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Research paper

Synthesis and antitumor activity of selenium-containing quinone-based triazoles possessing two redox centres, and their mechanistic insights

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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.

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

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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 1.4–1.9 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.

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].

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.

From nor-lapachol (**17**), the bromo intermediate **18** was synthesized following the methodology described by Pinto and coworkers (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).

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, compounds **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).

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