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PII: S0223-5234(16)30497-4

DOI: 10.1016/j.ejmech.2016.06.019

Reference: EJMECH 8680

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 27 November 2015

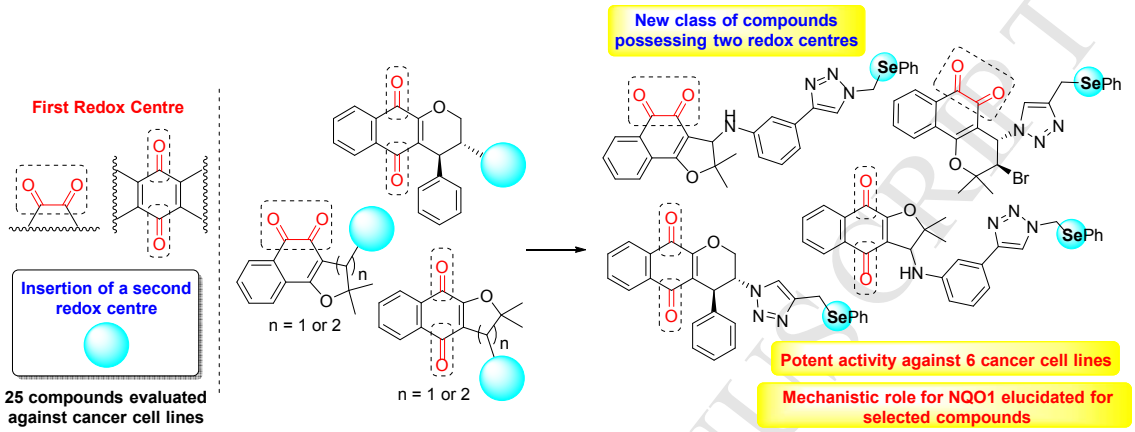
Revised Date: 2 June 2016

Accepted Date: 11 June 2016

Please cite this article as: 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, E.N. da Silva Júnior, Synthesis and antitumor activity of selenium-containing quinone-based triazoles possessing two redox centres, and their mechanistic insights, *European Journal of Medicinal Chemistry* (2016), doi: 10.1016/j.ejmech.2016.06.019.

Graphical abstract

Selenium-containing quinones were designed and synthesized by click chemistry reaction and evaluated against several human cancer cell lines showing, in some cases, IC_{50} values below 0.3 µM.



Synthesis and Antitumor Activity of Selenium-Containing Quinone-based Triazoles Possessing Two Redox Centres, and their Mechanistic Insights

Eduardo H. G. da Cruz,[a] Molly A. Silvers,[b] Guilherme A. M. Jardim,[a] Jarbas M. Resende,[a] Bruno C. Cavalcanti,[c] Igor S. Bomfim,[c] Claudia Pessoa,[c,d] Carlos A. de Simone,[e] Giancarlo V. Botteselle,[f] Antonio L. Braga,[f] Divya K. Nair,[g] Irishi N. N. Namboothiri,[g] David A. Boothman,[b] and Eufrânio N. da Silva Júnior[a]*

[a]Institute of Exact Sciences, Department of Chemistry, Federal University of Minas Gerais, CEP 31270-901, Belo Horizonte, MG, Brazil; [b]Departments of Pharmacology and Radiation Oncology, Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, 6001 Forest Park Road, Dallas, TX 75390-8807, USA; [c]National Laboratory of Experimental Oncology, Department of Physiology and Pharmacology, Federal University of Ceará, CEP 60180-900, Fortaleza, CE, Brazil; [d]Fiocruz-Ceará, CEP 60180-900, Fortaleza, CE, Brazil; [e]Institute of Physics, University of São Paulo, 13560-160, São Carlos, SP, Brazil; [f]Department of Chemistry, Federal University of Santa Catarina, 88040-900, Florianópolis, SC, Brazil; [g]Department of Chemistry, Indian Institute of Technology Bombay, Mumbai 400 076, India.

Corresponding author: E.N. da Silva Júnior: E-mail: eufranio@ufmg.br; Fax: +55 31 34095700; Tel.: +55 31 34095720.

Abstract: Selenium-containing quinone-based 1,2,3-triazoles were synthesized using click chemistry, the copper catalyzed azide-alkyne 1,3-dipolar cycloaddition, and evaluated against six types of cancer cell lines: HL-60 (human promyelocytic leukemia cells), HCT-116 (human colon carcinoma cells), PC3 (human prostate cells), SF295 (human glioblastoma cells), MDA-MB-435 (melanoma cells) and OVCAR-8 (human ovarian carcinoma cells). Some compounds showed IC_{50} values < 0.3 μM. The cytotoxic potential of the quinones evaluated was also assayed using non-tumor cells, exemplified by peripheral blood mononuclear (PBMC), V79 and L929 cells. Mechanistic role for NAD(P)H:Quinone Oxidoreductase 1 (NQO1) was also elucidated. These compounds could provide promising new lead derivatives for more potent anticancer drug development and delivery, and represent one of the most active classes of lapachones reported.

Keywords: β-Lapachone; Quinone; Click chemistry, Chalcogens, Selenium

1. Introduction

Development of diverse therapeutics is of paramount importance in the fight against different types of cancer [1,2]. Quinones are considered as privileged structures and are among the most important drugs used against cancer [3]. Although single-target drugs successfully inhibit or activate a specific target [4], drugs that are able to act simultaneously on diverse biological targets are more attractive in the design of new effective drugs [5]. In this context, quinoidal structures represent an essential multi-target class of compounds [6].

Naturally occurring naphthoquinones such as lapachol and β-lapachone (β-lap), isolated from the heartwood of *Tabebuia*, are among the most studied for their potential anti-tumor activity [7]. Docampo et al. [8] found significant activity for β-lap against Sarcoma 180 ascites tumor cells (S-180 cells) *in vitro*, and in mice bearing S-180 tumors. Although the antitumor activity of β-lap against Yoshida sarcoma and Walker 256 carcinoma cells in culture has been investigated [8,9], the exact mechanism of action was not known until recently [10].

β-Lapachone specifically destroys cancer cells with elevated endogenous levels of NAD(P)H:quinone oxidoreductase 1 (NQO1) [11] regardless of p53, caspase, or cell cycle status [12]. While in clinical trials, β-lap (i.e., ARQ 501) has been inaccurately touted as a cell cycle checkpoint activator [13], the major determinant of cell death is through NQO1 expression [11,12a,14]. The drug is not a substrate for known multidrug resistance or drug pumps [15,16] and β-lap cell death is not affected by changes in cell cycle position, oncogenic drivers, or pro- or anti-apoptotic factors [11,12a]. Finally, the drug targets (i.e., is 'bioactivated' by) NQO1, a Phase II, carcinogen-inducible enzyme that is also induced by ionizing radiation (IR) in some cancer, but not normal, cells [17,18].

β-Lap's use as a chemotherapeutic agent is curtailed by its high hydrophobicity which causes methemoglobinemia in patients [19]. When mixed with the carrier hydroxypropyl-β-cyclodextrin, the carrier itself can contribute to hemolysis [20]. Recently, Ohayon and coworkers [21] shed light on the hypothesis of β-lap being able to act nonreversibly for inhibition of deubiquitinases. These authors suggested that the therapeutic effect of β-lap could be also related to ubiquitin specific peptidase 2 (USP2) oxidation, which is likely a downstream effect of reactive oxygen species (ROS) generation via NQO1 futile cycle metabolism of β-lap. NQO1 is the unique gene, that

when deleted, leads to resistance to β-lap and other NQO1 bioactivatable drugs [22,23], strongly suggesting that most downstream effects cited by others are a result of upregulation of NQO1-derived ROS and Ca^{2+} intracellular increases, as well as rapid and dramatic losses in NAD^+/ATP caused by PARP1 hyperactivation ending in NAD^+-Keresis [24].

In the last few years, our group has been intensively dedicated to the synthesis and evaluation of lapachones against cancer cell lines [25,26]. We discovered a series of lapachones with modified C-rings (Scheme 1a) with potent activity against cancer lineages [27]. Lately, interest in preparing quinone-based triazoles has been stimulated by our discovery of bioactive compounds endowed with unique subunits in their chemical structures [28]. Recently, Perumal et al. [29] prepared amino-1,4-naphthoquinone-appended triazoles with antimycobacterial activity designed by the same molecular hybridization strategy [30]. β-Lapachone-based 1,2,3-triazoles possess significant activity with IC_{50} values below 2 μM for MDA-MB-435 cancer cells. These compounds promoted cell death by an apparent apoptotic cell death mechanism associated with significant ROS production [31]. The approach of inserting a triazole moiety in 1,4-naphthoquinones was also effective, since this unit is known as a potent pharmacophoric group [32]. Recently, 1,4-naphthoquinone-based 1,2,3-triazoles (Scheme 1a) were reported as having high activity in the range of 1.4 to 1.9 μM in HL-60 human promyelocytic leukemia cells [33].

From another perspective, organoselenium compounds show antitumor, antimicrobial, anti-neurodegenerative and antiviral properties [34]. A series of selenoproteins are involved in important physiological processes [35]. Jacob and co-workers [36] demonstrated the potential antitumor activity of selenium-containing quinones (Scheme 1b) capable of mimicking the enzymatic activity of the human enzyme, glutathione peroxidase (GPx). GPx targets redox sensitive thiol proteins, while simultaneously generating reactive oxygen species at a critical threshold. Thus, these drugs act as ROS-users and ROS-enhancers to affect downsteam targets [37]. This action would complement the mechanism of action of β-lap, since death caused by this agent relies upon the hyperactivation of PARP1, which is stimulated by ROS (H_2O_2) [24].

In continuation of our program for obtaining novel potent antitumor naphthoquinones and based on recent findings reported by our group, we discovered

potent chalcogen-containing β-lapachones (Scheme 1b) [38]. Here, we describe fifteen novel selenium-containing quinones and our strategy was based on inserting this pharmacophoric group, generating 1,2,3-triazole selenium-containing lapachones (Scheme 1c). Selected naphthoquinones with a structural framework with recognized activity against several types of cancer cell lines were used in the preparation of the new compounds. The structures were designed as multi-target ligands potentially giving rise to NQO1 cell death mechanisms of action.

Scheme 1.

2. Results and Discussion

2.1 Chemistry

The first class of compounds prepared possessing two redox centres was selenium-containing dihydropyran naphthoquinones obtained from lapachol (**1**) (Scheme 2). α-Lapachone **2** was prepared by acid catalyzed cyclization from **1**, and then two selenium-containing derivatives, **6** and **7**, were synthesized from **2**. Compound **6**, was prepared in moderate yield (75%) by copper(I) catalyzed click reaction [39] between compound **4** and (azidomethyl)(phenyl)selane. The intermediate compound **4** was obtained by the reaction of 3-ethynylaniline and the bromo derivative **3**. As previously reported [40], 4-azide-α-lapachone (**5**) was easily synthesized from the reaction of **3** with sodium azide in dichloromethane. The reaction of **5** and phenyl propargyl selenide affords selenium-containing α-lapachone 1,2,3-triazole **7**. Finally, from the azide derivative **9**, prepared as reported by us [41], β-lapachone-based 1,2,3-triazole **10** containing the chalcogen was obtained as a red solid. Compounds **3**-**10** are racemic. However, compounds **9** and **10** are single diastereomers, the relative stereochemistry is *trans*. The *trans*-stereochemistry was confirmed by comparison with previously reported data [41,47a].

Scheme 2.

We began the synthesis of selenium-containing dihydrofuran naphthoquinones, the second class of compounds, initially by synthesizing nor-α-lapachone derivatives **15** and **16** (Scheme 3). Since the synthesis of arylamino substituted lapachones and

azidoquinones are well established in our group [25,40,42], compounds **13** and **14** were prepared as shown in Scheme 3. Following click methodology, compounds **13** and **14** were reacted with selenium-containing azide and alkyne, respectively, to furnish the naphthoquinones **15** and **16** in 70% and 80% yield, respectively.

Scheme 3.

From nor-lapachol (**17**), the bromo intermediate **18** was synthesized following the methodology described by Pinto and co-workers (Scheme 4) [26,43]. Synthesis of various antitumor compounds from **18** was reported, as for instance, arylamino and alkoxy substituted nor-β-lapachone [26], lapachones in the presence of 1,2,3-triazole moiety [44] and hybrids with chalcones [45]. The unpublished arylamino substituted lapachone **19** bearing a terminal alkyne group was prepared based on the previously described compounds possessing activity against cancer cell lines [26]. The formation of the selenium-containing 1,2,3-triazole **21** from **19** herein described, allowed us to access the product designed with two redox centres. Using the same strategy discussed above, compound **22** was obtained from the azide derivative **20**, previously reported by our group [46] (Scheme 4).

Scheme 4.

At this juncture, we described the synthesis of lapachones obtained from lapachol (**1**) and nor-lapachol (**17**), their inferior homologue. Recently, we reported the synthesis of a new class of naphthoquinone compounds, containing a pendant 1,2,3-triazole motif from C-allyl lawsone (**23**) [47]. The iodination of **23** affords compounds **24** and **27** in 68% yield and 1:1 ratio (Scheme 5), which were easily separated by column chromatography. With these compounds in hand, the respective azide derivatives, compunds **25** and **28**, were synthesized by reaction of sodium azide in dimethylformamide. The respective selenium derivatives, compounds **26** and **29**, were prepared by Cu-catalyzed azide-alkyne cycloaddition (Scheme 5).

Scheme 5.

1,4-naphthoquinone coupled to selenium-containing 1,2,3-triazole was also a subject of our study. From compounds **33**-**35** and **39**, the respective triazolic derivatives, compounds **36**-**38** and **40**, were prepared using methodology discussed previously (Scheme 6). Suitable crystals of compounds **35** and **38** were obtained, and the structures were solved by crystallographic methods.

Scheme 6.

Recently, we reported a straightforward approach for the obtention of enantio-enriched α-lapachone derivatives [48]. In order to identify new enantio-enriched antitumor compounds, lawsone (**41**) was used to prepare the nitro-derivatives **42** and **43** via organocatalysis with a chiral squaramide (Scheme 7). The nitro group of the quinones was reduced to the amino group using $NiCl_2{\cdot}6H_2O/NaBH_4$ to afford aminoquinones **44** and **45**. These compounds were used immediately after synthesis, due to their instability, for the preparation of azidoquinones **46** and **47**, after a diazotransfer reaction. In the last step, we prepared the chalcogenium-containing 1,2,3-triazoles **48** and **49** for reaction with phenyl propargyl selenide by using a classical click procedure via catalysis with sodium ascorbate and copper(II) sulfate in a 1:1 mixture of dichloromethane and water.

Scheme 7.

Structures of the novel compounds **4**, **6**, **7**, **10**, **13**, **15**, **16**, **19**, **21**, **22**, **26**, **29**, **36**, **37**, **38**, **40**, **48** and **49** were determined by ^{1}H and ^{13}C NMR and 2D NMR spectra (COSY, HMBC and HSQC). Electrospray ionization mass spectra were also obtained to confirm compound identities.

2.2. Biological Studies

All of the selenium-containing quinone-based 1,2,3-triazoles described (Schemes 2-7) and their synthetic precursors were evaluated *in vitro* using the MTT assay against six cancer cell lines: HL-60 (human promyelocytic leukemia cells, NQO1-), HCT-116 (human colon carcinoma cells, NQO1), PC3 (human prostate cells,

NQO1+), SF295 (human glioblastoma cells, NQO1+), MDA-MB-435 (melanoma cells, NQO1+) and OVCAR-8 (human ovarian carcinoma cells, NQO1+). β-Lapachone and doxorubicin were used as positive controls (Table 1). NQO1- normal cells, human peripheral blood mononucluear (PBMC), and murine fibroblast immortalized cell lines (V79 and L929) were used to evaluate the selectivity of the compounds. Mechanistic aspects of selected compounds were also studied for NQO1-dependency using the fairly specific NQO1 inhibitor, dicoumarol. As previously described [49], 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).

The results showed most of compounds were highly active against all cancer cell lines evaluated, with IC_{50} values < 2 μM. In general terms, *ortho*-quinoidal compounds were more active than *para*-quinones. However, α-lapachone derivatives with potent antitumor activities were also identified.

Naphthopyranquinones **5-7**, **9** and **10** presented high to moderate activities (IC_{50} in the range of 0.92 to 5.46 μM) and the non-active compound **4** was the exception of this class. For the selenium-containing quinones **6** and **10**, the strategy of insertion of a second redox centre was a success and these derivatives were more active than their naphthoquinoidal precursors. Recently, we reported the synthesis and antitumor activities of several α-lapachone-based 1,2,3-triazoles [33]. It is important to highlight that the selenium-containing-based 1,2,3-triazole **7** displayed better activity than the compounds without the chalcogen.

Naphthofuranquinones were the second class of compounds evaluated. *Para*-naphthoquinones **15** and **16** were active against all cancer cell lines studied. In the last few years, we described nor-α-lapachone-based 1,2,3-triazoles obtained from lapachol (**1**) with IC_{50} values > 2 μM [33]. The strategy herein used to prepare compounds **15** and **16** with the presence of selenium improved the activities of nor-α-lapachone-based 1,2,3-triazoles, and these derivatives presented IC_{50} values ranging from 0.68-1.71 μM for **15**, and 1.59-2.95 μM for **16**.

Nor-β-lapachone and derivatives are among the most potent compounds from the lapachol group [25,26]. Recently, we demonstrated [50] the cytotoxicity and genetic toxicity of nor-β-lapachone in human lymphocytes, HL-60 leukaemia, and immortal normal murine V79 fibroblasts ranging from 2.5 and 5 μM. This compound failed to induce DNA damage in nontumor cells, but at the highest concentrations, it induced DNA single and double strand breaks and increased the frequency of chromosomal

aberrations. The biological effects of nor-β-lapachone is related to its ability to deplete reduced glutathione (GSH), which leads to a GSSG-dominant pro-oxidant cellular status that contributes to its antiproliferative properties.

In this context, we described the potent antitumor activities of nor-β-lapachones [26,28]. Importantly, C-ring modified, nor-β-lapachone with arylamino groups were the most active lapachones described [26]. These compounds present significant antiproliferative effects in human myeloid leukaemia cell lines and induce oxidative DNA damage by ROS generation. They also somehow impair DNA repair activity, while triggering cell death, which may be apoptosis [51]. Compound **21** was designed based on prior experience with these bioactive lapachones. This compound contains the structural framework of the 3-arylamino-nor-β-lapachone derivatives reported before, but with a second redox chalcogen centre inserted by click chemistry reaction. This substance was highly active against all cancer cell lines evaluated, with IC_{50} values ranging from 0.07 to 0.38 μM. Moreover, compound **21** exhibited a high selectivity index (SI represented by the ratio of cytotoxicities between normal cells and different lines of cancer cells). For instance, PBMC *vs* HL-60 = 19.8. By comparison, doxorubicin, a standard clinically used drug against various types of cancers, contained a selectivity index value of 10.6. In the same way, compound **22** (IC_{50} in the range of 1.06 to 2.56 μM) was more active than nor-β-lapachone-based 1,2,3-triazole without the chalcogen atom. Herein, two important examples of successful preparation of potent antitumor quinones with two redox centres are reported. Compound **26**, another nor-lapachone derivative obtained from C-allyl lawsone (**23**), also exhibited potent antitumor activity. This drug was considered highly active with IC_{50} values ranging from 0.07 to 0.29 μM, suggesting a highly active structure. Compounds **21** and **26** presented similar antitumor activities, showing the importance of the *ortho*-naphthofuranquinone moiety and the triazole selenium-containing group that potentially works together in the same two redox centre structures.

1,4-Naphthoquinones **36**-**40** were also evaluated and the compounds were considered moderately active with exception of compound **40**, which was inactive against all cancer cells examined. The last compounds evaluated were asymmetric α-lapachone **48** and **49** and these substances were inactive. As recently described by us [45], the asymmetric α-lapachone derivatives are inactive against several cancer cell lines evaluated. Herein, we tried to improve their activities using the approach of

insertion of the chalcogen in these quinones, but the strategy failed. Selectivity index for the most active compounds was summarized in Table 2.

Table 1.

Table 2.

Previous studies from our laboratory revealed that compounds **50**-**53** (Figure 1) can be considered as prototypes possessing potent antitumor activities against diverse cancer cells [25,26]. To deepen our knowledge about the mechanism of action of compounds **50**-**53** and compare the previously reported structures with selenium-containing lapachone-based 1,2,3-triazoles, we examined their potential NQO1-dependent cytotoxicities using a set two-hour exposure, with or without the NQO1 inhibitor, dicoumarol. Such exposures take advantage of elevated NQO1 levels specifically in most solid cancers compared to associated normal tissue [11]. Within the class of selenium-containing quinones, we selected compounds **21** and **22** to evaluate their characteristics by an NQO1-dependent mechanism.

Figure 1.

NQO1-dependency assessments. Within the drug concentration range tested, the compounds showed activity against human A549 non-small cell lung adenocarcinoma, an alveolar basal epithelial cell line. These cells express high levels of NQO1 (3000 + 300 enzymatic units). Cell death for many of the compounds tested were NQO1-specific, since addition of dicoumarol (DIC, an NQO1 inhibitor) spared their lethality. Based on the survival curves (Figure 2), previously reported arylamine substituted nor-β-lapachones have predicted IC_{50} as follows, compounds: **50** = 2.6 μM, **51** = 1.8 μM, **52** = 2.4 μM and **53** = 1.3 μM. Compound **53** showed the most dramatic lethality within a narrow therapeutic window, going from 93% viability at 0.8 μM to 11% viability at 1.6 μM. Overall, compounds **50**-**53** were NQO1-specific drugs exhibiting similar or lower IC_{50} values than β-lapachone. Compounds **21** and **22**, selenium-containing quinones, with IC_{50} values = 0.64 and 1.2 μM, respectively, were the most active of this series and were NQO1-dependent (Table 3). They showed

tremendous therapeutic windows using DIC treatment as a surrogate for responses to NQO1- cells, such as that found for nearly all human normal tissue [12a]. These responses are indicative of NQO1-dependent futile redox cycling of these drugs that create massive ROS, specifically H_2O_2, that ultimately cause PARP1 hyperactivation and programmed necrosis [23,24,52].

Figure 2.

Table 3.

The externalization of phosphatidylserine is considered an important marker in the apoptotic process. After treatments, selected compounds **21** and **22** induced a significant increase on populations of PC3 cells with phosphatidylserine expressed on the cell surface (Figure 3). On the other hand, phosphatidylserine externalization was not observed in cultures pre-treated with NAC before **21** and **22** exposure or co-treated with dicoumarol (Figure 3). Our data show that cytotoxic mechanisms of tested compounds may involve drug bioreduction by quinone reductase NQO1 as well emphasizing the ROS contribution on the cytotoxicity suggesting that tested compounds-induced apoptosis is associated with ROS production. Finally, corroborating these studies, we observed that a short exposure (1 h) to compound **21** led to the generation of intracellular ROS. In other hand, in cultures pre-exposed with NAC, compound **21** was not able to generate ROS, which may be explained due the antioxidant protection exercised by NAC (See Figure S1 in the Supporting Information).

Figure 3.

3. Conclusions

By using the strategy of conjoining two redox centres, a quinoidal moiety and the atom of selenium, we prepared and evaluated the activities of novel and diverse selenium-containing quinone-based 1,2,3-triazole compounds against cancer versus normal cell lines. We assessed these drugs for lethality overall (Table 1) as well as for specific role of NQO1. In general, our approach was efficient and we identified

compounds with IC_{50} values below 0.3 µM that were more potent than β-lapachone or doxorubicin, a standard clinically used agent against several types of cancers. We also studied the efficacy of two more active compounds **21** and **22** and found that these are specifically bioactivated by NQO1 with higher potency than nor-β-lapachone, previously published by us. Interestingly, we found that several of these drugs contained glutathione peroxidase (GPx)-like activities with selected selenium-containing nor-β-lapachone-based 1,2,3-triazoles being the most potent, and functionally acting on these two targets. Annexin V cytometry assay was also used to visualize the cell population in viable, early and late apoptosis stage for compounds **21** and **22**. The cytotoxic mechanisms of **21** and **22** are intrinsically related with ROS contribution on the cytotoxicity suggesting that apoptosis is associated with ROS production. In order, we have described different classes of quinones, *ortho*- and *para*-quinoidal systems with potent antitumor activity. For example, compound **29** (*para*-quinone) has IC_{50} in the range of 0.62 to 2.42 µM in the cancer cell lines evaluated. *Ortho*-quinones, exemplified for compounds **10**, **21**, **22** and **26**, presented IC_{50} among 0.07 to 2.52 µM. Finally, we have described potent antitumor naphthoquinone compounds that emerge as promising molecules for the therapeutic use of cancers overexpressing NQO1.

4. Experimental Section

4.1. Chemistry

4.1.1. General procedures

Lawsone were acquired from Sigma-Aldrich (St. Louis, MO, USA). Lapachol (**1**) (2-hydroxy-3-(3'-methyl-2'-butenyl)-1,4-naphthoquinone) was extracted from the heartwood of *Tabebuia* sp. (Tecoma). C-allyl lawsone (**23**) was prepared from lawsone as previously reported [53]. A saturated aqueous sodium carbonate solution was added to the sawdust of ipe tree. Upon observing rapid formation of lapachol sodium salt, hydrochloric acid was added, allowing the precipitation of lapachol. Then, the solution was filtered and a yellow solid was obtained. This solid was purified by recrystallizations with hexane. All chemicals were obtained from commercial sources and used without further purification. Solvents were distilled and when required were dried by distillation according to standard procedure [54].

For the synthesis of (azidomethyl)(phenyl)selane ($PhSeCH_2N_3$): Initially (chloromethyl)(phenyl)selane was prepared by the reaction of a solution of diphenyl diselenide (3.0 mmol) in THF (6.0 mL) with $NaBH_4$ (2 eq.) in EtOH (6.0 mL) and CH_2Cl_2 (30 mL). The mixture was kept under reflux in inert atmosphere and stirred for 12 h. After this period and subsequently extraction with H_2O, the organic phase were combined, dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel using hexane as the eluent [55]. To a solution of (chloromethyl)(phenyl)selane ($PhSeCH_2Cl$) (3.0 mmol) in CH_3CN (5.0 mL), sodium azide (4.5 mmol) and 18-crown-6 (0.60 mmol) were added at room temperature. Then the reaction mixture was stirred for 48 h under nitrogen atmosphere. After this time, 30 mL of H_2O was added and the organic phase was extracted with CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel using hexane as the eluent. The product was obtained in 90% yield [56].

For the synthesis of phenyl propargyl selenide: To a solution of diphenyl diselenide (1.0 mmol) in THF (8.0 mL) with $NaBH_4$ (2 eq.) in EtOH (4 mL). The mixture was kept under agitation at temperatue of 0 ºC under inert atmosphere and, then, propargyl bromide (2.0 mmol) in THF (4 mL) was added. After 10 minutes, 30 mL of H_2O was added and the organic phase was extracted with ethyl acetate. The organic phases were combined, dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel using hexane as the eluent. The product was obtained in 85% yield [57].

Melting points were obtained on a Thomas Hoover and are uncorrected. 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 and DRX400 MHz, in the solvents indicated, with tetramethylsilane (TMS) as internal reference. Chemical shifts (δ) are given in parts per million (ppm) and coupling constants (J) in Hertz (Hz). The mass spectrometer was operated in the positive ion mode. A standard atmospheric pressure photoionization (APPI) source was used to generate the ions. The sample was injected using a constant flow (3 µL/min). The solvent was an acetonitrile/methanol mixture. The APPI-Q-TOF MS instrument was calibrated in the mass range of 50-3000 m/z using an internal calibration standard (low concentration tuning mix solution) supplied by Agilent Technologies. Data were

processed employing Bruker Data Analysis software version 4.0. Compounds were named following IUPAC rules as applied by ChemBioDraw Ultra (version 12.0).

4.1.2. Procedures to prepare arylamino lapachones 4, 13 and 19

To prepare 4 and 13: Compounds **3** and **12** (1.0 mmol) were dissolved in 25 mL of CH_2Cl_2 and an excess of 3-ethynylaniline (117 mg, 1.2 mmol) was added. The mixture was left under stirring overnight, followed by the addition of 50 mL of water. The organic phase was extracted with CH_2Cl_2, washed with 10% HCl (3 x 50 mL), dried over sodium sulfate, and filtered. The solvent from the crude was evaporated under reduced pressure and it was purified by column chromatography on silica-gel, using eluents with an increasing polarity gradient mixture of hexane and ethyl acetate (9/1 to 7/3).

To prepare 19: To a solution of nor-lapachol (**17**) (228 mg, 1.0 mmol) in 25 mL of chloroform, 2 mL of bromine was added. The bromo intermediate **18** precipitated immediately as an orange solid. After removal of bromine, by adding dichloromethane and then removing the organic solvent with dissolved bromine by rotary evaporator, an excess of 3-ethynylaniline (117 mg, 1.2 mmol) was added in CH_2Cl_2 and the mixture was stirred overnight. The crude reaction mixture was poured into 50 mL of water. The organic phase was separated and washed with 10% HCl (3 x 50 mL), dried over sodium sulfate, filtered, and evaporated under reduced pressure. The product **19** was obtained after purification by column chromatography in silica-gel, eluted with an increasing polarity gradient mixture of hexane and ethyl acetate (9/1 to 7/3).

4.1.3. General procedures to prepare the azide derivatives

The previously published azido derivatives **5**, **9**, **14**, **20**, **25**, **39**, **46** were prepared as described in the literature [40-42,46-48]. Compound **28** (1.0 mmol) was prepared from **27** in the presence of sodium azide (120 mg, 1.85 mmol) in 2 mL of dimethylformamide (DMF). The mixture was stirred at room temperature until product formation was complete as determined by thin layer chromatography. After extraction with CH_2Cl_2, the residue was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Compound **28** was obtained after purification by column chromatography on silica gel eluting with a gradient mixture of hexane:ethyl acetate

with increasing polarity. The unpublished azide derivative **47** was prepared following the same procedure previously described [48].

4.1.4. General procedures for the preparation of 1,2,3-triazole derivatives

The azidoquinones (1.0 mmol) or quinone with terminal alkynes (1.0 mmol) were reacted with $CuSO_4{\cdot}5H_2O$ (0.04 mmol) and sodium ascorbate (0.11 mmol) and the phenyl propargyl selenide (195 mg, 1.0 mmol) or (azidomethyl)(phenyl)selane (212 mg, 1.0 mmol) in a mixture of CH_2Cl_2:H_2O (12 mL, 1:1, v/v). The mixture was stirred at room temperature until product formation was complete as determined by thin layer chromatography. The aqueous phase was extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with a gradient mixture of hexane:ethyl acetate with increasing polarity.

4.1.5. 4-((3-ethynylphenyl)amino)-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione (4). Yield: 70%; mp 196-197 ºC; Brown solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.11-8.03 (m, 2H), 7.75-7.68 (m, 2H), 7.16 (t, *J* = 7.5 Hz, 1H), 6.95-6.85 (m, 2H), 6.71 (d, *J* = 8.0 Hz, 1H), 4.68 (t, *J* = 3.7 Hz, 1H), 3.03 (s, 1H), 2.27 (dd, *J* = 3.5 and 14.4 Hz, 1H), 2.02 (dd, *J* = 5.5 and 14.4 Hz, 1H), 1.54 (s, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 183.7, 180.0, 155.5, 146.3, 134.6, 133.3, 132.1, 131.0, 129.4, 126.5, 126.3, 123.0, 122.8, 118.4, 117.1, 115.2, 84.1, 79.2, 43.7, 37.4, 28.7, 26.3; HRMS (ES^+) calculated for $C_{23}H_{20}NO_3$ $[M+H]^+$: 358.1443; found: 358.1488.

4.1.6. 2,2-dimethyl-4-((3-(1-((phenylselanyl)methyl)-1*H*-1,2,3-triazol-4-yl)phenyl)amino)-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione (6). Yield: 75%; mp 101-102 ºC; Red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (dd, *J* = 7.1 and 1.7 Hz, H_9), 8.07 (dd, *J* = 7.1 and 1.6 Hz, H_6), 7.72 (td, *J* = 7.1, 7.1 and 1.6 Hz, H_8), 7.68 (td, *J* = 7.1, 7.1 and 1.7 Hz, H_7), 7.62 (s, H_5-triazole), 7.50 (d, *J* = 7.4 Hz, $H_{2'',6''}$), 7.33-7.39 (m, $H_{4''}$), 7.26-7.28 (m, $H_{2'}$), 7.28-7.33 (m, $H_{3'',5''}$), 7.20-7.26 (m, $H_{5'}$), 7.05 (d, *J* = 7.7 Hz, $H_{4'}$), 6.69 (dd, *J* = 8.0 and 1.8 Hz, $H_{6'}$), 5.71 (s, H_{12}), 4.78 (dd, *J* = 5.6 and 3.4 Hz, H_4), 2.32 (dd, *J* = 14.3 and 3.4 Hz, H_{3a} or H_{3b}), 2.06 (dd, *J* = 14.3 and 5.6 Hz, H_{3a} or H_{3b}), 1.55 (s, $H_{11'}$ or $H_{11''}$), 1.54 (s, $H_{11'}$ or $H_{11''}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 183.5 (C_5), 180.0 (C_{10}), 155.3 (C_{10a}), 148.5 (C_4-triazole), 147.4 ($C_{1'}$), 134.8 ($C_{2'',6''}$), 134.3

(C_8), 133.1 (C_7), 132.1 (C_{9a}), 131.3 ($C_{3'}$), 130.9 (C_{5a}), 129.8 ($C_{5'}$), 129.6 ($C_{3'',5''}$), 129.0 ($C_{4''}$), 127.4 ($C_{1''}$), 126.4 (C_9), 126.2 (C_6), 119.4 (C_5-triazole), 118.9 (C_{4a}), 115.7 ($C_{4'}$), 113.6 ($C_{6'}$), 110.7 ($C_{2'}$), 79.1 (C_2), 44.8 (C_{12}), 43.2 (C_4), 37.6 (C_3), 29.0 ($C_{11'}$ or $C_{11''}$), 26.1 ($C_{11'}$ or $C_{11''}$); HRMS (ES^+) calculated for $C_{30}H_{27}N_4O_3Se$ $[M+H]^+$: 571.1248; found: 571.1234.

4.1.7. 2,2-dimethyl-4-(4-((phenylselanyl)methyl)-1*H*-1,2,3-triazol-1-yl)-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione (7). Yield: 70%; mp 168-169 ºC; Yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.18-8.09 (m, 1H), 8.04-7.96 (m, 1H), 7.80-7.68 (m, 2H), 7.50-7.39 (m, 2H), 7.24-7.10 (m, 4H), 5.71 (t, *J* = 6.3 Hz, 1H), 4.13 (s, 2H), 2.73 (dd, *J* = 5.5 and 14.4 Hz, 1H), 2.29 (dd, *J* = 6.3 and 14.4 Hz, 1H), 1.50 (s, 3H), 1.21 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 182.7, 179.5, 156.3, 145.3, 134.7, 133.7, 131.8, 131.1, 129.7, 129.1, 127.5, 126.8, 126.6, 122.0, 115.0, 79.4, 49.6, 39.0, 27.0, 26.5, 20.8; HRMS (ES^+) calculated for $C_{24}H_{21}N_3O_3SeNa$ $[M+Na]^+$: 502.0646; found: 502.0643.

4.1.8. 3-bromo-2,2-dimethyl-4-(4-((phenylselanyl)methyl)-1*H*-1,2,3-triazol-1-yl)-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione (10). Yield: 90%; mp 105-106 ºC; Orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (dd, *J* = 7.6 and 1.4 Hz, H_7), 7.90 (dd, *J* = 7.6 and 1.2 Hz, H_{10}), 7.73 (td, *J* = 7.6, 7.6 and 1.2 Hz, H_8), 7.63 (td, *J* = 7.6, 7.6 and 1.4 Hz, H_9), 7.48-7.52 (m, $H_{2',6'}$), 7.48 (s, H_5-triazole), 7.22-7.25 (m, $H_{3',5'}$), 7.22-7.25 (m, $H_{4'}$), 5.55 (d, *J* = 8.9 Hz, H_4), 4.93 (d, *J* = 8.9 Hz, H_3), 4.17 (d, *J* = 13.3 Hz, H_{12a}), 4.13 (d, *J* = 13.3 Hz, H_{12b}), 1.71 (s, $H_{11'}$ or $H_{11''}$), 1.64 (s, $H_{11'}$ or $H_{11''}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.8 (C_6), 176.4 (C_5), 162.7 (C_{10b}), 144.5 (C_4-triazole), 135.2 (C_8), 134.1 ($C_{2',6'}$), 132.3 (C_9), 130.6 (C_{10a}), 130.5 (C_{6a}), 129.4 ($C_{1'}$), 129.2 (C_7), 129.1 ($C_{3',5'}$), 127.6 ($C_{4'}$), 125.3 (C_{10}), 125.0 (C_5-triazole), 110.3 (C_{4a}), 83.4 (C_2), 54.4 (C_3), 58.9 (C_4), 20.7 ($C_{11'}$ or $C_{11''}$), 27.4 ($C_{11'}$ or $C_{11''}$), 20.7 (C_{12}); HRMS (ES+) calculated for $C_{24}H_{21}BrN_3O_3Se$ $[M+H]^+$: 557.9931; found: 557.9923.

4.1.9. 3-((3-ethynylphenyl)amino)-2,2-dimethyl-2,3-dihydronaphtho[2,3-*b*]furan-4,9-dione (13). Yield: 65%; mp 165-167 ºC; Brown solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.14-8.04 (m, 2H), 7.78-7.66 (m, 2H), 7.15 (t, *J* = 8.0 Hz, 1H), 6.91 (d, *J* = 7.5 Hz, 1H), 6.74 (s, 1H), 6.62 (dd, *J* = 2.1 and 8.0 Hz, 1H), 4.87 (s, 1H), 3.03 (s, 1H), 2.27 (s, 1H), 1.65 (s, 3H), 1.54 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 181.7, 178.7, 159.9, 146.8, 134.6, 133.2, 133.1, 131.5, 129.4, 126.5, 126.3, 123.0, 122.4, 122.1, 116.3,

114.0, 94.9, 83.9, 62.2, 29.5, 27.1, 21.5; HRMS (ES^+) calculated for $C_{22}H_{17}NO_3Na$ $[M+Na]^+$: 366.1106; found: 366.1257.

4.1.10. 2,2-dimethyl-3-((3-(1-((phenylselanyl)methyl)-1*H*-1,2,3-triazol-4-yl)phenyl)amino)-2,3-dihydronaphtho[2,3-*b*]furan-4,9-dione (15). Yield: 70%; mp 120-122 ºC; Red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (dd, *J* = 7.4 and 1.4 Hz, H_8), 8.07 (dd, *J* = 7.4 and 1.6 Hz, H_5), 7.73 (td, *J* = 7.4, 7.4 and 1.6 Hz, H_7), 7.68 (td, *J* = 7.4, 7.4 and 1.4 Hz, H_6), 7.60 (s, H_5-triazole), 7.50 (d, *J* = 7.5 Hz, $H_{2'',6''}$), 7.27-7.39 (m, $H_{3'',5''}$), 7.27-7.39 (m, $H_{4''}$), 7.22 (t, *J* = 7.8 Hz, $H_{5'}$), 7.20 (s, $H_{2'}$), 7.04 (d, *J* = 7.8 Hz, $H_{4'}$), 6.61 (dd, *J* = 7.8 and 1.8 Hz, $H_{6'}$), 5.71 (s, H_{11}), 4.96 (s, H_3), 4.05 (sl, N<u>H</u>), 1.67 (s, $H_{10'}$ or $H_{10''}$), 1.57 (s, $H_{10'}$ or $H_{10''}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.8 (C_4), 178.6 (C_9), 159.8 (C_{9a}), 148.3 (C_4-triazole), 147.5 ($C_{1'}$), 134.8 ($C_{2'',6''}$), 134.5 (C_7), 133.2 (C_{8a}), 133.1 (C_6), 131.9 ($C_{3'}$), 131.6 (C_{4a}), 129.9 ($C_{5'}$), 129.6 ($C_{3'',5''}$), 129.0 ($C_{4''}$), 127.4 ($C_{1''}$), 126.5 (C_8), 126.2 (C_5), 122.3 (C_{3a}), 119.4 (C_5-triazole), 115.9 ($C_{4'}$), 113.1 ($C_{6'}$), 110.5 ($C_{2'}$), 95.5 (C_2), 62.3 (C_3), 44.7 (C_{11}), 27.2 ($C_{10'}$ or $C_{10''}$), 21.6 ($C_{10'}$ or $C_{10''}$); HRMS (ES^+) calculated for $C_{29}H_{25}N_4O_3Se$ $[M+H]^+$: 557.1092; found: 557.1101.

4.1.11. 2,2-dimethyl-3-(4-((phenylselanyl)methyl)-1*H*-1,2,3-triazol-1-yl)-2,3-dihydronaphtho[2,3-*b*]furan-4,9-dione (16). Yield: 80%; mp 172-174 ºC; Yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (dd, *J* = 7.4 and 1.6 Hz, H_8), 8.08 (dd, *J* = 7.4 and 1.6 Hz, H_5), 7.80 (td, *J* = 7.4, 7.4 and 1.6 Hz, H_7), 7.76 (td, *J* = 7.4, 7.4 and 1.6 Hz, H_6), 7.40 (dd, *J* = 7.9 and 1.4 Hz, $H_{2'/6'}$), 7.08-7.19 (m, $H_{3'/5'}$), 7.08-7.19 (m, $H_{4'}$), 6.98 (s, H_5-triazole), 5.92 (s, H_3), 4.14 (d, *J* = 13.5 Hz, H_{11}), 4.09 (d, *J* = 13.5 Hz, H_{11}), 1.67 (s, $H_{10'}$ or $H_{10''}$), 1.02 (s, $H_{10'}$ or $H_{10''}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.5 (C_4), 177.8 (C_9), 151.1 (C_{9a}), 145.8 (C_4-triazole), 134.9 (C_7), 133.9 ($C_{2'/6'}$), 133.6 (C_6), 132.7 (C_{8a}), 131.6 (C_{4a}), 129.13 ($C_{1'}$), 129.06 ($C_{3'/5'}$), 127.5 ($C_{4'}$), 126.8 (C_8), 126.5 (C_5), 121.2 (C_5-triazole), 118.3 (C_{3a}), 94.5 (C_2), 67.3 (C_3), 27.4 ($C_{10'}$ or $C_{10''}$), 20.7 ($C_{10'}$ or $C_{10''}$), 20.5 (C_{11}); HRMS (ES^+) calculated for $C_{23}H_{19}N_3O_3SeNa$ $[M+Na]^+$: 488.0489; found: 488.0486.

4.1.12. 3-((3-ethynylphenyl)amino)-2,2-dimethyl-2,3-dihydronaphtho[1,2-*b*]furan-4,5-dione (19). Yield: 70%; mp 205-206 ºC; Red solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.10 (dd, *J* = 2.0 and 8.0 Hz, 1H), 7.76-7.58 (m, 3H), 7.12 (t, *J* = 7.8 Hz, 1H), 6.88 (d, *J* = 7.8 Hz, 1H), 6.69 (s, 1H), 6.58 (dd, *J* = 2.0 and 8.0 Hz, 1H), 4.79 (s, 1H), 3.02 (s, 1H),

1.68 (s, 3H), 1.57 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 175.4, 169.7, 147.1, 134.7, 132.7, 131.2, 129.6, 129.4, 128.6, 125.2, 122.9, 122.2, 116.1, 115.0, 114.1, 96.8, 84.1, 61.5, 27.4, 21.8; HRMS (ES^+) calculated for $C_{22}H_{17}NO_3Na$ $[M+Na]^+$: 366.1106; found: 366.1108.

4.1.13. 2,2-dimethyl-3-((3-(1-((phenylselanyl)methyl)-1*H*-1,2,3-triazol-4-yl)phenyl)amino)-2,3-dihydronaphtho[1,2-*b*]furan-4,5-dione (21). Yield: 50%; mp 135-137 ºC; Red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, *J* = 7.4 Hz, H_6), 7.70 (td, *J* = 7.4, 7.4 and 1.1 Hz, H_7), 7.74 (dd, *J* = 7.4 and 1.1 Hz, H_9), 7.64 (td, *J* = 7.4, 7.4 and 1.6 Hz, H_8), 7.59 (s, H_5-triazole), 7.50 (d, *J* = 6.9 Hz, $H_{2'',6''}$), 7.28-7.40 (m, $H_{3'',5''}$), 7.28-7.40 (m, $H_{4''}$), 7.14 (s, $H_{2'}$), 7.20 (dd, *J* = 8.1 and 7.7 Hz, $H_{5'}$), 7.02 (d, *J* = 7.7 Hz, $H_{4'}$), 6.56 (dd, *J* = 8.1 and 2.2 Hz, $H_{6'}$), 5.51 (s, H_{11}), 4.89 (s, H_3), 3.70-4.37 (sl, N<u>H</u>), 1.60 (s, $H_{10'}$ or $H_{10''}$), 1.17 (s, $H_{10'}$ or $H_{10''}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.0 (C_5), 175.4 (C_4), 169.6 (C_{9b}), 148.3 (C_4-triazole), 147.8 ($C_{1'}$), 134.8 ($C_{2'',6''}$), 134.6 (C_7), 132.5 (C_8), 131.3 ($C_{3'}$), 131.2 (C_{9a}), 129.8 ($C_{5'}$), 129.6 ($C_{3'',5''}$), 129.5 (C_6), 128.9 ($C_{4''}$), 127.5 (C_{5a}), 127.4 ($C_{1''}$), 125.1 (C_9), 119.5 (C_5-triazole), 115.5 ($C_{4'}$), 115.1 (C_{3a}), 113.0 ($C_{6'}$), 110.2 ($C_{2'}$), 96.9 (C_2), 61.6 (C_3), 44.8 (C_{11}), 27.5 ($C_{10'}$ or $C_{10''}$), 21.8 ($C_{10'}$ or $C_{10''}$); HRMS (ES^+) calculated for $C_{29}H_{24}N_4O_3SeNa$ $[M+Na]^+$: 579.0911; found: 579.0890.

4.1.14. 2,2-dimethyl-3-(4-((phenylselanyl)methyl)-1*H*-1,2,3-triazol-1-yl)-2,3-dihydronaphtho[1,2-*b*]furan-4,5-dione (22). Yield: 80%; mp 186-188 ºC; Yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.19 (d, *J* = 7.3 Hz, H_6), 7.68-7.82 (m, H_7), 7.68-7.82 (m, H_8), 7.68-7.82 (m, H_9), 7.41 (d, *J* = 7.1 Hz, $H_{2'/6'}$), 7.08-7.21 (m, $H_{3'/5'}$), 7.08-7.21 (m, $H_{4'}$), 7.00 (s, H_5-triazole), 5.86 (s, H_3), 4.05-4.16 (m, H_{11}), 1.70 (s, $H_{10'}$ or $H_{10''}$), 1.05 (s, $H_{10'}$ or $H_{10''}$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 180.0 (C_5), 174.5 (C_4), 171.1 (C_{9b}), 145.5 (C_4-triazole), 134.9 (C_7), 134.0 ($C_{2'/6'}$), 133.4 (C_8), 131.4 (C_{9a}), 129.9 (C_6), 129.2 ($C_{1'}$), 129.1 ($C_{3'/5'}$), 127.5 ($C_{4'}$), 126.6 (C_{5a}), 125.6 (C_9), 121.2 (C_5-triazole), 111.2 (C_{3a}), 95.9 (C_2), 66.7 (C_3), 27.6 ($C_{10'}$ or $C_{10''}$), 20.9 ($C_{10'}$ or $C_{10''}$), 20.6 (C_{11}); HRMS (ES^+) calculated for $C_{23}H_{19}N_3O_3SeNa$ $[M+Na]^+$: 488.0489; found: 488.0482.

4.1.15. 2-((4-((phenylselanyl)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)-2,3-dihydronaphtho[1,2-*b*]furan-4,5-dione (26). Yield: 85%; mp 163-164 ºC; Orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (dd, *J* = 7.4 and 1.5 Hz, H_6), 7.59 (td, *J* = 7.4,

7.4 and 1.4 Hz, H_7), 7.53 (td, *J* = 7.4, 7.4 and 1.5 Hz, H_8), 7.44 (dd, *J* = 7.4 and 1.1 Hz, H_9), 7.33-7.41 (m, $H_{2'/6'}$), 7.31 (s, H_5-triazole), 7.13-7.18 (m, $H_{3'/5'}$), 7.13-7.18 (m, $H_{4'}$), 5.33-5.42 (m, H_2), 4.55 (dd, *J* = 14.6 and 7.3 Hz, H_{10a}), 4.64 (dd, *J* = 14.6 and 3.9 Hz, H_{10b}), 4.11 (s, H_{11}), 3.23 (dd, *J* = 15.8 and 10.2 Hz, H_{3a}), 2.83 (dd, *J* = 15.8 and 7.0 Hz, H_{3b}); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.4 (C5), 175.2 (C4), 186.6 (C9b), 146.4 (C4-triazole), 134.7 (C7), 132.9 (C2'/6'), 132.3 (C8), 130.6 (C9a), 129.8 (C6), 129.8 (C1'), 129.2 (C3'/5'), 127.5 (C4'), 126.9 (C5a), 124.4 (C9), 122.7 (C5-triazole), 114.7 (C3a), 84.3 (C2), 53.3 (C10), 29.6 (C3), 20.2 (C11); HRMS (ES^+) calculated for $C_{22}H_{18}N_3O_3Se$ $[M+H]^+$: 452.0513; found: 452.0896.

4.1.16. 2-(azidomethyl)-2,3-dihydronaphtho[2,3-*b*]furan-4,9-dione (28). Yield: 90%; mp 116-117 ºC; Yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.06-7.94 (m, 2H), 7.71-7.57 (m, 2H), 5.23-5.09 (m, 1H), 3.75 (dd, *J* = 3.7 and 13.3 Hz, 1H), 3.56 (dd, *J* = 5.0 and 13.3 Hz, 1H), 3.26 (dd, *J* = 10.8 and 17.4 Hz, 1H), 3.02 (dd, *J* = 7.5 and 17.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 181.8, 177.1, 159.4, 134.1, 133.0, 132.6, 131.2, 126.1, 125.8, 124.0, 83.6, 53.5, 30.0; HRMS (ES^+) calculated for $C_{13}H_{10}N_3O_3$ $[M+H]^+$: 256.0722; found: 256.0716.

4.1.17. 2-((4-((phenylselanyl)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)-2,3-dihydronaphtho[2,3-*b*]furan-4,9-dione (29). Yield: 90%; mp 169-171 ºC; Yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (dd, *J* = 7.4 and 1.5 Hz, H_8), 7.93 (dd, *J* = 7.4 and 1.6 Hz, H_5), 7.66 (td, *J* = 7.4, 7.4 and 1.6 Hz, H_7), 7.62 (td, *J* = 7.4, 7.4 and 1.5 Hz, H_6), 7.32-7.37 (m, H_5-triazole), 7.32-7.37 (m, $H_{2'/6'}$), 7.11-7.17 (m, $H_{3'/5'}$), 7.11-7.17 (m, $H_{4'}$), 5.23-5.32 (m, H_2), 4.64 (dd, *J* = 14.7 and 3.9 Hz, H_{10b}), 4.57 (dd, *J* = 14.7 and 5.6 Hz, H_{10a}), 4.05 (s, H_{11}), 3.26 (dd, *J* = 17.4 and 10.6 Hz, H_{3b}), 2.95 (dd, *J* = 17.4 and 8.1 Hz, H_{3b}); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 181.6 (C_4), 177.2 (C_9), 159.0 (C_{9a}), 146.4 (C_4-triazole), 134.4 (C_7), 133.1 ($C_{2'/6'}$), 132.7 (C_{8a}), 133.2 (C_6), 131.3 (C_{4a}), 129.7 ($C_{1'}$), 129.1 ($C_{3'/5'}$), 127.4 ($C_{4'}$), 126.4 (C_8), 126.2 (C_5), 124.1 (C_{3a}), 123.1 (C_5-triazole), 83.0 (C_2), 52.9 (C_{10}), 30.1 (C_3), 20.3 (C_{11}); HRMS (ES^+) calculated for $C_{22}H_{18}N_3O_3Se$ $[M+H]^+$: 452.0513; found: 452.0512.

4.1.18. 2-chloro-3-(((1-((phenylselanyl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)-1,4-naphthoquinone (36). Yield: 65%; mp 127-128 ºC; Orange solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.14 (dd, *J* = 1.2 and 7.5 Hz, 1H), 8.03 (dd, *J* = 1.2

and 7.5 Hz, 1H), 7.97-7.57 (m, 2H), 7.51-7.40 (m, 3H), 7.33-7.23 (m, 3H), 6.49 (sl, 1H), 5.68 (s, 2H), 5.10 (d, *J* = 6.3 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 180.0, 176.7, 144.8, 143.6, 134.8, 134.7, 132.5, 132.3, 129.7, 129.5, 129.0, 126.9, 126.8, 126.7, 121.8, 111.6, 43.6, 40.0; HRMS (ES^+) calculated for $C_{20}H_{16}ClN_4O_2Se$ $[M+H]^+$: 459.0127; found: 459.0128.

4.1.19. 2-bromo-3-(((1-((phenylselanyl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)-1,4-naphthoquinone (37). Yield: 60%; mp 124-126 ºC; Orange solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.13 (dd, *J* = 1.2 and 8.0 Hz, 1H), 8.01 (dd, *J* = 1.2 and 8.0 Hz, 1H), 7.77-7.57 (m, 2H), 7.48 (s, 1H), 7.46-7.41 (m, 2H), 7.36-7.20 (m, 3H), 6.50 (sl, 1H), 5.68 (s, 2H), 5.11 (d, *J* = 6.3 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 180.1, 176.7, 146.2, 145.0, 135.0, 132.8, 132.3, 130.0, 129.8, 129.3, 127.2, 127.1, 122.1, 44.6, 40.7; HRMS (ES+) calculated for $C_{20}H_{16}BrN_4O_2Se$ $[M+H]^+$: 502.9622; found: 502.9612.

4.1.20. 2-(((1-((phenylselanyl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)-1,4-naphthoquinone (38). Yield: 60%; mp 162-164 ºC; Red solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.10 (dd, *J* = 7.6 and 1.3 Hz, H_8), 8.06 (dd, *J* = 7.6 and 1.3 Hz, H_5), 7.74 (td, *J* = 7.6, 7.6 and 1.3 Hz, H_6), 7.64 (td, *J* = 7.6, 7.6 and 1.3 Hz, H_7), 7.46 (dd, *J* = 7.8 and 1.5 Hz, $H_{2'/6'}$), 7.39 (s, H_5-triazole), 7.27-7.35 (m, $H_{3'/5'}$), 7.27-7.35 (m, $H_{4'}$), 6.22-6.35 (m, N<u>H</u>), 5.77 (s, H_3), 5.68 (s, H_{10}), 4.45 (d, *J* = 5.8 Hz, H_9); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 183.0 (C_1), 181.6 (C_4), 147.4 (C_4-triazole), 143.2 (C_2), 134.82 ($C_{2'/6'}$), 134.78 (C_6), 133.4 (C_{4a}), 132.2 (C_7), 130.4 (C_{8a}), 129.7 ($C_{3'/5'}$), 129.1 ($C_{4'}$), 127.0 ($C_{1'}$), 126.3 (C_5), 126.2 (C_8), 121.7 (C_5-triazole), 101.7 (C_3), 44.7 (C_{10}), 38.1 (C_9); HRMS (ES+) calculated for $C_{20}H_{17}N_4O_2Se$ $[M+H]^+$: 425.0517; found: 425.0512.

4.1.21. 2-(4-((phenylselanyl)methyl)-1*H*-1,2,3-triazol-1-yl)-1,4-naphthoquinone (40). Yield: 60%; mp 117-120 ºC; Brown solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.36 (s, 1H), 8.24-8.12 (m, 2H), 7.88-7.79 (m, 2H), 7.72 (s, 1H), 7.59-7.48 (m, 2H), 7.29-7.26 (m, 2H), 4.25 (s, 2H), 1.6 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 183.6, 179.1, 146.8, 139.1, 134.9, 134.2, 133.4, 131.3, 130.9, 129.3, 129.1, 127.6, 127.1, 126.4, 126.2, 123.8, 20.2; HRMS (ES^+) calculated for $C_{19}H_{14}N_3O_2Se$ $[M+H]^+$: 396.0251; found: 396.0242.

4.1.22. (3*R*,4*S*)-3-azido-4-(naphthalen-1-yl)-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione (47). Yield: 78%; mp 115-117 ºC; Light yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.27 (d, *J* = 8.3 Hz, 1H), 8.23-8.20 (m, 1H), 8.03-8.00 (m, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.80 (d, *J* = 8.1 Hz, 1H), 7.76-7.68 (m, 3H), 7.59 (t, *J* = 8.1 Hz, 1H), 7.35 (t, *J* = 7.3 Hz, 1H), 7.11 (d, *J* = 7.3 Hz, 1H), 5.14 (dd, *J* = 2.3 and 1.8 Hz, 1H), 4.46 (dt, *J* = 12.5 and 2.3 Hz, 1H), 4.19 (d, *J* = 12.5 Hz, 1H), 4.13 (d, *J* = 1.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 183.2, 179.1, 155.7, 136.0, 134.6, 133.6, 132.3, 131.3, 130.6, 129.7, 129.0, 127.6, 126.8, 126.7, 126.5, 126.2, 125.4, 122.2, 119.7, 64.6, 56.9, 36.4; HRMS (ES^+) calculated for $C_{23}H_{15}N_3O_3Na$ $[M+Na]^+$: 404.1006; found: 404.1007.

4.1.23. (3*R*,4*S*)-4-phenyl-3-(4-((phenylselanyl)methyl)-1*H*-1,2,3-triazol-1-yl)-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione (48). Yield: 70%; mp 187-188 ºC; Yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.22-8.16 (m, 1H), 8.02-7.98 (m, 1H), 7.78-7.73 (m, 2H), 7.40-7.26 (m, 7H), 7.15 (s, 1H), 7.13-7.02 (m, 3H), 4.98-4.96 (m, 1H), 4.70-4.65 (m, 2H), 4.50-4.44 (m, 1H), 4.10 (s, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 182.3, 178.4, 154.6, 145.8, 140.0, 134.5, 133.5, 133.4, 131.7, 130.7, 129.2, 128.9, 128.0, 127.7, 127.4, 126.5, 120.3, 119.7, 64.6, 58.3, 40.7, 20.2; HRMS (ES+) calculated for $C_{28}H_{21}N_3O_3SeH$ $[M+H]^+$: 528.0826; found: 528.0821.

4.1.24. (3*R*,4*S*)-4-(naphthalen-1-yl)-3-(4-((phenylselanyl)methyl)-1*H*-1,2,3-triazol-1-yl)-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione (49). Yield: 65%; mp 185-188 ºC; Yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, *J* = 8.6 Hz, $H_{9''}$), 8.12 (dd, *J* = 6.9 and 1.9 Hz, H_9), 7.89 (dd, *J* = 6.8 and 2.0 Hz, H_6), 7.84 (d, *J* = 8.1 Hz, $H_{6''}$), 7.72 (d, *J* = 8.2 Hz, $H_{4''}$), 7.64-7.70 (m, H_7), 7.64-7.70 (m, H_8), 7.62 (dd, *J* = 8.6 and 7.5 Hz, $H_{8''}$), 7.50 (dd, *J* = 8.1 and 7.5 Hz, $H_{7''}$), 7.31 (d, *J* = 7.4 Hz, $H_{2'/6'}$), 7.25 (dd, *J* = 8.2 and 7.1 Hz, $H_{3''}$), 7.20 (s, H_5-triazole), 7.06 (d, *J* = 7.1 Hz, $H_{2''}$), 7.00 (t, *J* = 7.4 Hz, $H_{3'/5'}$), 6.92 (t, *J* = 7.4 Hz, $H_{4'}$), 5.42 (s, H_4), 5.00 (s, H_3), 4.54 (d, *J* = 12.8 Hz, H_{2a}), 4.36 (dl, *J* = 12.8 Hz, H_{2b}), 4.04 (s, H_{11}); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.2 (C_5), 178.4 (C_{10}), 155.0 (C_{10a}), 145.9 (C_4-triazole), 135.9 ($C_{1'}$), 134.5 (C_8), 134.3 ($C_{5''}$), 133.5 ($C_{2'/6'}$), 133.6 (C_7), 131.9 (C_{9a}), 130.9 (C_{5a}), 130.4 ($C_{10''}$), 129.3 ($C_{1'}$), 129.2 ($C_{6''}$), 129.1 ($C_{4''}$), 128.9 ($C_{3'/5'}$), 127.8 ($C_{8''}$), 127.5 ($C_{4'}$), 126.7 (C_6), 126.6 (C_9), 126.5 ($C_{7''}$), 125.7 ($C_{2''}$), 124.9 ($C_{3''}$), 122.7 ($C_{9''}$), 120.3 (C_5-triazole), 120.0 (C_{4a}), 64.4 (C_2), 56.4 (C_3), 37.2 (C_4), 20.3 (C_{11}); HRMS (ES^+) calculated for $C_{32}H_{24}N_3O_3Se$ $[M+H]^+$: 578.0983; found: 578.0982.

4.2. Crystallographic data

The structures of the compounds **35** and **38** were determined from X-ray diffraction on an Enraf-Nonius Kappa-CCD diffractometer (95 mm CCD camera on κ-goniostat) using graphite monochromated MoKα radiation (0.71073 Å), at room temperature. Data collections were carried out using the COLLECT software [58] up to 50° in 2θ. Final unit cell parameters were based on 6482 reflections for compound **35** and 10161 reflections for compound **38**. Integration and scaling of the reflections, correction for Lorentz and polarization effects were performed with the HKL DENZO-SCALEPACK system of programs [59]. The structures were solved by direct methods with SHELXS-97 [60]. The models were refined by full-matrix least squares on F^2 using SHELXL-97 [61]. The program ORTEP-3 [62]. was used for graphic representation and the program WINGX [63] to prepare materials for publication. All H atoms were located by geometric considerations placed (C–H = 0.93-0.97 Å; N-H = 0.86 Å) and refined as riding with $U_{iso}(H) = 1.5U_{eq}$(C-methyl) or $1.2U_{eq}$(other). An Ortep-3 diagram of compounds **35** and **38** are shown in Scheme 6 and the main crystallographic data are listed as following:

For compound 35: Empirical formula: $C_{13}H_7BrNO_2$; Formula weight: 289.11; Temperature: 293(2) K; Wavelength: 0.71073 Å; Crystal system: triclinic; Space group: P-1; Unit cell dimensions: a = 7.2940(3) Å; b = 7.9110(3) Å; c = 9.7940(4) Å; α= 77.133(2)°; β= 89.477(2)°; γ = 84.157(2)°; Volume: 548.03(4) $Å^3$; Z: 2; Density (calculated): 1.75 Mg/m^3; Absorption coefficient: 2.140 mm^{-1}; F(000): 286; Crystal size: 0.32 x 0.22 x 0.10 mm^3; Theta range for data collection: 3.5 to 27.5°; Index ranges: -9≤h≤9, -9≤k≤10, -12≤l≤12; Reflections collected: 8533; Independent reflections: 2498 [R(int) = 0.092]; Refinement method: Full-matrix least-squares on F^2 ; Data / restraints / parameters: 2190 / 0 / 154; Goodness-of-fit on F^2: 1.06; Final R indices [I>2sigma(I)]: R1 = 0.042, wR2 = 0.109; R indices (all data): R1 = 0.048, wR2 = 0.114.

For compound 38: Empirical formula: $C_{20}H_{16}N_4O_2Se$; Formula weight: 419.3; Temperature: 293(2) K; Wavelength: 0.71073 Å; Crystal system: monoclinic; Space group: C2/c Unit cell dimensions: a = 34.0429(8) Å; b = 4.7831(2) Å; c = 23.3921(7) Å; α = 90°; β = 111.3°; γ = 90° ; Volume: 3548.6(2) $Å^3$; Z: 8; Density (calculated):1.57 Mg/m^3; Absorption coefficient: 2.140 mm^{-1}; F(000): 1680; Crystal size: 0.28 x 0.11 x

0.10 mm^3; Theta range for data collection: 2.6 to 27.54°; Index ranges: -43≤h≤44, -5≤k≤6, -30≤l≤30; Reflections collected: 16701; Independent reflections: 4026 [R(int) = 0.063]; Refinement method: Full-matrix least-squares on F^2; Data / restraints / parameters: 2822 / 0 / 244; Goodness-of-fit on F^2: 1.12; Final R índices [I>2sigma(I)]: R1 = 0.047, wR2 = 0.128; R indices (all data): R1 = 0.073, wR2 = 0.144.

Crystallographic data for the structures were deposited in the Cambridge Crystallographic Data Centre, with numbers CCDC 1063533 and 1063534.

4.3. Antitumor activity

Compounds were tested for antitumor activity in cell culture *in vitro* using several human cancer cell lines obtained from the National Cancer Institute, NCI (Bethesda, MD). The L929 cells (mouse fibroblast L cells NCTC clone 929) were obtained from the American Type Culture Collection (Manassas, VA), MDCK cells were purchased from the Rio de Janeiro Cell Bank (Rio de Janeiro, Brazil), and the Chinese hamster lung fibroblasts (V79 cells) were kindly provided by Dr. JAP Henriques (UFRGS, Brazil). 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. The L929, MDCK and V79 cells were cultivated under standard conditions in DMEM with Earle's salts. All culture media were supplemented with 20% (PBMC) or 10% (cancer, L929, MDCK and V79 cells) fetal bovine serum, 2 mM L-glutamine, 100 IU/mL penicillin, 100 µg/mL streptomycin at 37 °C with 5% CO_2. PBMC cultures were also supplemented with 2% phytohaemagglutinin. In cytotoxicity experiments, cells were plated in 96-well plates (0.1×10^6 cells/well for leukaemia cells, 0.7×10^5 cells/well for solid tumor as well V79, L929 and MDCK cells, and 1×10^6 cells/well for PBMC). 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, 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 [64]. At the end of the incubation time (72 h), the plates were centrifuged and the medium was replaced by fresh medium (200 μL) containing 0.5 mg/mL MTT. Three hours later, the MTT formazan product was dissolved in DMSO (150 μL) and the absorbance was measured using a multiplate reader (Spectra Count, Packard, Ontario, Canada). Drug effect was quantified as the percentage of control absorbance of the reduced dye at 550 nm. All cell treatments were performed with three replicates. All cells were mycoplasma-free.

4.4. DNA Survival Assays

A549 cells were plated into a 48-well plate with 10,000 cells/well in 500 μL of DMEM containing 10% FBS. The cells were allowed to attach and grow overnight. A stock of 5 mM of compounds or 10 mM β-lapachone, and 5 mM dicoumarol were made for the experiment. The 8 drug concentrations (0-3.2 μM) were prepared separately in 15 mL conical tubes with 7 mL of media each. The untreated control is DMSO. The media was removed from each well and 500 μL of each drug concentration was added to 6 wells (to produce sextuplet replicates for each concentration). After aliquoting the drug-containing media, 40 μL of dicoumarol was added to each remaining 15 mL conical tube (there remains 4 mL left of each drug concentration) to give a final concentration of 50 μM dicoumarol. The media was removed from a 2nd 48-well plate and 500 μL of the remaining drug + DIC media was aliquoted/well. The plates were gently shaken to mix and placed in the incubator for 2 h. After 2 h, all media was aspirated from the wells and 1 mL of fresh media was aliquoted into each well. The plates were then left in the incubator for 7 days, or until there was 100% confluency for the untreated control. Once the control was confluent, the media was discarded and 500 μL/well of 1X PBS was added to wash the wells. The PBS was discarded and 250 μL of dH_2O/well was added. The plates were then put in the -80 °C freezer overnight. The next day, the plates were thawed completely and 500 μL of Hoechst staining buffer (50 μL of Hoechst 33258 in 50 mL of 1X TNE buffer) was added to each well. The plates were incubated in the dark at RT for 2 h. After two hours, the plates were read on a PerkinElmer Victor X3 plate reader and the readings were plotted as the treated/control (T/C) ± SEM.

4.5. Annexin V/PI detection

The Annexin V cytometry assay was used to detect cell population in viable, early and late apoptosis stage as described by Cavalcanti and coworkers [65]. After short exposure time (6 h) with compounds **21** and **22** at 5 µM, PC3 cells were stained with fluorescein isothiocyanate (FITC) conjugated Annexin V (Guava Nexin kit, Guava Technologies, Inc., Hayward, CA, USA) and PI (necrotic-cell indicator), and then they were subjected to flow cytometry (Guava EasyCyte Mini). Cells undergoing early and late apoptosis were detected by the emission of the fluorescence from only FITC and, both FITC and PI, respectively. To determine whether ROS are involved with tested compounds-induced cytotoxicity, cultures were pre-exposed (24 h) to 5 mM *N*-acetylcysteine (NAC), a widely used thiol-containing antioxidant that is a precursor of GSH which protects against oxidative stress-induced cell death. Also, cultures were co-treated with dicoumarol (50 µM) in order to evaluate the role of NQO1 on compounds bioactivation. A total of 10,000 events was evaluated per experiment (n = 3) and cellular debris was omitted from analysis.

Supporting Information

^{1}H and ^{13}C NMR spectra for all the unpublished compounds and 2D NMR spectra (COSY, HMBC and HSQC) for compounds **6**, **10**, **15**, **16**, **21**, **22**, **26**, **29**, **38** and **49**. This material is available free of charge via the Internet at http://pubs.acs.org.

Notes

The authors declare no competing financial interest.

Acknowledgements

This research was funded by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), numbers: 480719/2012-8, PVE 401193/2014-4, 449348/2014-8, 474797/2013-9 and 454171/2014-5. We would also like to thank FAPEMIG (APQ-02478-14), FUNCAP, FAPESP, FAPESC, CAPES, INCT-catálise and also the Physics Institute of USP (São Carlos) for kindly allowing the use of the

KappaCCD diffractometer. Finally, these studies were also supported by NIH/NCI grant# CA102792 to DAB.

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Table 1. Cytotoxic activity expressed by IC_{50} μM (95% CI) of compounds **4-7**, **9-10**, **13-16**, **19-22**, **25**, **26**, **28**, **29**, **36-38**, **39**, **40**, **48** and **49** in cancer and normal cell lines after 72 h exposure, obtained by nonlinear regression for all cell lines from three independent experiments.

Compd	HL-60	HCT-116	PC3	SF295	MDA-MB-435	OVCAR-8	PBMC	V79	L929
4	>13.99	>13.99	>13.99	>13.99	>13.99	>13.99	>13.99	>13.99	>13.99
5	1.66 (1.09-2.22)	2.96 (2.19-3.81)	2.15 (1.94-2.36)	2.05 (1.52-2.54)	2.72 (2.33-3.28)	1.48 (1.38-1.62)	6.11 (5.65-6.88)	3.67 (2.89-4.45)	4.13 (3.28-4.87)
6	2.00 (1.74-2.25)	4.90 (4.62-5.76)	2.60 (2.18-2.86)	1.88 (1.47-2.26)	1.49 (1.30-1.70)	3.09 (1.98-4.85)	4.16 (3.93-4.44)	3.65 (3.21-3.81)	3.30 (2.81-3.58)
7	5.46 4.87-6.10)	2.24 (1.71-2.53)	3.89 (3.18-4.47)	4.20 (3.93-4.89)	3.85 (3.49-4.33)	2.55 (2.05-2.95)	4.45 (3.91-5.14)	5.20 (4.62-5.43)	5.46 (4.97-6.00)
9	1.43 (1.32-1.54)[a]	2.48 (2.32-2.68)	1.68 (1.52-2.02)	1.93 (1.76-2.12)[a]	1.54 (1.46-1.62)[a]	2.24 (1.99-2.70)	3.58 (3.34-3.86)[a]	2.40 (2.29-2.51)	2.04 (1.74-2.43)
10	1.22 (1.13-1.35)	1.11 (0.84-1.26)	1.90 (1.60-2.01)	1.52 (1.44-1.67)	1.31 (1.22-1.52)	0.92 (0.88-1.22)	1.87 (1.45-2.26)	2.17 (2.03-2.31)	1.74 (1.67-1.94)
13	2.59 (2.30-2.94)	4.60 (3.78-5.59)	5.15 (4.63-5.80)	>14.56	3.93 (3.44-4.40)	7.13 (6.12-8.30)	6.46 (5.50-7.10)	6.06 (5.36-6.55)	5.33 (5.15-5.53)
14	2.23 (1.78-2.67)	3.30 (2.90-3.68)	4.09 (3.49-4.57)	3.04 (2.64-3.82)	2.52 (2.23-2.75)	3.53 (3.34-3.75)	3.16 (2.86-3.49)	4.20 (3.82-4.90)	3.68 (3.45-4.34)
15	1.08 (0.86-1.49)	1.28 (1.04-1.57)	1.71 (1.44-2.03)	1.08 (0.94-1.46)	0.88 (0.76-1.06)	0.68 (0.43-1.06)	3.33 (2.94-3.56)	3.78 (3.73-3.94)	3.38 (3.06-3.65)
16	1.59 (1.34-1.87)	2.54 (1.55-2.88)	2.95 (2.22-3.47)	2.65 (2.39-3.34)	2.15 (2.00-2.32)	2.80 (2.45-3.34)	3.23 (2.43-3.96)	4.03 (3.29-4.63)	3.77 (3.06-4.48)
19	0.26 (0.20-0.35)	1.48 (1.34-1.69)	1.86 (1.69-2.39)	2.59 (2.36-2.85)	2.04 (1.78-2.18)	2.53 (2.33-2.74)	2.80 (2.30-3.35)	2.16 (1.83-2.68)	2.50 (2.33-2.77)
20	3.08 1.48-6.46[b]	0.89 (0.82-1.00)	1.74 (1.22-2.15)	3.23 2.38-4.38[b]	1.19 1.08-1.34[b]	1.34 (1.15-1.45)	nd	3.27 (2.97-3.53)	2.67 (2.26-3.49)
21	0.07 (0.02-0.16)	0.14 (0.11-0.25)	0.38 (0.29-0.67)	0.34 (0.20-0.45)	0.23 (0.16-0.38)	0.20 (0.14-0.43)	1.39 (1.12-1.53)	1.13 (1.03-1.26)	0.94 (0.86-1.15)
22	1.29 (1.03-1.59)	2.52 (2.00-2.88)	2.43 (1.87-3.21)	1.31 (1.10-1.59)	1.06 (0.99-1.12)	2.26 (1.66-3.06)	1.76 (1.44-2.11)	2.65 (1.76-3.16)	2.30 (1.89-2.71)
25	0.82 (0.55-1.45)	0.63 (0.43-0.86)	0.51 (0.24-0.59)	0.39 (0.31-0.43)	0.39 (0.27-0.59)	0.35 (0.12-0.47)	1.80 (1.57-2.15)	0.82 (0.67-1.14)	1.06 (0.94-1.18)
26	0.13 (0.04-0.29)	0.24 (0.18-0.31)	0.24 (0.20-0.27)	0.22 (0.20-0.24)	0.29 (0.22-0.33)	0.07 (0.02-0.13)	1.31 (1.11-1.40)	0.44 (0.22-0.60)	0.62 (0.49-0.78)
28	0.31 (0.20-0.43)	0.24 (0.16-0.39)	0.39 (0.16-0.63)	0.35 (0.12-0.47)	0.20 (0.04-0.27)	0.35 (0.27-0.43)	3.21 (2.58-4.27)	2.47 (2.27-3.02)	2.15 (1.88-2.70)
29	0.82 (0.58-1.09)	2.42 (1.80-3.26)	1.40 (1.13-1.71)	0.98 (0.69-1.44)	0.62 (0.42-1.02)	0.91 (0.75-1.11)	2.80 (2.53-3.04)	2.02 (1.64-2.44)	2.15 (2.04-2.53)
36	2.14 (1.99-2.49)	3.25 (3.01-3.76)	2.25 (1.92-2.40)	2.93 (2.77-3.08)	2.01 (1.64-2.47)	2.58 (2.21-2.97)	6.34 (5.70-6.66)	2.51 (2.03-2.86)	3.43 (3.01-3.78)
37	1.11 (0.96-1.23)	1.67 (1.43-2.05)	2.29 (2.07-2.53)	1.61 (1.43-1.89)	1.43 (1.17-1.81)	2.37 (1.87-2.49)	3.86 (3.78-4.04)	3.03 (2.87-3.40)	3.30 (2.97-3.62)
38	1.13 (0.78-1.20)	1.72 (1.42-1.98)	2.08 (1.84-2.41)	0.97 (0.85-1.28)	3.09 (2.72-3.76)	1.89 (1.46-2.67)	4.75 (4.44-5.06)	3.99 (3.40-4.49)	3.83 (3.76-3.97)
39	2.21 (1.91-2.56)	4.27 (4.22-4.52)	2.16 (1.76-2.71)	3.41 (3.11-3.62)	2.36 (1.91-2.91)	3.51 (3.16-4.27)	8.64 (7.53-9.69)	5.77 (4.62-6.73)	5.17 (4.27-5.97)
40	>12.68	>12.68	>12.68	>12.68	>12.68	>12.68	>12.68	>12.68	>12.68
48	8.26 (8.00-9.17)	7.22 (6.78-7.56)	7.79 (7.46-8.07)	5.58 (5.41-6.00)	6.82 (6.23-7.16)	7.90 (7.37-8.19)	7.45 (7.24-7.71)	6.74 (6.04-7.05)	7.16 (6.91-7.50)
49	>8.67	>8.67	>8.67	>8.67	>8.67	>8.67	>8.67	>8.67	>8.67
β-lap.	1.57 1.11-1.69	0.87 0.74-0.95	1.65 1.40-1.94	0.95 0.70-1.03	0.25 0.16-0.33	1.16 0.97-1.25	>20.63	-	-
DOXO	0.06 0.02-0.09	0.15 0.09-0.21	0.02 0.02-0.04	0.51 0.43-0.58	0.96 0.87-1.10	0.41 0.36-0.49	0.55 0.41-0.58	0.28 0.21-0.36	0.23 0.15-0.30

(a) Ref. 41; (b) Ref. 25. nd, not determined.

Table 2. Selectivity index for the most active compounds (only IC_{50} values < 2 μM for cancer cell lines were considered) [Selectivity index, represented by the ratio of cytotoxicities between normal cells and different lines of cancer cells].

Compd	PBMC, V79 and L929 *vs.* HL-60	PBMC, V79 and L929 *vs.* HCT-116	PBMC, V79 and L929 *vs.* PC3	PBMC, V79 and L929 *vs.* SF295	PBMC, V79 and L929 *vs.* MDA-MB-435	PBMC, V79 and L929 *vs.* OVCAR-8
5	3.6, 2.2 and 2.4	-	-	2.9, 1.7 and 2.0	-	4.1, 2.4 and 2.7
6	2.0, 1.8 and 1.6	-	-	2.2, 1.9 and 1.7	2.8, 2.4 and 2.2	-
9	2.5, 1.6 and 1.4	-	2.1, 1.4 and 1.2	1.8, 1.2 and 1.0	2.3, 1.5 and 1.3	-
10	1.5, 1.7 and 1.4	1.6, 1.9 and 1.5	0.9, 1.1 and 0.9	1.2, 1.4 and 1.1	1.4, 1.6 and 1.3	2.0, 2.3 and 1.8
15	3.0, 3.5 and 3.1	2.6, 2.9 and 2.6	1.9, 2.2 and 1.9	3.0, 3.5 and 3.1	3.7, 4.2 and 3.8	4.8, 5.5 and 4.9
19	10.7, 8.3 and 9.6	1.89, 1.45 and 1.68	1.50, 1.1 and 1.3	-	-	-
21	19.8, 16.1 and 13.4	9.9, 8.0 and 6.7	3.6, 2.9 and 2.4	4.0, 3.3 and 2.7	6.0, 4.9 and 4.0	6.9, 5.6 and 4.7
22	1.3, 2.0 and 1.7	-	-	1.3, 2.0 and 1.75	1.6, 2.5 and 2.1	-
25	2.1, 1.0, and 1.2	2.8, 1.3 and 1.6	3.5, 1.6 and 2.0	4.6, 2.1 and 2.7	4.6, 2.1 and 2.7	5.1, 2.3 and 3.0
26	10.0, 3.3 and 4.7	5.4, 1.8 and 2.5	5.4, 1.8 and 2.5	5.9, 2 and 2.8	4.5, 1.5 and 2.1	18.7, 6.2 and 8.8
28	10.3, 7.9 and 6.9	13.3, 10.2 and 8.9	8.2, 6.3 and 5.5	9.1, 7.0 and 6.1	16.0, 12.3 and 10.7	9.1, 7.0 and 6.1
29	3.4, 2.4 and 2.6	-	2.0, 1.4 and 1.5	2.8, 2.0 and 2.1	4.5, 3.2 and 3.4	3.0, 2.2 and 2.3
37	3.4, 2.7 and 2.9	2.3, 1.8 and 1.9	-	2.3, 1.8 and 2.0	2.6, 2.1 and 2.3	-
38	4.2, 3.5 and 3.3	2.7, 2.3 and 2.2	-	4.8, 4.1 and 3.9	-	-
Doxorubicin	9.1, 4.6 and 3.8	3.6, 1.8 and 1.5	27.5, 14.0 and 11.5	1.0, 0.5 and 0.4	0.5, 0.2 and 0.2	1.3, 0.6 and 0.5

Table 3. NQO1-dependent lethal responses of various compounds. IC_{50} values are reported for cells exposed for two hours in the absence or presence of dicoumarol (DIC).

Compound	Activity	NQO1 Specific	IC_{50} (μM)	IC_{50} (μM) + DIC	DIC protection
21	Yes	Yes	0.64	>3.2	Yes
22	Yes	Yes	1.2	>3.2	Yes
50	Yes	Yes	2.6	>3.2	Yes
51	Yes	Yes	1.8	>3.2	Yes
52	Yes	Yes	2.4	>3.2	Yes
53	Yes	Yes	1.3	>3.2	Yes
β-lapachone	Yes	Yes	3.4	>10	Yes

Legends to Figures and Schemes

Scheme 1. Overview.

Scheme 2. Synthesis of selenium-containing β-lapachone and α-lapachone-based 1,2,3-triazoles.

Scheme 3. Synthesis of selenium-containing nor-α-lapachone-based 1,2,3-triazoles.

Scheme 4. Synthesis of selenium-containing nor-β-lapachone-based 1,2,3-triazoles.

Scheme 5. Synthesis of **26** and **29** from C-allyl-lawsone (**23**).

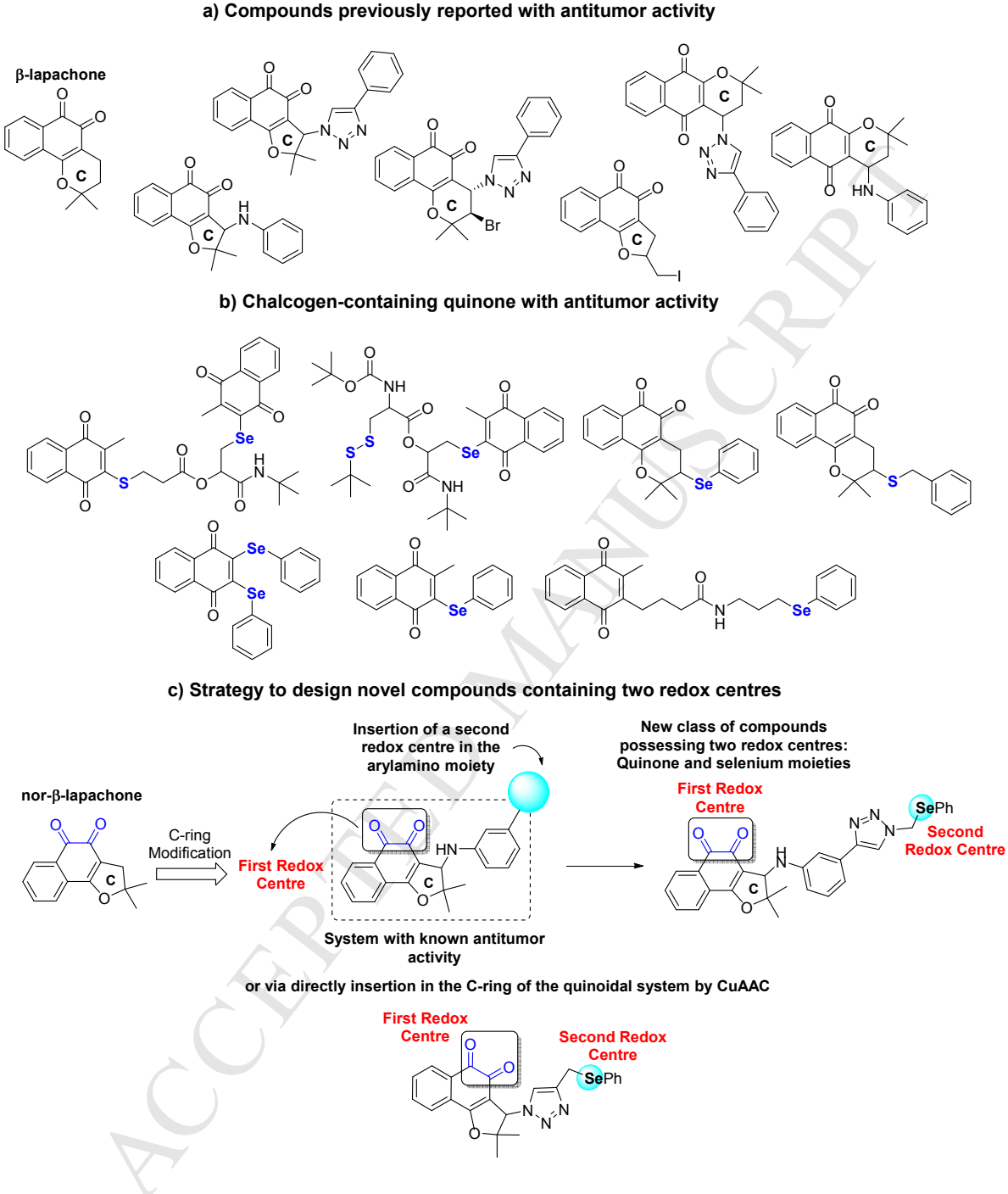
Scheme 6. X-ray structures of compounds **35** and **38**, and synthesis of selenium-containing *para*-quinone-based 1,2,3-triazoles.

Scheme 7. Synthesis of enantio-enriched selenium-containing α-lapachone derivatives-based 1,2,3-triazoles.

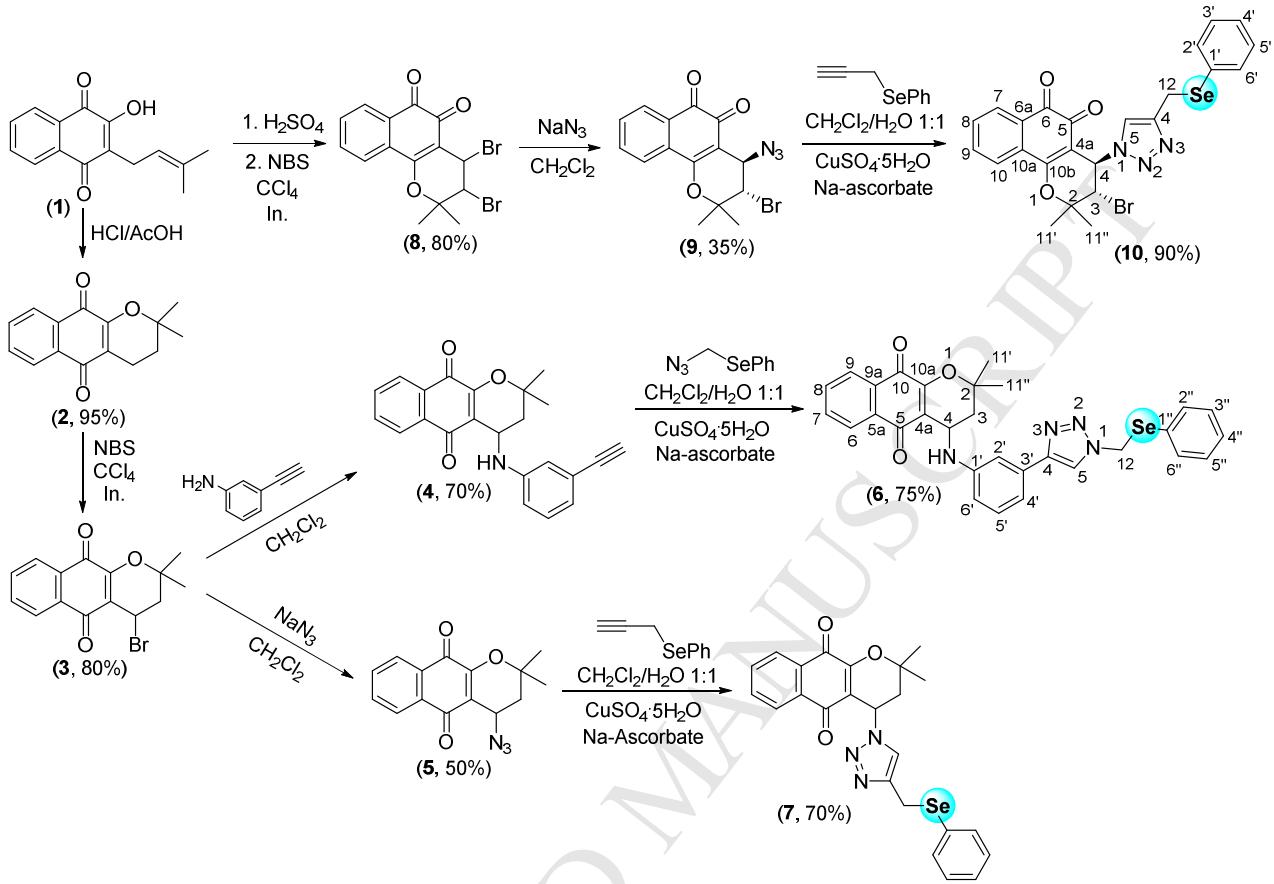
Figure 1. Selected compounds for NQO1- and GPx-specific studies.

Figure 2. Compounds **21**, **22**, **50**-**53** and β-lapachone evaluated for NQO1-dependence.

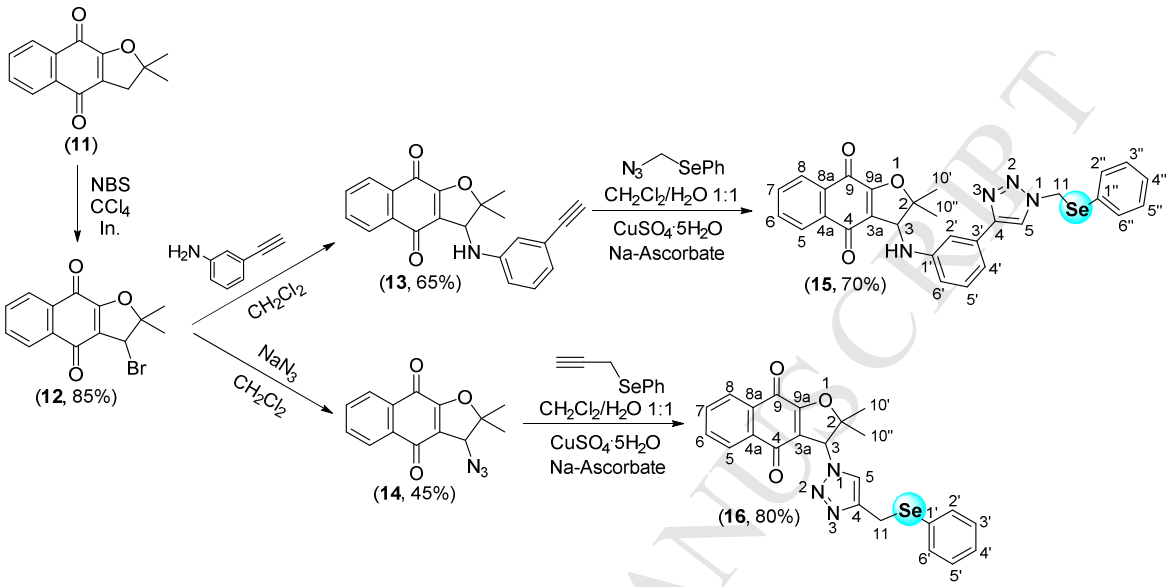
Figure 3. Effect on *phosphatidylserine* externalization after 6 h-treated PC3 with 5 μM of tested compounds. The *phosphatidylserine* externalization was determined by flow cytometry using AnnV-FITC (YLW-HLog) and PI (RED-HLog). Viable cells are plotted at lower left quadrant, cells in early and late apoptosis with *phosphatidylserine* externalized are plotted at lower right and upper right quadrants, respectively, and necrotic cells are plotted at upper left quadrant. $*p < 0.05$ compared to control by ANOVA followed by Newman-Keuls test. Data are presented as mean values ± S.E.M. from three independent experiments in triplicate.



Scheme 1. Overview.

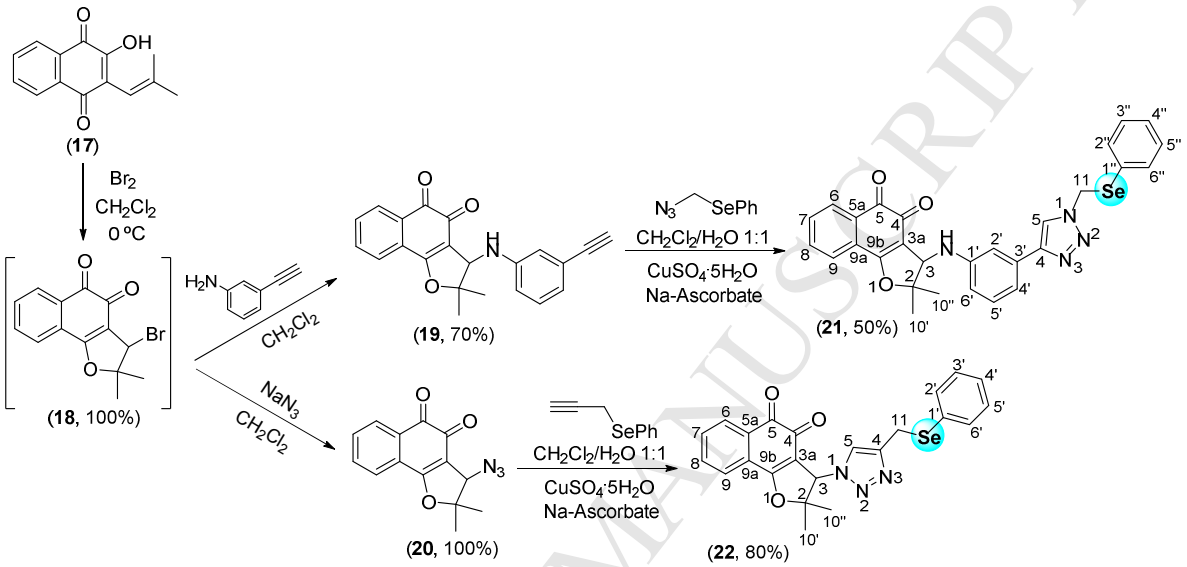


Scheme 2. Synthesis of selenium-containing β-lapachone and α-lapachone-based 1,2,3-triazoles. Radical initiator (In.) = benzoyl peroxide.

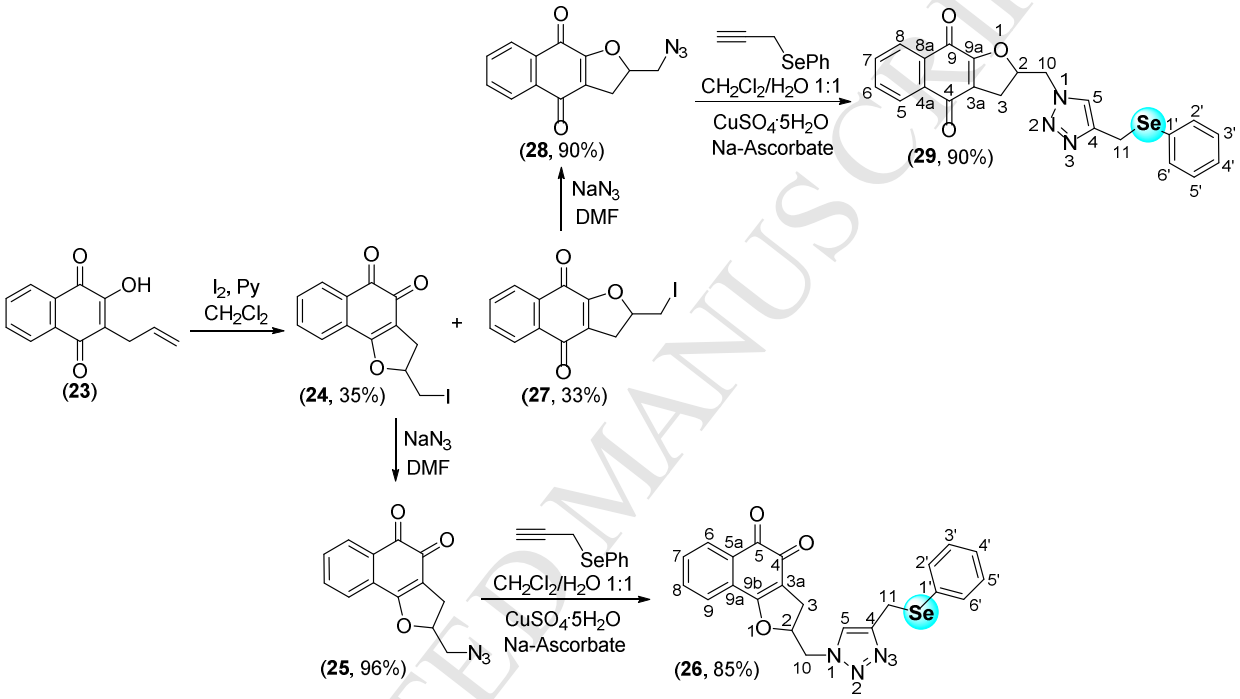


Scheme 3. Synthesis of selenium-containing nor-α-lapachone-based 1,2,3-triazoles.

Radical initiator (In.) = benzoyl peroxide



Scheme 4. Synthesis of selenium-containing nor-β-lapachone-based 1,2,3-triazoles.



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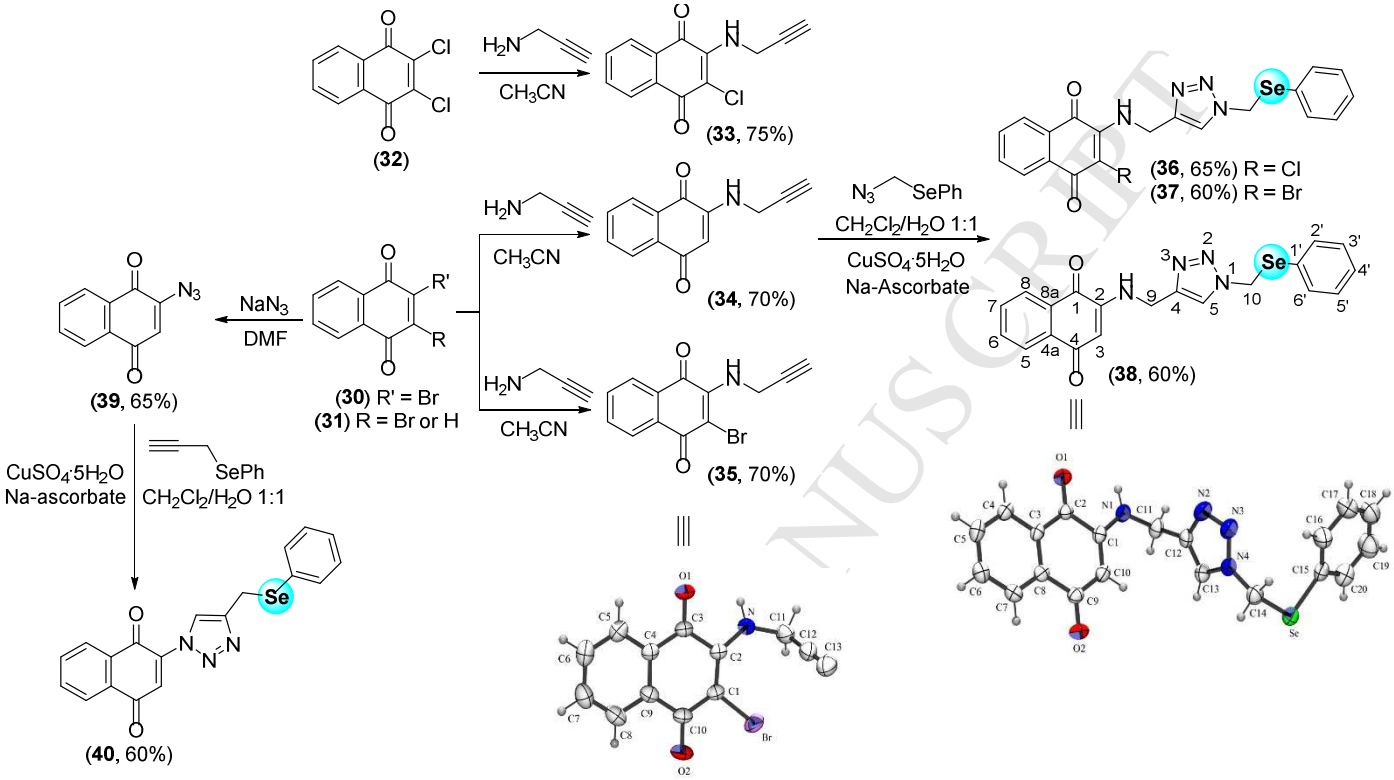
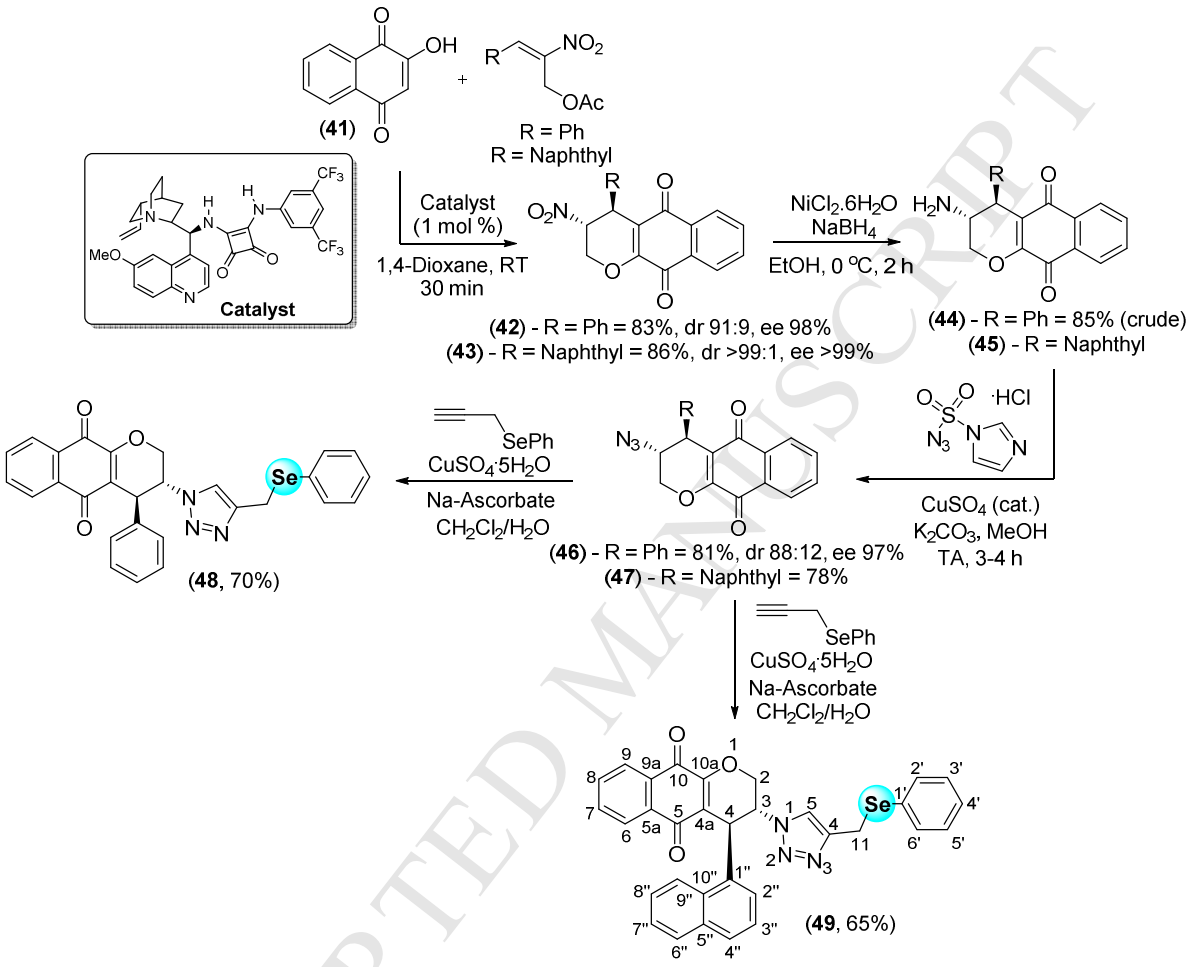


Scheme 6. X-ray structures of compounds **35** and **38**, and synthesis of selenium-containing *para*-quinone-based 1,2,3-triazoles.



Scheme 7. Synthesis of enantio-enriched selenium-containing α-lapachone derivatives-based 1,2,3-triazoles.

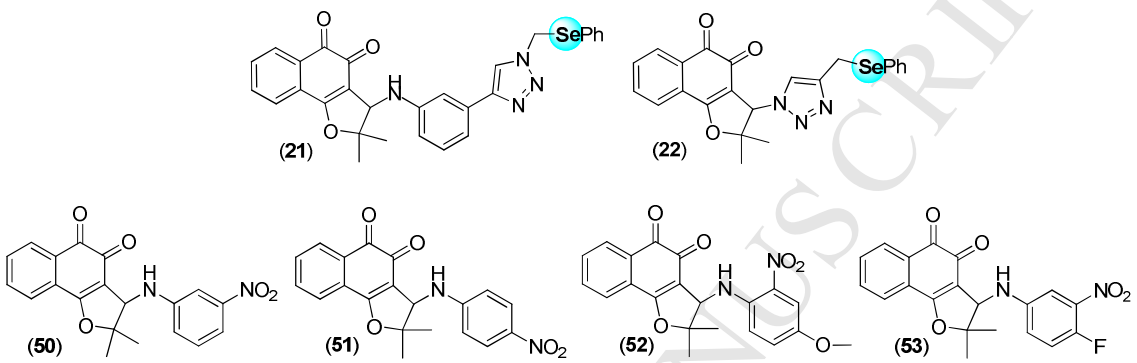
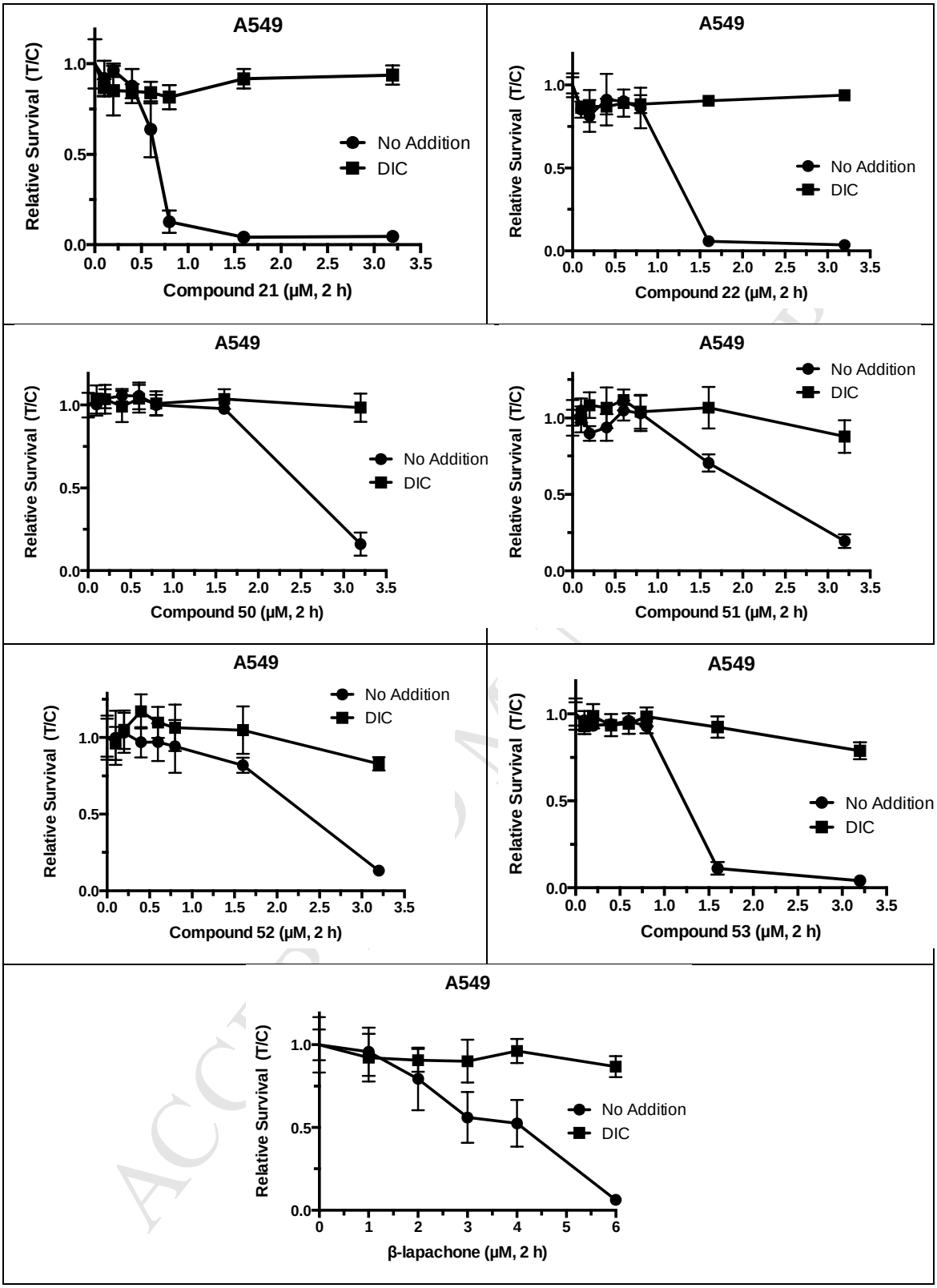


Figure 1. Selected compounds for NQO1 studies.



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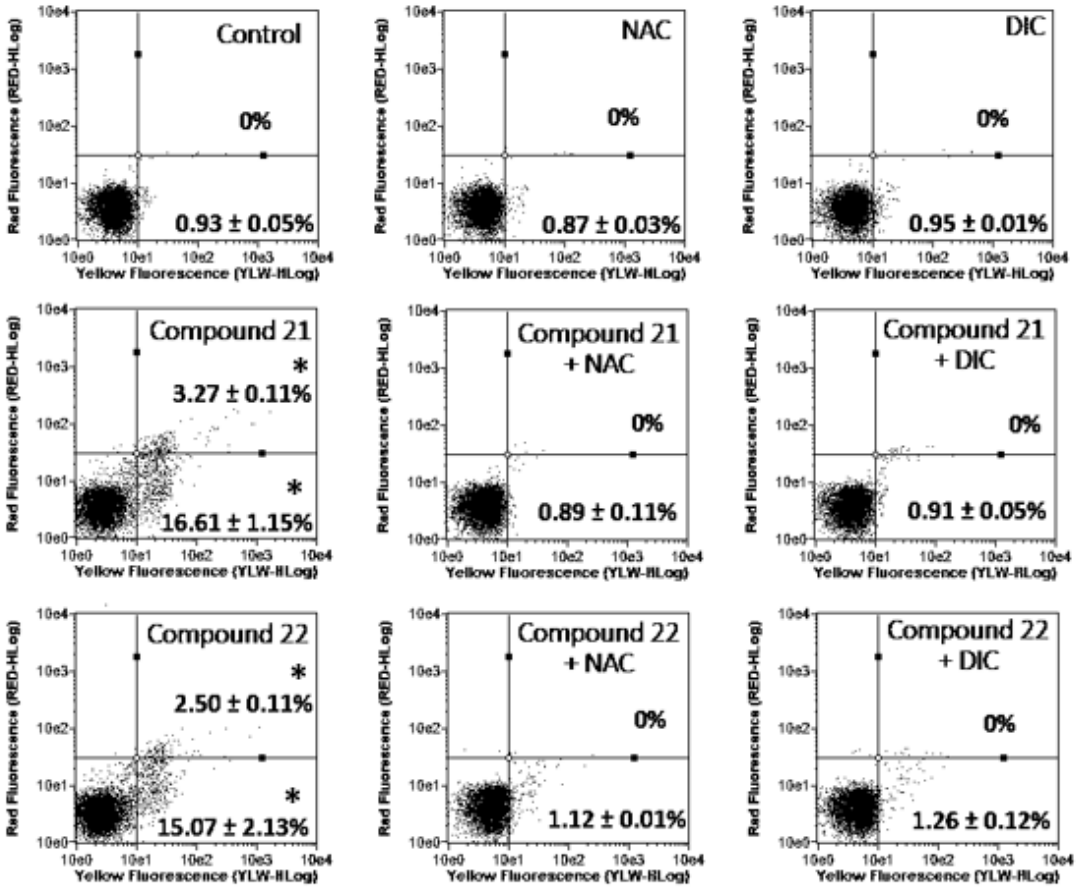

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Research highlights

1. Selenium-containing quinones were obtained with potent antitumor activity.

2. Click chemistry was used for the synthesis of selenium -containing quinones.

3. Mechanistic insights involving NQO1-target were studied.