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Cytotoxicity Activity of Semisynthetic Naphthoquinone-1-oximes against Cancer Cell Lines

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ABSTRACT

The cancer is among the most important diseases of our time, being the second leading cause of death in developed countries. The development of more effective chemotherapeutic drugs is one of the challenges of medicinal chemistry. Considering the cytotoxicity of quinones, the lapachol 1 and the β-lapachone 2 are attractive molecules for the development of analogues with antitumor activity. Then, the lapachol 1 was used in the synthesis of the 1,4-naphthalenodione,2-hydroxy-3-(3-methyl-2-butenyl),1-oxime 3, the 1,4-naphthalenodione,2-(acetyloxy)-3-(3-methyl-2-butenyl)-1- (O-acetyloxime) 4 and the 2H-naphtho[1,2-b]pyran-5,6-dione, 3,4-dihydro-2,2-dimethyl-6-oxime 5 with yields of 60%, 90% and 62%, respectively. The MTT reduction method was used for determine the cytotoxicity of the molecules 3, 4 and 5 against cancer cells lines: HCT-116 (colon); SF-295 (central nervous system); NCI-1975 (lung) and HL-60 (leukemia). The compound 4 showed no cytotoxic activity against these tumor cells, with IC_{50}> 10 µg/ml. The lapachol oxime 3 and the β-lapachone oxime 5 showed significant antitumor activity, in particular against HL-60 cells with IC_{50} of 10.20 and 3.84 µM, respectively. It is known that one of mechanisms of cytotoxicity demonstrated in quinones is by formation of reactive oxygen species, where the intermediate is the semiquinone radical. The most cytotoxic derivative, the β-lapachone oxime 5 may not generate semiquinone radical. Thus, this paper displays a new probable moiety with relevant antitumor activity, the nucleus 1,2-naphthoquinone-1-oxime.

Keywords: Antitumor; Lapachol; Cytotoxic activity; Naphthoquinone-1-oxime; Semisynthesis.

INTRODUCTION

Cancer or malignant neoplasms is characterized by abnormal cell division, as a result of the diversion of control mechanisms of cell proliferation and differentiation. This is among the most important diseases of our time, being the second leading cause of death in developed countries. The National Cancer Institute (NCI) estimates that, in 2015, 576,000 new cases arose in Brazil [1].

Quinone was identified as an important pharmacophoric element for anticancer activity, which justifies the numerous studies in the literature on the synthesis and evaluation of either natural quinones or their analogues as potential antitumor agents [2]. There are several modes of action by which quinones promote cytotoxic activity, however, it is unclear what actions are most important to induce cell damage, but the main target of cytotoxicity is the DNA. Some quinones act as intercalating agents in the DNA molecule or as inhibitors of enzymes essential for DNA replication and biosynthesis of nucleotides. There are also can be activated in situ by their reduction, leading to conjugated intermediates which are powerful alkylating agents [3 - 4]. The cytotoxicity of quinones is related to the ability of this group be reduced for flavoenzymes generating semiquinone radicals.

Theses radicals are capable of donate electrons to molecular oxygen and generate reactive species of oxygen (ROS) [5].

The lapachol 1 (2-Hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione) is a natural naphthoquinone that occurs in the grain of several wooden trees of the Bignoniaceae family. Since the discovery that lapachol 1 proved to have antitumor activity against carcinoma Walker 256 [6], many other natural and synthetic naphthoquinones were reported as potent antitumoral compounds. The β-lapachone 2 (3,4-Dihydro-2,2-dimethyl-2H-naphtho[1,2-b]pyran-5,6-dione) has demonstrated significant antineoplastic activity against human cancer cell lines of leukemia [7], hepatoma [8], prostate [9], colon [10] and others. Nevertheless, there are no reports of clinical use of these naphthoquinones.

The natural products have been of great importance for the development of new drugs. However, due to their low amount, difficulties in obtaining them, or even to the improvement of their pharmacological action, the organic synthesis has been shown to be critical to the development of more selective and effective compound. Considering the cytotoxicity of quinones, the lapachol 1 and the β-lapachone 2 are attractive molecules for the development of analogues with antitumor activity. Thus, the present work shows the semisynthesis of the 1,4-naphthalenodione, 2-hydroxy-3-(3-methyl-2-butenyl)-,1-oxime 3, the 1,4-naphthalenodione,2-(acetyloxy)-3-(3-methyl-2-butenyl)-1-(O-acetyloxime) 4 and the 2*H*-naphtho[1,2-b]pyran-5,6-dione,3,4-dihydro-2,2-dimethyl-6-oxime 5 from the lapachol 1 and subsequent evaluation of their cytotoxicity in different human tumor cell lines (Figure 1).

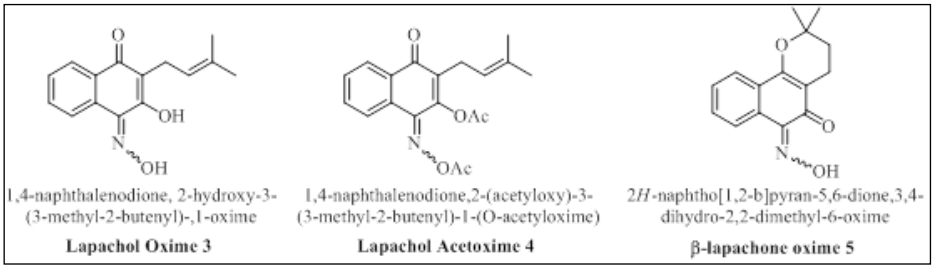

Figure 1: Structures of naphthoquinone-1-oxime synthesized, the 1,4-naphthalenodione, 2-hydroxy-3-(3-methyl-2-butenyl)-,1-oxime 3, the 1,4-naphthalenodione,2-(acetyloxy)-3-(3-methyl-2-butenyl)-1-(O-acetyloxime) 4 and the 2*H*-naphtho[1,2-b]pyran-5,6-dione,3,4-dihydro-2,2-dimethyl-6-oxime 5

EXPERIMENTAL SECTION

Chemistry

All solvents and reagents of analytical grade were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) using aluminum sheets coated with silica gel 60 with fluorescent indicator UV_{254} and compounds visualized using UV light or iodine. The purification by column chromatography was performed using silica gel 60 (70-230 mesh). Melting points were determined on a Kofler hot stage apparatus. ^{1}H-NMR spectra were recorded in deuterated solvent using Bruker – 400 MHz spectrometer and chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. ^{13}C-NMR spectra were recorded in deuterated solvent using Bruker – 100 MHz spectrometer and chemical shifts (δ) are reported in parts per million (ppm) relative to solvent as an internal standard.

Synthesis

The lapachol 1 was obtained by extraction of heartwood of *Tabebuia sp* and purified by recrystallization using water/ethanol and characterized by melting point (m.p.) 138-139 °C (lit. 139.5 °C) [11]. The β-lapachone 2 was prepared according to procedures described by Souza et al. (2008) [12] employing the lapachol 1, previously extracted and purified, as a starting material. Before use, purification by column chromatography was necessary. The synthesis of the β-lapachone 2, was confirmed by determining melting point and later compared with data available in scientific literature, experimental m.p. 154-155 °C (lit. 154-155 °C) [13].

Synthesis of 1,4-naphthalenodione, 2-hydroxy-3-(3-methyl-2-butenyl)-,1-oxime 3

To a magnetically-stirred mixture of lapachol 1 (0.24 g, 1.0 mmol) and solution 10% (v/v) of Et_3N in ethanol, $NH_2OH.HCl$ (0.20 g, 3.0 mmol) was added. The reaction mixture was then allowed to stand at room temperature for 24 h. Water was added to the reaction and it was acidified with glacial acetic acid until the formation of a yellow solid. The solid was filtered, washed with deionized water, allowed to dry at room temperature. Yield: 90%, m.p. 156-158 ºC [14, 15]. ^{1}H-RMN (Acetone-d_6, δ): 9.0 (m, 1H, Ar-H), 8.2 (m, 1H, Ar-H), 7.6 (m, 2H, Ar-H), 5.2 (m, 1H, H-C=C$(CH_3)_2$), 3.3 (dd, *J* = 6.7, 2H, C$\underline{H_2}$-CH= C$(CH_3)_2$), 1.8 (s, 3H, CH_3), 1.6 (s, 3H, CH_3). ^{13}C-RMN (Acetone-d_6, δ): 183.1 (C=O and OH$\underline{C}$=C), 139.9 (C=NOH), 132.1(C-Ar),

131.6 (C-Ar), 131.1 (C-Ar), 130.7(C-Ar), 129.7 (CH= $\underline{C}(CH_3)_2$), 126.3 (C-Ar), 126.0(C-Ar), 121.9 ($\underline{C}$=COH), 117.1($\underline{C}$H= $C(CH_3)_2$), 25.0 (CH_2), 21.9 (CH_3), 17.0 (CH_3).

Synthesis of 1,4-naphthalenodione,2-(acetyloxy)-3-(3-methyl-2-butenyl)-1-(O-acetyloxime) 4

To a magnetically-stirred solution of compound 3 (0.26 g, 1.0 mmol) and acetic anhydride (3.0 mL), anhydrous sodium acetate (0.75 g) was added. The reaction mixture was then allowed to stand at room temperature for 4 h. Then water was added until complete precipitation of solid, the solid was filtered and washed with deionized water. Then the product was purified by column chromatography. Yield: 60%, m.p. 100 – 105 ºC [14, 15]. ^{1}H-RMN ($CDCl_3$, δ): 8.8 (m, 1H, Ar-H), 8.3 (m, 1H, Ar-H), 7.7 (m, 2H, Ar-H), 5.1 (tt, *J* = 1.4, 1H, H-C=C$(CH_3)_2$), 3.3 (dd, *J* = 7.3, 2H, C$\underline{H_2}$-CH= $C(CH_3)_2$), 2.4 (s, 6H, $OCOCH_3$), 1.8 (s, 3H, CH_3), 1.7 (s, 3H, CH_3). ^{13}C-RMN ($CDCl_3$, δ): 183.8 (C=O), 168.6 (O$\underline{C}$OCH_3), 167.5 (O$\underline{C}$OCH_3), 152.1 (C=NOH), 144.4 (C=$\underline{C}$OCOCH_3), 133.9(C-Ar), 133.4 (C-Ar), 133.2 (C-Ar), 132.0 (C-Ar), 130.9 (CO$\underline{C}$=C), 127.7 (C-Ar), 126.1 (C-Ar), 119.2 ($\underline{C}$=$C(CH_3)_2$), 25.7 (CH_2), 23.5 (OCO$\underline{C}H_3$), 20.6 (OCO$\underline{C}H_3$), 19.7 (C=C($\underline{C}H_3$)$_2$), 17.9 (C=C($\underline{C}H_3$)$_2$).

Synthesis of 2*H*-naphtho[1,2-b]pyran-5,6-dione,3,4-dihydro-2,2-dimethyl-6-oxime 5

The $NH_2OH.HCl$ (0.14g, 1.98 mmol) was added to solution of the β-lapachone 2 (0.16 g, 0.66 mmol) in solution 10% (v/v) of Et_3N in ethanol. The reaction mixture was then allowed to stand at room temperature for 2.5 h. The gold yellow solid formed was filtered and washed with deionized water. Yield: 62%, , m.p. 147 ºC [15, 16]. ^{1}H-RMN ($CDCl_3$, δ): 8.3 (dd, *J* = 1.5, 1H, Ar-H), 7.9 (m, 1H, Ar-H), 7.5 (m, 2H, Ar-H), 2.6 (t, *J* = 6.7, 2H, CH_2), 1.9 (t, *J* = 6.7, 2H, CH_2), 1.5 (s, 6H, CH_3). ^{13}C-RMN ($CDCl_3$, δ): 180.1 (C=O), 164.1(C=$\underline{C}$-O), 143.2 (C=NOH), 130.6 (C-Ar), 130.2 (C-Ar), 129.0 (C-Ar), 125.6 (C-Ar), 123.5 (C-Ar), 122.5 (C-Ar), 109.9 ($\underline{C}$=C-O), 79.4 (CH_2-CH_2-$\underline{C}O(CH_3)_2$), 31.45 (CH_2-$\underline{C}H_2$-$CO(CH_3)_2$), 26.8 (CH_2-CH_2-CO($\underline{C}H_3$)$_2$), 15.5 ($\underline{C}H_2$-CH_2-$CO(CH_3)_2$).

Anticancer assay

Chemistry:

Fetal bovine serum was purchased from Cutilab (Campinas, SP, Brazil), and RPMI 1640 medium, trypsine EDTA, penicillin and streptomycin were purchased from GIBCO (Invitrogen, Carlsbad, CA, USA).

Cytotoxicity assay against cancer cell lines:

Compounds (5-25 µg/mL) were tested for cytotoxic activity against four cancer cell lines: SF-295 (central nervous system), HCT- 116 (colon), NCI-1975 (lung), HL-60 (leukemia) and L929 (normal fibroblast) from National Cancer Institute (EUA). Normal murine fibroblast cell lines (L929) were used to evaluate the selectivity of the compounds. All cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, 100 mg/mL streptomycin at 37 ºC with 5% CO_2. Each compound was dissolved with 1% DMSO to obtain a concentration of 5-25 µg/mL. They were incubated with the cells for 72 h. The negative control received the same amount of DMSO (0.1 % in the highest concentration). The cell viability was determined by reduction of the yellow dye 3-(4,5-dimethyl-2- thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a blue formazan product as described by Mosmann [17].

Statistical analysis

The experiments were analyzed according to the mean ± standard deviation (SEM) of the percentage of inhibition of cell growth using the GraphPad Prism program.

RESULTS AND DISCUSSION

In the synthesis route proposed for the preparation the naphthoquinone-1-oximes the lapachol oxime 3, the lapachol acetoxime 4 and the β-lapachone oxime 5, the lapachol 1 was the starting compound. Then, the natural naphtoquinone 1 was obtained from the heartwood of *Tabebuia sp.* through an acid-base extraction and purified by a series of recrystallizations. The β-lapachone 2 was obtained from the cyclization reaction of the naftoquinone 1, employing acetic anhydride /H_2SO_4 [12].

The lapachol oxime 3 and the β-lapachone oxime 5 were prepared by oximation reaction of the lapachol 1 and the β-lapachone 2, respectively, using hydroxylamine hydrochloride. This is the most important method of synthesis used for the preparation of oximes. The reaction occurs in two steps, starting with the attack hydroxylamine to carbonyl. The carbinolamine generating is dehydrates and the intended oxime is synthesize. In this method was required acidify with H_3CCOOH because the formation rate of the oxime depends on the pH, and the pH 4 is ideal for promote the dehydration of carbinolamine [18]. Finally the lapachol oxime 3 was acetylated to obtain the oxime ester 4 [14-16] (Figure 2).

The lapachol oxime 3, the lapachol acetoxime 4 and the β-lapachone oxime 5 were evaluated *in vitro* against four cancer cell lines, SF-295 (central nervous system), HCT- 116 (colon), NCI-1975 (lung) and HL-60 (leukemia). Thus, the compound 4 was low activity against the tumor cell lines tested. The lapachol oxime 3

showed moderate cytotoxicity activity, being more active against HL-60, with IC_{50} of 10.20 µM and selective for tumor cells (IC_{50} against fibroblasts > 41.28 µM), Table 1.

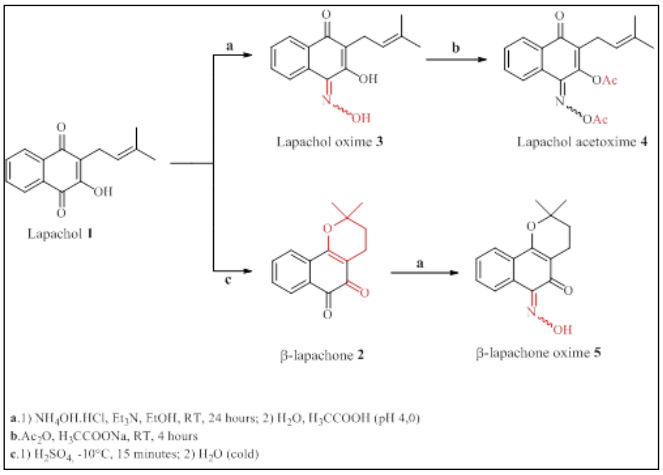

Figure 2: Synthetic scheme of the lapachol oxime 3, the lapachol acetoxime 4, the β-lapachone 2 and the β-lapachone oxime 5

The best results were obtained with β-lapachone oxime 5. High activity was observed against HL-60 (IC_{50} of 3.84 µM) and SF-295 (IC_{50} of 3.47 µM). In addition, this compound showed six times more selective for tumor cells when compared to fibroblasts cells. Comparing the results obtained with the β-lapachone oxime 5 and the naphthoquinone precursor 2 one notes that the oxime is less potent, but more selectivity against cancer cells, Table 1. This increase related to selectivity may be oxime grouping in this naftoquinoa-1-oxime 5, since this is the only structural difference between the synthetic derivative 5 and its precursor 2, the β-lapachone. The β-lapachone oxime 5 proved to be active against all tested tumor cell lines, as to be considered active the National Cancer Institute (NCI), the substance must provide IC_{50} less than 10 or 15 µM [19]. In addition, there is the potential of this derivative to be employed as cytotoxic agent against cancer cells lineage SF-295, which presented its lower IC_{50} value (3.47 µM).

This study demonstrates that the nucleus 1,2-naphthoquinone-1-oxime is a promising moiety with potential antitumor activity, once the derivative 5 showed better cytotoxic activity and selectivity for tumor cells, have the nucleus present in its structure. It is known that one of mechanisms of cytotoxicity demonstrated in quinones is by formation of reactive oxygen species, where the intermediate is the semiquinone radical; compound 5 may not generate semiquinone radical. Thus, this paper displays a new probable moiety with relevant antitumor activity, the nucleus 1,2-naphthoquinone-1-oxime, that requires further examination.

Table 1: Cytotoxic activity expressed as IC50 in µg/ml (µM) of compounds against cancer cell lines

Compounds	Cancer cell lines				
	HCT - 116	HL – 60	SF-295	NCI - 1975	L929
β-lapachone 2	0.20 (0.83)[a, b]	0.40 (1.65) [a]	0.22 (0.91) [a]	-	-
Lapachol oxime 3	4.804 (18.67)	2.625 (10.20)	7.494 (29.13)	8.143 (31.65)	> 10 (41.28)
Lapachol acetoxime 4	11.76 (39,33)	12.46 (41,60)	> 25	14.66 (49,03)	15.36 (51,37)
β-lapachone oxime 5	1.801 (7.00)	0.989 (3.84)	0.894 (3.47)	5.551 (21.57)	6.337 (24.63)

a – SILVA-JÚNIOR et al. 2007 [2] and ROCHA et al. 2011 [20].
b – Cancer cell lines HCT-8.

CONCLUSION

Among the compounds synthesized, it was found that the lapachol oxime 3 and the β-lapachone oxime 5 show significant cytotoxic activity against all the four tested tumor cell lines, as well as be obtained with satisfactory yields (90 and 62%, respectively). According to the INC, β-lapachone oxime 5 can be classified as active across all tested tumor cell lines (IC_{50} <10 µM), in addition to displaying selective for tumor cells tested. Further studies will be necessary to determine the relationship between the nucleus naphthoquinone-1-oxime and the cytotoxicity activity these new molecules.

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