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New pterocarpanquinones: Synthesis, antineoplasic activity on cultured human malignant cell lines and TNF-α modulation in human PBMC cells

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ABSTRACT

A new pterocarpanquinone (**5a**) was synthesized through a palladium catalyzed oxyarylation reaction and was transformed, through electrophilic substitution reaction, into derivatives **5b**–**d**. These compounds showed to be active against human leukemic cell lines and human lung cancer cell lines. Even multidrug resistant cells were sensitive to **5a**, which presented low toxicity toward peripheral blood mononuclear cells (PBMC) cells and decreased the production of TNF-α by these cells. In the laboratory these pterocarpanquinones were reduced by sodium dithionite in the presence of thiophenol at physiological pH, as NAD(P)H quinone oxidoredutase-1 (NQO1) catalyzed two-electron reduction, and the resulting hydroquinone undergo structural rearrangements, leading to the formation of Michael acceptors, which were intercepted as adducts of thiophenol. These results suggest that these compounds could be activated by bioreduction.

1. Introduction

Naturally occurring *para*-quinones and their synthetic analogs are an important source of antineoplasic products[1–4] and some of these compounds have been used in clinic as anticancer[5–10] and antiparasitic[5] drugs. Anthraquinones such as daunorubicin and doxorubicin, used in cancer chemotherapy, act through DNA intercalation. These and other xenobiotic *para*-quinones can be reduced at the mitochondria to the corresponding semiquinone radicals through action of cytochrome P450 reductases (one electron reduction)[1,2,5,6,10] and the resulting semiquinones can transfer one electron to molecular oxygen. The resulting superoxide anion can be transformed into hydroxyl radical which initiates a cascade of events leading to oxidative stress. Alternatively, enzymes such as NAD(P)H quinone oxidoredutase-1 (NQO1) catalyze two-electron reduction of xenobiotic *para*-quinones to the corresponding hydroquinones, which are subsequently conjugated (phase II metabolism) and them excreted.[10–12]

However, some hydroquinones can undergo structural rearrangements leading to the formation of Michael acceptors, that can alkylate essential nucleophiles in the target cells. This process is known as bioreductive activation.[10,13–18]

As part of a program directed at the discovery of new anticancer drugs, we previously synthesized the natural pterocarpan **1** (Fig. 1) and derivatives.[19,20] We showed that the compound **1** was active against different leukemic cell lines, including MDR cell lines, and presented low cytotoxicity against lymphocytes activated by the mitogen phytohemagglutinin (PHA).[20] However, once cathecols can be transformed in vivo into the corresponding *ortho*-quinones, compound **2** was considered as a possible metabolite of **1** and was prepared by the oxidation of the catechol system at ring A in **1**.[20] *ortho*-Quinone **2** was more potent against leukemic cells but presented high cytotoxicity against lymphocytes activated by PHA.[20] In order to prepare more active and less toxic compounds, we designed new derivatives, named pterocarpanqunones, in which the pro-toxic cathecol group was changed by a naphthoquinone group. These compounds can also be seen as analogs of Kalafungin, an antimicrobial agent obtained from the culture broth of a soil isolate of *Streptomyces tanashiensis*, sharing in their structures the dihydrofuran moiety.[21,22]

Pterocarpanquinones **4a**–**g** were synthesized and presented antineoplasic effect on human leukemic cell lines mentioned above[23] and MCF-7 breast cancer cell line.[24] Some of these pterocarpanquinones were also active in fresh leukemic cell samples obtained from patients and overexpressing ATP-binding cassette transporters (ABC) proteins.[23] On the other hand, these pterocarpanquinones showed low cytotoxicity to peripheral blood

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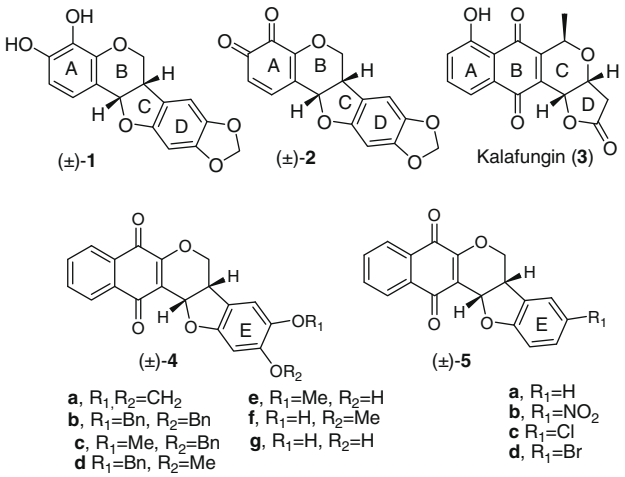


Figure 1. Pterocarpans, lapachol, and pterocarpanquinones.

mononuclear cells (PBMC) activated by PHA.[23] While in MCF-7 cells the antineoplasic activity was stronger for compounds bearing a phenol group at the E ring, the antileukemic profile was independent on the oxygenation pattern at this ring.[24] So, in order to know more about the structure–activity relationships, we decided to synthesise a new derivative (**5a**) without substitution at the E ring. Related non phenolic derivatives **5b–d** were also prepared and their antineoplasic activity was also evaluated. Antineoplasic effect of these new pterocarpanquinones on leukemia and lung human cell lines, and TNF-α modulation in PBMC cells was studied.

2. Results

2.1. Chemistry

Although very promising, pterocarpanquinones **4a–d** were synthesized[24] through the oxyarylation of chromenquinone **6** by *ortho*-mercuryphenols **8a–d** under the conditions developed by Horino and Cols[25] (stoichiometric $PdCl_2$, LiCl in acetone, condition A, Scheme 2, Table 1, entries 1–4). However, this procedure is expensive and the use of organomercurial reagents are not recommended to prepare drug candidates.

In contrast to the Heck reaction, only few examples of catalytic oxyarylation (oxa-Heck reaction) of olefins are described in the literature, and in these cases *ortho*-iodophenols[26–29] were used instead *ortho*-mercuryphenols and we decided to try these more adequate procedures using **9** as source of the organopalladium species to prepare the pterocarpanquinone **4d**. Once arylbromides are also substrates for Heck reactions, compound **10** was also studied.[30]

In contrast with the easy preparation of oxygenated *ortho*-mercury phenols by direct mercuration of the corresponding phenols,[19,24,25] oxygenated *ortho*-iodo phenols are more difficult to obtain and only few examples describing the iodination of these very reactive substrates are reported in the literature. In our hands, phenol **7** could not be transformed into the corresponding *ortho*-iodophenol **9** when allowed to react with *N*-iodosuccinimide in trifluoroacetic acid or acetonitrile.[31,32] In both cases a complex mixture of products was formed. However, **9** could be prepared from the corresponding *ortho*-mercuryphenol **8d** by reaction with I_2. On the other hand, the *ortho*-bromophenol **10** was prepared from phenol **7** by reaction with NBS (Scheme 1).[33,34]

Unfortunately, attempts to accomplish the oxyarylation of **6** by **9** or **10** under the conditions favoring the neutral mechanism ($Pd(OAc)_2$, Et_3N, Condition B)[26,27] or under conditions favoring the cationic mechanism ($Pd(OAc)_2$, Ag_2CO_3, Condition C)[28] did not lead to the desired adduct **4d** (Table 1, entries 5 and 6).

Probably, under the conditions employed it was not possible to accomplish the oxidative addition of Pd(0) in the C–I bond in *ortho*-iodophenol **9** and *ortho*-bromophenol **10**. This step is disfavored by the presence of electron releasing groups in the aromatic ring.

Next, we decided to accomplish the catalytic oxyarylation reaction of **6** using *ortho*-iodophenol (**11**). Compound **5a** was not formed in condition B (Scheme 3, Table 2, entry 1), but in condition C this pterocarpanquinone was obtained in 41% yield (entry 2). A similar yield was obtained in the absence of PPh_3 (entry 3).

Pterocarpanquinones **5b–d** were obtained from **5a** by electrophilic substitution, taking advantage of the great reactivity of E-ring for electrophilic aromatic substitution over the A-ring, which is deactivated due to the conjugation with the carbonyl groups of the quinone moiety (Scheme 4).[33,35]

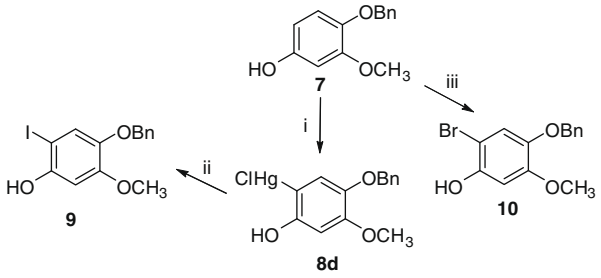
2.2. Pharmacology

The pterocapanquinones **5a–d** were evaluated on two human leukemic cell lines, K562 and HL-60. K562 cells, from a chronic

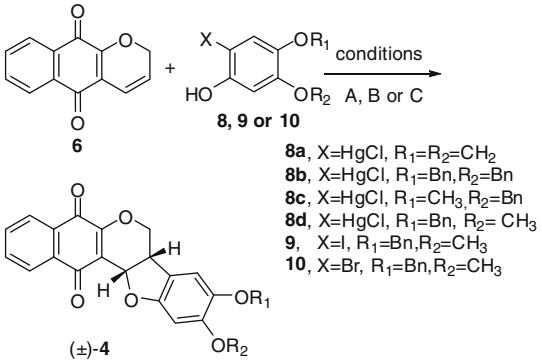
Table 1
Yields and major conditions for reactions showed in Scheme 1

Entry	(Ar–X)	Conditions	**4** (yield%)
1	**8a**	A	**4a** (57)
2	**8b**	A	**4b** (50)
3	**8c**	A	**4c** (58)
4	**8d**	A	**4d** (55)
5	**9**	C	—
6	**10**	B or C	—

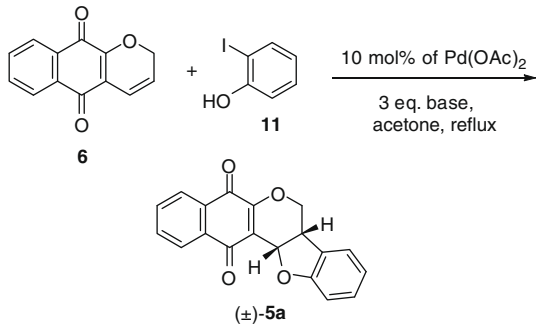
Conditions A = stoichiometric $PdCl_2$, LiCl in acetone at room temperature for 12 h. Conditions B = 10 mol % $Pd(OAc)_2$, 20 mol % PPh_3, 3.0 equiv Et_3N, acetone, reflux for 12 h. Conditions C = 10 mol % $Pd(OAc)_2$, 20 mol % PPh_3, 3.0 equiv Ag_2CO_3, acetone, reflux for 12 h.



Scheme 1. Synthesis of *o*-halophenols. Reagents and conditions: (i) $Hg(OAc)_2$/LiCl/rt/quantitative yield; (ii) I_2, THF, rt, quantitative yield; (iii) NBS, CH_3CN, −30 °C, 75%.



Scheme 2. Synthesis for pterocarpanquinones **4a–d**.

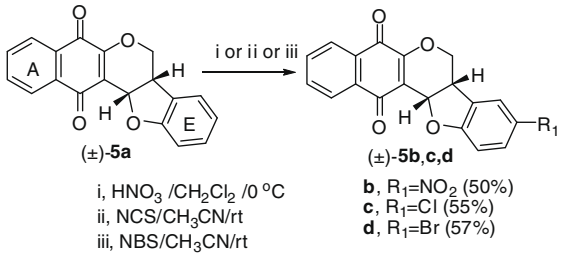


Scheme 3. Synthesis of pterocarpanquinone **5a** by catalytic oxy-arylation of **6**.

Table 2
Yields and major conditions for reactions showed in Scheme 3

Entry	Condition	PPh_3	**5a** (yield%)
1	B	0.2 equiv	–
2	C	0.2 equiv	41
3	C	–	40

Conditions B = 10 mol % $Pd(OAc)_2$, 20 mol % PPh_3, 3.0 equiv Et_3N, acetone, reflux for 12 h. Conditions C = 10 mol % $Pd(OAc)_2$, 20 mol % PPh_3, 3.0 equivAg_2CO_3, acetone, reflux for 12 h.



Scheme 4. Synthesis of pterocarpanquinones **5b**–**d**.

myeloid leukemia, contains high levels of intracellular glutathione (GSH) and are resistant to oxidative stress.[36] Cell viability greater than 90% was observed, even after treatment of these cells with H_2O_2 100 μM.[37] In contrast, HL-60 cells, a pro-myelocytic leukemia, presents a low level of antioxidant defense and is sensible to oxidative stress.[36] In Table 3 are presented the results obtained, showing that these new pterocarpanquinones are as active as compounds **1** and **4f**, used as reference in this study. Mitomycin C was used as reference too. Since **5a** was the more potent in K562 and (excepted for **5b**) HL-60 cell lines (Table 3), we decided to investigate its pharmacological properties in more details in other selected leukemic cells.

As multidrug resistant (MDR) is one of the most important problems in cancer chemotherapy, the antineoplasic effect of **5a** was tested on Lucena-1 (Table 4). This cell line, derived from K562 and originally selected for resistance to the vinca alkaloid vincristine, is a MDR cell and overexpresses ATP-binding cassette sub-family B member 1 protein (ABCB1), a transmembrane protein of 170 KDa which belongs to the ABC superfamily of transporters and is codified by MDR-1 gene.[38] This protein is responsible for removing xenobiotics from the cell, being related to the process of MDR.[38]

Table 4 shows the IC_{50} obtained for **5a** on Lucena-1, Raji, Jurkat and Daudi human leukemic cell lines. Lucena-1 is also resistant to oxidative stress[39] and was slightly more resistant than K562. Jurkat, Raji and Daudi are human lymphocytic cell lines. Jurkat, a T cell leukemia with high levels of Bcl-2 expression, was more resistant than the other leukemic cell lines. Compound **5a** was very bioselective and did not show significant cytotoxicity for PBMC activated by PHA (IC_{50} > 20 μM). Mitomycin C (Mit. C) was used as reference.

Not only leukemic cells were sensitive to quinone **5a**. Table 5 shows that this quinone was very active against a small cell lung cancer cell line (GLC-4), and to a lesser extent to non-small cell lung cancer cell lines (A549 and H460). It is worth to mention that GLC-4 and A549 cell lines present high expression of MRP-1 protein (MDR phenotype).[40,41]

Currently it is known that inflammation and cancer are often associated. The neoplastic microenvironment is rich in cytokines, chemokines and inflammatory enzymes that can become it more favorable to the tumor development.[42] One of the most important inflammatory molecule is tumor necrosis factor (TNF-α), a cytokine that drives inflammatory reactions. Despite its name, TNF-α mediates inflammation-induced tumor growth and can act as an endogenous tumor promoter.[43,44] To determine whether **5a** could modulate the production of TNF-α, we incubated human PBMC with lipopolysaccharide (LPS) and this quinone in different concentrations for 2 h. The levels of TNF-α in PBMC supernatants showed that **5a** significantly reduced TNF-α liberation at 25 μM concentration (Fig. 2). Moreover, the highest concentration tested (100 μM)

Table 3
Antineoplasic effect of pterocarpanquinones **5a,b,d,e** in K562 and HL-60 cell lines (IC_{50} in μM)[a]

Quinone	K562	HL-60
5a	1.67	2.00
5b	3.48	0.40
5c	6.77	4.87
5d	5.70	4.87
1	2.95	2.10
3	16.04	ND
4f	2.18	ND
Mitomycin C	0.47	ND

ND = not done.
[a] Results are reported as IC_{50} values (concentration required to inhibit cell growth by 50%) in micromolar. Data represent the means of three independent experiments, with each concentration tested in triplicate and SD values were lower than 15%. Assays were performed as described in Section 5.

Table 4
Antineoplasic effect of pterocarpanquinone **5a** in leukemia cancer cell lines and PBMC (IC_{50} in μM)[a]

Quinone	Lucena-1	Raji	Jurkat	Daudi	PBMC
5a	2.75	3.32	6.77	3.10	>20
Mit. C	2.75	ND	ND	0.45	4.03

ND = not done.
[a] Results are reported as IC_{50} values (concentration required to inhibit cell growth by 50%) in micromolar. Data represent the means of three independent experiments, with each concentration tested in triplicate and SD values were lower than 15%. Assays were performed as described in Section 5.

Table 5
Antineoplasic effect of pterocarpanquinones **5a** on lung cancer cell lines, A549, H460, and GLC-4 cell lines (IC_{50}, μM)[a]

Quinone	A549	H460	GLC-4
5a	11.21	12.86	5.17

ND = not done.
[a] Results are reported as IC_{50} values (concentration required to inhibit cell growth by 50%) in micromolar. Data represent the means of three independent experiments, with each concentration tested in triplicate and SD values were lower than 15%. Assays were performed as described in Section 5.

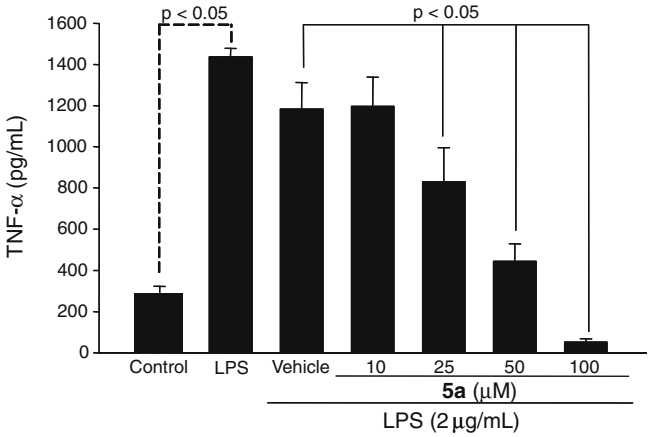
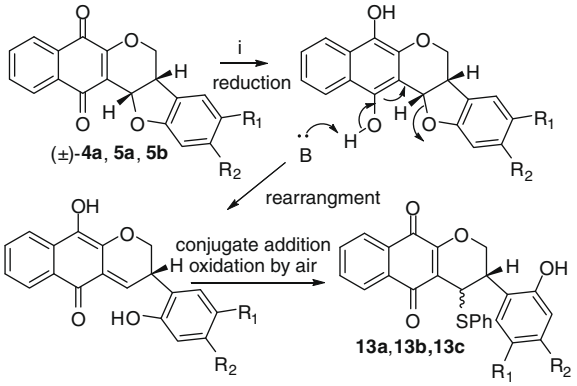


Figure 2. Effect of **5a** on LPS-stimulated TNF-α secretion by PBMC incubated for 2 h. Data are representative of three experiments and are mean ± SEM.



Scheme 5. Reductive activation of pterocarpanquinones **4a**, **5a**, and **5b**. Reagents and conditions: (i) sodium dithionite, Tris/HCl (pH 7.4), PhSH, 22% for **13a**, quantitative for **13b** and 67% for **13c**. Compound **13a**: $R_1,R_2 = -OCH_2O-$; **13b**: $R_1 = R_2 = H$; **13c**, $R_1 = NO_2$, $R_2 = H$.

was able to inhibit almost 100% of TNF-α liberation when compared to LPS-stimulated PBMC treated with vehicle.

Its worth to note that under this condition **5a** was still less toxic to PBMC activated by PHA. In PBMC stimulated by LPS, more than 70% of cell viability was observed even in the presence of 100 μM of **5a** (data not shown).

3. Discussion

Similar to what has been observed for other *para*-naphthoquinones,[1,2,5,6,10] it seems reasonable to accept that pterocarpanquinones **4a–d** and **5a–d** can be transformed through one-electron reduction into the corresponding semiquinone radicals. These radicals can initiate a cascade of reactions leading to oxidative stress of the cell. The antineoplasic effect of these compounds on HL-60, Raji, and Daudi cell lines could be associated with this mechanism of action. However, Jurkat, K562 and Lucena-1 cell lines are resistant to oxidative stress and, at least in these cases, an alternative mechanism of action should be operating. Pterocarpanquinones **4** and **5** share with the pyranonaphthoquinone Kalafungin (**3**) an important structural feature, the presence of a C–O bond at D-ring (Fig. 3). It was proposed that Kalafungin acts as antibiotic through a mechanism[13] similar to that suggested for Mitomycin C and analogs,[5–7,17] and antitumor drugs model quinomethanes[14,15] (bioreductive activation), being activated after reduction by NQO1 (two electron reduction). The resulting hydroquinone undergo a structural rearrangement, furnishing a Michael acceptor which react with essential nucleophiles in the cell, as proposed by Moore and Czeniak.[13,16] The first experimental evidence for this metabolic pathway was supported by Brimble and Nairn through the reduction of the *O*-methyl ether of **3** by sodium dithionite in the presence of sulfur nucleophiles, leading to the incorporation of these nucleophiles.[13]

A similar result was obtained in our laboratory for **4a**, **5a** and **5b** (Scheme 5). The reduction of these compounds by sodium dithionite at buffered pH 7.4 in the presence of thiophenol led to the adducts of thiophenol **13a**, **13b**, and **13c**, respectively, strongly suggesting that these compounds could be activated by bioreduction, acting as alkylating agents (Scheme 5). This mechanism of action could explain the potency of the antineoplasic effect of these compounds on cell lines resistant to oxidative stress.

It is interesting to mention that K562 and probably Lucena-1 expresses a high level of NQO2 (an isoform of NQO1), the enzyme responsible for two electron reduction of quinones to hydroquinones.[45–47]

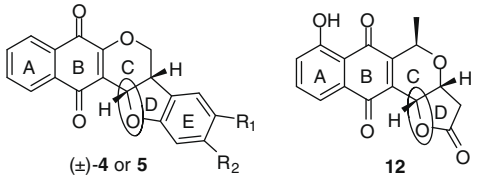


Figure 3. Pterocarpanquinones **4** and **5** and Kalafungin (**12**).

4. Conclusions

Although pterocarpanquinones **4a–d** and **5a–d** have similar pharmacological profile, the conditions used to prepare **5a–d** are less expensive, using catalytic amount of $Pd(OAc)_2$ and avoiding the use of organomercurial reagents.

Pterocarpanquinone **5a** showed potent antineoplasic effect against leukemic cell lines, including those with the MDR phenotype, suggesting that this compound is not a substrate to ABCB1, the transporter that confers resistance to Lucena-1 cells. Although to a lesser degree, **5a** was also effective against non-small lung cancer cell lines (A549 and H460) known to express different levels of the MDR transporters ABCB1 and ABCC1.[48,49] The small cell lung cancer cell line GLC-4 was, however, more sensitive to **5a**.

One of the most important pro-inflammatory cytokines, TNF-α has a critical role in carcinogenesis too.[50] Interestingly, several studies have demonstrated endogenous TNF-α as a tumor promoter and metastatic factor.[51–53] Significant levels of TNF-α was found in tumor microenvironment of various human cancers, including those of breast, ovarian, prostate, lymphoma, melanoma and leukemia.[54] We demonstrated that **5a** inhibited TNF-α liberation in human PBMC cells. This effect revealed **5a** as potentially even more effective against cancer, since it can act by two mechanisms, directly by killing tumor cells and indirectly, resolving the inflammatory environment that supports tumor development.

The key feature to the understanding the cytotoxic effect of these compounds on cell lines resistant to oxidative stress seems to be the bioreductive activation mechanism, transforming these compounds into potent Michael acceptors, especially in cells that overexpress NQO1. It is possible that this mechanism of action overcomes the antioxidant defenses present in these cells. The low toxicity in vitro (PBMC activated by PHA) and the efficient synthesis of **5a**, show that this compound is a good candidate to further evaluations in vivo.

5. Experimental

5.1. Pharmacology

5.1.1. Cell lines

All cell lines used in this work were maintained in RPMI-1640 medium (Sigma–Aldrich Corp. St. Louis, MO, USA), supplemented with 50 μM β-mercaptoethanol, 25 mM Hepes, pH adjusted to 7.4 with NaOH, 60 mg/L penicillin, 100 mg/L streptomycin and 10% fetal calf serum (FCS) (Gibco, Grand Island, NY, USA), inactivated at 56 °C for 1 h. Daudi cells were supplemented with 20% FCS. Vincristine sulfate (60 nM) (Sigma–Aldrich) was added to Lucena-1 in order to maintain the MDR phenotype.

5.1.2. Isolation of PBMC

Peripheral blood mononuclear cells (PBMC) were obtained by fractionating heparinized blood from healthy volunteers. Blood was heparinized and fractionated on Ficoll–hypaque (Hystopaque) (GE Healthcare, Uppsala, Sweden) by density gradient centrifugation. The PBMC fraction was washed twice and resuspended in RPMI-1640, supplemented as described above, and the cell density was adjusted to 10^6 cells/mL. Cells were incubated with 5 μg/mL of the mitogen phytohemagglutinin (PHA) (Sigma–Aldrich), in the presence or absence of the compounds being tested.

5.1.3. Cell treatment

Leukemic cell lines K562, Lucena-1, Daudi, Raji and Jurkat were exposed to **5a** in culture for 24 h, 48 h, or 72 h. Lung cancer cells A549, H460 and GLC-4 were let to adhere for 24 h before being exposed to **5a**. Briefly, 2×10^4 cells/mL in 200 μL were seeded in 96-well microtiter plates in drug-free medium or in medium containing different concentrations of **5a** and maintained for 72 h at 37 °C in an atmosphere of 5% CO_2, and cell viability was then measured.

5.1.4. Cell viability

Cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) colorimetric assay. MTT can be reduced by dehydrogenases present in active mitochondria of living cells. After incubation in the presence or absence of the compound being tested, 20 μL of MTT (5 mg/mL) (Sigma–Aldrich) was added to each well. Plates were then kept at 37 °C in 5% CO_2 for 3 h. After centrifugation, 200 μL of DMSO (Sigma–Aldrich) was added to all wells in order to dissolve the dark blue crystals formed by the reduction of MTT. Absorbance was measured with a Tecan Sunrise ELISA reader at 490 nm. Absorbance was directly proportional to the amount of formazan (reduction product) present, indicating the percentage of living cells. The IC_{50} values were obtained by nonlinear regression on the GraphPad Prism v4.0 program (GraphPad Software, San Diego, CA, USA).

5.1.5. TNF-α release assay

PBMC were cultured in 24-well plates at 10^6 cells/mL. Stimulation of PBMC was induced by 2 μg/mL of Escherichia Coli 055:B5 LPS (Sigma, Chemical Co., St Louis, MO, USA) for 2 h (37 °C, 5% CO_2). Additionally, groups of cells were incubated at same time with 0.5% DMSO in RPMI 1640 (vehicle) with or without **5a** in different concentrations (10, 25, 50 and 100 μM). After stimulation, supernatants were collected and analyzed by ELISA. TNF-α Assay-TNF-α levels in PBMC supernatants were determined using sandwich-ELISA kits (R&D Systems, USA), with sensitivities of 4 pg/mL.

5.2. Chemistry

Melting points were determined with a Thomas–Hoover apparatus and are uncorrected. Column chromatography was performed on silica gel 230–400 mesh (Aldrich). ^{1}H NMR spectrum was recorded on a Bruker Avance 400 (400.013 MHz) spectrometer at ambient temperature. All *J* values are given in Hz. Chemical shifts are expressed in parts per million downfield shift from tetramethylsilane as an internal standard, and reported as position (δH) (relative integral, multiplicity (s = singlet, d = doublet, dd = double doublet, dt = double triplet, m = multiplet), coupling constant (*J* Hz) and assignment. ^{13}C NMR spectrum was recorded on a Bruker Avance 400 (100.003 MHz) spectrometer at ambient temperature with complete proton decoupling. Data are expressed in parts per million downfield shift from tetramethylsilane as an internal standard and reported as position (δC).

5.2.1. Synthesis of pterocarpanquinone 5a

To a stirred solution of **6** (106 mg, 0.5 mmol) in acetone (10 ml), 2-iodophenol **11** (83 mg, 0.75 mmol), silver-carbonate (413 mg, 1.5 mmol), PPh_3 (26.2 mg, 0.1 mmol, 20 mol %) and $Pd(OAc)_2$ (11.2 mg, 0.05 mmol, 10 mol %) were added. The reaction mixture was refluxed for 16 h and filtered in celite with ethyl acetate. The organic layer was washed with brine, dried over anhyd Na_2SO_4 and concentrated. The crude product was washed in *n*-hexane and purified by flash chromatography on silica. After column chromatography using *n*-hexane/ethyl acetate (95:5) as eluant, this compound was obtained as a yellow solid in 41% yield, mp 145 °C. A 40% yield was observed in absence of PPh_3.

^{1}H NMR ($CDCl_3$) δ 8.21–8.10 (m, 2H); 7.78–7.68 (m, 2H); 7.30–7.18 (m, 2H); 6.98–6.91 (m, 2H); 5.66 (d, *J* = 6.69 Hz; 1H); 4.59 (dd, *J* = 11.08, 5.03 Hz; 1H); 3.81 (t, *J* = 11.08 Hz; 1H); 3.64–3.53 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 183.25 (C); 179.28 (C); 158.73 (C); 157.01 (C); 134.54 (CH); 133.38 (CH); 130.58 (C); 130.57 (C); 129.58 (CH); 126.49 (CH); 126.42 (CH); 125.11 (C); 124.51 (CH); 121.23 (CH); 118.16 (C); 110.80 (CH); 72.32 (CH); 67.09 (CH_2); 38.37 (CH); LRMS (EI) *m/z 304*.

5.2.2. Eletrophilic substitution reactions on 5a. Synthesis of 5b, 5c and 5d

5.2.2.1. Pterocarpanquinone 5b. To a solution of **5a** (100 mg, 0.33 mmol) in 3.5 mL of $CHCl_3$ was added fuming HNO_3 (0.5 ml) at −30 °C. The reaction mixture was stirred for 2 h at same temperature. After this time, the TLC showed the formation of a product more polar than start material. The reactional mixture was dropped into cold water and then extracted with ethyl acetate. The organic layer was washed with brine, dried over anhyd Na_2SO_4 and concentrated in vacuum. The crude product was purified by flash chromatography on silica to furnish a yellow solid (57.6 mg, 0.165 mmol) in 50% yield, mp 250 °C.

^{1}H NMR ($CDCl_3$) δ 8.24–8.16 (m, 4H); 7.86–7.77 (m, 2H); 7.02 (d, 1H, *J* = 8.6 Hz); 5.90 (d, 1H, *J* = 6.3 Hz); 4.67 (d, 1H, *J* = 4.1 and 10.9 Hz); 3.90 (t, 1H, 10.8 Hz); 3.78–3.75 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 179.0 (C); 164.2 (C); 157.4 (C); 142.6 (C); 134.9 (CH); 133.9 (CH); 131.8 (C); 130.7 (C); 127.2 (CH); 126.8 (CH); 126.8 (CH); 121.2 (CH); 117.3 (C); 110. 9 (CH); 74.8 (CH); 66.6 (CH2); 38.1 (CH). LRMS (EI) *m/z 349*.

5.2.2.2. Pterocarpanquinone 5c. To a solution of **5a** (50 mg, 0.165 mmol) in 5 mL of CH_3CN was added NCS (100 mg, 0.75 mmol) at room temperature. The reaction mixture was stirred for 24 h. After the reaction was complete, the solvent was evaporated under reduced pressure and ethyl acetate was added. The organic layer was washed with brine, dried over anhyd Na_2SO_4 and concentrated. The crude product was purified by flash chromatography on silica to furnish a yellow solid (30.8 mg, 0.091 mmol) in 55% yield, mp 228 °C.

^{1}H NMR ($CDCl_3$) δ 8.18 (d, 1H, *J* = 7.5 Hz); 8.13 (d, 1H, *J* = 7.5 Hz); 7.81–7.71(m, 2H); 7.25 (d, 1H, *J* = 2.1 Hz); 7.17 (dd, 1H, *J* = 8.5, 2.1 Hz) 6.85 (d, 1H, *J* = 8.5 Hz); 5.69 (d, 1H, *J* = 6.7 Hz); 4.57 (dd, 1H, *J* = 11.2, 5.1 Hz); 3.81 (t, 1H, *J* = 11.1 Hz); 3.61- 3.55 (m, 1H);

^{13}C NMR ($CDCl_3$, 75 MHz) δ 183.31 (C); 179.3 (C); 157.63 (C); 157.20 (C); 134.8 (CH); 133.66 (CH); 131.98 (C); 130.76 (C); 129.71 (CH); 127.14 (C); 126.72 (CH); 126.17 (C); 124.87 (CH); 117.96 (C); 111.96 (CH); 73.16 (CH); 66.92 (CH_2); 33.67 (CH); LRMS (EI) *m/z* 338.

5.2.2.3. Pterocarpanquinone 5d. To a solution of **5a** (50 mg, 0.165 mmol) in 5 mL of CH_3CN was added NBS (106.5 mg, 0.6 mmol) at room temperature. The reaction mixture was stirred for 24 h. After the reaction was complete, the solvent was evaporated under reduced pressure and ethyl acetate was added. The organic layer was washed with brine, dried over anhyd Na_2SO_4 and concentrated. The crude product was purified by flash chromatography on silica to furnish a yellow solid (36.3 mg, 0.095 mmol) in 57% yield, mp 228 °C.

^{1}H NMR ($CDCl_3$) δ 8.2 (m, 2H);7.8 (m, 2H); 7.4 (d, 1H, *J* = 1.97 Hz); 7.35 (dd, 1H, J = 8.5 and 2.14 Hz); 6.85 (d, 1H, *J* = 8.45 Hz) 5.7 (d, 1H, J = 5.9 Hz); 4.6 (dd, 1H, *J* = 11.3, 6.0 Hz); 3.8 (t, 1H, *J* = 11.0 Hz); 3.6 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 183.16 (C); 179.15 (C); 158.0 (C); 157.06 (C); 134.67 (CH); 133.54 (CH); 132.48 (CH); 131.82 (C); 130.6 (C); 127.6 (CH); 126.58 (CH); 117.78 (C); 113.04 (C); 112.43 (C); 112.2 (CH); 73.0 (CH); 66.8 (CH_2); 38.44 (CH); LRMS (EI) *m/z* 382 (100%), 383, 384, 385, 386.

5.2.2.4. General procedure for reductive thioalkylation. A solution of pterocarpanquinone (0.06 mmol) in 3:1 THF–MeOH (8.0 mL) and Tris–HCl buffer (pH 7.4, 3.0 mL) was degassed for 15 min with dry nitrogen. To this solution was added sodium dithionite (254.04 mg 1.46 mmol) to effect hydroquinone formation, followed by a solution of the thiophenol (24.6 μL, 0.24 mmol) in degassed 3:1 THF–MeOH (2.0 mL). The reaction was stirred at room temperature under nitrogen atmosphere and monitored periodically by TLC. The reaction mixture was extracted by ethyl acetate and the organic layer was washed with brine and dried over sodium sulfate. The solvent was removed under reduced pressure. The crude product was purified by flash chromatography using 9:1 hexane/ethyl acetate as eluant.

5.2.2.5. Reductive thioalkylation of 4a. Treatment of **4a** (21.2 mg; 0.06 mmol) with sodium dithionite and thiophenol following the general procedure furnished **13a** 6.04 mg (0.0132 mmol; 22% yield) of a yellow oil.

^{1}H NMR ($CDCl_3$) δ 8.15 (m, 2H); 7.70 (m, 4H); 7.30 (m, 3H); 6.78 (s, 1H); 6.70 (s, 1H); 6.25 (s, 1H); 5.80 (s, 1H); 5.79 (s, 1H); 4.98 (dd, *J* = 11.73, 3.47 Hz; 1H); 4.85 (d, J = 4.85 Hz; 1H); 4.64 (s, 1H); 3.62 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ C 14.52 (CH); 30.12 (C); 36.55 (CH), 42.41(CH); 67.73 (CH_2), 98.62 (CH), 101.53 (CH_2), 106.96 (C), 116.60 (C), 118.05 (C), 119.64 (C); 126.92 (CH), 126.97 (CH), 128.60 (CH); 129.47 (CH); 131.36 (CH), 132.63 (C), 133.68 (CH), 133.90 (CH); 134.74 (CH), 142.08 (C), 147.43 (C), 148.10 (CH); 155.36 (C), 179.55 (C), 183.05 (C).

5.2.2.6. Reductive thioalkylation of 5a. Treatment of **5b** (18.2 mg; 0.06 mmol) with sodium dithionite and thiophenol following the general procedure furnished **13b** 26.5 mg (0.06 mmol; quantitative yield) of a yellow oil.

^{1}H NMR ($CDCl_3$) δ 8.15 (m, 2H); 7.70 (m, 4H); 7.30 (m, 3H); 7.0 (m, 2H); 6.70 (m, H); 5.1 (s, 1H); 4.9 (m, 1H); 4.8 (s, 1H); 3.7 (s, 1H).

5.2.2.7. Reductive thioalkylation of 5b. Treatment of **5b** (21 mg; 0.06 mmol) with sodium dithionite and thiophenol following the general procedure furnished **13c** 17.8 mg (0.04 mmol; 67%) as yellow oil.

^{1}H NMR ($CDCl_3$) δ 8.15 (m, 2H); 7.75 (m, 4H); 7.35 (m, 3H); 6.45 (m, 3H); 4.98 (m, 2H); 4.7 (s, 1H); 3.65 (s, 1H).

5.2.3. Synthesis of *ortho*-halophenols 9 and 10

5.2.3.1. *ortho*-Iodophenol 9. To a stirred 0.1 M solution of organomercurial **8d** in THF at −78 °C was slowly added a 1 M solution of I_2 in THF until the reaction mixture was purple. Immediately was added a solution of $NaHSO_3$ (20% w/v). The organic layer was extracted with ethyl acetate, dried over anhyd Na_2SO_4. The solvent was removed under reduced pressure to furnish a dark oil. The product was used in crude form.

^{1}H NMR ($CDCl_3$) δ 7.48–7.25 (m, 5H), 7.10 (s, 1H), 6.60(s, 1H), 5.00 (s, 2H), 3.82 (s, 3H).

5.2.3.2. *ortho*-Bromophenol 10. To a stirred solution of phenol **7** (920 mg, 4 mmol) in 15 mL of CH_3CN at 0 °C was added NBS (784 mg, 4.4 mmol). After the reaction was complete (10 min) the reaction mixture was extracted with ethyl acetate, washed with brine, dried over anhyd Na_2SO_4. The solvent was removed under reduced pressure. The crude product was purified by flash chromatography (hexane/ethyl acetate 7:3) on silica to furnish a dark solid in 75% yield.

^{1}H NMR ($CDCl_3$) δ 7.50–7.20 (m, 5H), 6.99 (s, 1H), 6.62 (s, 1H), 5.02 (s, 2H), 3.82 (s, 3H).

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