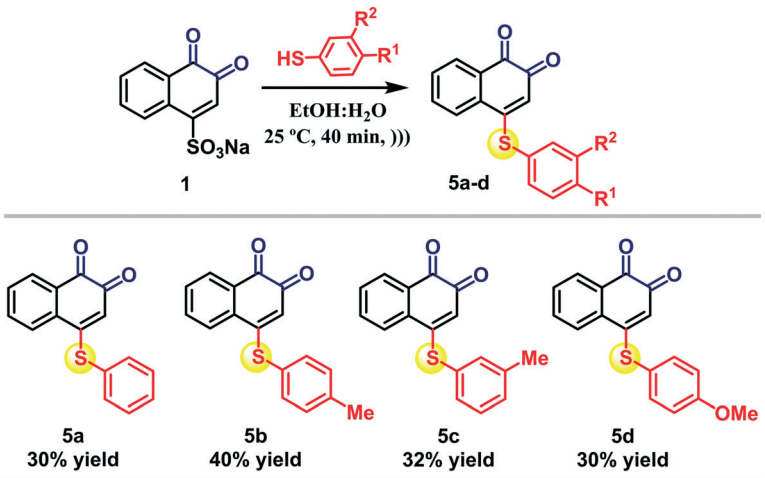
2.2. Redox transformations

The process of substituting the sulfonate group in compound **1** for the nitrogen or sulphur in **2** and **5**, respectively, and also by *O*-alkylation when moving from **2** to **3** and **4**, changes the "redox core" of these molecules, as an additional redox active element is added to the initial *ortho*-quinone system. Some of the resulting redox systems therefore appear to differ significantly from the original structures, and these differences should also be reflected in the redox behaviour and biological activities associated with them. As such, cyclic voltammetry (CV) was employed to study redox changes, as it represents a readily applicable, fast, informative and also fairly reliable method to obtain initial information about reversible and irreversible reduction and oxidation processes associated with such molecules. CV was performed in mixed medium, *i.e.* phosphate buffer + 30% methanol, on a glassy carbon working electrode, with *E vs.* Ag/AgCl as a reference electrode (SSE), at 200 mV s^{-1}. In the presence of a protic organic solvent (methanol), the reduction occurs in two monoelectronic steps, different from the mechanism in totally aqueous medium, where the reduction occurs through the capture of $2e^{-} + 2H^{+}$.

The cyclic voltammograms of selected compounds belonging to class 1 (compounds **1** and **2**), class 3 (compound **3a**) and class 5 (compound **5b**) were obtained and are shown in Fig. S39 (see the ESI† file), together with the values for the relevant reduction signals (E_{pc}) and corresponding oxidation (E_{pa}) ones for various quinones, in Table 1. We have focused here on the prime signals associated with the quinone redox centre, as these waves are important to rationalize the relevant biological activities (see below).

By analysis of the first reduction potential (E_{pc1}), the ease of reduction is the following: **5c** > **5b** > **1** ≈ **5d** > **3e** > **3h** ≈ **3b** > **3a** > **2a**.

As anticipated, the redox behaviour changes significantly, once the original *ortho*-quinone **1** is modified by a nitrogen substituent in the *para*-position to yield compounds of class **2**, due to the electron donating character of the amino group. Interestingly, the subsequent *O*-alkylation converting **2** into **3** also – quite dramatically – changes the entire redox system, since there is a direct modification on the redox system. This significant change from an *ortho*-quinone to a *para*-quinone imine is also observed electrochemically, and is associated with a shift of the first reduction potentials to more negative potentials. Generally, the potentials for the *para*-quinone imine are 150 to 200 mV more negative, when compared to those for the *ortho*-quinones, pointing towards a less electrophilic compound, being reduced at more negative potentials. Modifying the compounds of class **2** (less electrophilic) to yield class **3** brings an anodic shift of the relevant potentials, however less intense, when compared to



Scheme 5 Synthesis of chalcogenic aryl *ortho*-quinones **5a–d**.

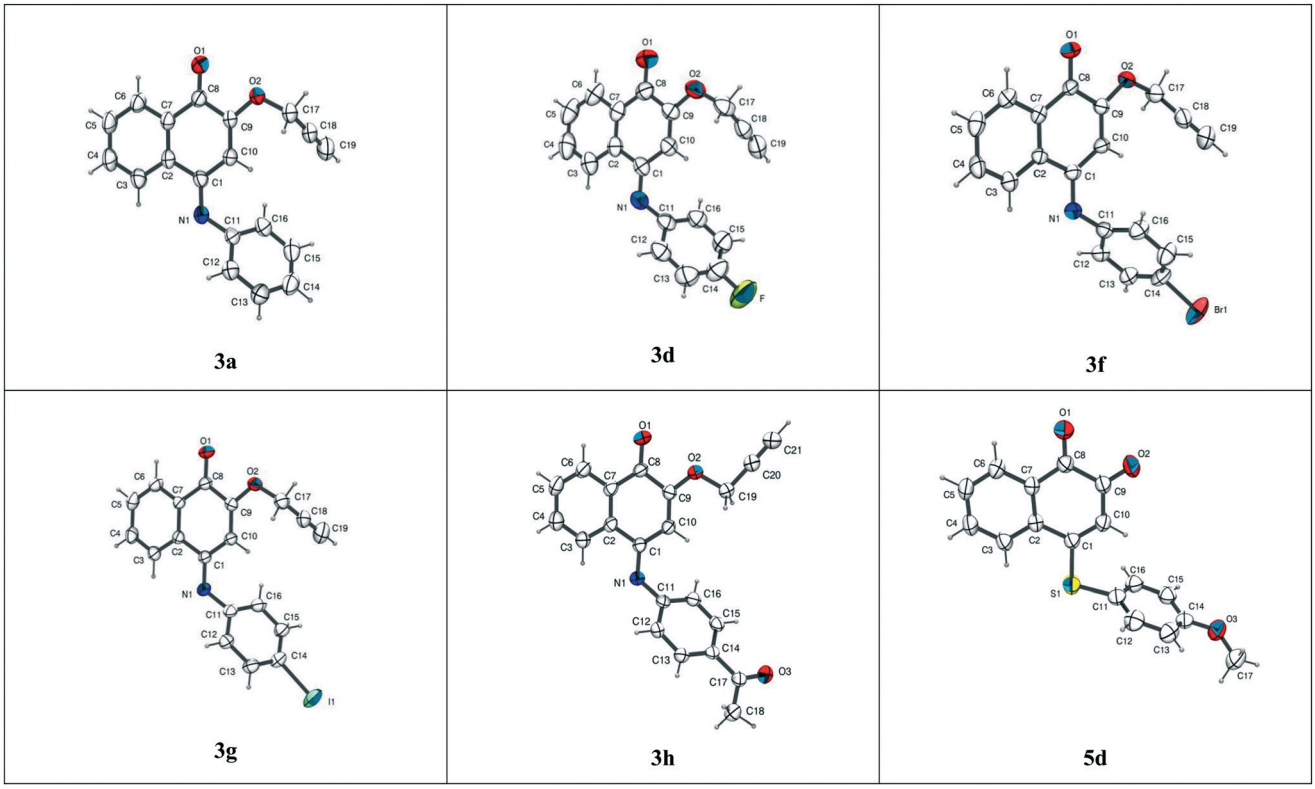


Fig. 1 ORTEP-3 projections of **3a**, **3d**, **3f**, **3g**, **3h** and **5d**, showing the atom-numbering and displacement ellipsoids at the 50% probability level.

the original compound **1**, and introducing another factor involved in the redox cycle with the quinone imines, once they are known to undergo a redox cycle through aminophenols. Another important fact should be considered: arylquinone imines can suffer from hydrolysis, to generate back the original quinone,[45] possibly behaving as a pro-drug.[40]

In contrast, the substitution of the initial "redox core" with a sulfur atom seems to change the original redox properties to a quasi-reversible electron transfer with potentials in the range of −168 mV up to −254 mV (E_{pc1}) and from −500 mV up to −574 mV (E_{pc2}). A significant change from an *ortho*-quinone to a chalcogenic quinone is observed electrochemically, and is associated with a shift of the relevant potentials to more positive potentials. Generally, the potentials for the chalcogenic quinone are 20 to 100 mV more positive, when compared to those for the *ortho*-quinone, pointing towards a more oxidizing species. Hence, its influence on the overall redox behaviour of these compounds is primarily due to electronic effects.

Table 1 Reduction potential values (E_{pc1} and E_{pc2}) and corresponding oxidation potentials (E_{pa1} and E_{pa2}), representing three different classes of compounds and their synthetic precursor (**1**)

	1st wave		2nd wave	
Compounds	E_{pc1}	E_{pa1}	E_{pc2}	E_{pa2}
1	−250 mV	−340 mV	−602 mV	−28 mV
2a	−490 mV	−420 mV	−716 mV	−202 mV
3a	−429 mV	−493 mV	−669 mV	−259 mV
3b	−387 mV	−475 mV	−687 mV	−292 mV
3e	−339 mV	−415 mV	−690 mV	−235 mV
3h	−384 mV	−414 mV	−614 mV	−190 mV
5b	−210 mV	−304 mV	−552 mV	−132 mV
5c	−168 mV	−324 mV	−500 mV	−110 mV
5d	−254 mV	−360 mV	−574 mV	−178 mV

2.3. Antiparasitic activity

Based on the strategy of modifying and hence modulating the initial "redox core" of the *ortho*-quinone, and the insights obtained by electrochemistry, it was then interesting to see if these modifications also translate into specific – changes in – biological activities. Here, two distinct models indicative of the activity of quinones have been selected, *i.e.* cancer cell lines and *T. cruzi* (see section 1 Introduction).

The selection of these models was based on studies previously described in the literature that demonstrate the antitumor and trypanocidal activity of quinoidal compounds containing the 1,2,3-triazole nucleus and the sulphur atom.[27,29,30] As discussed in the Introduction, our research group demonstrated that quinone-based *N*-sulfonyl-1,2,3-triazoles exhibited cytotoxicity against various tumour cell lines. These compounds were not evaluated against *T. cruzi*, the parasite that causes Chagas disease, but it is well documented that active redox systems are potential candidates to present

Table 2 Activity of the naphthoquinones on trypomastigote forms of *T. cruzi*[a]

Compounds	IC_{50}/24 h (μM)	LC_{50}/24 h[b] (μM)	SI
4a	>500	80.8 ± 6.5	>6.19
4b	>500	44.3 ± 6.4	>11.29
4c	>500	88.5 ± 3.5	>5.65
4d	>500	26.9 ± 1.2	>18.59
4e	>500	>100	nd
4f	>500	>100	nd
4g	>500	>100	nd
4h	>500	40.6 ± 2.5	>12.31
5a	**24.3 ± 7.9**	**36.9 ± 2.6**	**1.52**
5b	**88.7 ± 10.1**	**81.4 ± 3.7**	**0.92**
5c	**65.6 ± 10.2**	**67.3 ± 6.9**	**1.03**
5d	112.0 ± 10.6	41.5 ± 0.2	0.37
Bz	103.6 ± 0.6	>4000	>38.6

[a] Mean ± SD of at least three independent experiments, 5% blood at 4 °C. [b] Cytotoxicity assays were performed using primary cultures of peritoneal macrophages obtained from Swiss Webster mice. nd: not determined. Bz: benznidazole. SI = selectivity index, represented by the ratio LC_{50}/IC_{50}.

trypanocidal activity, since in general such activity may be intrinsically related to the generation of ROS.

Whilst none of the *N*-sulfonyl triazoles **4a–4h** appears to be particularly active against *T. cruzi*, with IC_{50} values higher than 500 μM, the sulphur-containing quinones **5a–5d** exhibit rather promising IC_{50} values between 24.3 and 112.0 μM (Table 2). It therefore appears that this particular microorganism is sensitive towards the substituted *ortho*-quinone series, and less so towards the quinone imines. From the perspective of redox chemistry, this may be explained by the fact that the *ortho*-quinones with their more positive electrochemical potentials are also generally more oxidizing and hence toxic, whilst the quinone imines, with their somewhat more negative potentials, are less oxidizing. Nonetheless, practical applications of such compounds may be compromised by a wider toxicity, as the selectivity index (SI) is rather low, with values ranging from 0.37 for compound **5d** to 1.52 for compound **5a**.

It is already established in the literature that naphthoquinones are inactivated in the presence of certain components present in human blood.[46,47] Compounds **5a–5d** were therefore also assayed in the absence of blood at 37 °C (Table 3).[46] These "blood-free" assays resulted in IC_{50} values between 1.3 μM for compound **5a** and 3.9 μM for compound **5d**. The SI was also improved, with values above 10 observed for virtually all compounds of class **5** under investigation. When compared to the standard drug benznidazole, which under these experimental conditions shows an IC_{50} of 9.7 μM, the quinones **5a–5d** were even more active. Nonetheless, the selectivity for the parasite is notoriously low for the quinones when compared to benznidazole (SI > 413.2), regardless of the assay conditions.

The sulphur-containing quinones **5a–5d** also exhibited some activity against macrophages with LC_{50} values between 36.9 and 81.4 μM. The quinones **5a–5d** were, however, more cytotoxic when compared to the standard drug benznidazole, which under these experimental conditions shows LC_{50} > 4000 μM, and it is worth noting here the correlation between the observed activity and the recorded electrochemical potentials for the chosen compounds.

2.4. Cytotoxic activity

In the case of the cancer cell lines, cytotoxicity was evaluated *in vitro* by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay representative of six cancer cell lines, *i.e.* colon cancer cells (HCT-116), lung cancer cells (NCI-H460), prostate cancer cells (PC3), human promyelocytic leukemia cells (HL-60), human immortalized myelogenous leukemia cells (K-562) and multidrug resistant leukemia cells (Lucena 1) (Table 4). The murine, non-tumorigenic fibroblast immortalized cell line (L929) was employed as a control to evaluate the selectivity index of the compounds for tumour cells, once its IC_{50} is compared to the corresponding values of neoplastic cell lines through selectivity index calculation. Doxorubicin was employed as a positive control and benchmark drug. The compounds were classified according to their activity as highly active (IC_{50} < 2 μM), moderately active (2 μM < IC_{50} < 10 μM), or inactive (IC_{50} > 10 μM).[48]

As expected and reflected in Table 4, the redox active quinones exhibit significant cytotoxicity against a range of cancer cell lines, with IC_{50} values for some compounds in the low micromolar range. For instance, some alkynes of class **3** are moderately active and generally present a non-selective cytotoxicity against the tumour cells evaluated. Compound **3d** is an exception, as it is particularly active and selective against HL-60 cells, with an IC_{50} of 9.20 μM (Table 4) and a

Table 3 Activity of the synthetic derivatives against bloodstream trypomastigotes of *T. cruzi* (Y strain) at 37 °C in the absence of blood[a]

Compounds	IC_{50}/24 h (μM) at 37 °C, absence of blood	LC_{50}/24 h[b] (μM) macrophages	SI at 37 °C, absence of blood
5a	1.3 ± 0.2	36.9 ± 2.6	28.38
5b	3.4 ± 1.0	81.4 ± 3.7	23.94
5c	4.6 ± 1.1	67.3 ± 6.9	14.63
5d	3.9 ± 1.4	41.5 ± 0.2	10.64
Bz	9.7 ± 2.4	>4000	>413.2

[a] Mean ± SD of at least three independent experiments, absence of blood at 37 °C. [b] Cytotoxicity assays were performed using primary cultures of peritoneal macrophages obtained from Swiss Webster mice. nd: not determined. Bz: benznidazole. SI = selectivity index, represented by the ratio LC_{50}/IC_{50}.

Table 4 Cytotoxic activity of the compounds expressed as IC_{50} (μM) in cancer and non-cancer cell lines after 72 h exposure, obtained by nonlinear regression for all cell lines from three independent experiments

	HCT-116	NCI-H460	PC-3	HL-60	K-562	Lucena 1	L929
3a	>20.00	>20.00	14.10 (12.18–16.29)	17.44 (14.79–20.50)	>20.00	>20.00	>20.00
3b	10.71 (9.61–12.35)	**9.45** (7.88–9.45)	**8.82** (8.04–9.67)	**7.18** (5.64–9.11)	>20.00	11.41 (9.36–13.90)	>20.00
3c	>20.00	16.92 (13.94–20.91)	18.45 (16.36–20.77)	11.98 (10.22–14.00)	>20.00	14.07 (12.51–15.80)	>20.00
3d	18.67 (16.51–21.13)	17.03 (15.07–19.32)	>20.00	**9.20** (7.73–10.97)	13.48 (11.69–15.57)	17.00 (13.92–20.74)	>20.00
3e	18.87 (16.29–21.88)	16.16 (13.99–18.65)	14.64 (11.66–18.37)	11.03 (9.39–13.02)	12.80 (10.38–15.78)	16.43 (13.44–20.11)	>20.00
3f	>20.00	>20.00	15.87 (14.25–17.69)	14.20 (11.25–17.94)	>20.00	12.52 (10.31–15.20)	>20.00
3g	16.46 (13.07–20.81)	>20.00	>20.00	11.90 (10.38–13.65)	16.96 (14.33–20.09)	15.13 (12.27–18.66)	>20.00
3h	>20.00	>20.00	12.51 (10.93–14.33)	>20.00	>20.00	16.25 (11.87–22.23)	>20.00
4a	**5.37** (4.75–6.40)	**7.22** (6.40–8.05)	>20.00	**6.71** (4.93–9.08)	12.24 (10.13–14.79)	**3.67** (3.01–4.46)	>20.00
4b	**7.43** (6.48–8.58)	**8.39** (7.24–9.72)	>20.00	**6.96** (5.75–8.46)	>20.00	**4.58** (3.55–5.93)	>20.00
4c	**3.61** (3.01–4.21)	**8.02** (7.62–8.62)	**2.65** (2.25–3.11)	**5.42** (4.42–6.64)	**6.22** (4.97–7.76)	**1.44** (0.98–2.11)	17.73 (13.91–22.61)
4d	**5.75** (4.96–6.69)	**7.36** (6.77–7.96)	**2.03** (1.65–2.49)	**7.52** (6.07–9.33)	**7.70** (6.39–9.27)	**6.58** (5.57–7.78)	>20.00
4e	**9.44** (8.48–10.79)	**12.14** (10.98–13.30)	>20.00	**6.92** (5.57–8.57)	**9.80** (7.64–12.58)	**3.16** (2.47–4.09)	>20.00
4f	**9.25** (8.55–9.99)	**9.41** (8.34–10.47)	**3.41** (2.88–4.05)	**4.22** (3.44–5.16)	**5.40** (4.06–7.15)	**3.32** (2.45–4.51)	>17.75
4g	>16.38	>16.38	>16.38	**6.31** (5.29–7.49)	10.76 (8.08–14.32)	**2.90** (2.44–3.44)	>16.38
4h	**6.27** (5.32–7.41)	15.76 (14.43–17.47)	**3.91** (3.42–4.50)	**5.30** (4.51–6.28)	**5.52** (4.77–6.39)	**6.46** (5.05–8.26)	10.75 (8.77–13.17)
5a	12.77 (10.14–16.15)	>20.00	>20.00	**6.08** (4.88–7.55)	15.71 (13.59–18.16)	15.58 (12.58–19.30)	>20.00
5b	>20.00	>20.00	>20.00	**4.14** (3.21–5.31)	14.91 (11.38–19.54)	12.84 (10.52–15.66)	>20.00
5c	19.62 (16.77–22.83)	>20.00	>20.00	**5.42** (4.21–6.99)	16.24 (12.86–19.87)	12.66 (10.20–15.73)	>20.00
5d	>20.00	>20.00	>20.00	**9.38** (7.49–11.78)	13.33 (9.97–17.84)	>20.00	>20.00
Dox	0.21 (0.16–0.29)	0.15 (0.13–0.18)	0.76 (0.59–0.93)	0.02 (0.01–0.02)	0.49 (0.47–0.51)	0.18 (0.15–0.22)	1.72 (1.58–1.87)

Table 5 Selectivity index for the most active compounds[a]

Compounds	HCT116	NCI-H460	PC3	HL-60	K-562	Lucena I
3a	1.2	1.6	**2.5**	**2.0**	1.0	1.5
3b	**2.9**	**3.3**	**3.6**	**4.4**	1.0	**2.8**
3c	1.6	**2.0**	1.8	**2.8**	1.5	**2.4**
3d	1.8	1.9	1.0	**3.6**	**2.4**	1.9
3e	1.6	1.9	**2.1**	**2.8**	**2.4**	1.9
3f	1.2	1.3	1.7	1.9	1.2	**2.2**
3g	1.5	1.0	1.0	**2.0**	1.4	1.6
3h	1.0	1.0	**24**	1.1	1.0	1.9
4a	**3.8**	**2.9**	1.0	**3.1**	1.7	**5.6**
4b	**2.9**	**2.6**	1.0	**3.3**	1.0	**5.4**
4c	**4.9**	**2.2**	**6.7**	**3.3**	**2.9**	**12.3**
4d	**3.5**	**2.7**	**9.8**	**2.6**	**2.6**	**3.0**
4e	**2.0**	1.6	1.0	**2.8**	**2.0**	**6.1**
4f	1.9	1.9	**5.2**	**4.2**	**3.3**	**5.3**
4g	1.0	1.0	1.0	**2.6**	1.5	**5.6**
4h	1.7	0.7	**2.7**	**2.0**	1.9	1.7
5a	**2.2**	0.7	0.7	**4.6**	1.8	1.8
5b	1.3	0.8	0.8	**6.6**	1.8	**2.1**
5c	1.8	1.0	1.0	**6.6**	**2.2**	**2.8**
5d	1.0	1.0	1.0	**3.6**	**2.5**	1.0

[a] Selectivity index, represented by the ratio of cytotoxicities between normal cells and different lines of cancer cells.

selectivity index (SI) superior to 3.6 (Table 5). Compound **3b** presents a broader cytotoxicity against NCI-H460 (IC_{50} = 9.45 μM), PC-3 (IC_{50} = 8.82 μM) and HL-60 (IC_{50} = 7.18 μM) cells with a SI higher than 1.8. Generally, the members of class **4** exhibit a more selective cytotoxic activity against all the cell lines, with IC_{50} values ranging from 1.44 to > 20 μM. For example, **4d** presents IC_{50} values between 2.03 and 7.70 μM (Table 4), with a SI higher than 2.6 (Table 5), while **4f** presents an IC_{50} from 3.32 to 9.41 μM (Table 4) and a SI higher than 1.9 (Table 5). Interestingly, the only difference between these molecules is the deactivating halogen substituent in the benzene ring, –F in **4d** and –Br in **4f**, suggesting that the more electronegative the halogen substituent, the more cytotoxic the compound. This pattern is followed by **4g**, which possesses an –I substituent and is a less cytotoxic compound against the cell lines tested, except for a particularly high selectivity for Lucena 1, *i.e.* IC_{50} of 2.90 μM and SI higher than 5.6 (Table 4).

Notable differences can be observed in the SI values shown in Table 5. The sulphur containing quinones seem to be fairly selective against certain cancer cell lines, such as HL-60, as observed in the case of compounds **5b** and **5c**. Indeed, compounds of class **5** are particularly attractive as far as observed activity and selectivity are considered, reflecting the potential of the redox core in concert with the sulfur atom added to this analogue. Compounds **5b** and **5c** present IC_{50} values of 4.14 μM and 5.42 μM against HL-60, respectively, with an SI of 6.6.

In general, generation of intracellular ROS is a key mechanism associated with the cytotoxic effects of quinones in tumor cells. Thus, the redox status of treated K-562 cells was monitored using the oxidation-sensitive fluorescent dye CM-H_2DCFDA after 1 and 3 hours of incubation. Fig. 2 shows that all three tested compounds induced a significant increase in intracellular ROS levels, reaching a higher ROS + cell count at 3 hours. Compound **5a** was the most potent in inducing significant changes ($p < 0.05$) in ROS levels, at both 1 and 3 hours. In contrast, compound **4f** generated lower levels of ROS, but still significant ($p < 0.05$) at 3 hours. As expected, menadione and hydrogen peroxide increased ROS generation in treated cells.

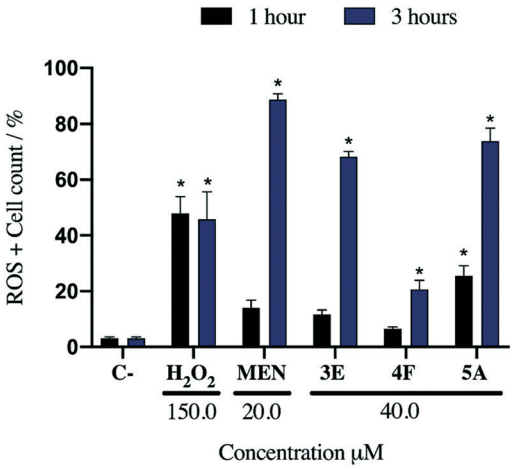


Fig. 2 Effect of compounds **3e**, **4f** and **5a** on ROS production in K-562 cells determined by flow cytometry using CM-H_2DCFDA, after 1 and 3 hours incubation. Menadione (MEN, 20 μM) and hydrogen peroxide (H_2O_2, 150 μM) were used as positive controls. A total of 10 000 events were analyzed per sample. Data are expressed as mean ± SEM from three independent experiments. *, $p < 0.05$ compared to the negative control by ANOVA followed by Tukey's test, performed using GraphPad Prism 8.

3. Conclusions

Considered together, the modification of the *ortho*-quinone lead **1** provides access to a wide range of different classes of

compounds with distinctive "redox cores", electrochemical potentials, biological activities and SI values. Here, the underlying mode(s) of action and also possible selectivity need to be investigated, together with potential applications against different human diseases. Whilst some of the compounds synthesised as part of this study already exhibit a combination of high activity and selectivity, these aspects may need to be further improved. It remains to be shown, for instance, against which cells or organisms these compounds are especially active, and if there is some selectivity associated with this activity, which may be exploited in practice.

Fortunately, the synthetic chemistry associated with these compounds is straightforward and provides wide opportunities for structural modifications, from the initial nucleophilic substitution and alkylation to the side chains and prospects provided by click chemistry reactions. Similarly, electrochemical investigations by cyclic voltammetry are fast and simple, and may provide initial information on the redox behaviour and activities one may expect.

4. Experimental section

4.1. Chemistry

Starting materials available from commercial suppliers were employed as received, unless stated otherwise. All other reagents requiring purification were purified by standard laboratory techniques, according to methods published by Perrin, Armarego and Perrin.[49] Catalytic reactions were performed under nitrogen or argon atmosphere. Glassware, syringes and needles were either flame-dried immediately prior to use or placed in an oven (200 °C), for at least 2 h, and allowed to cool either in a desiccator or under an atmosphere of nitrogen or argon. Liquid reagents, solutions or solvents were added *via* a syringe through rubber septa. Melting points of solid compounds were measured on a Thomas Hoover melting point apparatus and are uncorrected. Column chromatography was performed on silica gel (SiliaFlash G60 Ultrapure 60–200 μm, 60 Å). ^{1}H and ^{13}C NMR spectra were recorded at room temperature on a Bruker AVANCE DRX200 and DRX400, in the solvents indicated. Chemical shifts (δ) are given in parts per million (ppm) and coupling constants (J) are reported in Hertz (Hz). MS analyses were performed on a LC-MS/ESI TOF spectrometer (Model Xevo G2-XS QTof, Waters). The compounds are named in accordance with IUPAC rules as applied by ChemBioDraw Ultra (version 12.0).

General procedure for the synthesis of compounds 2a–h. The synthesis of compounds **2a–h** was performed according to the methodology described in the literature by the Potter and Chen groups with minor modifications.[36,37] To a solution of sodium 3,4-dioxo-3,4-dihydronaphthalene-1-sulfonate (**1**) (2.5 mmol) in EtOH : H_2O 1 : 1 (75 mL), the respective aniline (2.7 mmol) was added. The reaction mixture was stirred at room temperature monitored by TLC until the total consumption of the starting material. After completion of the reaction, the solvent was removed *in vacuo* and the resulting residue was purified by column chromatography over silica gel, using as the eluent a gradient mixture of hexane/ethyl acetate with increasing polarity. In some cases, ultrasound can be used to promote the reaction. The analytical data for compounds **2a–h** are in accordance with those reported in the literature.[36–38]

General procedure for the synthesis of compounds 3a–h. To a stirred solution of a type **2** compound (2.0 equiv.) in DMF (4 mL), K_2CO_3 (250 mg, 0.18 mmol, 1.1 equiv.) was added and the reaction mixture was stirred at room temperature. After 10 min, propargyl bromide (80 mg, 0.7 mmol, 2.1 equiv.) was added dropwise and stirred under ultrasound for 40 min. The solvent was evaporated *in vacuo* and water (5 mL) was added to the crude reaction mixture. The aqueous phase was extracted with ethyl acetate (3 × 10 mL) and the organic phases were combined and dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography by eluting with an increasing polarity gradient mixture of hexane and ethyl acetate to afford the respective alkynes (**3a–h**).

(*E*)-4-(Phenylimino)-2-(prop-2-yn-1-yloxy)naphthalen-1(4*H*)-one (3a). The product was obtained as an orange solid (99% yield); mp 139–141 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (1H, d, J = 7.8 Hz), 8.24 (1H, d, J = 7.8 Hz), 7.74 (1H, t, J = 7.7 Hz), 7.67 (1H, t, J = 7.7 Hz), 7.43 (2H, t, J = 7.8 Hz), 7.21 (1H, t, J = 7.4 Hz), 6.96 (2H, d, J = 7.4 Hz), 6.60 (1H, s), 4.61 (2H, d, J = 2.2 Hz), 2.55 (1H, t, J = 2.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.3, 154.8, 154.3, 150.5, 134.8, 133.5, 131.3, 131.3, 129.2, 126.7, 125.5, 125.0, 120.6, 104.6, 77.5, 76.6, 56.2; IR ν_{max} (cm^{-1}, KBr): 3264, 3067, 2974, 2117, 1670, 1612, 1483, 1383, 1332, 1255, 1189, 1044, 1019, 949, 857, 769, 694, 648; EI/HRMS (m/z) $[M + H]^+$: 288.1023. Cald. for $[C_{19}H_{14}NO_2]^+$: 288.1024.

(*E*)-4-((4-Methoxyphenyl)imino)-2-(prop-2-yn-1-yloxy)-naphthalen-1(4*H*)-one (3b). The product was obtained as a red solid (97% yield); mp 149–150 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.51 (1H, d, J = 7.8 Hz), 8.22 (1H, d, J = 7.8 Hz), 7.73 (1H, t, J = 7.9 Hz), 7.65 (1H, t, J = 7.9 Hz), 6.98 (4H, s), 6.72 (1H, s), 4.65 (2H, d, J = 2.3 Hz), 3.78 (3H, s), 2.59 (1H, t, J = 2.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.4, 157.7, 154.3, 154.1, 143.6, 135.1, 133.4, 131.2, 131.0, 126.6, 125.3, 122.7, 114.6, 104.6, 77.5, 76.8, 56.2, 55.7; IR ν_{max} (cm^{-1}, KBr): 3264, 2930, 2843, 2128, 1675, 1597, 1497, 1461, 1354, 1320, 1260, 1243, 1201, 1044, 1022, 847, 777, 682, 653; EI/HRMS (m/z) $[M + H]^+$: 318.1122. Cald. for $[C_{20}H_{16}NO_3]^+$: 318.1125.

(*E*)-2-(Prop-2-yn-1-yloxy)-4-(*p*-tolylimino)naphthalen-1(4*H*)-one (3c). The product was obtained as an orange solid (95% yield); mp 148–150 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.50 (1H, d, J = 7.6 Hz), 8.21 (1H, d, J = 7.6 Hz), 7.71 (1H, t, J = 7.2 Hz), 7.64 (1H, t, J = 7.2 Hz), 7.22 (2H, d, J = 7.4 Hz), 6.88 (2H, d, J = 7.4 Hz), 6.65 (1H, s), 4.62 (2H, s), 2.59 (1H, s), 2.40 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.1, 154.3, 153.9, 147.7, 134.8, 134.6, 133.2, 131.1, 130.9, 129.6, 126.4, 125.2, 120.7,

104.4, 76.8, 76.6, 56.0, 21.0; IR ν_{max} (cm^{-1}, KBr): 3269, 2117, 1672, 1614, 1597, 1500, 1335, 1257, 1192, 1044, 1019, 772; EI/HRMS (*m*/*z*) $[M + H]^+$: 302.1170. Cald. for $[C_{20}H_{16}NO_2]^+$: 302.1176.

(*E*)-4-((4-Fluorophenyl)imino)-2-(prop-2-yn-1-yloxy)-naphthalen-1(4*H*)-one (3d). The product was obtained as an orange solid (79% yield); mp 147–149 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (1H, d, J = 7.7 Hz), 8.18 (1H, d, J = 7.7 Hz), 7.70 (1H, t, J = 7.4 Hz), 7.63 (1H, t, J = 7.4 Hz), 7.10 (2H, t, J = 8.6 Hz), 6.93–6.90 (2H, m), 6.56 (1H, s), 4.61 (2H, d, J = 2.1 Hz), 2.57 (1H, t, J = 2.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.1, 161.6, 159.2, 155.2, 154.3, 146.4, 134.6, 133.5, 131.3, 131.2, 126.6, 125.3, 122.2, 122.1, 116.1, 115.9, 104.1, 76.5, 56.2; IR ν_{max} (cm^{-1}, KBr): 3296, 2990, 2123, 1667, 1624, 1597, 1497, 1339, 1264, 1221, 1204, 1184, 1044, 1019, 862, 772, 697, 648; EI/HRMS (*m*/*z*) $[M + H]^+$: 306.0919. Cald. for $[C_{19}H_{13}FNO_2]^+$: 306.0925.

(*E*)-4-((4-Chlorophenyl)imino)-2-(prop-2-yn-1-yloxy)-naphthalen-1(4*H*)-one (3e). The product was obtained as an orange solid (77% yield); mp 145–147 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (1H, dd, J = 1.5 and 7.8 Hz), 8.19 (1H, dd, J = 1.5 and 7.8 Hz), 7.75–7.59 (2H, m), 7.36 (2H, d, J = 8.6 Hz), 6.89 (2H, d, J = 8.6 Hz), 6.51 (1H, s), 4.62 (2H, d, J = 2.4 Hz), 2.58 (1H, t, J = 2.4 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 180.0, 155.2, 154.4, 148.8, 134.5, 133.5, 131.4, 131.1, 130.4, 129.3, 126.7, 125.4, 122.0, 104.0, 77.6, 76.5, 56.2; IR ν_{max} (cm^{-1}, KBr): 3275, 1670, 1614, 1483, 1337, 1252, 1192, 1044, 1019, 854, 772, 639; EI/HRMS (*m*/*z*) $[M + H]^+$: 322.0629. Cald. for $[C_{19}H_{13}ClNO_2]^+$: 322.0629.

(*E*)-4-((4-Bromophenyl)imino)-2-(prop-2-yn-1-yloxy)-naphthalen-1(4*H*)-one (3f). The product was obtained as a red solid (72% yield); mp 156–158 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.48 (1H, d, J = 7.8 Hz), 8.23 (1H, d, J = 7.8 Hz), 7.75 (1H, t, J = 7.4 Hz), 7.68 (1H, t, J = 7.4 Hz), 7.54 (2H, d, J = 8.4 Hz), 6.85 (2H, d, J = 8.4 Hz), 6.54 (1H, s), 4.65 (2H, d, J = 2.1 Hz), 2.58 (1H, t, J = 2.1 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 180.1, 155.3, 154.6, 149.4, 134.6, 133.6, 132.3, 131.5, 131.3, 126.8, 125.5, 122.4, 118.2, 104.2, 77.6, 76.5, 56.3; IR ν_{max} (cm^{-1}, KBr): 3275, 2117, 1667, 1612, 1478, 1337, 1255, 1192, 1046, 1024, 852, 772, 685, 641; EI/HRMS (*m*/*z*) $[M + H]^+$: 366.0120. Cald. for $[C_{19}H_{13}BrNO_2]^+$: 366.0124.

(*E*)-4-((4-Iodophenyl)imino)-2-(prop-2-yn-1-yloxy)-naphthalen-1(4*H*)-one (3g). The product was obtained as a red solid (68% yield); mp 123–125 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (1H, d, J = 7.8 Hz), 8.21 (1H, d, J = 7.8 Hz), 7.73–7.65 (4H, m), 6.72 (2H, d, J = 8.2 Hz), 6.52 (1H, s), 4.64 (2H, s), 2.59 (1H,s); ^{13}C NMR (50 MHz, $CDCl_3$): δ 179.8, 154.9, 154.2, 149.7, 137.9, 134.3, 133.3, 131.2, 130.9, 126.5, 125.2, 122.5, 103.8, 88.6, 77.4, 76.2, 56.1; IR ν_{max} (cm^{-1}, KBr): 3280, 1668, 1609, 1473, 1476, 1253, 1187, 1044, 1020, 770; EI/HRMS (*m*/*z*) $[M + H]^+$: 413.9989. Cald. for $[C_{19}H_{13}INO_2]^+$: 413.9985.

(*E*)-4-((4-Acetylphenyl)imino)-2-(prop-2-yn-1-yloxy)-naphthalen-1(4*H*)-one (3h). The product was obtained as a yellow solid (48% yield); mp 172–174 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.49 (1H, dd, J = 1.0 and 7.7 Hz), 8.25 (1H, dd, J = 1.0 and 7.7 Hz), 8.05 (2H, d, J = 8.6 Hz), 7.77 (1H, t, J = 7.5 Hz), 7.70 (1H, t, J = 7.5 Hz), 7.01 (2H, d, J = 8.6 Hz), 6.44 (1H, s), 4.62 (2H, d, J = 2.3 Hz), 2.65 (3H, s), 2.56 (1H, t, J = 2.3 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 197.0, 179.8, 155.1, 154.8, 154.5, 134.1, 133.6, 133.5, 131.4, 131.1, 129.7, 126.7, 125.6, 120.1, 104.0, 77.5, 76.1, 56.1, 26.5; IR ν_{max} (cm^{-1}, KBr): 3264, 2925, 2134, 1679, 1614, 1592, 1560, 1359, 1267, 1209, 1019, 726, 685; EI/HRMS (*m*/*z*) $[M + H]^+$: 330.1122. Cald. for $[C_{21}H_{16}NO_3]^+$: 330.1125.

General procedure for preparing the 1,2,3-triazole derivatives. To a stirred solution of copper(I) thiophene-2-carboxylate (CuTC, 8 mg, 0.04 mmol, 0.08 equiv.) in toluene (5 mL), the respective propargylated aminonaphthoquinones (0.5 mmol, 1 equiv.) were added and the reaction mixture was cooled in an ice–water bath. Subsequently, the sulfonyl azide (0.5 mmol, 1 equiv.) was added dropwise. The reaction mixture was then allowed to warm to room temperature and stirred until the reaction was complete (as evidenced by TLC). The reaction mixture was extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over sodium sulfate and filtered through Celite®. The eluent was concentrated under vacuum and the product was purified by silica gel column chromatography using eluents with an increasing polarity gradient mixture of hexane and ethyl acetate to afford compounds **4a–h**.

(*E*)-4-(Phenylimino)-2-((1-tosyl-1*H*-1,2,3-triazol-4-yl)-methoxy)naphthalen-1(4*H*)-one (4a). The product was obtained as a red solid (60% yield); mp 153–155 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.48 (1H, d, J = 7.5 Hz), 8.22 (1H, s), 8.19 (1H, d, J = 7.5 Hz), 7.99 (2H, d, J = 8.0 Hz), 7.72 (1H, t, J = 7.5 Hz), 7.65 (1H, t, J = 7.5 Hz), 7.43–7.37 (4H, m), 7.19 (1H, t, J = 7.1 Hz), 6.84 (2H, d, J = 8.0 Hz), 6.52 (1H, s), 5.02 (2H, s), 2.44 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.0, 154.6, 154.5, 150.2, 147.7, 141.8, 134.6, 133.4, 132.7, 131.1, 131.0, 130.5, 129.2, 129.2, 126.5, 125.3, 124.9, 123.2, 120.2, 120.1, 103.8, 61.7, 21.8; IR ν_{max} (cm^{-1}, KBr): 3132, 2922, 1668, 1607, 1392, 1254, 1193, 1014, 667, 587; EI/HRMS (*m*/*z*) $[M + H]^+$: 485.1208. Cald. for $[C_{26}H_{21}N_4O_4S]^+$: 485.1278.

(*E*)-4-((4-Methoxyphenyl)imino)-2-((1-tosyl-1*H*-1,2,3-triazol-4-yl)methoxy)naphthalen-1(4*H*)-one (4b). The product was obtained as a red solid (55% yield); mp 123–125 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (1H, d, J = 7.8 Hz), 8.26 (1H, s), 8.22 (1H, d, J = 7.8 Hz), 8.02 (2H, d, J = 7.5 Hz), 7.74 (1H, t, J = 7.3 Hz), 7.66 (1H, t, J = 7.3 Hz), 7.41 (2H, d, J = 7.5 Hz), 6.98 (2H, d, J = 7.2 Hz), 6.86 (2H, d, J = 7.2 Hz), 6.67 (1H, s), 5.09 (2H, s), 3.89 (3H, s), 2.47 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.0, 157.5, 154.4, 154.0, 147.7, 143.3, 142.0, 134.8, 133.3, 132.8, 131.0, 130.9, 130.5, 128.9, 126.5, 125.2, 123.1, 122.2, 114.6, 104.0, 61.7, 55.5; IR ν_{max} (cm^{-1}, KBr): 3139, 2958, 2922, 1659, 1601, 1499, 1391, 1261, 1193, 1020, 670, 583; EI/HRMS (*m*/*z*) $[M + H]^+$: 515.1372. Cald. for $[C_{27}H_{23}N_4O_5S]^+$: 515.1384.

(*E*)-4-(*p*-Tolylimino)-2-((1-tosyl-1*H*-1,2,3-triazol-4-yl)-methoxy)naphthalen-1(4*H*)-one (4c). The product was obtained as a yellow solid (50% yield); mp 157–159 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (1H, d, J = 7.8 Hz), 8.23–8.21 (2H, m), 8.03 (2H, d, J = 8.2 Hz), 7.75 (1H, t, J = 7.6 Hz), 7.67 (1H, t, J = 7.6 Hz), 7.41 (2H, d, J = 8.2 Hz), 7.23 (2H, d, J = 8.0

Hz), 6.78 (2H, d, J = 8.0 Hz), 6.59 (1H, s), 5.06 (2H, s), 2.47 (3H, s), 2.42 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ; 180.2, 154.8, 154.6, 147.9, 142.3, 135.0, 135.0, 133.6, 133.1, 131.3, 131.2, 130.8, 130.1, 129.1, 126.7, 125.5, 123.3, 120.6, 104.1, 62.0, 22.1, 21.2; IR ν_{max} (cm^{-1}, KBr): 3132, 2917, 1665, 1597, 1396, 1260, 1197, 1015, 670, 587; EI/HRMS (m/z) $[M + H]^+$: 499.1421. Cald. for $[C_{27}H_{23}N_4O_4S]^+$: 499.1435.

(*E*)-4-((4-Fluorophenyl)imino)-2-((1-tosyl-1*H*-1,2,3-triazol-4-yl)methoxy)naphthalen-1(4*H*)-one (4d). The product was obtained as a yellow solid (79% yield); mp 162–164 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (1H, d, J = 7.6 Hz), 8.23 (1H, s), 8.19 (1H, d, J = 7.6 Hz), 8.00 (2H, d, J = 7.8 Hz), 7.72 (1H, t, J = 7.2 Hz), 7.65 (1H, t, J = 7.2 Hz), 7.39 (2H, d, J = 7.8 Hz), 7.11 (2H, t, J = 8.2 Hz), 6.83–6.81 (2H, m), 6.55 (1H, s), 5.06 (2H, s), 2.45 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.9, 155.0, 154.6, 147.7, 141.7, 134.5, 133.5, 132.6, 131.2, 131.0, 130.6, 128.9, 126.5, 125.3, 123.3, 121.8, 121.8, 116.2, 116.0, 61.5, 21.8; IR ν_{max} (cm^{-1}, KBr): 3127, 2925, 1670, 1607, 1495, 1388, 1260, 1197, 1015, 854, 670, 587; EI/HRMS (m/z) $[M + H]^+$: 503.1154. Cald. for $[C_{26}H_{20}FN_4O_4S]^+$: 503.1184.

(*E*)-4-((4-Chlorophenyl)imino)-2-((1-tosyl-1*H*-1,2,3-triazol-4-yl)methoxy)naphthalen-1(4*H*)-one (4e). The product was obtained as an orange solid (95% yield); mp 155–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.47 (1H, d, J = 7.6 Hz), 8.25 (1H, s), 8.21 (1H, d, J = 7.6 Hz), 8.03 (2H, d, J = 7.5 Hz), 7.75 (1H, t, J = 7.2 Hz), 7.68 (1H, t, J = 7.2 Hz), 7.43–7.39 (4H, m), 6.82 (2H, d, J = 7.2 Hz), 6.53 (1H, s), 5.07 (2H, s), 2.47 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.1, 155.3, 155.1, 148.9, 147.9, 142.0, 134.7, 133.8, 133.0, 131.6, 131.3, 130.8, 130.6, 129.7, 129.2, 126.9, 125.6, 123.5, 121.9, 61.7, 21.9; IR ν_{max} (cm^{-1}, KBr): 3138, 2919, 1670, 1609, 1391, 1260, 1194, 1088, 1010, 852, 670, 583; EI/HRMS (m/z) $[M + H]^+$: 519.0883. Cald. for $[C_{26}H_{20}ClN_4O_4S]^+$: 519.0888.

(*E*)-4-((4-Bromophenyl)imino)-2-((1-tosyl-1*H*-1,2,3-triazol-4-yl)methoxy)naphthalen-1(4*H*)-one (4f). The product was obtained as a yellow solid (92% yield); mp 175–177 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.47 (1H, d, J = 7.7 Hz), 8.24 (1H, s), 8.21 (1H, d, J = 7.7 Hz), 8.03 (2H, d, J = 8.4 Hz), 7.75 (1H, t, J = 7.4 Hz), 7.68 (1H, t, J = 7.4 Hz), 7.54 (2H, d, J = 8.4 Hz), 7.42 (2H, d, J = 8.2 Hz), 6.76 (2H, d, J = 8.2 Hz), 6.52 (1H, s), 5.07 (2H, s), 2.48 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.8, 154.9, 154.8, 149.1, 147.7, 141.7, 134.4, 133.5, 132.7, 132.3, 131.3, 131.0, 130.6, 128.9, 126.6, 125.4, 123.2, 122.0, 118.1, 103.4, 61.7, 21.9; IR ν_{max} (cm^{-1}, KBr): 3127, 1670, 1609, 1393, 1260, 1192, 1015, 852, 774, 670, 587, 540; EI/HRMS (m/z) $[M + H]^+$: 563.0364. Cald. for $[C_{26}H_{20}BrN_4O_4S]^+$: 563.0383.

(*E*)-4-((4-Iodophenyl)imino)-2-((1-tosyl-1*H*-1,2,3-triazol-4-yl)-methoxy)naphthalen-1(4*H*)-one (4g). The product was obtained as a yellow solid (89% yield); mp 173–175 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (1H, d, J = 7.8 Hz), 8.21 (1H, s), 8.19 (1H, d, J = 7.8 Hz), 8.01 (2H, d, J = 8.2 Hz), 7.75–7.69 (3H, m), 7.66 (1H, t, J = 7.4 Hz), 7.39 (2H, d, J = 8.2 Hz), 6.62 (2H, d, J = 8.3 Hz), 6.79 (1H, s), 5.05 (2H, s), 2.45 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.8, 154.9, 149.7, 147.7, 141.7, 138.2, 134.4, 133.5, 132.7, 131.3, 131.0, 130.6, 128.9, 126.6, 125.4, 123.2, 122.3, 103.4, 88.8, 61.7, 21.9; IR ν_{max} (cm^{-1}, KBr): 3138, 2919, 1670, 1609, 1396, 1260, 1194, 1017, 668, 585; EI/HRMS (m/z) $[M + H]^+$: 611.0246. Cald. for $[C_{26}H_{20}IN_4O_4S]^+$: 611.0244.

(*E*)-4-((4-Acetylphenyl)imino)-2-((1-tosyl-1*H*-1,2,3-triazol-4-yl)methoxy)naphthalen-1(4*H*)-one (4h). The product was obtained as a yellow solid (87% yield); mp 174–176 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (1H, d, J = 7.6 Hz), 8.22–8.20 (2H, m), 8.04–7.99 (4H, m), 7.76 (1H, t, J = 7.3 Hz), 7.68 (1H, t, J = 7.3 Hz), 7.40 (2H, d, J = 8.1 Hz), 6.91 (2H, d, J = 8.1 Hz), 6.39 (1H, s), 5.03 (2H, s), 2.65 (3H, s), 2.46 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 197.0, 179.7, 155.0, 154.6, 147.7, 141.6, 134.1, 133.7, 133.6, 132.7, 131.5, 131.1, 130.6, 129.8, 128.9, 126.6, 125.5, 123.2, 119.9, 103.6, 61.8, 29.7, 26.5, 21.8; IR ν_{max} (cm^{-1}, KBr): 3133, 2919, 2859, 1670, 1594, 1393, 1269, 1194, 1019, 670, 590; EI/HRMS (m/z) $[M + H]^+$: 527.1378. Cald. for $[C_{28}H_{23}N_4O_5S]^+$: 527.1384.

General procedure for the synthesis of compounds 5a–d. The synthesis of compounds **5a–d** was performed according to the methodology described in the literature by the Potter and Chen groups with minor modifications.[36,37] To a 25 mL round bottom flask, 5 mL of ethanol and 5 mL of water, and then sodium 3,4-dioxo-3,4-dihydronaphthalene-1-sulfonate (**1**) (1 mmol) and the corresponding thiophenol (2 mmol) were added. The reaction mixture was stirred at room temperature under ultrasound energy, and monitored by TLC until the total consumption of the starting material. After completion of the reaction, the organic phase was extracted with dichloromethane and dried over sodium sulphate. The solvent was removed under reduced pressure to afford the crude product, which was purified by column chromatography over silica gel, using as the eluent a gradient mixture of hexane/ethyl acetate with increasing polarity. The analytical data for compounds **5a** are in accordance with those reported in the literature.[17b]

4-(*p*-Tolylthio)naphthalene-1,2-dione (5b). The product was obtained as an orange solid (40% yield); mp 205–206 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.09 (1H, d, J = 7.4 Hz), 7.87 (1H, d, J = 7.6 Hz), 7.65 (t, 1H, J = 7.2 Hz), 7.52 (1H, t, J = 7.4 Hz), 7.37 (2H, d, J = 8.0 Hz), 7.25 (2H, d, J = 8.0 Hz), 5.80 (s, 1H), 2.36 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): 179.5, 176.6, 161.9, 141.7, 135.8, 135.0, 133.4, 131.1, 130,3, 129.4, 125.0, 122.6, 121.1, 21.3; EI/MS (m/z) $[M + H]^+$: 281.0. Cald. for $[C_{17}H_{13}O_2S]^+$: 281.0.

4-(*m*-Tolylthio)naphthalene-1,2-dione (5c). The product was obtained as an orange solid (32% yield); mp 210–212 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (1H, d, J = 7.4 Hz), 7.97 (1H, d, J = 7.6 Hz), 7.74 (1H, t, J = 7.2 Hz), 7.62 (1H, t, J = 7.4 Hz), 7.44–7.35 (m, 4H), 5.92 (s, 1H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 179.5, 176.6, 161.9, 140.6, 136.5, 135.0, 133.4, 131.1, 130,3, 129.4, 125.0, 122.6, 121.1, 21.3; EI/MS (m/z) $[M + H]^+$: 281.0. Cald. for $[C_{17}H_{13}O_2S]^+$: 281.0.

4-((4-Methoxyphenyl)thio)naphthalene-1,2-dione (5d). The product was obtained as a red solid (30% yield); mp 215–216 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 (1H, d, J = 7.6 Hz), 7.95 (1H, d, J = 7.7 Hz), 7.73 (1H, t, J = 7.6 Hz), 7.60 (1H, t, J = 7.5 Hz), 7.48 (2H, d, J = 8.4 Hz), 7.03 (2H, d, J = 8.4 Hz), 5.88 (1H, s), 3.89 (3H,

s); ^{13}C NMR (100 MHz, $CDCl_3$): 179.5, 176.6, 162.0, 137.5, 135.8, 135.0, 133.4, 131.1, 130,3, 129.4, 125.0, 122.6, 121.1, 55.6; EI/MS (m/z) $[M + H]^+$: 297.0. Cald. for $[C_{17}H_{13}O_3S]^+$: 297.0.

4.2. Biological assays

4.2.1. Animals. All experiments dealing with animals were performed in accordance with the Brazilian Law 11.794/2008 and regulations of the National Council of Animal Experimentation Control under the license L038/2018 from the Ethics Committee for Animal Use of the Oswaldo Cruz Institute (CEUA/IOC).

4.2.2. Cytotoxicity assays. The compounds were tested for cytotoxic activity in cell culture *in vitro* employing several human cancer cell lines obtained from the National Cancer Institute, NCI (Bethesda, MD). Cytotoxicity was investigated against six cancer cell lines, *i.e.* HCT-116 (human colon carcinoma cells), NCI-H460 (human lung cancer cells), PC3 (human prostate cells), HL-60 (human promyelocytic leukemia cells), K-562 (myelogenous leukemia cell line) and Lucena 1 (MDR-cell line). The murine fibroblast immortalized cell line (L929) was used as control lineage. All culture media were supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 IU mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin at 37 °C with 5% CO_2. In cytotoxicity experiments, cells were plated in 96-well plates (0.1×10^6 cells per well for leukaemia cells, 0.7×10^5 cells per well for HCT-116, and 0.1×10^6 cells per well for PC3, L929 and NCI-H460). All the compounds tested were dissolved in DMSO. The final concentration of DMSO in the culture medium was kept constant (0.1%, v/v). Doxorubicin (0.001–1.10 μM) served as positive control, and negative control groups received the same amount of vehicle (DMSO). The cell viability was determined by reduction of the yellow dye 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to a blue formazan product as described by Mosmann.[50a] At completion of the incubation after 72 h, the plates were centrifuged and the medium was replaced by fresh medium (200 μL) containing 0.5 mg mL^{-1} MTT. Three hours later, the MTT formazan product was dissolved in DMSO (150 μL) and the absorbance was measured on a multiplate reader (SpectraCount, Packard, Ontario, Canada). The influence of the compound on cell proliferation and survival was quantified as the percentage of control absorbance of the reduced dye at 550 nm. The results were obtained by nonlinear regression for all the cell lines from three independent experiments. All cell treatments were performed with three replicates. All cells were mycoplasma-free.

4.2.3. Measurement of intracellular reactive oxygen species levels. Intracellular reactive oxygen species (ROS) accumulation was monitored using 5-(6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA), which is converted to the highly fluorescent dichlorofluorescein (DCF) in the presence of intracellular ROS.[50b] K-562 cells (myelogenous leukemia cell line) were pre-loaded with 10 μM CM-H_2DCFDA and incubated for 1 hour, in the dark, at 37 °C/5% CO_2. After that time, the cells were centrifuged, the medium containing CM-H_2DCFDA was removed and the cells were washed with PBS buffer. From this stage, the cells were always protected from light. Fresh medium containing compounds **3e**, **4f** and **5a** was added and the cells were incubated at the times of interest (1 and 3 hours). After the incubation time, the cells were centrifuged, washed and re-suspended in PBS containing propidium iodide (PI) to a final concentration of 1 μg mL^{-1}. Tubes were placed on ice and immediately analyzed by flow cytometry. Living cells, which are PI negative, were selected by gating. In those living cells, the DCF fluorescence was recorded using excitation and emission wavelengths of 490 and 525 nm, respectively. Menadione (MEN, 20 μM) and hydrogen peroxide (H_2O_2, 150 μM) were used as positive controls. A total of 10 000 events were analyzed per sample. Data are expressed as mean ± SEM from three independent experiments.

4.2.4. Trypanocidal assay and selectivity index. Stock solutions of the compounds were prepared in dimethyl sulfoxide (DMSO), with the final concentration of the latter in the experiments never exceeding 0.4%. Preliminary experiments showed that DMSO has no deleterious effect on the parasites when its concentration is up to 4%. *T. cruzi* bloodstream trypomastigotes (Y strain) were obtained at the peak of parasitaemia from infected albino mice, purified by differential centrifugation and resuspended in RPMI to a parasite concentration of 10^7 cells per mL in the presence of 10% mouse blood. This suspension (100 μL) was added in the same volume of each compound previously prepared at twice the desired final concentrations for 24 h at 4 °C. Cell quantification was performed in a Neubauer chamber and the trypanocidal activity was expressed as IC_{50}/24 h, corresponding to the concentration that leads to lysis of 50% of the parasites. In order to determine the SI, cytotoxicity assays were performed with primary cultures of peritoneal macrophages obtained from Albino Swiss mice. For the experiments, 2.5×10^4 cells in 200 μL of RPMI-1640 medium (pH 7.2 plus 10% foetal bovine serum and 2 mM glutamine) were added to each well of a 96-well microtitre plate and incubated for 24 h at 37 °C. The treatment of the cultures was performed in fresh supplemented medium (200 μL per well) for 24 h at 37 °C. After this period, 110 μL of the medium was discarded and 10 μL of PrestoBlue (Invitrogen) was added to complete the final volume of 100 μL. Thus, the plate was incubated for 2 h and the measurement was performed at 560 and 590 nm, as recommended by the manufacturer. The results were expressed as the difference in the percentage of reduction between treated and untreated cells being the LC_{50} value, corresponding to the concentration that leads to damage of 50% of the mammalian cells.

5. X-ray crystallography

X-ray diffraction data collection for the compounds was performed on an Enraf-Nonius Kappa-CCD diffractometer (95

mm CCD camera on a κ-goniostat) and XtaLAB Mini (ROW) two-circle diffractometer employing graphite monochromated MoK_radiation (0.71073 Å), at room temperature. Data collection was carried out using the COLLECT software and CrysAlisPro.[51,52] Integration and scaling of the reflections, and correction for Lorentz and polarization effects were performed with the HKL DENZO-SCALEPACK and the CrysAlisPro system of programs.[53] The structure of the compounds was solved by direct methods with SHELXS-97.[54] The models were refined by full-matrix least squares on F^2 using SHELXL-97.[54] The program ORTEP-3 was used for graphic representation and the program WINGX was used to prepare materials for publication.[55,56] All H atoms were located by geometric considerations placed (C–H = 0.93–0.96 Å; N–H = 0.86 Å) and refined as riding with U_{iso}(H) = 1.5U_{eq}(C-methyl) or 1.2U_{eq}(other). Crystallographic data for the structures were deposited in the Cambridge Crystallographic Data Centre, with CCDC numbers 1928261 (**3a**), 1928296 (**3d**), 1928326 (**3f**), 1928361 (**3g**), 1928382 (**3h**) and 1928407 (**5d**).

6. Electrochemical studies

Cyclic voltammetry was performed on a 100B/W electrochemical workstation (BASI®, West Lafayette, USA) at ambient temperature. Cyclic voltammograms of the compounds (1 mM) were recorded in phosphate buffer (pH 7.4), employing a glassy carbon working electrode cleaned and polished with Al_2O_3 after each scan, an Ag/AgCl reference electrode (SSE) and a platinum wire counter electrode, at a potential range between −1 and +1 V. Quinone derivatives required 30% methanol due to limited solubility in aqueous media. Buffers were purged with nitrogen for 30 min prior to use. The sensitivity of the cell was adjusted to 10 μA V^{-1} and the compounds were scanned at the rate of 200 mV s^{-1}.

Conflicts of interest

Authors declare no conflict of interest.

Acknowledgements

This research was funded by grants from CNPq (404466/2016-8 and PQ 305741/2017-9, PDS 110818/2016-4 and PQ SR 302613/2013-9), Chamada Universal MCTI/CNPq No 01/2016, FAPEMIG (Edital 01/2014 APQ-02478-14 and Programa Pesquisador Mineiro – PPM-00638-16 and PPM-00635-18), FAPERJ (CNE Proc. E-26/203.083/2016), CAPES and INCT-Catalysis. ENSJ would like to thank CAPES and DAAD for the PROBRAL exchange program (99999.008126/2015-01). ENSJ thanks the Capes-Humboldt research fellowship for experienced researchers (Proc. No. 88881.145517/2017-01), Return Fellowship of the Alexander-von-Humboldt foundation and the Royal Society of Chemistry for the research fund grant (R19-9781). CAS would like to thank the Institute of Physics of the University of São Paulo (São Carlos). The authors would like to thank the Multi-User Facility of Drug Research and Development Center of the Federal University of Ceará for technical support. AK also thanks the University of Saarland, CAPES and DAAD for the PROBRAL exchange program that provided financial support for his exchange period at the Federal University of Minas Gerais. The authors are grateful to Prof. Claus Jacob from the University of Saarland for his excellent suggestions during the preparation of this manuscript.

References

1 J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D. M. Parkin, D. Forman and F. Bray, *Int. J. Cancer*, 2015, **136**, E359–E386.
2 WHO, *Weekly epidemiological record.*, 2015, **90**, 33–43.
3 J. C. P. Dias, A. N. Ramos Jr., E. D. Gontijo, A. Luquetti, M. A. Shikanai-Yasuda, J. R. Coura, R. M. Torres, J. R. D. C. Melo, E. A. D. Almeida, W. D. Oliveira Jr., A. C. Silveira, J. M. D. Rezende, F. S. Pinto, A. W. Ferreira, A. Rassi, A. A. F. Filho, A. S. D. Sousa, D. C. Filho, A. M. Jansen, G. M. Q. Andrade, C. F. D. P. D. C. Britto, A. Y. D. N. Pinto, A. Rassi Jr., D. E. Campos, F. Abad-Franch, S. E. Santos, E. Chiari, A. M. Hasslocher-Moreno, E. F. Moreira, D. S. D. O. Marques, E. L. Silva, J. A. Marin-Neto, L. M. D. C. Galvão, S. S. Xavier, S. A. D. S. Valente, N. B. Carvalho, A. V. Cardoso, R. A. E. Silva, V. M. D. Costa, S. M. Vivaldini, S. M. Oliveira, V. D. C. Valente, M. M. Lima and R. V. Alves, *Epidemiol. Serv. Saude*, 2016, **25**, 7–86.
4 G. A. Schmunis and Z. E. Yadon, *Acta Trop.*, 2010, **115**, 14–21.
5 A. Requena-Méndez, S. Bussion, E. Aldasoro, Y. Jackson, A. Angheben, D. Moore, M.-J. Pinazo, J. Gascón, J. Muñoz and E. Sicuri, *Lancet*, 2017, **5**, e439–e447.
6 J. Gascon, C. Bern and M. J. Pinazo, *Acta Trop.*, 2010, **115**, 22–27.
7 S. Antinori, L. Galimberti, R. Bianco, R. Grande, M. Galli and M. Corbellino, *Eur. J. Intern. Med.*, 2017, **43**, 6–15.
8 K. Salomão, R. F. Menna-Barreto and S. L. de Castro, *Curr. Top. Med. Chem.*, 2016, **16**, 2266–2289.
9 R. P. Verma, *Anti-Cancer Agents Med. Chem.*, 2006, **6**, 489–499.
10 (*a*) S. L. de Castro, F. S. Emery and E. N. da Silva Junior, *Eur. J. Med. Chem.*, 2013, **69**, 678–700; (*b*) E. N. da Silva Júnior, G. A. M. Jardim, C. Jacob, U. Dhawa, L. Ackermann and S. L. de Castro, *Eur. J. Med. Chem.*, 2019, **179**, 863–915.
11 E. I. Parkinson and P. J. Hergenrother, *Acc. Chem. Res.*, 2015, **48**, 2715–2723.
12 H. Hussain and I. R. Green, *Expert Opin. Ther. Pat.*, 2017, **27**, 1111–1121.
13 H. Y. Qiu, P. F. Wang, H. Y. Lin, C. Y. Tang, H. L. Zhu and Y. H. Yang, *Chem. Biol. Drug Des.*, 2018, **91**, 681–690.
14 A. V. Pinto and S. L. de Castro, *Molecules*, 2009, **14**, 4570–4590.
15 D. Trachootham, J. Alexandre and P. Huang, *Nat. Rev. Drug Discovery*, 2009, **8**, 579–591.
16 S. Shaaban, R. Diestel, B. Hinkelmann, Y. Muthukumar, R. P. Verma, F. Sasse and C. Jacob, *Eur. J. Med. Chem.*, 2012, **58**, 192–205.

17 (*a*) Y. G. Paiva, T. L. Silva, A. F. A. Xavier, M. F. C. Cardoso, F. C. da Silva, M. F. S. Silva, D. P. Pinheiro, C. Pessoa, V. F. Ferreira and M. O. F. Goulart, *J. Braz. Chem. Soc.*, 2019, **30**, 658–672; (*b*) A. K. Mishra and J. N. Moorthy, *J. Org. Chem.*, 2016, **81**, 6472–6480.

18 C. J. Li, Y. Z. Li, A. V. Pinto and A. B. Pardee, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 13369–13374.

19 J. I. Lee, D. Y. Choi, H. S. Chung, H. G. Seo, H. J. Woo, B. T. Choi and Y. H. Choi, *Exp. Oncol.*, 2006, **28**, 30–35.

20 M. R. Gunner, J. Madeo and Z. Zhu, *J. Bioenerg. Biomembr.*, 2008, **40**, 509.

21 M. Doering, L. A. Ba, N. Lilienthal, C. Nicco, C. Scherer, M. Abbas, A. A. Zada, R. Coriat, T. Burkholz, L. Wessjohann, M. Diederich, F. Batteux, M. Herling and C. Jacob, *J. Med. Chem.*, 2010, **53**, 6954–6963.

22 G. I. Giles, K. M. Tasker, R. J. K. Johnson, C. Jacob, C. Peers and K. N. Green, *Chem. Commun.*, 2001, 2490–2491.

23 G. I. Giles, N. M. Giles, C. A. Collins, K. Holt, F. H. Fry, P. A. S. Lowden, N. J. Gutowski and C. Jacob, *Chem. Commun.*, 2003, 2030–2031.

24 E. N. da Silva Júnior, M. C. de Souza, A. V. Pinto, C. Pinto Mdo, M. O. Goulart, F. W. Barros, C. Pessoa, L. V. Costa-Lotufo, R. C. Montenegro, M. O. de Moraes and V. F. Ferreira, *Bioorg. Med. Chem.*, 2007, **15**, 7035–7041.

25 E. N. da Silva Júnior, M. A. B. F. D. Moura, A. V. Pinto, M. D. C. F. R. Pinto, M. C. B. V. D. Souza, A. J. Araújo, C. Pessoa, L. V. Costa-Lotufo, R. C. Montenegro, M. O. D. Moraes, V. F. Ferreira and M. O. F. Goulart, *J. Braz. Chem. Soc.*, 2009, **20**, 635–643.

26 E. N. da Silva Júnior, C. F. de Deus, B. C. Cavalcanti, C. Pessoa, L. V. Costa-Lotufo, R. C. Montenegro, M. O. de Moraes, M. D. C. F. R. Pinto, C. A. de Simone, V. F. Ferreira, M. O. F. Goulart, C. K. Z. Andrade and A. V. Pinto, *J. Med. Chem.*, 2010, **53**, 504–508.

27 (*a*) E. N. da Silva Júnior, B. C. Cavalcanti, T. T. Guimaraes, C. Pinto Mdo, I. O. Cabral, C. Pessoa, L. V. Costa-Lotufo, M. O. de Moraes, C. K. de Andrade, M. R. Dos Santos, C. A. de Simone, M. O. Goulart and A. V. Pinto, *Eur. J. Med. Chem.*, 2011, **46**, 399–410; (*b*) S. Shabaan, L. A. Ba, M. Abbas, T. Burkholz, A. Denkert, A. Gohr, L. A. Wessjohann, F. Sasse, W. Weber and C. Jacob, *Chem. Commun.*, 2009, 4702–4704; (*c*) S. Mecklenburg, S. Shaaban, L. A. Ba, T. Burkholz, T. Schneider, B. Diesel, A. K. Kiemer, A. Roseler, K. Becker, J. Reichrath, A. Stark, W. Tilgen, M. Abbas, L. A. Wessjohann, F. Sasse and C. Jacob, *Org. Biomol. Chem.*, 2009, **7**, 4753–4762.

28 E. H. G. da Cruz, M. A. Silvers, G. A. M. Jardim, J. M. Resende, B. C. Cavalcanti, I. S. Bomfim, C. Pessoa, C. A. de Simone, G. V. Botteselle, A. L. Braga, D. K. Nair, I. N. N. Namboothiri, D. A. Boothman and E. N. da Silva Júnior, *Eur. J. Med. Chem.*, 2016, **122**, 1–16.

29 (*a*) G. A. M. Jardim, E. H. G. da Cruz, W. O. Valença, D. J. B. Lima, B. C. Cavalcanti, C. Pessoa, J. Rafique, A. L. Braga, C. Jacob and E. N. da Silva Júnior, *Molecules*, 2018, **23**, 83; (*b*) G. A. M. Jardim, W. X. C. Oliveira, R. P. de Freitas, R. F. S. Menna-Barreto, T. L. Silva, M. O. F. Goulart and E. N. da Silva Júnior, *Org. Biomol. Chem.*, 2018, **16**, 1686–1691.

30 W. O. Valença, T. V. Baiju, F. G. Brito, M. H. Araujo, C. Pessoa, B. C. Cavalcanti, C. A. de Simone, C. Jacob, I. N. N. Namboothiri and E. N. da Silva Júnior, *ChemistrySelect*, 2017, **2**, 4300–4307.

31 E. N. da Silva Júnior, R. F. Menna-Barreto, M. C. Pinto, R. S. Silva, D. V. Teixeira, M. C. de Souza, C. A. De Simone, S. L. De Castro, V. F. Ferreira and A. V. Pinto, *Eur. J. Med. Chem.*, 2008, **43**, 1774–1780.

32 E. N. da Silva Júnior, G. A. M. Jardim, R. F. S. Menna-Barreto and S. L. D. Castro, *J. Braz. Chem. Soc.*, 2014, **25**, 1780–1798.

33 (*a*) R. L. de Carvalho, G. A. M. Jardim, A. C. C. Santos, M. H. Araujo, W. X. C. Oliveira, A. C. S. Bombaça, R. F. S. Menna-Barreto, E. Gopi, E. Gravel, E. Doris and E. N. da Silva Júnior, *Chem. – Eur. J.*, 2018, **24**, 15227–15235; (*b*) G. G. Dias, T. Rogge, R. Kuniyil, C. Jacob, R. F. S. Menna-Barreto, E. N. da Silva Júnior and L. Ackermann, *Chem. Commun.*, 2018, **54**, 12840–12843.

34 S. B. B. B. Bahia, W. J. Reis, G. A. M. Jardim, F. T. Souto, C. A. de Simone, C. C. Gatto, R. F. S. Menna-Barreto, S. L. de Castro, B. C. Cavalcanti, C. Pessoa, M. H. Araujo and E. N. da Silva Júnior, *MedChemComm*, 2016, **7**, 1555–1563.

35 E. N. da Silva Júnior, T. T. Guimarães, R. F. S. Menna-Barreto, M. C. F. R. Pinto, C. A. de Simone, C. Pessoa, B. C. Cavalcanti, J. R. Sabino, C. K. Z. Andrade, M. O. F. Goulart, S. L. de Castro and A. V. Pinto, *Bioorg. Med. Chem.*, 2010, **18**, 3224–3230.

36 M. J. Hatfield, J. Chen, E. M. Fratt, L. Chi, J. C. Bollinger, R. J. Binder, J. Bowling, J. L. Hyatt, J. Scarborough, C. Jeffries and P. M. Potter, *J. Med. Chem.*, 2017, **60**, 1568–1579.

37 C. H. Tseng, C. M. Cheng, C. C. Tzeng, S. I. Peng, C. L. Yang and Y. L. Chen, *Bioorg. Med. Chem.*, 2013, **21**, 523–531.

38 (*a*) C. H. Tseng, C. K. Lin, Y. L. Chen, C. K. Tseng, J. Y. Lee and J. C. Lee, *Eur. J. Med. Chem.*, 2018, **143**, 970–982; (*b*) T. P. Fragoso, J. W. M. Carneiro and M. D. Vargas, *J. Mol. Model.*, 2010, **16**, 825–830.

39 P. H. Di Chenna, V. Benedetti-Doctorovich, R. F. Baggio, M. T. Garland and G. Burton, *Eur. J. Med. Chem.*, 2001, **44**, 2486–2489.

40 K. E. Reinicke, E. A. Bey, M. S. Bentle, J. J. Pink, S. T. Ingalls, C. L. Hoppel, R. I. Misico, G. M. Arzac, G. Burton, W. G. Bornmann, D. Sutton, J. Gao and D. A. Boothman, *Clin. Cancer Res.*, 2005, **11**, 3055–3064.

41 G. A. M. Jardim, I. A. O. Bozzi, W. X. C. Oliveira, C. Mesquita-Rodrigues, R. F. S. Menna-Barreto, R. A. Kumar, E. Gravel, E. Doris, A. L. Braga and E. N. da Silva Júnior, *New J. Chem.*, 2019, **43**, 13751–13763.

42 J. Raushel and V. V. Fokin, *Org. Lett.*, 2010, **12**, 4952–4955.

43 A. A. Vieira, I. R. Brandao, W. O. Valença, C. A. de Simone, B. C. Cavalcanti, C. Pessoa, T. R. Carneiro, A. L. Braga and E. N. da Silva Júnior, *Eur. J. Med. Chem.*, 2015, **101**, 254–265.

44 F. H. Allen, O. Kennard, D. G. Watson, L. Brammer, A. G. Orpen and R. Taylor, *J. Chem. Soc., Perkin Trans. 2*, 1987, **12**, S1–S19.

45 R. I. Misico and E. S. Forzani, *Electrochem. Commun.*, 2003, **5**, 449–454.

46 K. Salomão, N. A. Santana, M. T. Molina, S. L. de Castro and R. F. S. Menna-Barreto, *BMC Microbiol.*, 2013, **13**, 196.
47 (*a*) J. N. Lopes, F. S. Cruz, R. Docampo, M. E. Vasconcellos, M. C. Sampaio, A. V. Pinto and B. Gilbert, *Ann. Trop. Med. Parasitol.*, 1978, **72**, 523–531; (*b*) G. A. M. Jardim, W. J. Reis, M. F. Ribeiro, F. M. Ottoni, R. J. Alves, T. L. Silva, M. O. F. Goulart, A. L. Braga, R. F. S. Menna-Barreto, K. Salomão, S. L. de Castro and E. N. da Silva Júnior, *RSC Adv.*, 2015, **5**, 78047–78060.
48 E. Perez-Sacau, R. G. Diaz-Penate, A. Estevez-Braun, A. G. Ravelo, J. M. Garcia-Castellano, L. Pardo and M. Campillo, *J. Med. Chem.*, 2007, **50**, 696–706.
49 D. D. Perrin, W. L. F. Armarego and D. R. Perrin, *Purification of Laboratory Chemicals*, 2nd edn, Pergamon, Oxford, 1980.
50 (*a*) T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55–63; (*b*) C. P. LeBel, H. Ischiropoulos and S. C. Bondy, *Chem. Res. Toxicol.*, 1992, **5**, 227–231.
51 B. V. Nonius, *Enraf-Nonius COLLECT*, Delft, The Netherlands, 1997–2000.
52 O. D. Rigaku, *CrysAlisPro*, 2015.
53 Z. Otwinowski and W. Minor, *Methods Enzymol.*, 1997, **276**, 307–326.
54 C. Schnitter, H. W. Roesky, T. Albers, H. G. Schmidt, C. Röpken, E. Parisini and G. M. Sheldrick, *Chem. – Eur. J.*, 1997, **3**, 1783–1792.
55 L. Farrugia, *J. Appl. Crystallogr.*, 1997, **30**, 565.
56 L. Farrugia, *J. Appl. Crystallogr.*, 1999, **32**, 837–838.