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1,2,3-Triazole-, arylamino- and thio-substituted 1,4-naphthoquinones: Potent antitumor activity, electrochemical aspects, and bioisosteric replacement of C-ring-modified lapachones

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ABSTRACT

1,2,3-Triazole-, arylamino- and thio-substituted naphthoquinones (24, 8, and 2 representatives, respectively) were synthesized in moderate yields and evaluated against several human cancer cell lines (blood, ovarian, breast, central nervous system, colon, and prostate cancers and melanoma), showing, for some of them, IC_{50} values below 2 μM. The cytotoxic potential of the tested naphthoquinones was also assayed on non-tumor cells such as human peripheral blood mononucluear cells (PBMC) and two murine fibroblast lines (L929 and V79 cells). α-Lapachone- and nor-α-lapachone-based 1,2,3-triazoles and arylamino-substituted naphthoquinones showed potent cytotoxicity against different cancer cell lines. The compounds may represent promising new lead derivatives for anticancer drug development. The electrochemical properties of selected compounds were evaluated in an attempt to correlate them with antitumor activity.

1. Introduction

Reactive oxygen species (ROS) are important participants in regulating normal cellular processes. A deregulated redox balance may contribute to the development of several human diseases, including cancers, as recently reported by Nogueira and Hay.[1] Several types of cancer cells exhibit disturbed intracellular redox balance, differentiating them from their non-cancerous counterparts.[2] ROS may act directly with DNA, lipids, and proteins to induce cell damage.[3] The alkylation of crucial proteins and nucleic acids can also cause cell damage.[4] Compared to non-cancerous cells, the reactive oxygen species (ROS) levels are considerably closer to the critical redox threshold at which cell death is induced.[2] These biochemical differences between healthy and malignant tissues are significant and may be exploited in the design of selective drugs.[2]

Compounds that are able to modulate the redox balance in cancer cells are potential candidates for the development of anticancer drugs.[5–7] In general, these compounds catalyze the oxidation of redox-sensitive, thiol-containing proteins and enzymes and/or significantly increase intracellular ROS levels. These features relate to subsequent processes that lead to apoptosis.[7] Quinones belong to this class of compounds, as they are capable of increasing intracellular ROS levels over a critical threshold and therefore may induce apoptosis in cancer cells.

Electrochemistry is the standard method for studying redox systems. Electrochemical techniques applied to biology are continuously described in the literature and provide both kinetic and thermodynamic information.[8] Electrochemical methods are useful in the characterization and ultimately the design of redox-modulating natural products and drugs, including potential antioxidants and anticancer agents.[9] Among these techniques, cyclic voltammetry can rapidly evaluate the redox properties of some of those compounds, including quinones. The usual parameters normally obtained and employed, especially in cyclic voltammetry, are the

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potentials of the oxidation (Epa) and reduction (Epc) peaks or Eredox (Epc + Epa)/2 (for reversible systems) or Epc − Epc/2 (for irreversible ones). The potential Eredox or similar parameters give a quantitative measure of the ease of reduction of an oxidant or electron acceptor, A, since the more positive the value of the potential of the couple E (A/A·), the more powerful the oxidant. Similarly, the more negative the value of E (A·/A^{2-}), the more powerful the reductant.[9] The Epc or the Eredox of the first peak or system, respectively, are the ones to be used in correlation of electrochemistry and biological activities.[8a] With this approach these data may even be useful in the rational design of such compounds as part of a special chemical structure–electrochemical potential–biological activity relationship. Nonetheless, one must bear in mind that electrochemical parameters such Epa and/or Epc are only one measure and other data, like abundance, distribution, accessibility are also decisive factors in vitro or cell environments.[8b,10]

The mechanism of action of quinonoid anti-tumor agents has been thoroughly investigated.[10] Under aerobic conditions, that is, in organs with sufficient blood supply, a one-electron reduction predominates, resulting in free-radical intermediates that undergo back oxidation in the presence of oxygen, releasing reactive oxygen species (ROS).[8,11] This may cause damage to the DNA of the tumor cell, but normal cells are also negatively affected. A two-electron reduction of the quinone function followed by its inactivation through subsequent glucuronidation and/or sulfation or by the conversion of the hydroquinone into an alkylating intermediate is an alternative pathway.[11] This mechanism is believed to predominate under anaerobic conditions.[12]

Recently, our research group has described the synthesis of redox modulating quinone compounds. To access substances with different redox behaviors, we have appended groups such as arylamines,[13] 1,2,3-triazoles,[14] azides,[14] among others, onto the quinone moiety.[15]

Through our efforts to design molecular scaffolds using hybridization protocols, we report herein the extension of our approach, which includes modification of 1,4-naphthoquinones with triazoles (**1**–**19**) and sugars (**20**–**21**) to enhance the bioavailability of the compounds, the insertion of a second quinone group (**22**–**24**) to potentially increase ROS release and through the use of phenylthiolated compounds to connect chalcogens[16] to the main structure. Additionally, we have synthesized compounds **28**–**35**, isomers of substituted arylamino-nor-β-lapachones,[13] which are widely recognized as highly active cytotoxic compounds, in order to compare the effect of the *para*-quinone moiety on their activity and electrochemical behavior. Among the thirty-four compounds assayed, sixteen are new and are fully characterized. Eleven of the cell lines used are cancerous, and three are non-cancerous.

2. Results and discussion

2.1. Chemistry

2-Bromo-1,4-naphthoquinone and 2-chloro-1,4-naphthoquinone were acquired from Sigma-Aldrich (St. Louis, MO, USA). Lapachol (**25**) (2-hydroxy-3-(3′-methyl-2′-butenyl)-1,4-naphthoquinone) was extracted from the heartwood of *Tabebuia* sp. (Tecoma) and purified by a series of recrystallizations.[17] Nor-lapachol (**38**) (2-hydroxy-3-(2′-methyl-propenyl)-1,4-naphthoquinone) was obtained as a crystalline orange solid by Hooker oxidation of quinone **25**.[18]

As previously described, the first group of compounds was prepared using click chemistry reaction[19] with the respective azide derivatives.[20] 4-Azido-α-lapachone and 3-azido-nor-α-lapachone were synthesized and used to prepare the 1,2,3-triazole analogues **1**–**6** in moderate yields, as shown in Figure 1.

The second group, 1,4-naphthoquinone-based 1,2,3-triazoles **7**–**19**, was previously described (Fig. 2).[21] Compounds **20**–**24** are herein described for the first time.

para-Naphthoquinones **7**–**19** were synthesized by clicking a series of *N*-phthalimidoalkylazides with the respective naphthoquinones. Compounds **18** and **19** were prepared by the same methodology using 1-azido-3-nitrobenzene (Fig. 2).

Unpublished compounds **20**–**24** were obtained by reaction of azide-substituted carbohydrate or naphthoquinone and the alkyne-substituted quinone. Compounds **20**–**24** were obtained in high yields using copper iodide(I) in acetonitrile as the reaction medium (Scheme 1).

The unpublished arylamino-substituted α-lapachones **28**–**35**, were prepared from lapachol (**25**), as shown in Scheme 2. The first step consisted of the simple cyclization of lapachol (**25**) to α-lapachone (**26**) using HCl/HOAc. Compound **27** reacted with various anilines containing electron donating and electron withdrawing groups to prepare **28**–**35**. The compounds were obtained in high yields, and their structures were determined by IR, ^{1}H and ^{13}C NMR and by comparing their spectroscopic data with those of already reported similar compounds.[22] Electrospray ionization mass spectra and combustion analysis data were also obtained for unpublished compounds.

The last class of compounds was obtained using similar methodology. The respective bromo derivatives were prepared and were reacted with thiophenol as shown in Scheme 3. Compounds **36** and **39** were isolated in moderate yields as yellow solids.

2.2. Electrochemistry

Several studies have investigated and reported on the electrochemical behavior of quinones. Some correlation has been observed between the electrochemical parameters (Epc1: potential of the first reduction peak) and biological activities.[23]

The single electron reduction of quinones by enzymes such as NADH-cytochrome P450 oxidoreductase initiates redox cycling and oxidative stress and then the relative one-electron reduction potentials of quinones control the position of the equilibrium defining the electron transfer between the semiquinone and oxygen.[8b] Also, the first reduction step of quinones can be correlated with the unoccupied molecular orbital of lower energy (LUMO).[23b]

In the context of cancer, there is a considerable number of positive correlations.[24]

The quinone/semiquinone/hydroquinone ($Q/SQ^{\cdot-}/H_2Q$) triad is an important component of many redox systems in biology. It is a vital link for the transfer of electrons through cells and tissues (Eqs. 1–3).[25]

$$Q + e^- \rightleftharpoons SQ^{\bullet-} \quad (1)$$

$$SQ^{\bullet-} + e^- + 2H^+ \rightleftharpoons H_2Q \quad (2)$$

$$\text{Net} \quad Q + 2e^- + 2H^+ \rightleftharpoons H_2Q \quad (3)$$

In typical measurements, cyclic voltammograms (CV) were recorded in aprotic medium (DMF + TBAP, 0.1 mol L^{-1}), at a scan rate of 100 mV s^{-1}. This enabled determination of the electrochemical reduction behavior of each compound. Cathodic and anodic peaks for each compound are listed in Table 1.

It was not feasible to study all of the quinones in the present work. Representative compounds **20** and **21** (Scheme 1), **28** and **34** (Scheme 2) and **39** (Scheme 3) were selected to encompass each of the structural classes. The present electrochemical study complements recently published results.[21] In Table 1, the previously reported electrochemical data of compounds **2**, **3** and **10** are presented in a more complete form to allow comparison and rationalization of the electrochemical behavior. It is out of the scope of

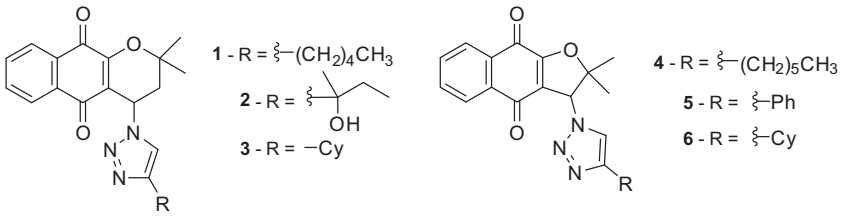


Figure 1. Nor-α-lapachone and α-lapachone-based 1,2,3-triazoles **1**–**6**.

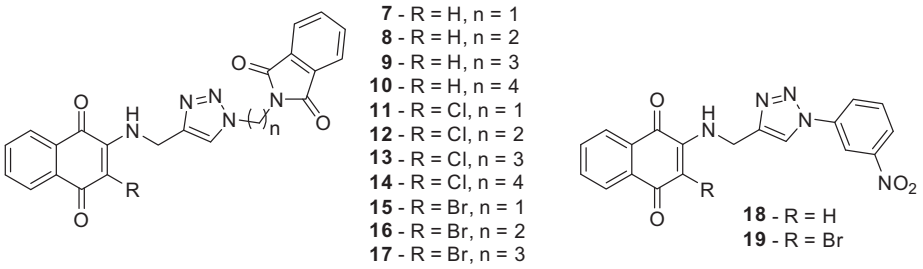
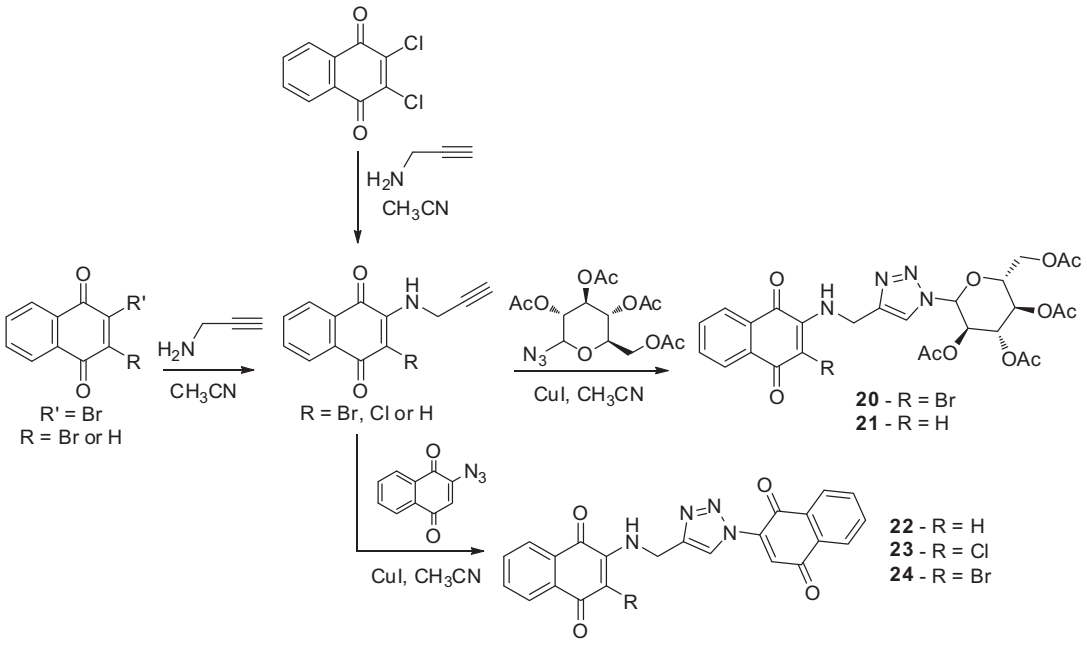


Figure 2. 1,4-Naphthoquinone-based 1,2,3-triazoles **7**–**19**.



Scheme 1. 1,4-Naphthoquinone-based 1,2,3-triazoles **20**–**24**.

the present paper, the rationalization of the electrochemical behavior.

As has been noted, the compounds described here can be divided among four structural classes: dihydropyran-*para*-naphthoquinones (**2** and **3**), *para*-naphthoquinone derivatives (**10**, **20** and **21**), nitroaniline-substituted α-lapachones (**28** and **34**) and a thiolated nor-α-lapachone (**39**). Figure 3 shows the cyclic voltammograms of the compounds **20**, **21**, **28**, **34** and **39**.

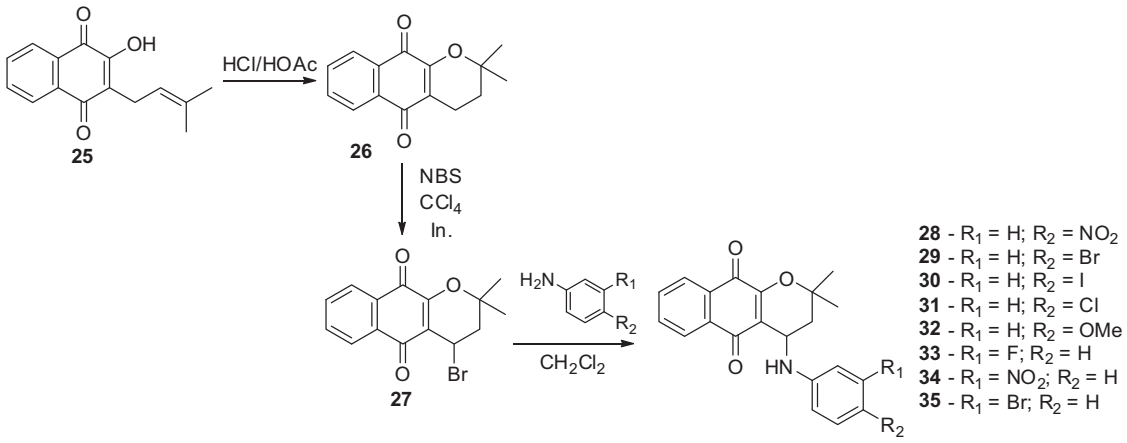
The first two quinones (**20** and **21**), similar to compounds **2** and **3**,[21] exhibited quasi-reversible reduction behavior (Fig. 3), which is typical of simple and well-behaved quinones (Eqs. 1 and 2). The CV profiles of the other four compounds (**10**,[21] **28**, **34** and **39**) are much more complex due to the presence of other electroactive groups.

The major electrochemical parameters for each compound are listed in Table 1. Epc1 values are most commonly used to compare biological activities. The ease of reduction is as follows:

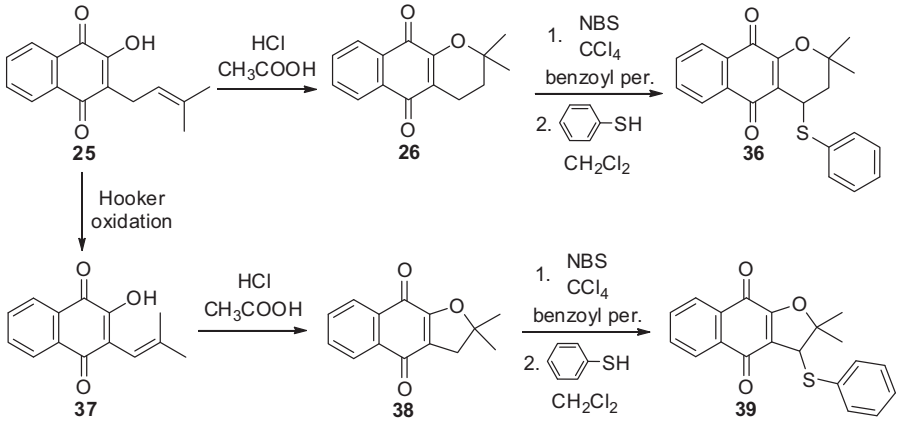
39 > **2** ~ **3** > **20** > **28** > **34** > **21** > **10**

Compounds **28** and **34** display electrochemical behavior similar to *m*-nitroaniline-substituted nor-β-lapachone.[26] As observed in Figure 3, four and three cathodic peaks are displayed for **28** (*para* derivative) and **34** (*meta* derivative), respectively. The first peak of the CV of **28** has a cathodic peak potential (Epc1) of −0.674 V, while for **34**, the value is −0.684 V. These cathodic peaks display two corresponding backward peaks, revealing quasi-reversible behavior.

For **34**, the first pair of peaks observed is consistent with an initial one-electron reduction of the *para*-quinone to form the semiquinone radical according to Eq. 4:



Scheme 2. Arylamino-substituted α-lapachones **28**–**35**.



Scheme 3. Thio derivatives **36** and **39**.

Table 1
Major electrochemical parameters of the quinones ($c = 1 \times 10^{-3}$ mol L^{-1}), in DMF/TBAP, 0.1 mol L^{-1}, versus Ag/AgCl, Cl^{-1}, v = 100 mV s^{-1}

Compounds	Epc1 (V)	Epc2 (V)	Epc3 (V)	Epc4 (V)	Epa1 (V)	Epa2 (V)	Epa3 (V)	Epa4 (V)
2[a]	−0.632	−1.116	—	—	−0.940	−0.540	—	—
3[a]	−0.630	−1.147	—	—	−0.967	−0.547	—	—
10[a]	−0.807	−1.348	−2.085	−2.471	−1.151	−0.779	—	—
20	−0.642	−1.281	—	—	−1.098	−0.544	—	—
21	−0.797	−1.378	—	—	—	−0.709	—	—
28	−0.674	−1.177	−1.392	−2.114	−1.333	−1.045	−0.596	—
34	−0.684	−1.169	−1.842	—	−1.050	−0.811	−0.607	—
39	−0.556	−1.132	−1.500	—	−1.385	−1.139	−0.479	−0.030

Epc1: potential of the first cathodic peak; Epc2: potential of the second cathodic peak; etc; Epa1: potential of the first anodic peak; etc.
[a] Ref. 21.

$$[Q]\text{-}PhNO_2 + e^- \leftrightarrows [Q^{\cdot -}]\text{-}PhNO_2. \quad (4)$$

According to a previous article reporting the reduction of a similar compound (nor-β-lapachone derivative) on mercury,[27] the second cathodic pair of peaks, twice as intense as the first peak and appearing at −1.169 V, should correspond to the subsequent one-electron reduction of the electrogenerated semiquinone and to the formation of the nitroanion radical (Eq. 5), leading to a dianion quinone-nitro radical, which is further reduced, in the third peak, in a conventional nitroaromatic reduction:[10]

$$[Q^{\cdot -}]\text{-}PhNO_2 + e^- \leftrightarrows [Q^{2-}]\text{-}PhNO_2^{\cdot -}. \quad (5)$$

For compound **28**, the two waves are separated (Epc2 = −1.177 V and EpIIIc = −1.392 V) and until now, it has not been possible to determine their nature. Studies on this subject are under way.

For compound **39**, the most readily reduced in the series and, to our knowledge, a previously unreported compound, a different electrochemical profile is observed. The first two redox systems correspond to Eqs. 1 and 2, and the third one, which has an irreversible nature, suggests the occurrence of a dissociative electron transfer with cleavage of the C–S bond. Studies to elucidate the electrochemical behavior are ongoing.

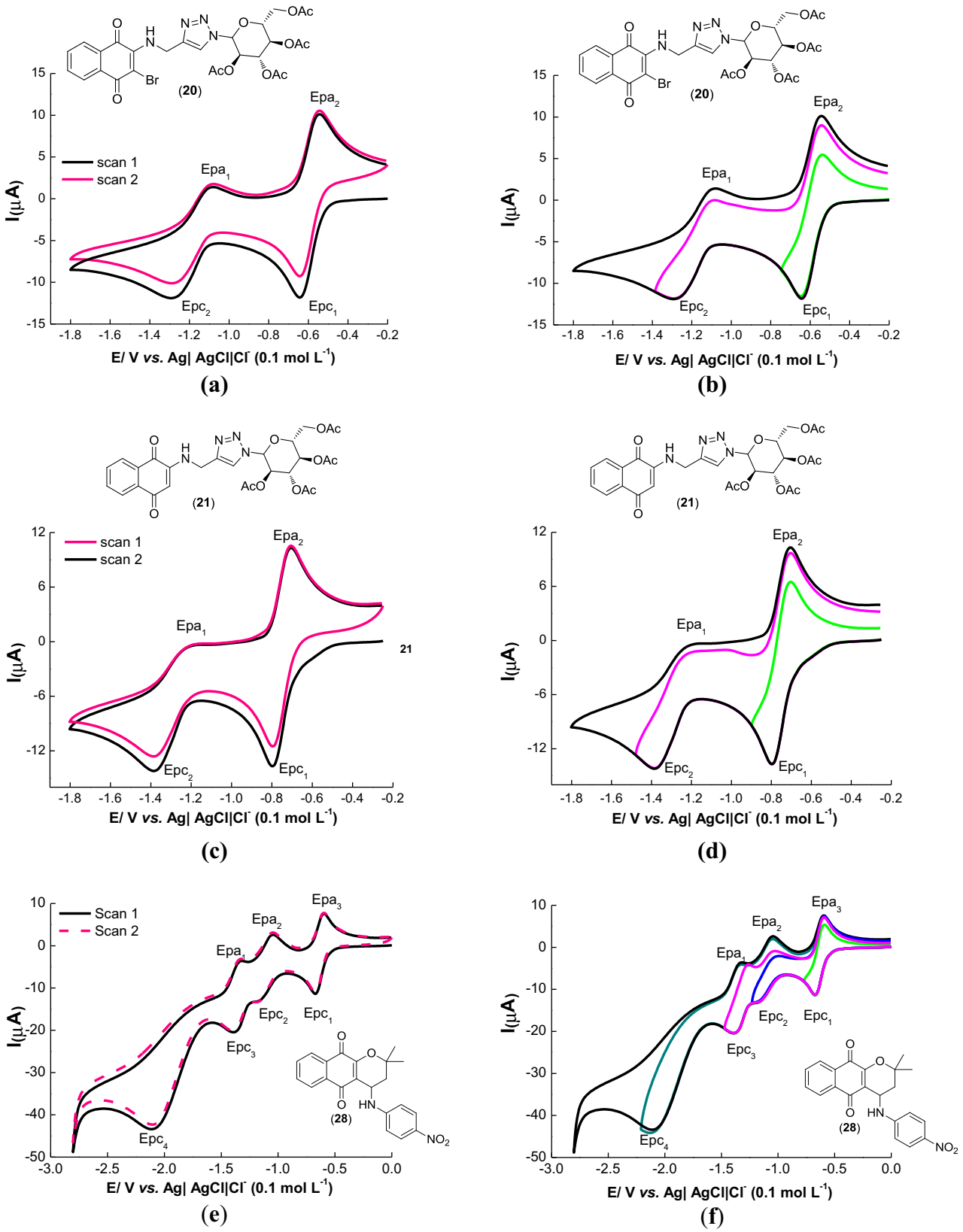


Figure 3. Cyclic voltammograms of naphthoquinones **20**, **21**, **28**, **34**, and **39** ($c = 1 \times 10^{-3}$ mol L^{-1}). DMF/TBAP (0.1 mol L^{-1}), glassy carbon electrode, versus Ag/AgCl, Cl^{-1}, cathodic direction, $\upsilon = 100$ mV s^{-1}.

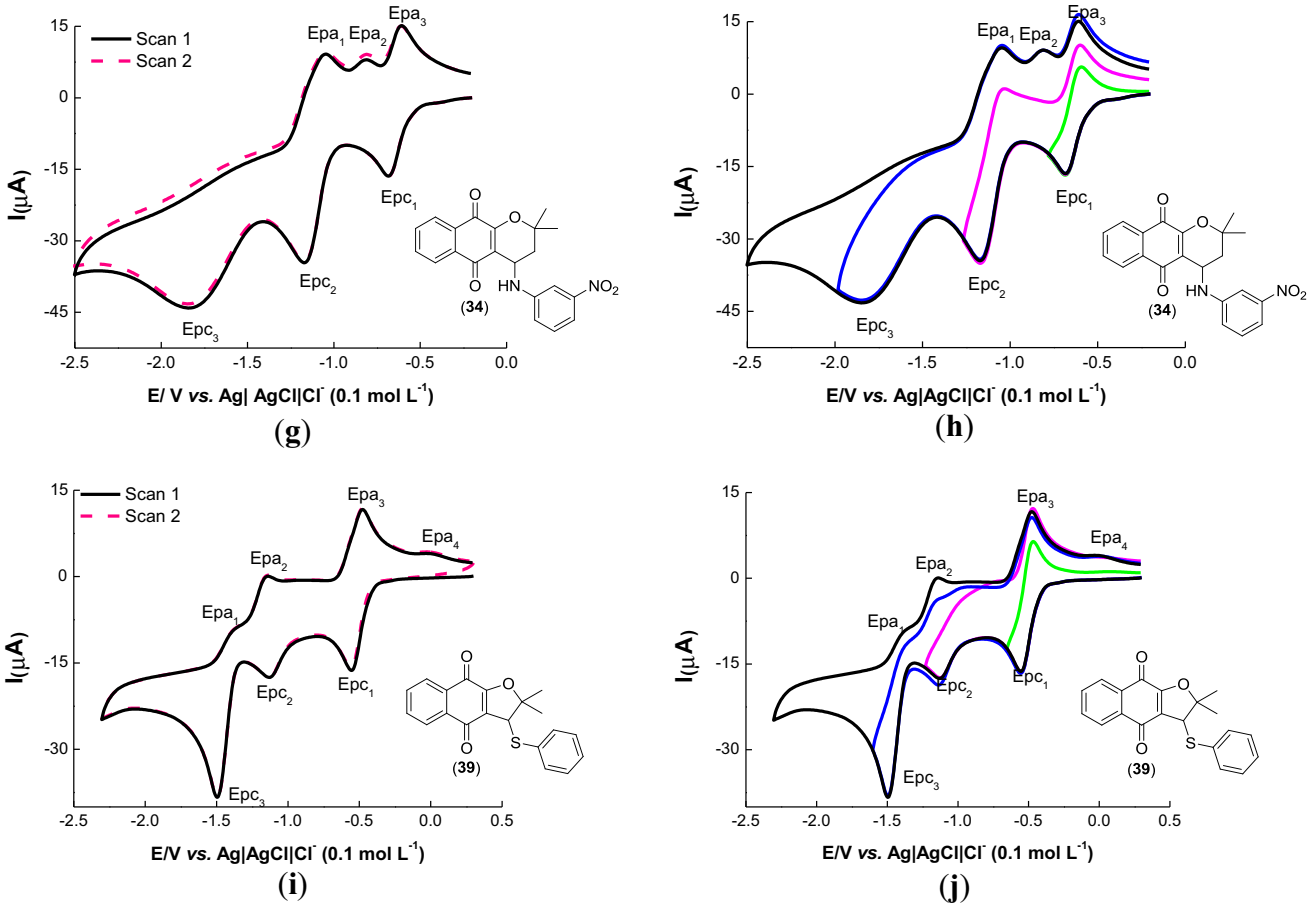


Fig. 3 (*continued*)

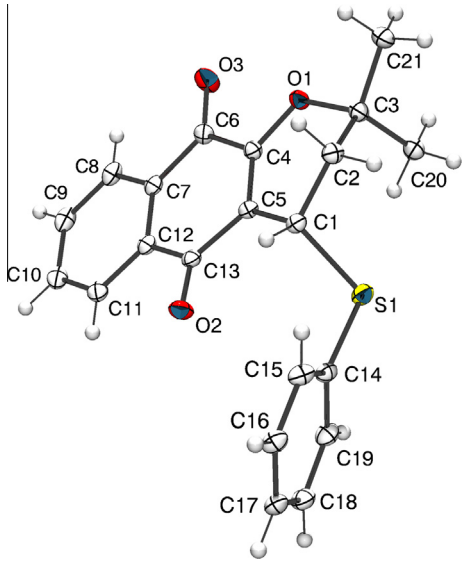


Figure 4. ORTEP-3 projection of compound **36**, showing atom labeling and displacement ellipsoids drawn at the 40% probability level.

Table 2
Crystal data and structure refinement for compound **36**

Empirical formula	$C_{21}H_{17}O_3S$	
Formula weight	349.41	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system, space group	Triclinic, $P\bar{1}$	
Unit cell dimensions	a = 9.1351(6) Å	α = 108.220(7)°
	b = 9.3814(8) Å	β = 100.266(6)°
	c = 10.4100(8) Å	γ = 97.646(6)°
Volume	816.66(11) Å^3	
Z, calculated density	2, 1.421 mg/m^3	
Absorption coefficient	0.216 mm^{-1}	
$F(000)$	366	
Crystal size	0.16 × 0.10 × 0.07 mm^3	
Theta range for data collection	2.12–29.55°	
Limiting indices	$-11 \leqslant h \leqslant 12$, $-7 \leqslant k \leqslant 12$, $-13 \leqslant l \leqslant 14$	
Reflections collected	6145	
Independent reflections	3797 [R(int) = 0.0274]	
Completeness to theta = 26.32°	100%	
Absorption correction	Analytical	
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/parameters	3797/0/228	
Goodness-of-fit on F^2	1.037	
Final R indices [$I > 2\sigma(I)$]	$R1$ = 0.0424, $wR2$ = 0.1036	
R indices (all data)	$R1$ = 0.0553, $wR2$ = 0.1128	
Largest diff. peak and hole	0.350 and −0.379 e Å^{-3}	

2.3. X-ray analysis

The Ortep-3 diagram of **36** is shown in Figure 4. The crystallographic data collection and refinement parameters are shown in Table 2. Compound **36** crystallizes in the triclinic space group $P\bar{1}$, with one molecule in the asymmetric unit. The bond lengths and angles are in good agreement with expected values, based on each atomic type. Two planes may be defined through the carbon skeleton of the molecule. Plane 1 is formed by the quinonoid ring

Table 3
Cytotoxic activity expressed by IC_{50} μg/mL [μM] (95% CI) of compounds **1**-**24**, **28**-**36** and **39** in cancer and normal cell lines after 72 h exposure, obtained by nonlinear regression for all cell lines from three independent experiments

Compounds	IC_{50} μg/mL [μM] (CI 95%)													
	HL-60	OVCAR-8	MDA-MB435	SF295	HCT-8	HCT-116	PC3	DU-145	MCF-7	MX1	HS578t	PBMC	L929	V79
1	0.52 [1.37] (0.39–0.61)	2.53 [6.67] (2.37–2.70)	1.05 [2.77] (0.94–1.12)	1.05 [2.77] (0.83–1.25)	1.69 [4.45] (1.46–1.73)	1.87 [4.93] (1.51–2.06)	1.39 [3.66] (1.22–1.48)	0.94 [2.48] (0.73–1.16)	1.20 [3.16] (1.08–1.37)	0.91 [2.40] (0.82–0.98)	1.17 [3.08] (1.02–1.30)	2.95 [7.77] (2.66–3.20)	1.89 [4.98] (1.53–2.07)	2.12 [5.59] (1.74–2.41)
2	0.81 [2.12] (0.69–0.96)	1.82 [4.77] (1.59–2.08)	0.80 [2.10] (0.61–0.93)	1.61 [4.22] (1.43–1.80)	1.04 [2.73] (0.83–1.21)	1.22 [3.20] (1.05–1.42)	1.17 [3.07] (1.08–1.24)	1.28 [3.36] (1.13–1.47)	1.19 [3.12] (1.10–1.28)	1.30 [3.41] (1.18–1.46)	1.16 [3.04] (0.92–1.27)	1.82 [4.77] (1.67–2.19)	1.47 [3.85] (1.13–1.70)	1.55 [4.06] (1.32–1.75)
3	4.08 [10.42] (3.79–4.23)	5.91 [15.10] (5.62–6.16)	4.17 [10.65] (3.78–4.39)	4.42 [11.29] (4.19–4.67)	4.81 [12.29] (4.79–4.85)	4.1 [10.47] (3.82–4.30)	4.38 [11.19] (4.04–4.77)	4.57 [11.67] (4.29–4.82)	>5 [>12]	>5 [>12]	4.25 [10.86] (4.11–4.48)	>5 [>12]	4.89 [12.49] (4.71–4.94)	4.70 [12.00] (4.61–4.87)
4	0.72 [1.90] (0.43–1.02)	2.03 [5.35] (1.91–2.25)	1.51 [3.98] (1.40–1.57)	4.24 [11.17] (3.94–4.51)	2.02 [5.32] (1.82–2.17)	1.85 [4.88] (1.57–2.17)	3.86 [10.17] (3.67–3.95)	3.75 [9.88] (3.62–3.81)	3.57 [9.41] (3.47–3.61)	2.78 [7.33] (2.55–2.93)	3.14 [8.28] (2.93–3.27)	3.42 [9.01] (3.20–3.58)	3.15 [8.30] (2.91–3.37)	2.74 [7.22] (2.59–2.93)
5	1.61 [4.34] (1.45–1.84)	2.68 [7.22] (2.46–2.81)	1.84 [4.95] (1.69–1.93)	1.28 [3.44] (0.93–1.45)	3.37 [9.07] (3.20–3.48)	3.07 [8.27] (2.61–3.47)	2.16 [5.82] (1.98–2.31)	1.74 [4.68] (1.52–1.90)	2.58 [6.95] (2.33–2.76)	2.20 [5.92] (2.07–2.41)	2.14 [5.76] (2.09–2.25)	2.98 [8.02] (2.74–3.14)	3.12 [8.40] (2.71–3.38)	2.83 [7.62] (2.57–3.04)
6	1.06 [2.70] (0.85–1.19)	2.03 [5.38] (1.95–2.17)	1.04 [2.76] (0.88–1.17)	1.2 [3.18] (1.08–1.37)	2.53 [6.70] (2.28–2.82)	2.08 [5.51] (1.81–2.20)	1.62 [4.29] (1.48–1.85)	2.07 [5.48] (1.83–2.29)	2.18 [5.78] (2.06–2.31)	1.59 [4.21] (1.38–1.74)	1.87 [4.95] (1.56–1.91)	2.78 [7.36] (2.59–2.94)	2.50 [6.62] (2.37–2.71)	2.31 [6.12] (2.16–2.54)
7	>5 [>12]	>5 [>12]	>5 [>12]	>5 [>12]	>5 [>12]	>5 [>12]	>5 [>12]	>5 [>12]	>5 [>12]	>5 [>12]	>5 [>12]	>5 [>12]	>5 [>12]	>5 [>12]
8	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]
9	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]
10	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]
11	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]
12	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]
13	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]
14	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]
15	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]
16	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]
17	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]	>5 [>9]
18	>5 [>13]	>5 [>13]	>5 [>13]	>5 [>13]	>5 [>13]	>5 [>13]	>5 [>13]	>5 [>13]	>5 [>13]	>5 [>13]	>5 [>13]	>5 [>13]	>5 [>13]	>5 [>13]
19	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]	>5 [>11]
20	0.79 [1.19] (0.43–1.36)	1.82 [2.80] (1.50–2.27)	1.77 [2.67] (1.42–2.01)	1.88 [2.83] (1.40–2.54)	1.46 [2.20] (1.14–1.82)	2.91 [4.39] (2.67–3.49)	>5 [>7]	2.7 [4.07] (2.28–3.10)	1.93 [2.91] (1.24–2.42)	2.38 [3.59] (2.07–2.63)	2.44 [3.68] (2.16–2.92)	3.16 [4.76] (2.90–3.27)	2.43 [3.66] (2.25–2.78)	2.38 [3.59] (1.81–2.67)
21	>5 [>8]	>5 [>8]	>5 [>8]	>5 [>8]	>5 [>8]	>5 [>8]	>5 [>8]	>5 [>8]	>5 [>8]	>5 [>8]	>5 [>8]	>5 [>8]	>5 [>8]	>5 [>8]
22	0.33 [0.80] (0.22–0.50)	1.18 [2.86] (0.97–1.42)	1.27 [3.09] (0.88–1.52)	1.57 [3.82] (1.07–2.29)	1.11 [2.70] (0.74–1.35)	>5 [>12]	2.06 [5.02] (1.58–2.37)	2.19 [5.34] (1.80–2.34)	1.51 [3.67] (1.17–1.93)	1.52 [3.70] (1.10–1.83)	2.33 [5.68] (2.04–2.56)	2.78 [6.77] (2.41–3.07)	2.14 [5.21] (1.91–2.43)	2.55 [6.21] (2.24–2.79)
23	1.52 [3.42] (1.15–1.82)	2.7 [6.02] (2.40–3.03)	1.48 [3.33] (1.12–1.93)	2.47 [5.55] (2.14–2.86)	3.15 [7.08] (2.75–3.62)	2.96 [6.65] (2.49–3.31)	2.41 [5.42] (2.07–2.84)	2.19 [4.92] (1.90–2.34)	3.45 [7.76] (3.28–3.81)	1.82 [4.02] (1.44–2.16)	2.08 [4.68] (1.73–2.31)	4.52 [10.16] (4.23–4.89)	3.04 [6.83] (2.72–3.41)	3.16 [7.10] (2.77–3.45)
24	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]	>5 [>10]
28	1.61 [4.26] (1.34–1.82)	2.52 [6.66] (2.18–2.80)	2.05 [5.42] (1.83–2.17)	2.25 [5.95] (2.01–2.48)	1.88 [4.97] (1.52–2.16)	2.14 [5.66] (1.84–2.32)	2.19 [5.79] (1.87–2.64)	2.32 [6.13] (1.88–2.85)	2.71 [7.16] (2.34–3.29)	2.09 [5.52] (1.76–2.45)	2.25 [5.95] (1.53–2.61)	0.76 [2.01] (0.60–0.97)	0.52 [1.37] (0.38–0.61)	0.61 [1.61] (0.47–0.73)

29	1.95 [4.73] (1.77–2.16)	2.28 [5.53] (2.07–2.40)	2.16 [5.24] (1.93–2.27)	2.63 [6.38] (2.49–2.84)	2.19 [5.31] (1.90–2.36)	2.41 [5.84] (2.27–2.61)	2.23 [5.41] (1.74–3.15)	2.77 [6.72] (1.97–3.88)	2.05 [4.97] (1.63–2.64)	2.61 [6.33] (2.17–3.06)	2.64 [6.40] (2.03–3.12)	1.19 [2.89] (0.87–1.47)	0.98 [2.38] (0.56–1.14)	1.05 [2.55] (0.70–1.25)
30	3.16 [6.88] (2.98–3.34)	>5 [>10]	4.14 [9.01] (3.75–4.47)	4.22 [9.19] (3.82–4.51)	>5 [>10]	>5 [>10]	4.16 [9.06] (3.81–5.08)	4.96 [10.80] (3.82–6.44)	3.46 [7.53] (3.15–4.10)	4.91 [10.69] (4.16–5.34)	4.21 [9.17] (3.59–5.37)	1.48 [3.22] (1.34–1.62)	1.06 [2.31] (0.73–1.20)	1.17 [2.55] (0.84–1.31)
31	0.36 [0.98] (0.23–0.45)	0.54 [1.47] (0.38–0.71)	0.37 [1.00] (0.22–0.50)	0.26 [0.71] (0.21–0.32)	0.24 [0.65] (0.10–0.31)	0.20 [0.54] (0.13–0.28)	0.37 [1.00] (0.28–0.53)	0.43 [1.17] (0.22–0.51)	0.36 [0.98] (0.14–0.48)	0.28 [0.76] (0.17–0.34)	0.22 [0.60] (0.13–0.29)	1.19 [0.52] (0.76–1.32)	0.68 (0.43–0.96) [1.85]	0.55 (0.33–0.69) [1.50]
32	0.07 [0.19] (0.03–0.15)	0.16 [0.44] (0.13–0.21)	0.21 [0.58] (0.10–0.22)	0.27 [0.74] (0.22–0.34)	0.17 [0.47] (0.11–0.20)	0.12 [0.33] (0.10–0.13)	0.20 [0.55] (0.11–0.25)	0.32 [0.88] (0.21–0.37)	0.37 [1.02] (0.32–0.42)	0.19 [0.52] (0.06–0.25)	0.25 [0.69] (0.18–0.30)	0.64 [1.76] (0.41–0.80)	0.27 [0.74] (0.19–0.31)	0.35 [0.96] (0.26–0.43)
33	nd	nd	nd	nd	nd	nd	2.73 [7.77] (2.22–3.16)	3.08 [8.76] (2.75–3.45)	2.43 [6.92] (2.11–2.94)	2.50 [7.11] (1.89–2.75)	2.70 [7.68] (2.46–3.07)	1.56 [4.44] (1.37–1.73)	1.14 [3.24] (0.91–1.25)	0.94 [2.68] (0.75–1.17)
34	0.57 [1.51] (0.43–0.75)	nd	0.55 [1.45] (0.40–0.78)	0.57 [1.51] (0.45–0.71)	2.43 [6.42] (2.31–2.55)	3.21 [8.48] (2.34–4.64)	nd	nd	nd	nd	nd	1.83 [4.84] (1.62–1.95)	1.75 [4.62] (1.59–1.82)	1.46 [3.86] (1.28–1.71)
35	3.16 [7.66] (2.87–3.41)	4.28 [10.38] (3.74–4.52)	3.96 [9.60] (3.70–4.16)	3.61 [8.76] (3.43–3.88)	>5 [>12]	>5 [>12]	4.07 [9.87] (3.51–4.92)	4.34 [10.53] (3.53–5.33)	4.12 [9.99] (3.44–5.18)	3.57 [8.66] (3.10–4.02)	4.72 [11.45] (4.10–5.61)	2.06 [5.00] (1.89–2.25)	1.57 [3.81] (1.39–1.76)	1.32 [3.20] (1.04–1.56)
36	1.02 [2.91] (0.77–1.35)	>5 [>14]	nd	[>14]	>5 [>14]	>5 [>14]	>5 [>14]	>5 [>14]	4.66 [13.29] (4.27–4.92)	4.13 [11.78] (3.88–4.30)	4.61 [4.61] (4.27–4.93)	>5 [>14]	>5 [>14]	>5 [>14]
39	0.16 [0.47] (0.11–0.25)	2.05 [6.09] (1.82–2.30	nd	3.08 [9.15] (2.92–3.25)	0.76 [2.25] (0.59–0.87)	1.11 [3.29] (0.93–1.33)	2.05 [6.09] (1.77–2.21)	2.35 [6.98] (2.11–2.56)	2.50 [7.43] (2.36–2.81)	2.17 [6.45] (1.91–2.25)	3.26 [3.26] (3.09–3.41)	>5 [>14.8]	4.64 [13.79] (4.26–5.10)	>5 [>14.8]
Doxorubicin	0.02 [0.03] (0.01–0.02)	0.19 [0.35] (0.14–0.25)	0.48 [0.83] (0.34–0.66)	0.23 [0.40] (0.19–0.25)	0.07 [0.13] (0.03–0.12	0.01 [0.02] (0.01–0.02)	0.24 [0.44] (0.21–0.27)	0.29 [0.53] (0.23–0.35)	0.07 [0.13] (0.17–0.24)	0.27 [0.50] (0.21–0.33)	0.20 [0.37] (0.15–0.26)	0.23[0.42] (0.10–0.38)	0.09 [0.16] (0.05–0.12)	0.13 [0.24] (0.10–0.17)

(atoms C4–C13), and C6 is the most distant atom from the mean plane of the ring (d(C6-plane equal to 0.524(13) Å). Notably, the oxygen atom O1 [d(O1-plane) = 0.0333(19) Å] lies in the plane as well. Atoms O2 [d(O2-plane) = 0.1932(19) Å] and O3 [d(O3-plane) = 0,1631(21) Å] are close to plane 1. Plane 2 is formed by the phenyl ring (C14–C19) and includes the sulfur atom, with d(S1-plane) equal to 0.0866(23) Å. Cremer and Pople ring puckering parameters[28] (Q = 0.4531(19) Å, θ = 52.7(2)°, Φ = 97.6(3)°) suggest that the pyran ring (C1, C2, C3, O1, C4 and C5) is in approximately a half-chair conformation.

2.4. Biological activity

All the substances described (Figs. 1 and 2 and Schemes 1–3), were evaluated *in vitro* using the MTT assay against eleven cancer cell lines: HL-60 (human promyelocytic leukemia), OVCAR-8 (ovarian adenocarcinoma), MDA-MB435 (human melanoma), SF295 (human glioblastoma), HCT-8 (Human colon carcinoma), HCT-116 (human colon carcinoma), PC3 (human prostate carcinoma), DU-145 (human prostate carcinoma), MCF-7 (human breast adenocarcinoma), MX1 (human breast adenocarcinoma) and HS578t (human breast adenocarcinoma). Doxorubicin was used as the positive control (Table 3).[29] The selectivity of the compounds toward a normal proliferating cell line was investigated using the MTT assay with human peripheral blood mononucluear cells (PBMC) after 72 h of drug exposure. To test with normal cells, two murine fibroblasts cell lines, L929 and V79, were used. As previously described,[30] 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).

In previous studies, we demonstrated that appending a 1,2,3-triazole group to nor-β-lapachone can intensify its antitumor[31] and trypanocidal[32] activities. Molecular hybridization[33] was used as a strategy to obtain new molecules by connecting a quinoidal moiety and a 1,2,3-triazole ring via click[19] chemistry reactions.

Herein, the antitumor activities of 1,4-naphthoquinone-based 1,2,3-triazoles were evaluated and ten compounds were observed to possess moderate to high activity.

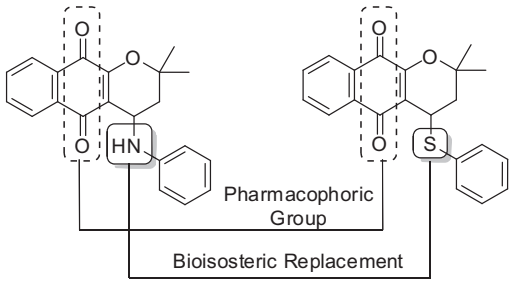
For compounds **7**–**19**, **21** and **24**, our approach was not successful and these compounds did not exhibit activity against any of the cancer cell lines evaluated.

For the quinone-based 1,2,3-triazoles, compounds **1**–**6** were considered moderately active with IC values in the range of 2.12–10.0 μM. However, high activity was observed against HL-60 with compounds **1** (IC_{50} = 1.37 μM) and **4** (IC_{50} = 1.90 μM).

For the 1,2,3-triazoles, the most significant activity was observed with compounds **1**, **4**, **21** and **22** against HL-60. They were highly active, with IC_{50} values of 1.37, 1.90, 1.19 and 0.80 μM, respectively.

Recently, our research group has described arylamino-substituted nor-β-lapachones with significant activity against cancer cell lines.[34] We observed that the presence of a methoxy group in the arylamino ring intensifies the antitumor activity. Herein, the evaluation of the arylamino substituted α-lapachones **28**–**35** was described. The compounds showed moderate to high activity against all cancer cell lines evaluated. Interestingly, compound **32**, with a methoxy group in the *para*-position, was found to be the most active, exhibiting IC_{50} values in the range of 0.19–1.76 μM. The cell line with the most sensitivity toward **32** was HL-60 (IC_{50} = 0.19 μM). Compound **31** has IC_{50} values < 2.00 μM and may also represent an important candidate for subsequent studies.

Compounds **36** and **39** were designed based on the bioisosteric replacement of the arylamino substituent in α-lapachone as shown in the Scheme 4. Our objective was to evaluate the influence of the sulfur atom (chalcogen) on activity. Compound **36** was highly



Scheme 4. Bioisosteric replacement strategy for **36** and **39**.

effective against the HL-60 cancer cell line (IC_{50} value = 2.91 μM) with a high selective index compared with PBMC, L929 and V79. Compound **39** was highly active against HL-60 and HCT-8.

Despite the small number of compounds, there was a positive correlation between the electrochemical parameters and cytotoxicity, allowing the discrimination of compounds **20** (active and more electrophilic) and **21** and the less cytotoxic compound **10** from the more selective compound **39**.

3. Conclusion

In this paper, we synthesized and evaluated the cytotoxicity of several 1,2,3-triazole-, arylamino- and thio-substituted naphthoquinones against cancer and normal cell lines. Of the described substances, compounds **31** and **32** were considered highly active (IC_{50} <2 μM). Considering the selectivity index, compounds **36** and **39** have been identified as lead compounds for further investigation. As previously shown by Claus Jacob's group, employing chalcogens is a productive approach. Our strategy to append arylamino groups to the 1,4-naphthoquinone structure was also successful, and several compounds with potent antitumor activity were presented in this manuscript that will be further investigated.

4. Experimental section

4.1. Chemistry

Melting points were obtained on Thomas Hoover and are uncorrected. Analytical grade solvents were used. Column chromatography was performed on silica gel (SiliaFlash G60 UltraPure—60–200 μm, 60 Å). Infrared spectra were recorded on an FTIR Spectrometer IR Prestige-21—Shimadzu. ^1H and ^{13}C NMR were recorded at room temperature using a Bruker AVANCE DRX200, Varian Mercury 300 and Varian Mercury 400 MHz, in the solvents indicated, with TMS as internal reference. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hertz. Mass spectra (electrospray ionization) were obtained using a MicroTOFIc—BrukerDaltonics. Elemental analysis was performed using a CE Instruments (Thermo-Fisher) EA 1110 CHNS-O elemental analyzer. All the compounds were nominated using the program CS ChemDraw Ultra version 10.0.

4.2. General procedures to prepare 1,4-naphthoquinone-based 1,2,3-triazoles

Method A: In a flask containing 3 mL acetonitrile, it was added 0.5 mmol of the appropriate azides, followed by 0.6 mmol of the appropriate alkynes and finally 11 mg (0.06 mmol) of copper(I) iodide. The reaction was left under magnetic stirring at 28 °C under argon atmosphere, for a reaction time ranging from 19 to 24 h. The end of the reaction was monitored by TLC with dichloromethane as eluent. The solvent was then removed under reduced pressure and the reaction mixture was purified on a silica gel column as a gradient mixture of hexane/ethyl acetate or dichloromethane/ethyl acetate with increasing polarity. *Method B*: The same procedures described in Method A, but 2 mL DMF, one drop Et_3N were added and the reaction was left under magnetic stirring for 60 min as monitored by TLC.

4.2.1. 2-(4-(4-(((3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)butyl)isoindoline-1,3-dione

Using method B, compound **14** was obtained as an orange solid (100 mg, 0.20 mmol, 78%); mp 230–232 °C. IR ν_{max} (cm^{-1}, KBr): 3312 (N–H). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.99–7.74 (m, 9H), 7.55 (br s, 1H, NH), 5.02 (d, 2H J = 6.6 Hz), 4.35 (t, 2H, J = 7.0 Hz), 3.58 (t, 2H, J = 7.1 Hz), 1.83 (qt, 2H, J = 7.0 Hz), 1.57 (qt, 2H, J = 6.6 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 179.8, 167.5, 144.6, 134.5, 134.0, 132.4, 131.4, 129.8, 126.2, 122.6, 122.3, 109.2, 48.6, 36.5, 26.8, 24.7. Anal. Calcd for $C_{25}H_{20}ClN_5O_4$ 0.65 × H_2O, C, 59.86, H, 4.28, N, 13.96. Found. C, 60.24, H, 4.22, N, 13.59.

4.2.2. (2*R*,3*R*,4*S*,5*R*)-2-(Acetoxymethyl)-6-(4-(((3-bromo-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate

Using method B, compound **20** was obtained as an orange solid (150 mg, 0.23 mmol, 87% yield); mp 152–153 °C. IR ν_{max} (cm^{-1}, KBr): 3339 (NH). ^1H NMR (400 MHz, acetone-d_6) δ: 8.23 (s, 1H), 8.07-8.02 (m, 2H), 7.83-7.74 (m, 2H), 7.19 (br s, 1H, NH), 6.22 (d, 1H, J = 9.0 Hz), 5.60 (dd, 1H, J= 9.4 and 9.4 Hz), 5.51 (dd, 1H, J = 9.0 and 9.0 Hz), 5.25-5.15 (m, 3H), 4.34–4.14 (m, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.93 (s, 3H). ^{13}C NMR (100 MHz, acetone-d_6) δ: 180.8, 180.2, 170.6, 170.1, 170.0, 169.1, 146.7, 135.4, 133.4, 132.9, 127.5, 127.1, 122.1, 85.8, 75.2, 73.3, 71.2, 68.7, 62.0, 20.5, 20.4, 20.0. Anal. Calcd for $C_{27}H_{27}BrN_4O_{11}$ 0.45 × H_2O, C, 48.29, H, 4.19, N, 8.34. Found: C, 48.65, H, 4.59, N, 8.01.

4.2.3. (2*S*,3*S*,4*R*,5*S*)-2-(Acetoxymethyl)-6-(4-(((1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate

Using method A, compound **21** was obtained as an orange solid (150 mg, 0.26 mmol, 62% yield); mp 209–210 °C. IR ν_{max} (cm^{-1}, KBr): 3351 (NH). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.08 (dd, 1H, J = 7.8 and 0.9 Hz), 8.05 (dd, 1H, J = 7.8 and 0.9 Hz), 7.80 (s, 1H), 7.73 (td, 1H, J = 7.6 and 1.5 Hz), 7.62 (td, 1H, J = 7.6 and 1.5 Hz), 6.35 (br s, 1H, NH), 5.88 (d, 1H, J= 8.5 Hz), 5.79 (s, 1H), 5.41 (t, 1H, J = 4.5 Hz), 5.24 (t, 1H, J = 5.1 Hz), 5.21 (t, 1H, J = 5.1 Hz), 4.52 (d, 1H, J = 5.1 Hz), 4.30 (dd, 1H, J = 12.6 and 4.8 Hz), 4.14 (dd, 1H, J = 12.6 and 4.8 Hz), 4.00 (dd, 1H, J = 9.9 and 3.3 Hz), 2.07 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 1.86 (s, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 183.0, 181.5, 170.5, 169.8, 169.3, 168.9, 151.4, 134.8, 132.2, 130.4, 126.4, 126.2, 107.6, 85.8, 75.2, 72.4, 70.2, 67.6, 61.4, 38.2, 20.7, 20.5, 20.4, 20.1. Anal. Calcd for $C_{27}H_{28}N_4O_{11}$ C, 55.48, H, 4.83, N, 9.58. Found: C, 55.46, 4.52, N, 9.25.

4.2.4. 2-(((1-(1,4-Dioxo-1,4-dihydronaphthalen-2-yl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)naphthalene-1,4-dione

Using method A, compound **22** was obtained as an orange solid (100 mg, 0.24 mmol, 78% yield); mp 228–229 °C. IR ν_{max} (cm^{-1}, KBr): 3344 (NH). 1605, 1572 (C=C). ^1H NMR (400 MHz, DMSO-d_6) δ: 8.65 (s, 1H), 8.13 (dd, 1H, J = 6.0 and 3.2 Hz), 8.06 (dd, 1H, J = 6.0 and 3.2 Hz), 8.00 (dd, 1H, J = 7.6 and 1.2 Hz), 7.96–7.93 (m, 4H), 7.82 (td, 1H, J = 7.6 and 1.6 Hz), 7.73 (td, 1H, J = 7.6 and 1.6 Hz), 7.48 (s, 1H), 5.87 (s, 1H), 4.64 (d, 2H, J = 6.0 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 183.8, 181.4, 181.3, 178.5, 148.0, 144.0, 140.0, 134.6, 134.5, 134.3, 132.8, 132.1, 131.2, 131.1, 130.1,

126.5, 126.1, 125.7, 125.6, 125.2, 125.1, 100.6, 36.9. Anal. Calcd for $C_{23}H_{14}N_4O_4$, 0.6 × H_2O, C, 65.98; H, 3.64; N, 13.3 Found: C, 65.98; H, 3.48; N, 12.90.

4.2.5. 2-Chloro-3-(((1-(1,4-dioxo-1,4-dihydronaphthalen-2-yl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)naphthalene-1,4-dione

Using method A, compound **23** was obtained as an orange solid (100 mg, 0.23 mmol, 68% yield); mp 219–221 °C. IR ν_{max} (cm^{-1}, KBr): 3325 (NH). 1606, 1574 (C═C).^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.59 (s, 1H), 8.11–7.94 (m, 7H), 7.83 (t, 1H, J = 7.8 Hz), 7.76 (t, 1H, J = 7.8 Hz), 7.47 (br s, 1H, NH), 5.14 (d, 2H, J = 6.6 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 184.0, 180.1, 178.7, 175.6, 146.4, 140.2, 134.9, 134.5, 132.9, 131.8, 131.2, 130.1, 129.0, 126.6, 125.8. Anal. Calcd for $C_{23}H_{13}ClN_4O_4$ 1.0 × H_2O, C, 58.87, H, 2.92, N, 12.48 Found: C, 58.52, H, 2.93, N, 12.19.

4.2.6. 2-Bromo-3-(((1-(1,4-dioxo-1,4-dihydronaphthalen-2-yl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)naphthalene-1,4-dione

Using method A, compound **24** was obtained as an orange solid (100 mg, 0.20 mmol, 99% yield); mp 168–170 °C. IR ν_{max} (cm^{-1}, KBr): 3301 (NH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.59 (s, 1H), 8.12 (dd, 1H, J = 5.6 and 3.2 Hz), 8.06 (dd, 1H, J = 6.0 and 3.2 Hz), 8.00–7.93 (m, 4H), 7.82 (td, 1H, J = 7.6 and 1.2 Hz) 7.75 (td, 1H, J = 7.6 and 1.2 Hz), 7.47 (s, 1H), 5.18 (d, 2H, J = 6.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 183.8, 179.6, 178.5, 175.2, 147.5, 146.0, 140.0, 134.6, 134.3, 132.6, 131.3, 131.1, 130.0, 126.5, 126.4, 126.1, 125.9, 125.6, 124.8. Anal. Calcd for $C_{23}H_{13}BrN_4O_4$, 1.1 × H_2O, C, 56.46; H, 2.68; N, 11.45 Found: C, 54.58; H, 2.69, H, 10.62.

4.2.7. General procedures to synthesis of α-lapachone arylamino derivatives

Compound **27** (321 mg, 1 mmol) was dissolved in 5 mL of CH_2Cl_2, the respective anilines were added (1.3 mmol) and the mixture was left under stirring overnight, followed by the addition of 50 mL of water. The organic phase was extracted with dichloromethane, washed with HCl 10% (3 × 50 mL), dried over sodium sulfate, and filtered. The solvent from the crude product was evaporated under reduced pressure and it was purified by column chromatography in silica-gel, using eluents with an increasing polarity gradient mixture of hexane and ethyl acetate (9/1 to 7/3).

4.2.8. 2,2-Dimethyl-4-((4-nitrophenyl)amino)-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione

The compound **28** was obtained as a red solid (227 mg, 0.6 mmol, 60% yield); mp 223–225 °C. IR ν_{max} (cm^{-1}, KBr): 3387 (NH), 1337 (N═O). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.13–7.92 (m, 2H), 7.78–7.59 (m, 2H), 7.23–7.02 (m, 1H), 6.60–6.32 (m, 3H), 4.64 (dd, 1H, J = 5.3 and 3.2 Hz), 2.28 (dd, 1H, J = 14.4 and 3.3 Hz), 1.99 (dd, 1H, J = 14.3 and 5.2 Hz), 1.55 (s, 3H), 1.53 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 183.7, 180.0, 155.6, 148.9, 134.5, 133.4, 132.2, 131.0, 130.7, 130.5; 126.6, 126.3, 118.7, 109.7, 109.6, 100.7, 79.3, 43.4, 37.6, 29.0, 26.3. EI/MS (m/z) $[M+Na]^+$: 401.1. Calcd for $[C_{21}H_{18}N_2O_5Na]^+$: 401.1.

4.2.9. 4-((4-Bromophenyl)amino)-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione

The compound **29** was obtained as a red solid (247 mg, 0.6 mmol, 60% yield); mp 174–176 °C. IR ν_{max} (cm^{-1}, KBr): 3386 (NH). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.22–7.99 (m, 2H), 7.82–7.65 (m, 2H), 7.29 (d, 2H, J = 8.6 Hz), 6.60 (d, 2H, J = 8.5 Hz), 4.64 (dd, 1H, J = 4.9 and 3.4 Hz), 2.25 (dd, 1H, J = 14.4 and 3.6 Hz), 2.00 (dd, 1H, J = 14.7 and 5.5 Hz), 1.54 (s, 3H), 1.53 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 183.8, 183.0, 155.5, 146.1, 134.6, 133.4, 132.2, 131.0, 126.6, 126.4, 118.8, 115.5, 110.2, 96.6, 79.3, 43.4, 37.6, 29.0, 26.4. EI/MS (m/z) $[M+H]^+$: 413.0. Calcd. for $[C_{21}H_{18}BrNO_3H]^+$: 413.0.

4.2.10. 4-((4-Iodophenyl)amino)-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione

The compound **30** was obtained as a red solid (229 mg, 0.5 mmol, 50% yield); mp 154–156 °C. IR ν_{max} (cm^{-1}, KBr): 3376 (NH). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.14–7.99 (m, 2H), 7.77–7.65 (m, 2H), 7.46 (d, 2H, J = 8.8 Hz), 6.50 (d, 2H, J = 8.6 Hz), 4.64 (dd, 1H, J = 5.3 and 3.5 Hz), 2.24 (dd, 1H, J = 14.3 and 3.2 Hz), 1.99 (dd, 1H, J = 14.5 and 5.4 Hz), 1.53 (s, 3H), 1.52 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 183.8, 180.0, 155.6, 146.5, 138.1, 134.6, 133.4, 132.1, 131.0, 126.6, 126.4, 123.3, 118.6, 116.1, 79.5, 79.3, 43.5, 37.5, 28.8, 24.4. EI/MS (m/z) $[M+H]^+$: 460.0. Calcd for $[C_{21}H_{18}INO_3H]^+$: 460.0.

4.2.11. 4-((4-Chlorophenyl)amino)-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione

The compound **31** was obtained as a red solid (184 mg, 0.5 mmol, 50% yield); mp 159–161 °C. IR ν_{max} (cm^{-1}, KBr): 3384 (NH). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.11–7.98 (m, 2H), 7.76–7.64 (m, 2H), 7.15 (d, 2H, J = 8.5 Hz), 6.64 (d, 2H, J = 8.6 Hz), 4.63 (dd, 1H, J = 5.1 and 2.7 Hz), 2.25 (dd, 1H, J = 14.4 and 3.5 Hz), 1.99 (dd, 1H, J = 14.4 and 5.1 Hz), 1.53 (s, 3H), 1.52 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 183.8, 180.0, 155.6, 145.6, 134.5, 133.4, 132.2, 131.1, 129.3, 126.6, 126.4, 123.3, 118.8, 115.2, 79.3, 43.8, 28.9, 26.4. EI/MS (m/z) $[M+H]^+$ 368.0. Calcd for $[C_{21}H_{18}ClNO_3H]^+$ 368.0.

4.2.12. 4-((4-Methoxyphenyl)amino)-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione

The compound **32** was obtained as a brown solid (137 mg, 0.37 mmol, 37% yield); mp 149–150 °C. IR ν_{max} (cm^{-1}, KBr): 3372 (NH). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.13–8.04 (m, 2H), 7.77–7.67 (m, 2H), 6.87–6.74 (m, 4H); 4.62–4.55 (m, 1H), 3.77 (s, 3H), 2.24 (dd, 1H, J = 14.2 and 4.0 Hz), 2.00 (dd, 1H, J = 14.1 and 5.6 Hz), 1.56 (s, 3H), 1.49 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 183.8, 179.9, 155.1, 153.1, 141.0, 134.2, 133.1, 132.0, 130.8, 126.3, 119.4, 116.6, 114.8, 79.2, 55.7, 44.7, 37.5, 28.6, 26.4. EI/MS (m/z) $[M+H]^+$: 364.0. Calcd for $[C_{22}H_{21}NO_4H]^+$: 364.0.

4.2.13. 4-((3-Fluorophenyl)amino)-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione

The compound **33** was obtained as a red solid (176 mg, 0.5 mmol, 50% yield); mp 152–154 °C. IR ν_{max} (cm^{-1}, KBr): 3382 (NH). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.15–7.99 (m, 2H), 7.77–7.64 (m, 2H), 7.19–7.06 (m, 2H), 6.55–6.40 (m, 2H), 4.67 (dd, 1H, J = 5.5 and 3.5 Hz), 2.27 (dd, 1H, J = 13.8 and 2.2 Hz), 2.02 (dd, 1H, J = 13.8 and 5.0 Hz), 1.54 (s, 3H), 1.53 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 183.8, 180.0, 172.7 155.6, 148.5 (d, J = 10.7 Hz), 134.6, 133.4, 132.2, 131.1, 126.5 (d, J = 10 Hz), 118.6, 109.8 (d, J = 2.3 Hz), 104.5 (d, J = 21.8 Hz), 100.5 (d, J = 24.9 Hz), 79.3, 43.6, 37.6, 28.9, 26.4 EI/MS (m/z) $[M+H]^+$: 352.0. Calcd for $[C_{21}H_{18}FNO_3H]^+$: 352.0.

4.2.14. 2,2-Dimethyl-4-((3-nitrophenyl)amino)-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione

The compound **34** was obtained as a orange solid (189 mg, 0.5 mmol, 50% yield); mp 152–154 °C. IR ν_{max} (cm^{-1}, KBr): 3386 (NH). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.10–7.95 (m, 2H), 7.75–7.58 (m, 2H), 7.09–6.55 (m, 4H), 4.87–4.61 (m, 1H), 2.35–2.17 (m, 1H), 2.14–1.98 (m, 1H), 1.58 (s, 3H), 1.56 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 183.6, 179.4, 155.5, 149.2, 134.3, 133.3, 131.8, 130.6, 130.5, 129.7, 126.5, 125.9, 125.8, 119.0, 117.8, 112.5, 79.1, 43.0, 37.1, 28.8, 26.0. EI/MS (m/z) $[M+H]^+$: 401.0. Calcd for $[C_{21}H_{18}N_2O_5Na]^+$: 401.0.

4.2.15. 4-((3-Bromophenyl)amino)-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione

The compound **35** was obtained as a red solid (247 mg, 0.6 mmol, 60% yield); mp 182–185 °C. IR ν_{max} (cm^{-1}, KBr): 3410 (NH). ^1H NMR (200 MHz, $CDCl_3$) δ: 8.10–7.95 (m, 2H); 7.75–7.58 (m, 2H), 7.09–6.55 (m, 4H), 4.17–4.59 (m, 1H), 2.26 (dd, 1H, J = 14.4 and 2.2 Hz), 1.99 (dd, 1H, J = 14.1 and 5.3 Hz), 1.54 (s, 3H), 1.52 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 183.4, 179.7, 155.3, 148.1, 134.3, 133,2, 131.9, 130.8, 130.5, 126.4, 126.1, 123.2, 121.0, 118.4, 116.0, 112.3, 79.0, 43.1, 37.3, 28.8, 26.1. EI/MS (m/z) $[M+H]^+$: 413.0. Calcd for $[C_{21}H_{18}BrNO_3H]^+$: 413.0.

4.2.16. General procedures to the synthesis of α-lapachone arylamino derivatives

The respective bromo derivatives (1 mmol) were dissolved in 5 mL of CH_2Cl_2, and thiophenol was added (1.3 mmol) and the mixture was left under stirring overnight, followed by the addition of 50 mL of water. The organic phase was extracted with dichloromethane, dried over sodium sulfate, and filtered. The solvent from the crude mixture was evaporated under reduced pressure and it was purified by column chromatography in silica-gel, using eluents with an increasing polarity gradient mixture of hexane and ethyl acetate (9/1 to 7/3).

4.2.17. 2,2-Dimethyl-4-(phenylthio)-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione

The compound **36** was obtained as a yellow solid (125 mg, 0.5 mmol, 50% yield); mp 182–185 °C. IR ν_{max} (cm^{-1}, KBr): 718 (C–S) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ: 8.12–8.00 (m, 2H), 7.90–7.75 (m, 2H), 7.70–7.62 (m, 2H), 7.46–7.31 (m, 3H), 4.58–4.50 (m, 1H), 2.10–2.00 (m, 2H), 1.61 (s, 3H), 1.48 (s, 3H). EI/HRMS (m/z) $[M+Na]^+$ 373.0905. Calcd for $[C_{21}H_{18}O_3SNa]^+$ 373.0874.

4.2.18. 2,2-Dimethyl-3-(phenylthio)-2,3-dihydronaphtho[2,3-*b*]furan-4,9-dione

The compound **39** was obtained as a yellow solid (150 mg, 0.45 mmol, 45% yield); mp 148–151 °C. IR (KBr): 718 (C–S) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ: 8.15–8.02 (m, 2H), 7.80–7.61 (m, 2H), 7.59–6.51 (m, 2H), 7.35–7.19 (m, 3H), 4.58 (s, 1H), 1.80 (s, 3H), 1.58 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 181.3, 178.2, 158.7, 135.0, 134.5, 133.4, 133.1, 132.7, 131.7, 129.3, 128.0, 126.5, 126.4, 123.6, 94.4, 59.2, 28.5, 24.3. EI/MS (m/z) $[M+Na]^+$: 359.0. Calcd for $[C_{20}H_{16}O_3SNa]^+$: 359.0.

4.3. Cytotoxicity against cancer and normal cell lines

Compounds (0.001–30 μM) were tested for cytotoxic activity against several human cancer cell lines obtained from National Cancer Institute, NCI (Bethesda, MD). The L929 cells (mouse fibroblast L cells NCTC clone 929) were obtained from American Type Culture Collection (Manassas, VA), and the Chinese hamster lung fibroblasts (V79 cells) were kindly provided by Dr. JAP Henriques (UFRGS). Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood from healthy, non-smoker donors who had not taken any medication at least 15 days prior to sampling by a standard method of density-gradient centrifugation on Histopaque-1077 (Sigma Aldrich Co., St. Louis, MO, USA). All cancer cell lines and PBMC were maintained in RPMI 1640 medium. L929 and V79 cells were cultivated under standard conditions in MEM with Earle's salts. All culture media were supplemented 20% (PBMC) or 10% (cancer, L929 and V79 cells) fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin at 37 °C with 5% CO_2. All tested compounds were dissolved with DMSO. The final concentration of DMSO in the culture medium was kept constant (0.1%, v/v). Doxorubicin (0.001–1.10 μM) was used as the positive control. 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.[29]

4.4. Electrochemical studies

Cyclic voltammetry (CV) experiments were performed with a conventional undivided three electrode cell using an Autolab PGSTAT-30 potentiostat (Echo Chemie, Utrecht, The Netherlands) coupled to a microcomputer, interfaced by GPES 4.9 software. Glassy carbon (GC) (diameter = 3 mm) as the working electrode, a Pt wire as the counter electrode and the reference electrode an Ag|AgCl, Cl^- (saturated) were used. The GC electrode was cleaned up by polishing with alumina on a polishing felt (BAS polishing kit). The solvent used in aprotic media studies, DMF from Acros, was used as received. In CV experiments, the scan rate varied from 10 to 500 mV s^{-1}. All experiments were conducted at room temperature (25 ± 2 °C) and purging an inert gas (Argon). Electrochemical reduction in aprotic media (DMF + TBAP 0.1 mol L^{-1}) was performed in the absence of oxygen. Each compound (1×10^{-3} mol L^{-1}) was added to the supporting electrolyte and the solution was deoxygenated with argon before the measurements by cyclic voltammetry.

4.5. X-ray analysis

X-ray data were collected at 150 K using MoKα (0.71073 Å) on an Agilent–Gemini diffractometer equipped with a CCD area detector. The CrysAlisPro software package[35] was used for data collection and data reduction. The data were corrected empirically for absorption using spherical harmonics using the SCALE3 ABSPACK[36] scaling algorithm. The structure was solved by direct methods using SHELXS-97[37] and refined by full-matrix least squares on F^2 using SHELXL-97.[38] All non-hydrogen atoms were successfully refined using anisotropic displacement parameters. Hydrogen atoms were found in the Fourier difference synthesis and fixed. Crystallographic data for the structure were deposited in the Cambridge Crystallographic Data Centre, with number CCDC 964308.

Conflict of interest

Authors declare no conflict of interest.

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