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Article

Antitumoral, Antileishmanial and Antimalarial Activity of Pentacyclic 1,4-Naphthoquinone Derivatives

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As pterocarpanquinonas **8a**-**c**, previamente sintetizadas em nosso laboratório, e uma série homóloga de derivados, substâncias **9a**-**c** preparadas neste trabalho, foram avaliadas em células de câncer de mama (MCF-7) e em cultura dos parasitas *Leishmania amazonensis* e *Plasmodium falciparum*. As substâncias **8a**-**c** foram mais potentes que **9a**-**c** nas células tumorais e em *Leishmania amazonensis*. Por outro lado, **9a**-**c** mostraram ser as mais ativas sobre o *Plasmodium falciparum*. Todas as substâncias estudadas foram biosseletivas, apresentando baixa citotoxicidade para linfócitos murinos frescos e linfócitos humanos ativados pelo mitógeno fitoemoaglutinina (PHA).

Pterocarpanquinones **8a**-**c**, previously synthesized in our laboratory, and an homologous series of derivatives, compounds **9a**-**c** prepared in this work, were evaluated on breast cancer cells (MCF-7) and on the parasites *Leishmania amazonensis* and *Plasmodium falciparum*, in culture. Compounds **8a**-**c** were more potent than **9a**-**c** on tumor cells and *Leishmania amazonensis*. On the other hand, **9a**-**c** showed to be more active on *Plasmodium falciparum*. All the compounds studied were bioselective, presenting negligible cytotoxicity against fresh murine lymphocytes and human lymphocytes activated by the mitogen phytohemaglutinin (PHA).

Keywords: antineoplasic, antiparasitic, pterocarpans, naphtoquinones, oxa-Heck reaction, antimalarial, leishmanicide

Introduction

Phytoalexins or phytotoxins are low molecular substances produced by plants in response to microorganism attacks.[1] These compounds inhibit the growth of bacteria and fungi *in vivo* and *in vitro*, and their production during an infection can induce resistance to subsequent infections. It has been shown that pterocarpans, among other group of natural products, act as phytoalexins. Phaseollidin for example (**1**, Figure 1), is present in higher concentration in species of Colombian beans resistant to *Colletotrichum lindemuthianum* fungus, the causal agent of anthrachnose disease, than in species sensitive to this fungus.[1] Pterocarpans present other interesting biological properties, depending on the pattern of substitution present at the A- and D-rings (Figure 1). For example, edunol (**2**), isolated from *Harpalyce braziliana*, a plant used in the northeast of Brazil as folk medicine, shows antiofidic activity *in vitro* and *in vivo* (mices).[2] Pterocarpan **3**, isolated from a plant of genus *Erythrina*, presents anti-HIV activity *in vitro*[3] and crotafuran B (**4**), isolated from *Crotalaria pallida*, has antiinflamatory properties *in vitro*.[4] In 1995, the catechol pterocarpan **5** was isolated from *Petalostemon purpureus* and showed to be active on KB cells, a human epidermoid carcinoma cell line.[5] This compound and four new derivatives were synthesized and their toxicities in

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leukemia cell lines, including cell lines with MDR (multi drug resistant) phenotype were evaluated.[6] Cathecol **5** and its possible metabolite *in vivo*, *ortho*-quinone **6**, showed to be the most active compounds. While **5** was bioselective, **6** presented high toxicity against human lymphocytes activated by the mitogen phytohemaglutinin (PHA).

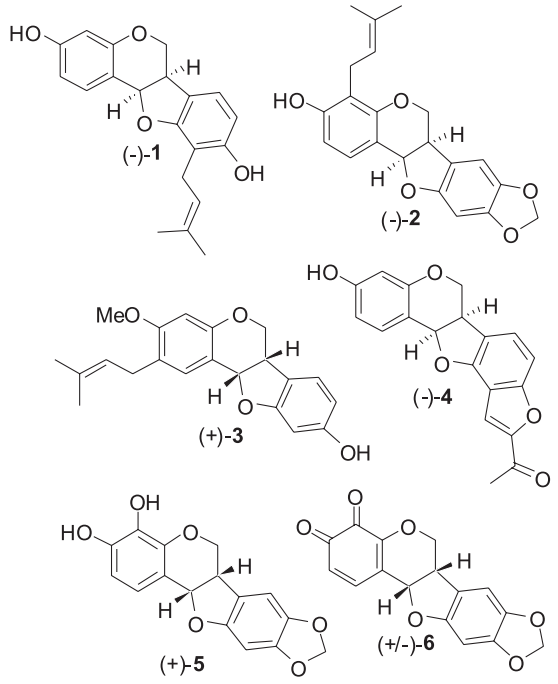
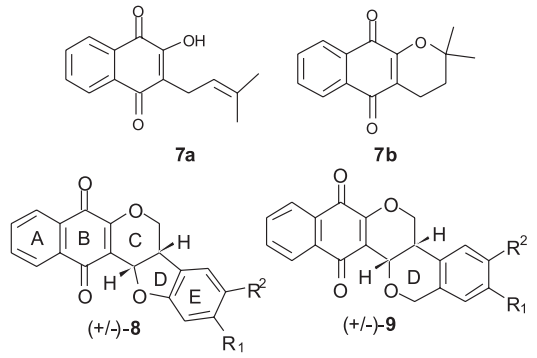


Figure 1. Bioactive naturally occurring pterocarpans (**1**-**5**) and derivative (**6**).

It is known that *ortho*-quinones lead to a depletion of physiological levels of NAD(P)H and mitochondrial membrane depolarization and react with DNA, leading to depurinating adducts.[7] This transformation has proven relevant in the toxicity of some hormones and steroids which are metabolized to *ortho*-quinones.[7] Thus we find most promising the design and synthesis of *para*-quinones derivatives, due to its lower toxicity and pterocarpanquinones **8a**-**c** (Figure 2) were chosen as new prototype molecules.[8] The new molecular architecture present in the structure of these compounds was designed through molecular hydridization between lapachol (**7a**) and α-lapachone (**7b**), two antineoplasic 1,4-naphthoquinones isolated from *Tabebuia* species[9] and pterocarpan **5** (Figure 1), which as mentioned before, is cytotoxic for KB and leukemia cells in culture (Figure 2).[5,6] The interesting pharmacological results obtained for **8a**-**c** prompted us to prepare an homologous series, compounds **9a**-**c** (Figure 2). Although the same groups are present at E-ring in compounds **8a**-**c** and **9a**-**c**, molecular modelling studies (*ab initio*) showed that the presence of the additional methylene group at the D-ring in **9a**-**c** alters the molecular shape.[10] In this paper we describe the preparation of the new homologous pterocarpanquinones **9a**-**c**. The effect of compounds **8a**-**c** and **9a**-**c** on MCF-7 cells (breast cancer),[11] cultured *Plasmodium falciparum*[12] and culture *Leishmania brasiliensis*[13] are compared, providing interesting information on the structural features required for bioactivity and bioselectivity.



a, R^1-R^2 = -OCH_2O-; **b**, R^1 = OMe, R^2 = OH; **c**, R^1 = OH, R^2 = OMe

Figure 2. Lapachol **7a**, α-lapahone **7** and pterocarpanquinones studied in this work.

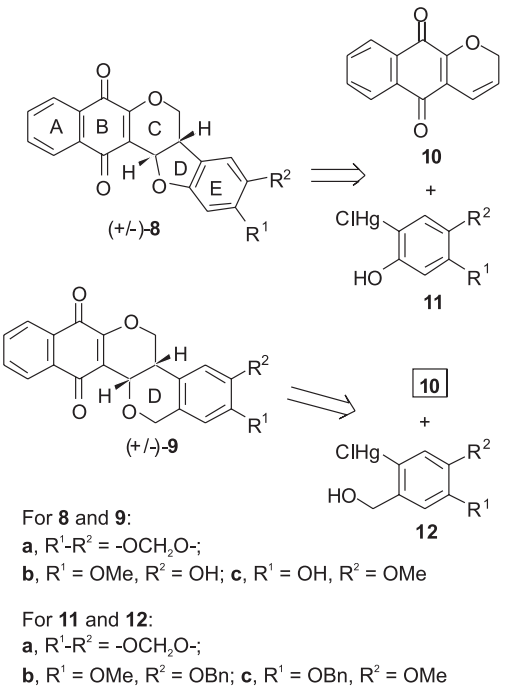
Results and Discussion

Several approaches are reported in the literature to prepare pterocarpans[14] but the oxy-arylation of chromenes, described for the first time in 1976 by Horino and co-workers, is the most used.[8,15] The choice of this approach seems to be related with the easy preparation of oxygenated *ortho*-mercury phenols through electrophilic mercuration and the transformation of these products into the corresponding *ortho*-palladium phenols by mercury-palladium exchange at room temperature. These species react with chromenes through an oxa-Heck reaction, allowing the introduction of different oxygenated aromatic moieties present at D-ring in the structure of pterocarpans. However, a severe limitation of this reaction is the use of organomercurials and $PdCl_2$ in stoichiometric amounts.

Some years ago Larock and co-workers[16] described a catalytic version for this reaction by using chromenes and dihydronaphthalen as olefins, and *ortho*-iodophenols instead *ortho*-mercury phenols, under conditions favoring the intervention of neutral palladium species (Et_3N, DMF, $Pd(OAc)_2$, *n*-Bu_4NCl). Kiss and co-workers[17] also reported the oxy-arylation of 7-(benzyloxy)-2*H*-chromene by *ortho*-iodophenol under conditions favoring a cationic mechanism (Ag_2CO_3, acetone, PPh_3, $Pd(OAc)_2$), but the scope of this reaction was not investigated by studying additional examples. In contrast with the easy preparation of oxygenated *ortho*-mercury phenols, oxygenated

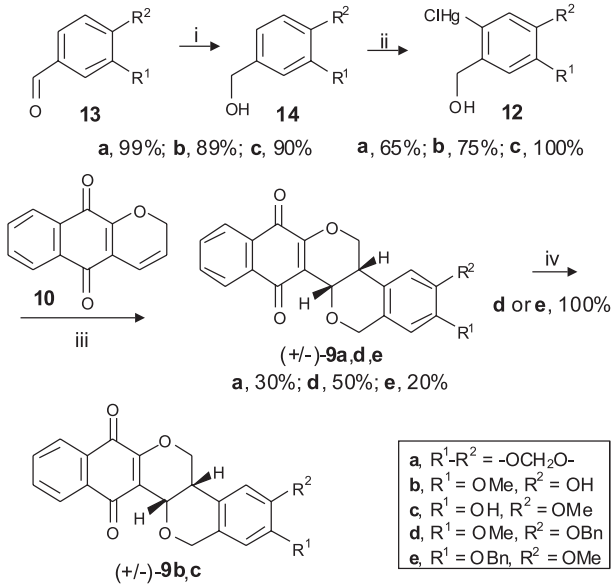
ortho-iodo phenols are more difficult to obtain and only few examples describing the iodination of these very reactive substrates are reported in the literature[18] and, maybe for this reason, until this moment natural products were not prepared by using Larock's and Kiss' approaches.

In view of the difficulty to prepare oxygenated *ortho*-iodophenols,[18] pterocarpanquinones **8a-c** were previously synthesized in our laboratory[8] through an oxa-Heck reaction between chromenequinone **10** and *ortho*-mercury phenols **11a-c**, under the original Horino's conditions[15] (Scheme 1). This is the sole report on the use of an electron poor chromene in an oxa-Heck reaction. While we are studying conditions to accomplish these reactions from *ortho*-iodo phenols in the presence of catalytic amounts of Pd reagents,[19] we decided to study the synthesis of pterocarpanquinones **9a-c** under the Horino's conditions, using *ortho*-mercury benzyl alcohols **12a-c** instead *ortho*-mercury phenols **11a-c** as source of organic species of palladium in this new oxy-arylation reaction (Scheme 1).



Scheme 1. Previous synthesis of **8a-c** and proposed synthesis of **9a-c** using Horino's protocol.

ortho-Mercury benzyl alcohols (**12a-c**) were prepared from easily available aldehydes **13a-c**, by reduction of the carbonyl group with $NaBH_4$ followed by *ortho*-mercuration of the resulting alcohols **14a-c** with $Hg(AcO)_2$/LiCl (Scheme 2). Reaction of chromene 10 with **12a-c** in the presence of stoichiometric $PdCl_2$ and LiCl in acetone at room temperature led to naphthoquinone **9a** and *O*-benzylated derivatives **9d**,**e**, respectively. Hydrogenolysis of the protecting benzyl groups in **9d**,**e** gave the naphthoquinones **9b**,**c**. In an attempt to get a catalytic version for this reaction, we performed an unsuccessfull experiment seeking the coupling of chromene **10** with the *ortho*-iodo benzyl alcohol.[19] This result has discouraged us to obtain *ortho*-iodo oxygenated benzylic alcohols.



i, $NaBH_4$ (1.25 eq), HCl (10%), MeOH, 0 °C, 4h; ii, $(AcO)_2Hg$ (3 eq), LiCl (3 eq), MeOH, 7 days; iii, $PdCl_2$, LiCl, acetone, iv H_2, Pd/C, acetone.

Scheme 2. Synthesis of pterocarpanquinones **9a-c**.

Our next goal was to check the relative stereochemistry in **9a-c** (Figure 2). Due to the overlapping of chemical shifts, nOe experiments involving hydrogens H4b and H12b could not be accomplished and we tried to establish the stereochemistry based on coupling constants. The dihedral angles calculated by molecular modelling[10] for hydrogens H4b and H12b in **9a** and the hypothetical corresponding *trans*-isomer 15 are 48.63^0 and 173.58^0 respectively, and the expected values for coupling constant between these hydrogens are 3.04 Hz and 10.16 Hz, respectively.[20] Once the observed coupling constant was 2.93 Hz, these data strongly suggest a *cis*-geometry around C and D-rings, in **9a** (Figure 3). The same trend in *J*H4b, H12b was observed for compounds **9b-e**. Recently, *cis*-coumarin-annulated sulfur heterocycles were described and the proposed *cis*-configuration between six-membered rings B and C was determined by ^{1}H NMR (J = 4.0 Hz).[21]

Pharmacology

The antitumoral effect on MCF-7 cells (breast cancer), antileishmanial effect on cultured *Leishmania*

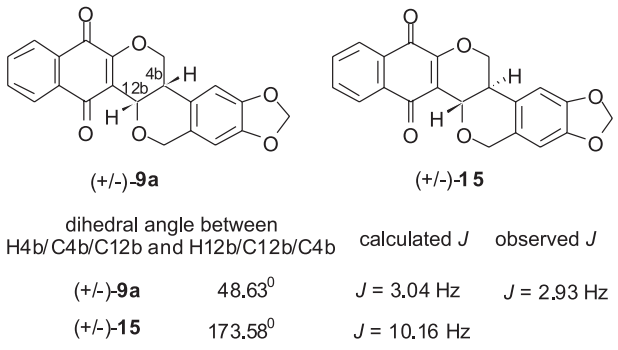


Figure 3. Epimeric pterocarpanquinones **9a** and **15**.

amazonensis promastigotes and antimalarial effect on cultured *Plasmodium falciparum* (FcB1 strain, resistant to chloroquine) are shown in Table 1. Naphthoquinone **8a**, bearing the apolar methylenedioxy group at the E-ring, was the least active in inhibiting the proliferation of MCF-7 cells (IC_{50} = 29.3 μmol L^{-1}). In contrast, the presence of the hydroxyl group in **8b** and **8c** increased the antitumoral potency by approximately four-fold (IC_{50} = 5.3 and 7.8 μmol L^{-1}, respectively). Similar to series **8**, in series **9** compound **9a** was also the least active on MCF-7 cells (IC_{50} = 19.3 μmol L^{-1}). Likewise, the presence of hydroxyl groups in E-ring also increased the antitumoral potency in *ca.* 2-fold (IC_{50} = 12.5 μmol L^{-1} for **9b** and 8.5 μmol L^{-1} for **9c**).

The antileishmanial activity of the series **8** compounds was also modulated by the pattern of substitution at the E-ring. The presence of free phenol groups in ring E in compounds **8b** and **8c** greatly increased the molecule activity (IC_{50} = 1.67 and 1.96 μmol L^{-1} respectively) as compared to **8a** (IC_{50} = 9.60 μmol L^{-1}). The ring expansion from 5 to 6 members in **9** substantially reduced the antileishmanial activity (IC_{50} > 20.0, 11.7 and 6.8 μmol L^{-1} for **9a**, **9b** and **9c**, respectively).

Similarly to the antileishmanial activity, naphthoquinone **8a** was the least active of the series **8** compounds on *Plasmodium falciparum* (IC_{50} > 10.0 μmol L^{-1}), while the presence of free phenol groups at E-ring in **8b** and **8c** resulted in a five to ten fold increase in activity (IC_{50} = 0.88 and 2.50 μmol L^{-1}, respectively). Contrary to observed with the antitumoral and antileishmanial activity, where the most potent compounds were of the series **8**, the antimalarial activity of series **9** compounds were superior to series **8**, effective in the sub-micromolar range (IC_{50} = 0.28, 0.03, and 0.04 μmol L^{-1} for **9a**, **9b** and **9c**, respectively). These results suggest that the molecular target(s) of series **9** compounds on *Plasmodium falciparum* is probably distinct from *Leishmania amazonensis* and MCF-7 cells. Chloroquine, α-lapachone (**7**) and amphotericin B were used as standard toward plasmodium, MCF-7 cells and leishmania, respectively.

Table 1. Activity of compounds **8a-c** and **9a-c** on MCF-7 cells, *Leishmania amazonensis* and *Plasmodium falciparum* (FcB1 strain)

Comp.	IC_{50} (μmol L^{-1} ± SD)		
	MCF-7	*Leishmania*	Plasmodium
8a	29.3 ± 3.3	9.60 ± 0.18	>10[a]
8b	5.3 ± 0.7	1.67 ± 0.19	0.88 ± 0.09
8c	7.8 ± 0.6	1.96 ± 0.23	2.50 ± 0.11
9a	19.3 ± 7.1	32.94 ± 0.35	0.28 ± 0.13
9b	12.5 ± 3.8	11.75 ± 0.25	0.03 ± 0.01
9c	8.5 ± 4.9	6.80 ± 0.14	0.04 ± 0.02
A	ND	ND	0.145± 0.011
B	>38.0	ND	ND
C	ND	0.82 ± 0.25	ND

(A), Chloroquine; (B), α-lapachone;, (C) Amphotericin B;[22] [a]Highest value.

All the compounds showed low toxicity to fresh murine lymphocytes (Table 2). Compound **8c** presented the best safety ratio for MCF-7 (Lymphocytes / MCF-7 = 10.6). Although compounds **8b** and **8c** displayed similar activities on *Leishmania amazonensis*, **8c** presented a higher level of therapeutic safety (Lymphocyte/*Leishmania amazonensis* ratio = 42.3). The new series **9a-c** was very selective against malarial parasites, with ratios ranging from 257 to 1,829.

Table 2. Cytotoxic effect of compounds **8a-c** and **9a-c** on fresh murine lymphocytes and their safety ratio toward MCF-7 tumor cells, *Leishmania* and *Plasmodium* as shown in Table1

Comp.	IC_{50} (μmol L^{-1} ± SD)	Safety ratio		
	Lymph.	Lymph / MCF-7	Lymph / *Leish.*	Lymph / *Plasm*
8a	53.1 ± 1.9	1.8	5.5	< 5.3
8b	33.0 ± 1.8	6.2	19.7	37.3
8c	83.1 ± 2.3	10.6	42.3	33.2
9a	72.3 ± 2.2	3.7	<3.6	257.1
9b	32.5 ± 1.9	2.6	2.7	969.6
9c	75.1 ± 2.1	3.8	11.0	1,829.2

Conclusion

New bioactive pterocarpanquinones were prepared through a new oxa-Heck reaction between chromenequinone **10** and *ortho*-mercury benzyl alcohols **12a-c**. Based on *in vitro* assays it was possible to discover active compounds with selective antitumoral, antileishmanial and antimalarial activities. The safety ratio for MCF-7 cells was lower than

observed for both parasites. This is not surprising, since lymphocytes are genetically more similar to mammalian carcinoma cells than to protozoan parasites.

Work is now in progress to identify the biological targets of these compounds and to prepare new derivatives for SAR (structure activity relationship) studies through palladium catalyzed oxa-Heck reaction. In particular, the development of new compounds with antimalarial properties sharing the new molecular architecture represented by series **9** compounds is a very promising possibility. Conversely, compounds of series **8** show a higher potential for the development of antileishmanial and antitumoral drugs.

Experimental

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).

*General procedure for the oxa-Heck or oxy-arylation reaction to naphthoquinone (**9d**), as an example*

To a mixture of $PdCl_2$ (28.31 mg, 0.16 mmol) and LiCl (13.6 mg, 0.32 mmol) in dry acetone (5 mL) was added chromene **10** (34 mg, 0.16 mmol) in dry acetone (10 mL). This mixture was stirred for 15 min at 0 °C and then 2-chloromercurio-4-benzyloxy-5-methoxy-benzylalcohol **12b** (79 mg, 0.16 mmol) in dry acetone (10 mL) was added. The suspension thus obtained was stirred for 12 h at 25 °C. After this time, brine (30 mL) was added and the reaction mixture was extracted with ethyl acetate (100 mL). The organic layer was dried (Na_2SO_4), evaporated and the resulting oil was purified by column chromatography, furnishing compound **9d** as a yellow solid (36 mg, 50%), mp 205 °C. ^{1}H NMR ($CDCl_3$) δ 2.84 (dt, *J* 12.18, 3.66 Hz, 1H), 3.88 (s, 3H), 4.16 (dd, *J* 12.40, 11.00 Hz, 1H), 4.36 (ddd, *J* 12.70, 4.65, 1.72 Hz, 1H), 4.78 (d, *J* 14.92 Hz, 1H), 4.88 (dd, *J* 2.93, 1.74 Hz, 1H), 5.00 (d, *J* 14.74 Hz, 1H), 5.16 (s, 2H), 6.60 (s, 1H), 6.66 (s, 1H), 7.38 (m, 5H), 7.74 (m, 2H), 8.14 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 34.01 (CH), 55.87 (CH_3), 63.28 (CH), 67.63 (CH_2), 68.06 (CH_2), 71.20 (CH_2), 107.69 (CH), 114.83 (CH), 118.85 (C), 121.39 (C), 126.29 (CH), 127.16 (CH), 127.83 (CH), 127.97 (C), 128.44 (CH), 130.65 (C), 131.94 (C), 133.09 (CH), 134.36 (CH), 136.63 (C), 146.90 (C), 149.39 (C), 156.25 (C), 179.77 (C), 182.87 (C).

*Naphthoquinone (**9a**).* After column chromatography this compound was obtained as a brown solid in 30% yield, mp 215 °C. ^{1}H NMR ($CDCl_3$) δ 2.90 (dt, *J* 12.45, 3.66 Hz, 1H), 4.24 (dd, *J* 12.46, 10.99 Hz, 1H), 4.52 (ddd, *J* 12.82, 4.76, 1.83 Hz, 1H), 4.78 (d, *J* 14.65 Hz, 1H), 4.90 (dd, *J* 2.93, 1.83 Hz, 1H), 4.80 (d, *J* 14.65 Hz, 1H), 5.96 (d, *J* 2.57 Hz, 1H), 5.98 (d, *J* 2.19 Hz, 1H), 6.57 (s, 1H), 6.70 (s, 1H), 7.78 (m, 2H), 8.19 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 34.74 (CH), 63.26 (CH), 67.99 (CH_2), 68.19 (CH_2), 101.04 (CH_2), 104.67 (CH), 108.67 (CH), 118.81 (C), 122.67 (C), 126.39 (CH), 128.55 (C), 130.71 (C), 131.99 (C), 133.19 (CH), 134.45 (CH), 146.62 (C), 147.23 (C), 156.30 (C), 179.82 (C), 182.95 (C). IR (KBr) ν_{max}/cm^{-1}: 2896 (aromatic H), 1684 (C=O), 1649 (C=O). High resolution mass spectrometry (M+H): calculated 363.0869, founded 363.1006.

*Naphthoquinone (**9e**).* After column chromatography this compound was obtained as a orange solid in 20% yield, mp 230 °C. ^{1}H NMR ($CDCl_3$) δ 2.87 (m, 1H), 3.92 (s, 3H), 4.28 (dd, *J* 12.45, 10.99 Hz, 1H), 4.55 (ddd, *J* 10.99, 4.39, 1.83 Hz, 1H), 4.74 (d, *J* 15.01 Hz, 1H), 4.92 (dd, *J* 2.93, 1.83 Hz, 1H), 4.98 (d, *J* 15.02 Hz, 1H), 5.14 (s, 2H), 6.60 (s, 1H), 6.72 (s, 1H), 7.40 (m, 5H), 7.74 (m, 2H), 8.16 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 34.25 (CH), 56.08 (CH_3), 63.21 (CH), 67.53 (CH_2), 68.12 (CH_2), 70.80 (CH_2), 109.64 (CH), 112.06 (CH), 118.84 (C), 122.08 (C), 126.28 (CH), 127.08 (CH), 127.22 (C), 127.79 (CH), 128.41 (CH), 130.62 (C), 131.90 (C), 133.10 (CH), 134.36 (CH), 136.49 (C), 147.77 (C), 148.59 (C), 156.25 (C), 179.76 (C), 182.89 (C).

*General procedure for hydrogenolysis to naphthoquinone (**9c**), as an example*

*Naphthoquinone (**9c**).* (76 mg, 0.17 mmol) in acetone was allowed to react with hydrogen (3 atm) in the presence of Pd/C (10%). After 3 h the catalyst was filtered and the product purified by flash chromatography using hexane:ethyl acetate (6:4) as eluant to give **9c** (62 mg) in quantitative yield, mp 150 °C. ^{1}H NMR (acetone-D) δ 2.96 (dt, *J* 12.09, 8.60, 3.48 Hz, 1H), 3.88 (s, 3H), 4.08 (dd, *J* 12.46, 10.63 Hz, 1H), 4.60 (ddd, *J* 8.06, 4.48, 1.74 Hz, 1H),

4.74 (d, *J* 13.92 Hz, 1H), 4.8 (m, 2H), 6.60 (s, 1H), 7.00 (s, 1H), 7.84 (m, 2H), 8.06 (m, 2H); ^{13}C NMR (acetone-D) δ 34.96 (CH), 56.30 (CH_3), 64.49 (CH), 68.17 (CH_2), 68.72 (CH_2), 111.67 (CH), 113.10 (CH), 118.53 (C), 120.00 (CH), 122.11 (CH), 126.67 (CH), 126.73 (CH), 128.86 (C), 131.97 (C), 132.99 (C), 134.10 (CH), 135.20 (CH), 146.94 (C), 147.50 (C), 157.12 (C), 180.17 (C), 183.19 (C). IR (KBr) ν_{max}/cm^{-1}: 3414 (OH), 2927 (aromatic H), 1681 (C=O), 1620 (C=O).

*Naphthoquinone (**9b**).* This compound was obtained after column chromatography (hexane:ethyl acetate (6:4)) as a orange solid in quantitative yield (62 mg), mp 145 °C. ^{1}H NMR (acetone-D) δ 2.96 (m 1H), 3.84 (s, 3H), 4.08 (dd, *J* 12.46, 10.72 Hz, 1H), 4.54 (ddd, *J* 12.39, 4.51, 1.66 Hz, 1H), 4.72 (d, *J* 14.65 Hz, 1H), 4.8 (m, 2H), 6.74 (s, 1H), 6.86 (s, 1H), 7.86 (m, 2H), 8.08 (m, 2H). IR (KBr) ν_{max}/cm^{-1}: 3414 (OH), 2928 (aromatic H), 1679 (C=O), 1650 (C=O).

Biological assays

Antimalarial activity. Plasmodium falciparum (FcB1-Columbia), a chloroquine-resistant strain (chloroquine IC_{50}: 145 ± 11.2 nmol L^{-1}), was cultured according to the method described by Trager and Jensen,[23] with modifications.[24] Cultures were synchronized by 5% D-sorbitol lysis (Merck, Darmstadt, Germany).[25] *In vitro* antimalarial activity testing was evaluated by [^{3}H]-hypoxanthine (ICN, France) incorporation as described by Desjardins *et al.*,[26] with modifications.[25] Incubation time between parasite culture and the drugs was 48 h.

Antitumoral activity. The antitumoral activity was estimated on human breast cancer cells (MCF-7). This cell line was cultured in the same conditions as *P. falciparum*, except for the 5% human serum in MCF-7 instead of 5% fetal calf serum (Boehringer) in *Plasmodium*. After addition of drugs in varying concentrations, cell growth inhibition was determined by [^{3}H]-hypoxanthine incorporation after a 48 h incubation.[27]

Antileishmanial activity. The antileishmanial activity was fluorimetrically determined against promastigote forms of *L. amazonensis* (Josefa strain) parasites transfected with the green fluorescence protein (GFP).[28,29] Fluorescent promastigotes were plated in triplicate at 10^5 parasites/well with varying concentrations of test compounds in a final volume of 200 µL of medium containing 5% fetal calf serum and 1% hybri-max dimethyl sulfoxide (DMSO, Sigma). After 72 h at 26 °C, the fluorescence intensity of the cultures was measured using a plate-reader fluorometer (Bio-Tek) set at 435 nm excitation/538 nm emission. The IC_{50} values were calculated by linear regression analysis.

Cytotoxicity against lymphocytes. For cytotoxicity against mammalian cells, total murine lymph node cells (mostly lymphocytes) were cultured for 48 h at 37 °C with varying concentrations of the test compounds. The release of the cytoplasmic enzyme lactate dehydrogenase (LDH) into the culture medium was measured using an assay kit (Doles Reagentes, Brazil). Maximum and minimum release values were obtained using cells cultured with 2% Triton X-100 or with no drugs, respectively. The IC_{50} values were calculated by linear regression analysis.

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Antitumoral, Antileishmanial and Antimalarial Activity of Pentacyclic 1,4-Naphthoquinone Derivatives

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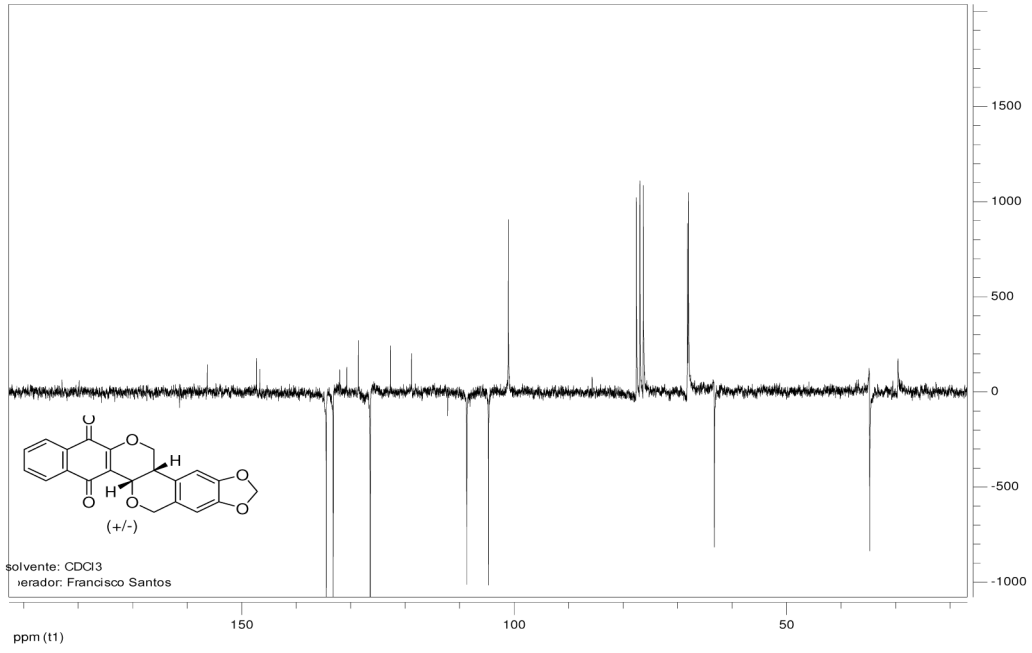

Figure S1. ^{13}C NMR (APT) spectrum for compound **9a**.

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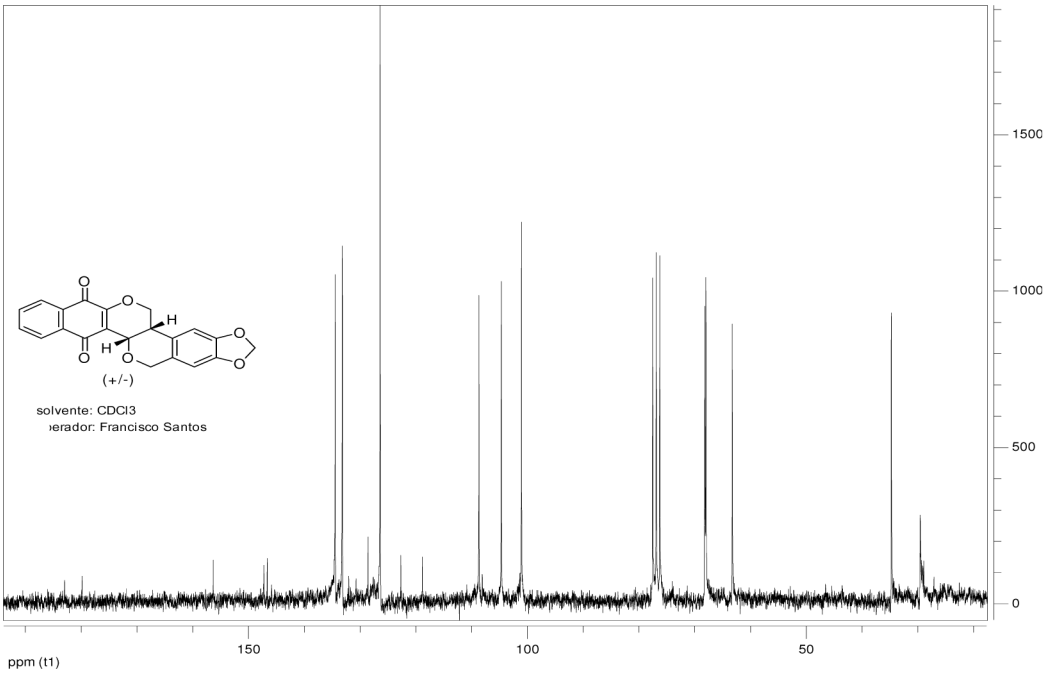

Figure S2. ^{13}C NMR spectrum for compound **9a**.

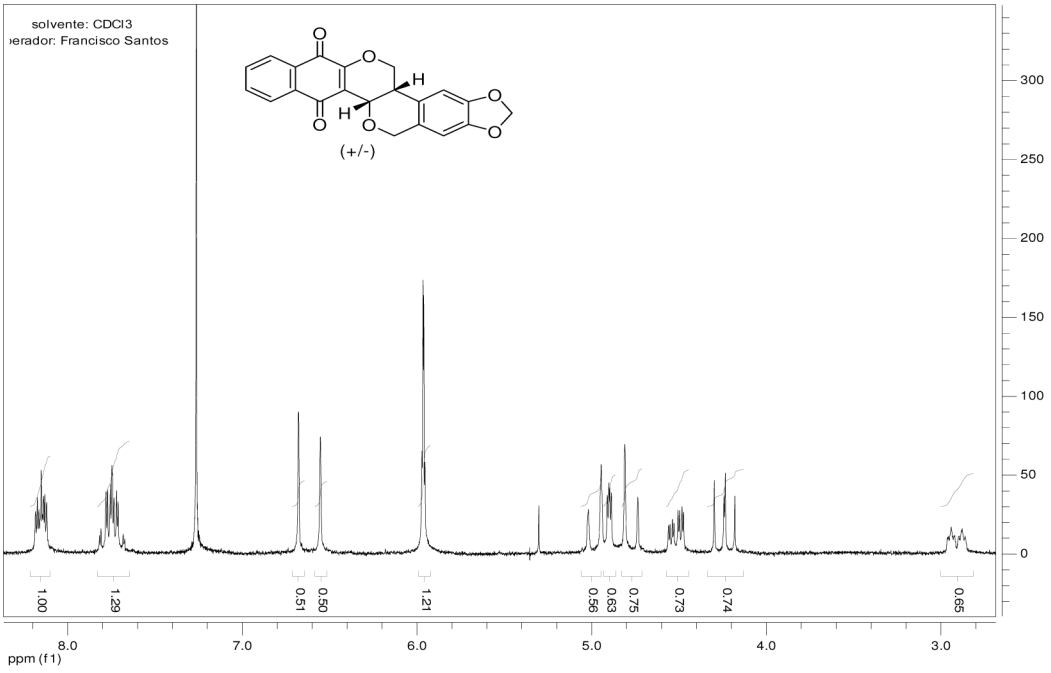

Figure S3. ^{1}H NMR spectrum for compound **9a**

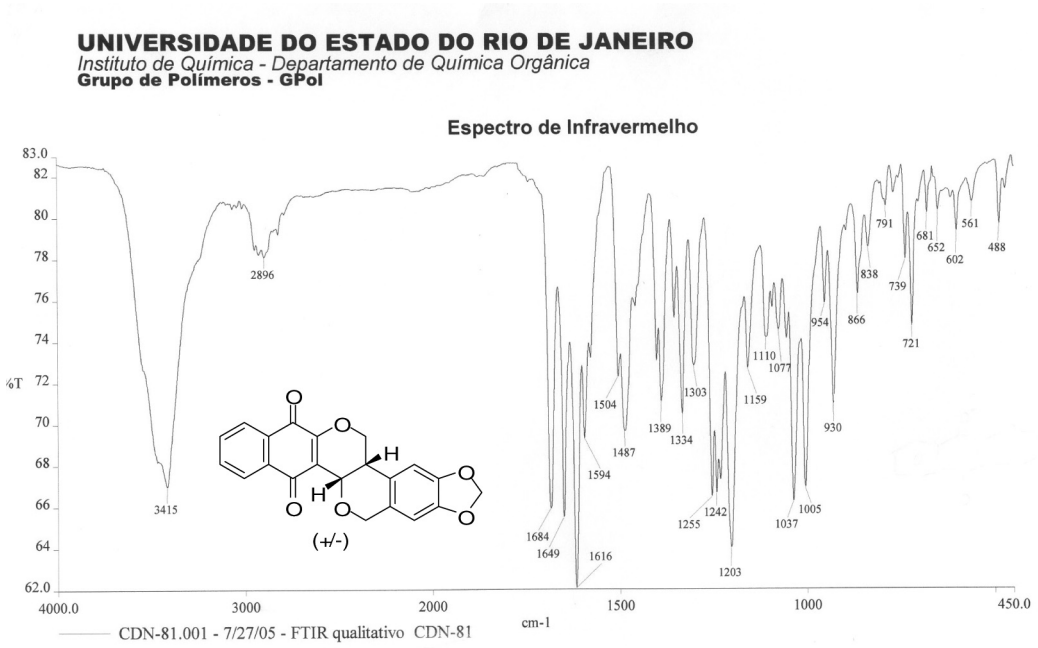

Figure S4. Infra Red spectrum for compound **9a**.

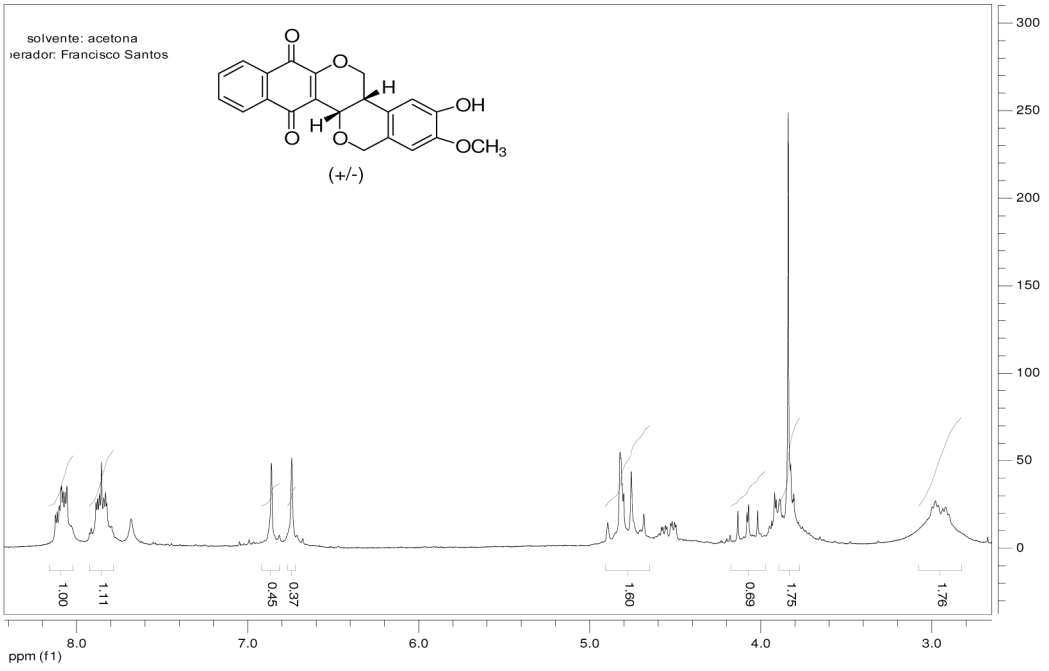

Figure S5. ^{1}H NMR spectrum for compound **9b**.

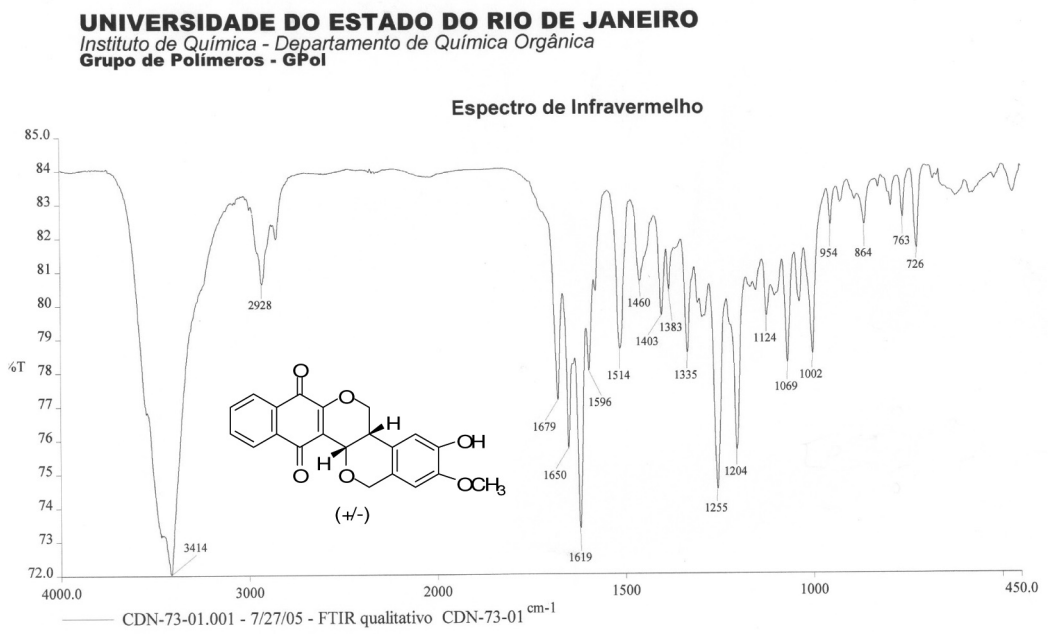

Figure S6. IR mass spectrum for compound **9b**.

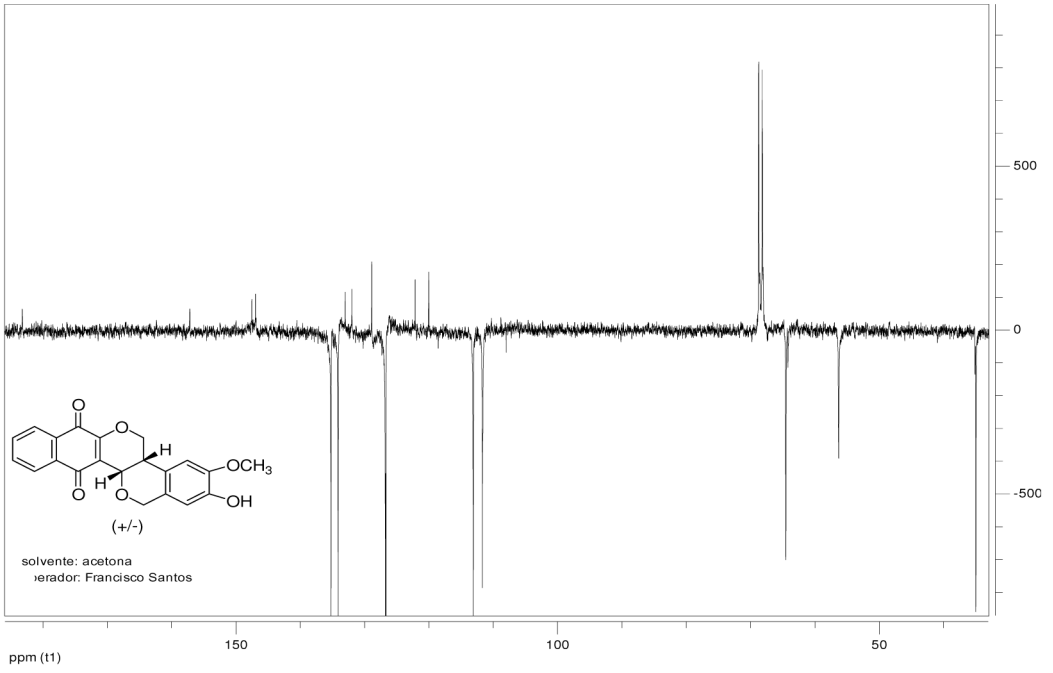

Figure S7. ^{13}C(APT) NMR spectrum for compound **9c**.

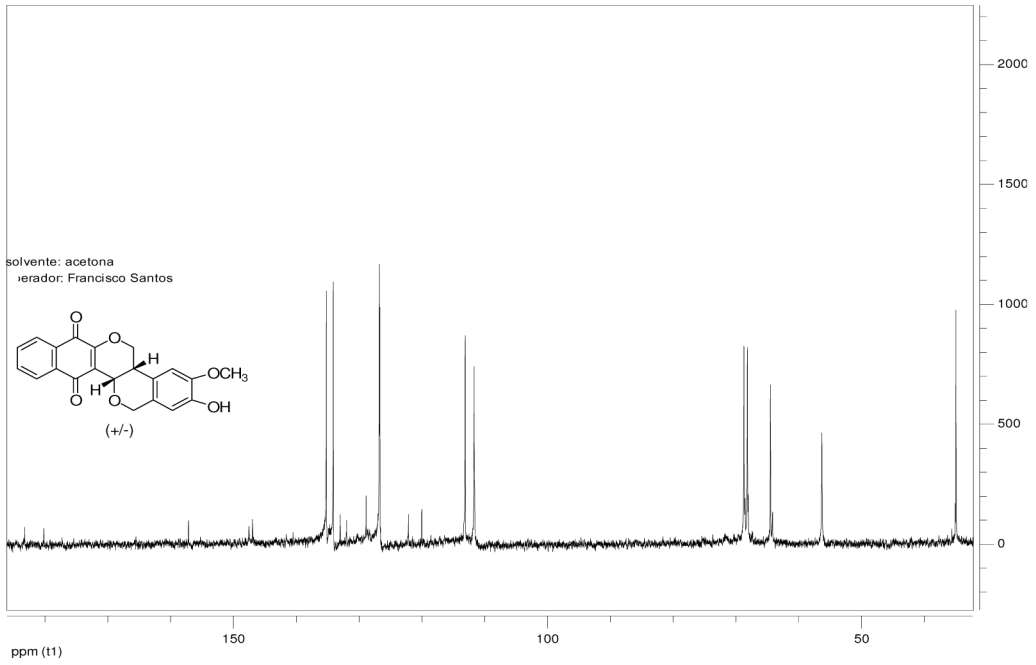

Figure S8. ^{13}C NMR spectrum for compound **9c**.

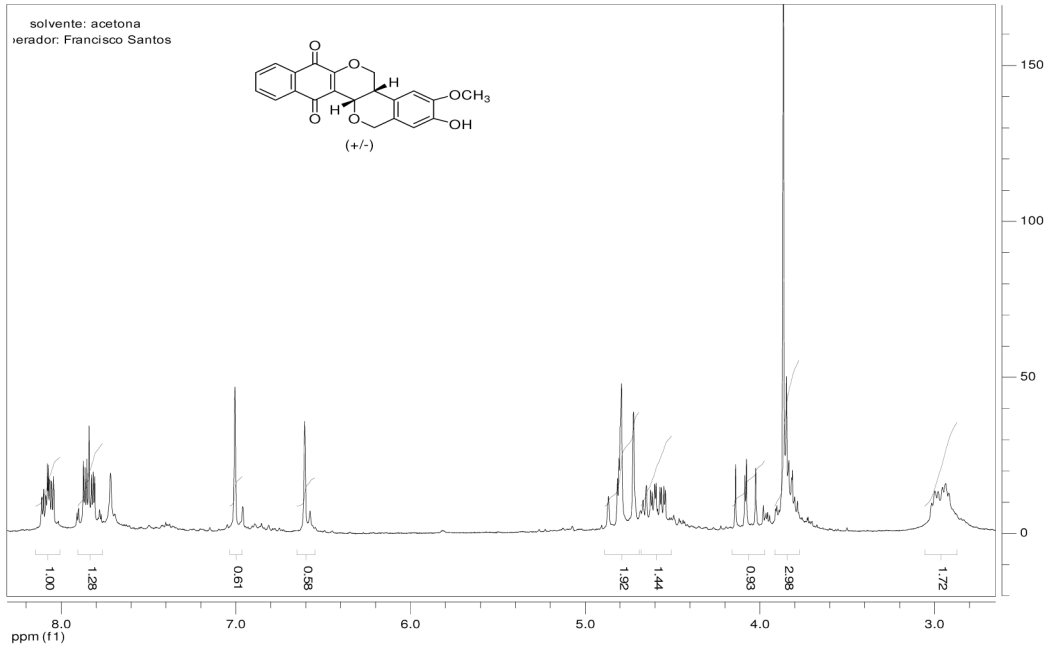

Figure S9. ^{1}H NMR spectrum for compound **9c**.

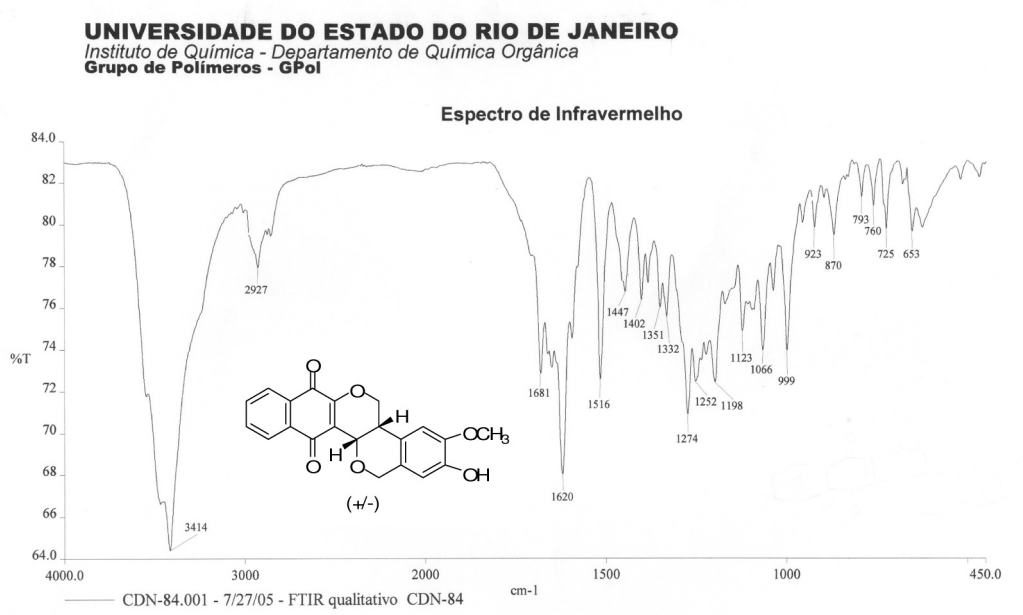

Figure S10. Infra Red spectrum for compound **9c**.