Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 44 (2009) 920–925
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Short communication

(±)-3,4-Dihydroxy-8,9-methylenedioxypterocarpan and derivatives: Cytotoxic effect on human leukemia cell lines

Chaquip D. Netto [a], Eduardo S.J. Santos [b], Carolina Pereira Castro [b], Alcides J.M. da Silva [a], Vivian M. Rumjanek [b,*], Paulo R.R. Costa [a,**]

[a] *Laboratório de Química Bioorgânica (LQB), Núcleo de Pesquisas de Produtos Naturais, Centro de Ciências da Saúde, Bloco H, Universidade Federal do Rio de Janeiro, RJ 21941-590, Brazil*
[b] *Laboratório de Imunologia Tumoral, Instituto de Bioquímica Médica, Centro de Ciências da Saúde, Bloco H, Universidade Federal do Rio de Janeiro, RJ 21941-590, Brazil*

Received 22 October 2007; received in revised form 10 January 2008; accepted 18 January 2008
Available online 2 February 2008

Abstract

Naturally occurring pterocarpans **1a**,**b**, pterocarpan **1c**, isoflavane **2** and *ortho*-quinone **3** were synthesized in the racemic form and their cytotoxic effect was evaluated on the human leukemia cell lines K562 (resistant to oxidative stress), Lucena-1 (MDR phenotype) and HL-60. *Ortho*-quinone **3** ($IC_{50} = 1.5$ μM, 1.8 μM and 0.2 μM, respectively) and catechol pterocarpan **1a** ($IC_{50} = 3.0$ μM, 3.7 μM and 2.1 μM, respectively) were the most active compounds on these cells and were also evaluated on other human leukemia cell lines (Jurkat and Daudi). *Ortho*-quinone **3** was 2 to 10 times more potent than pterocarpan **1a**, depending on the cell line considered, however, showed a greater toxicity for lymphocytes activated by PHA.

Keywords: Pterocarpans; Oxa-Heck reaction; Cytotoxic activity; MDR phenotype; Human leukemia; Quinones

1. Introduction

Pterocarpans are isoflavonoids [1] isolated mainly from Leguminosae. These compounds act as phytoalexins, being biosynthesized as a defense mechanism of these plants against pathogens [2]. Some phytoalexins, such as phaseollidin and erybraedins A, B and C, possess relevant antibiotic properties [3,4]. Compounds prenylated at the A-ring, such as cabenegrins A-I and A-II [5], edunol [6] and analogues [7], are potent neutralizers of the effects of snake venoms, acting through the inhibition of phospholipases and proteases [7,8].

Pterocarpan (+)-**1a** (Fig. 1), isolated from *Petalostemon purpureus*, showed to be cytotoxic for KB cells, a human epidermoid carcinoma cell line [9,10]. Its isomer, pterocarpan (−)-**1b**, was isolated from *Cladrastis platycarpa* but its cytotoxic effect was not evaluated [11,12]. Maakian (+)-**1d** was also isolated from *P. purpureus* and showed to be less potent than (+)-**1a** for KB cells [9]. Compound (+)-**1e**, recently isolated from *Platymiscium floribundum*, induced apoptotic cell death in HL-60 leukemia cells [13]. Pterocarpan (±)-**1c** was not isolated from natural sources. Compounds **1a**, **1d** and **2** had been previously synthesized by our group [14,15], whereas compounds **3** and **1c** are new and **1b**, although a natural product, has never been synthesized previously.

Independent of their mode of action, the cytotoxicity exerted by anti-tumor compounds tends to involve the induction of apoptosis. Therefore, tumor cells presenting various mechanisms capable of interfering with the apoptotic process are more resistant to treatment. In the present work the cytotoxic effect of compounds **1a**–**d**, **2** and **3** was evaluated on human leukemia cell lines selected based on their special characteristics. All compounds were tested on the K562 cell line, derived from a chronic myeloid human erythroleukemia, its multidrug

* Corresponding author. Tel.: +55 21 2562 6791; fax: +55 21 2562 6512.
** Corresponding author.
E-mail address: lqb@nppn.ufrj.br (V.M. Rumjanek).

0223-5234/$ - see front matter © 2008 Published by Elsevier Masson SAS.
doi:10.1016/j.ejmech.2008.01.027

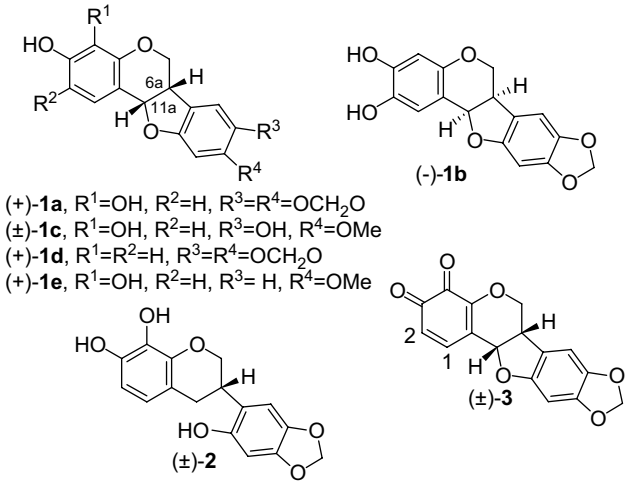
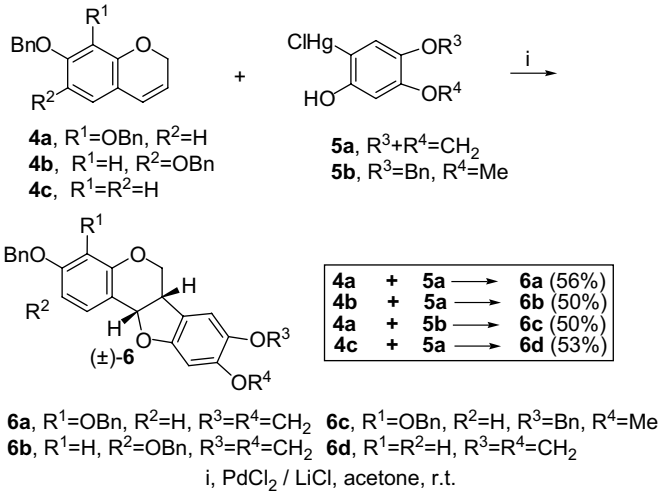


Fig. 1. Pterocarpans **1a**–**e**, isoflavane **2** and *ortho*-quinone **3**.



Scheme 1. Synthesis of benzylated pterocarpans **6a**–**d**.

resistant counterpart K562-Lucena-1 cell line and HL-60, derived from a pro-myelocytic leukemia. The more active compounds were also tested on lymphoid cell lines: Daudi, a Burkitt's lymphoma, and Jurkat, derived from T lymphoblastic leukemia. These cell lines show varying resistance to oxidative stress (Table 1).

2. Chemistry

Compounds **1a**,**d** and **2** were prepared in the racemic form, as previously described by our group [14, 15], while the synthesis of catechol pterocarpans **1b**,**c** and *ortho*-quinone **3** is reported herein for the first time. The benzylated pterocarpans **6a**–**d**, intermediates required to prepare these target compounds, were synthesized through a $PdCl_2$ mediated oxa-Heck or oxy-arylation reaction [25] between chromens **4a**–**c** and organomercurials **5a**,**b** in moderated yields (Scheme 1) [14,15,26,27].

Debenzylation of **6a**–**d** with H_2/Pd–C (3 atm) led to the pterocarpans **1a**–**d** in excellent yields (Scheme 2). In the presence of excess of Pd/C the benzylic C–O bond in **6a** was also cleaved and isoflavane **2** was formed in quantitative yield.

Once phenols and catechols can be oxidized to quinones in biological medium, acting as pro-drugs [28–32], we tried to prepare quinones through the oxidation of **1a**,**b**,**d** (Scheme 2) [33]. In the presence of excess of DDQ, **1a** was transformed into *ortho*-quinone **3**, but under the same conditions **1b** led to a non-identified mixture of products, while **1d** led to the corresponding coumestan (not shown).

Table 1
Cell line panel

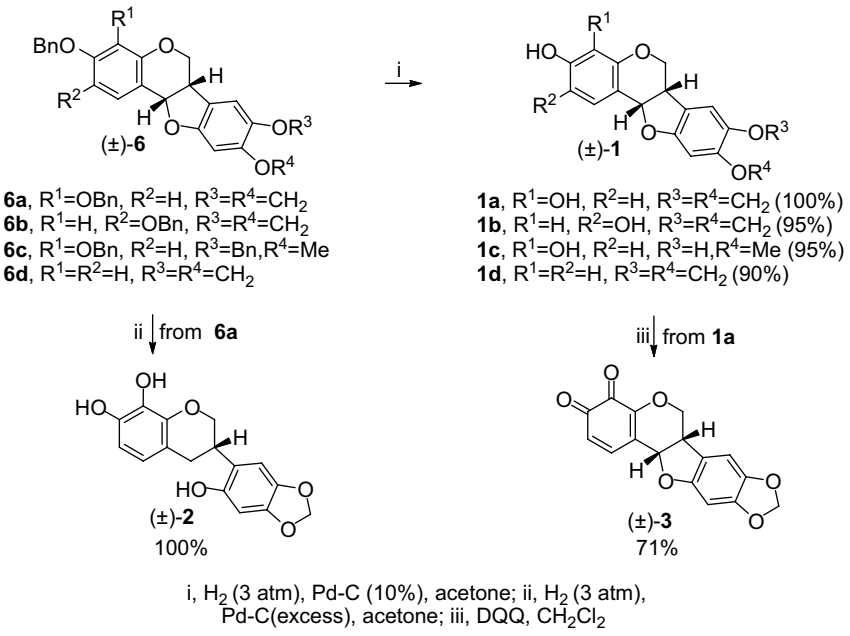
Cell lines	Characteristics	Response to oxidative stress	References
K562	High levels of intracellular GSH High levels of catalase	Resistant	[16,17]
Lucena-1	Overexpression of P-glycoprotein Increased levels of catalase	Resistant	[18,19]
HL-60	Moderate levels of Bcl-2	Sensitive	[16,20,21]
Daudi	No expression of Bcl-2	Sensitive	[22]
Jurkat	High levels of Bcl-2	Moderately resistant	[23,24]

3. Results and discussion

The cytotoxic effect of compounds **1a**–**d**, **2** and **3** on leukemic cells is summarized in Table 2. The pterocarpan **1a** exhibited antiproliferative activity on K562 ($IC_{50} = 3.0$ μM), Lucena-1 ($IC_{50} = 3.7$ μM) and HL-60 ($IC_{50} = 2.1$ μM) to a similar extent. In contrast, **1b** and maakian **1d** were inactive on those cell lines ($IC_{50} \sim 20$–50 μM). Pterocarpan **1c** and isoflavane **2** were almost inactive compared to **1a** on K562 and Lucena-1 cells ($IC_{50} \sim 10$–20 μM), however, were active in HL-60 cells ($IC_{50} = 6.4$ μM and 5.7 μM, respectively), a much more sensitive cell line. The *ortho*-quinone **3** was twice as effective compared to compound **1a** on K562 ($IC_{50} = 1.5$ μM) and Lucena-1 ($IC_{50} = 1.8$ μM) and almost 10 times more active on HL-60 ($IC_{50} = 0.2$ μM).

The cytotoxic effect of the more active compounds, **1a** and **3**, was also studied in Jurkat and Daudi cells (Table 2). Compound **1a** did also show a potent effect on Daudi cell lines ($IC_{50} = 2.8$ μM) being ~2 times less active on Jurkat cells ($IC_{50} = 7.6$ μM). As mentioned in Section 1, Jurkat cells present a high expression of Bcl-2, which confers a certain degree of resistance to mitochondria induced oxidative stress. The compound **3**, already shown to be more active than **1a** on K562, Lucena-1 and HL-60 cells, was also nearly 3–9-fold more potent on Jurkat ($IC_{50} = 1.9$ μM) and Daudi ($IC_{50} = 0.9$ μM) cell lines.

However, as shown in Fig. 2, despite the fact that compound **3** was very active against leukemia cells it presented high toxicity against normal lymphocytes ($IC_{50} = 2.4$ μM) activated by the mitogen phytohemaglutinin (PHA). In contrast, pterocarpan **1a**, although less potent for leukemia cells, was 10



Scheme 2. Transformation of **6a-d** into the target compound **1a–d**, **2** and **3**.

times less toxic for lymphocytes when compared to **3** ($IC_{50} = 24.1$ μM).

Among the pterocarpans, the potency on leukemia cells was dependent on the pattern of substitution at the A- and D-rings. Since **1a** was active on K562 and Lucena-1, cell lines very resistant to the oxidative stress, a mechanism of action through the redox cycle seems unlikely. It has been suggested that *ortho*-quinones are formed by oxidation of catechols and these metabolites are responsible for the toxicity of these compounds [28–31]. However, when comparing IC_{50} towards lymphocytes, this conversion seems unlikely. Should this mechanism be operating, similar levels of toxicity should be expected. When the position of the catechol group in A-ring was changed (**1b**), the activity on all cell lines studied was abolished. In addition, the absence of the catechol group on maakian (**1d**) dramatically reduced the effect on all cell lines tested, confirming the importance of this group for the cytotoxic effect. On the other hand, although **1c** has the catechol group at the A-ring like in **1a**, the presence of a polar phenol group at the D-ring led to a decrease in the cytotoxic effect on K562 and Lucena-1 cells. Due to the *cis*-fusion between B and C rings, compound **1a** is conformationally constrained, while in isoflavane **2**, inactive on the K562 and Lucena-1 cell lines, the D-ring is conformational free. However, these compounds were active against the oxidative stress sensitive cell HL-60.

It has been shown that *ortho*-quinones can be reduced to the corresponding semiquinones radical by P450 reductases, leading to the formation of reactive oxygen species [34–36]. However, *ortho*-quinone **3** was very active on either oxidative stress resistant or sensitive cell lines. As the Cβ (C1) in **3** is highly electrophilic, this *ortho*-quinone could act as a Michael acceptor and we would like to suggest this as an alternative mechanism of action.

Table 2
Cytotoxic effect (IC_{50}, μM) of compounds **1a–c**, **2** and **3** on human leukemia cell lines

	1a	1b	1c	1d	2	3
Compounds (IC_{50}, μM)[a]						
K562	3.0	>20	>10	>50	>10	1.5
Lucena I	3.7	>50	>20	>50	>20	1.8
HL-60	2.1	>20	6.4	>50	5.7	0.2
Jurkat	7.6	nd	nd	nd	nd	1.9
Daudi	2.8	nd	nd	nd	nd	0.9

[a] Effect of compounds **1a–d**, **2** and **3** on the viability of various leukemic cell lines was determined by MTT assay 72 h after incubation. Each point of the experiments was performed in triplicate and results are expressed as the mean of at least three experiments. nd (not done).

4. Conclusions

The cytotoxic effect shown by compound **1a** toward several human leukemia cell lines, including K562 (oxidative stress resistant), Lucena-1 (multidrug resistance phenotype – MDR) and, to a lesser extent, Jurkat (Bcl-2 high), in addition to its low toxicity against lymphocytes activated by PHA, make this compound an attractive target. Furthermore, multidrug resistance is the major cause of chemotherapy failure in our days and we present evidence, herein, that MDR cells overexpressing Pgp are sensitive to compound **1a**, suggesting it is not a substrate to this pump. On the other hand, compound **3**, despite its high potency and broad spectrum of anti-tumor activity, probably could not be used in humans because of its high toxicity.

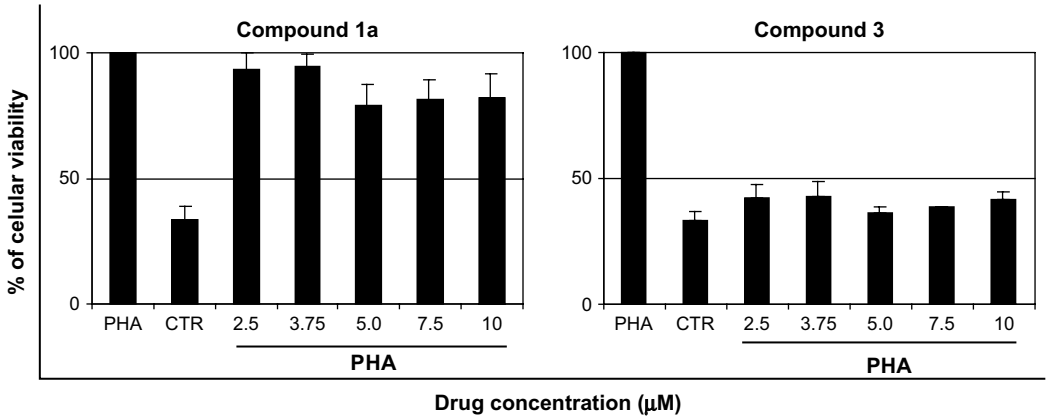


Fig. 2. Effect of compounds **1a** and **3** on the viability of normal human peripheral lymphocytes. Viability was determined by MTT assay 72 h after incubation. Lymphocytes were stimulated with the mitogen PHA (5 μg/mL). CTR − control cells were treated with diluent and no PHA. Each point of the experiment was performed in triplicate and results are expressed as the mean + SD of at least three experiments.

5. Experimental protocols

5.1. Biology

5.1.1. Cell lines

Human leukemic cell lines: Jurkat, Daudi, HL-60, K562 and its resistant variant, Lucena I, were all maintained in RPMI 1640 medium, supplemented with 5×10^{-5} M 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), inactivated at 56 °C for 1 h. Daudi cells were supplemented with 20% fetal calf serum (FCS). Lucena I cultures had also 60 nM of vincristine sulfate added in order to maintain the resistant phenotype. All cells, except Jurkat, were passaged at a concentration of 2×10^4 cells/mL after 3 days in culture and kept at 37 °C in 5% CO_2 humidified environment. Jurkat cells were passaged at a concentration of 10^6 cells/mL.

5.1.2. Preparation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC) were obtained by fractionating heparinized blood from healthy volunteers on ficoll-hypaque (Hystopaque), density gradient centrifugation. The PBMC fraction was washed twice and resuspended in RPMI 1640, supplemented as described above. The cell number was adjusted at 10^6 cells/mL and incubated with 5 μg/mL of the mitogen PHA, in the presence or absence of compounds being tested.

5.1.3. Treatment with the different compounds

Cell lines were exposed to the compounds **1**–**3** (0.1–50 μM) in culture for 72 h. Briefly, 2×10^4 cells/mL were seeded in 96 well microtiter plates in drug-free medium or in medium containing different concentrations of compounds **1**–**3** and kept for 72 h at 37 °C in an atmosphere of 5% CO_2. After that period cell viability was measured.

5.1.4. Cell viability

Cell viability was accessed by MTT colorimetric assay. MTT is a substance capable of being reduced by dehydrogenase enzymes present inactive mitochondria of living cells. After 72 h of incubation 20 μL of MTT (5 mg/mL) were added to each well. Plates were kept at 37 °C, 5% CO_2 for 3 h. After centrifugation, 200 μL DMSO was added to all wells in order to dissolve the dark blue crystals formed by the reduction of MTT. Absorbance was then read in an ELISA reader at wavelength of 490 nm. The absorbance was directly proportional to the amount of formazan (reduction product) present, indicating the percentage of living cells. All drugs remained in the media during incubation.

5.2. Chemistry

Column chromatography was performed on silica gel 230–400 mesh (Aldrich). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini 200 and Bruker ARX 400 instruments using tetramethylsilane (TMS) as standard, $CDCl_3$ and acetone-d_6 as solvents. *J* values are given in Hertz.

*5.2.1. Oxy-Heck reaction between **4b** and **5a**: synthesis of **6b***

To a mixture of $PdCl_2$ (87 mg, 0.49 mmol) and LiCl (42 mg, 1.0 mmol) in acetone (5 mL) was added chromen **4b** (158 mg, 0.46 mmol) in acetone (10 mL). This mixture was stirred for 15 min at 0 °C and then 2-chloromercurio-4,5-methylenedioxyphenol **5a** (172 mg, 0.42 mmol) in acetone (10 mL) was added. The suspension thus obtained was stirred for 12 h at 25 °C. After this time, brine (150 mL) was added to it and the mixture was extracted with acetyl acetate (3×50 mL), the organic extract dried (Na_2SO_4), and submitted to column chromatography to give the compound **6b** as a solid (101.0 mg, 50%).

^{1}H NMR δ 7.37 (10H, m), 7.07 (1H, s), 6.70 (1H, s), 6.53 (1H, s), 6.43 (1H, s), 5.90 (1H, d, $J = 5.49$ Hz), 5.89 (1H, d, $J = 5.49$ Hz), 5.41 (1H, d, $J = 6.87$ Hz), 5.13 (2H, s), 5.11

(2H, s), 4.18 (1H, dd, $J = 10.8$, 4.63 Hz), 3.59 (1H, t, $J = 10.75$ Hz), 3.46 (1H, m).

^{13}C NMR δ 153.90 (C), 150.53 (C), 150.23 (C), 147.84 (C), 143.71 (C), 141.82 (C), 137.15 (C), 136.56 (C), 128.31–126.97 (10 CH), 117.67 (C), 116.60 (CH), 111.31 (C), 104.51 (CH), 103.18 (CH), 101.06 (CH_2), 93.53 (CH), 78.30 (CH), 72.01 (CH_2), 70.59 (CH_2), 66.31 (CH_2), 40.00 (CH).

*5.2.2. Oxa-Heck reaction between **4b** and **5b**: synthesis of **6c***

To a mixture of $PdCl_2$ (98 mg, 0.56 mmol) and LiCl (47 mg, 1.12 mmol) in acetone (5 mL) was added chromen **4b** (193 mg, 0.56 mmol) in acetone (10 mL). This mixture was stirred for 15 min at 0 °C and then 2-chloromercurio-4-benzyloxy,5-methoxyphenol **5b** (250 mg, 0.56 mmol) in acetone (10 mL) was added. The suspension thus obtained was stirred for 12 h at 25 °C. After this time, brine (150 mL) was added to it and the mixture was extracted with acetyl acetate (3×50 mL), the organic extract dried (Na_2SO_4), and submitted to column chromatography to give the compound **6c** as a solid (160.6 mg, 50%).

^{1}H NMR ($CDCl_3$) δ 7.45–7.25 (m, 15H), 7.17 (d, $J = 8.43$ Hz, 1H), 6.81 (s, 1H), 6.69 (d, $J = 8.42$ Hz, 1H), 6.51 (s, 1H), 5.46 (d, $J = 6.23$ Hz, 1H), 5.13 (s, 2H), 5.11 (s, 2H), 5.04 (s, 2H), 4.22 (m, 1H), 3.83 (s, 3H), 3.51 (m, 2H).

^{13}C NMR ($CDCl_3$) δ 39.99 (CH), 55.92 (CH_3), 66.43 (CH_2), 70.86 (CH_2), 72.70 (CH_2), 74.99 (CH_2), 78.12 (CH), 95.62 (CH), 107.80 (CH), 112.85 (CH), 114.39 (C), 116.79 (C), 125.37 (CH), 127.14–128.31 (15 CH), 136.65 (C), 136.73 (C), 137.27 (C), 137.42 (C), 142.31 (C), 149.74 (C), 151.20 (C), 152.67 (C), 154.30 (C).

*5.2.3. Hydrogenolysis of **6b**: synthesis of **1b***

(±)-2,3-di-*O*-benzyl-pterocarpan **6b** (31.8 mg, 0.07 mmol) in acetone was hydrogenated (3 atm) in the presence of Pd–C (10% by weight). After 30 min the catalyst was filtered to give **1b** (21.0 mg) in 95% yield.

^{1}H NMR: 6.91 (s, 1H), 6.87 (s, 1H), 6.38 (s, 1H), 6.37 (s, 1H), 5.91 (2d, $J = 5.49$ Hz, 2H), 5.44 (d, $J = 6.59$ Hz, 1H), 4.21 (dd, $J = 8.42$ Hz and 2.93 Hz, 1H), 3.57 (m, 2H).

^{13}C NMR: 41.25 (CH), 67.09 (CH_2), 79.52 (CH), 93.90 (CH), 102.07 (CH_2), 104.31 (CH), 105.89 (CH), 112.11 (CH), 117.01 (C), 119.55 (C), 140.81 (CH), 142.38 (C), 147.63 (C), 148.83 (C), 150.19 (C), 155.26 (C).

IR (KBr) ν_{max}/cm^{-1}: 3369 (OH), 2924 (aromatic H), 1674–1602 (aromatic ring).

*5.2.4. Hydrogenolysis of **6c**: synthesis of **1c***

(±)-2,3-di-*O*-benzyl-pterocarpan **6c** (35.0 mg, 0.06 mmol) in acetone was hydrogenated (3 atm) in the presence of Pd–C (10% by weight). After 3 h the catalyst was filtered to give **1c** (18.2 mg) in 95 % yield.

^{1}H NMR: 6.85 (d, $J = 8{,}42$, 1H), 6.84 (s, 1H), 6.56 (d, $J = 8.42$, 1H), 6.47 (s, 1H), 5.46 (d, $J = 6.59$ Hz, 1H), 4.31 (dd, $J = 10.44$, 4.48 Hz, 1H), 3.79 (s, 1H), 3.70 (t, $J = 10.53$ Hz, 1H), 3.55 (m, 1H).

IR (KBr) ν_{max}/cm^{-1}: 3392 (OH), 2923 (aromatic H), 1629–1602 (aromatic ring).

*5.2.5. Hydrogenolysis of **6a**: synthesis of **2***

A solution of *cis*-(±)-3,4-di-*O*-benzyl-pterocarpan **6a** [24] (31.8 mg = 0.07 mmol) in acetone (5 mL) was hydrogenated (3 atm) in the presence of Pd–C (100% by weight). After 3 h the catalyst was filtered and the reaction mixture was concentrated in vacuo to furnish **2** (21.0 mg, 0.07 mmol) in 100% yield. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 6.7 (1H, s, aromatic), 6.52 (1H, s), 6.46 (1H, d, $J = 8.33$ Hz), 6.38 (1H, d, $J = 8.33$ Hz), 5.87 (2H, dd, $J = 2.1$ Hz, 1.0 Hz), 4.28 (1H, ddd, $J = 1.70$ Hz, 3.48 Hz, 10.21 Hz), 4.04 (1H, t, $J = 10.0$ Hz), 3.54 (1H, m), 2.9 (2H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 32.69 (CH) 70.42 (CH_2), 98.57 (CH), 101.70 (CH_2), 107.74 (CH). 108.69 (CH), 114.84 (C), 119.90 (C), 120.34 (CH), 133.69 (C), 141.77 (C), 143.56 (C), 144.61 (C), 147.27 (C), 150.33 (C).

*5.2.6. Oxidation of **1a**: synthesis of **3***

To a solution of **1a** (24.7 mg, 0.08 mmol) in methylene chloride (25 mL) was added DDQ (four portions of 20.85 mg, 0.37 mmol) at room temperature. After 24 h the reaction was quenched with brine and extracted with ethyl acetate, dried with sodium sulfate and concentrated. Flash chromatography (50:50 EtOAc/hexane) furnished a yellow solid (17.9 mg, 0.06 mmol) in 71% yield. The chemical shifts were based on COSY spectrum. ^{1}H NMR ($CDCl_3$ 6.96 (d, 2H, J = 9.52 Hz), 200 MH_Z), 6.49 (s, 1H), 6.41 (s, 1H), 6.15 (d, 1H, $J = 10.26$ Hz), 5.89 (d, 1H, $J = 4.40$ Hz), 5.88 (d, 1H, $J = 4.76$ Hz), 4.72 (d, 1H, $J = 8.79$), 4.30 (dd, 1H, $J = 8.97$, 2.38), 3.28–3.22 (m, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 69.31(CH), 165.50 (CH), 150.27 (CH), 135.61 (CH), 128.79 (CH_2), 128.05 (CH), 101.31 (CH_2), 67.05 (CH); IR (KBr) ν_{max}/cm^{-1}: 1689 (C=O), 1638 (C=O), 1484–1384 (aromatic ring).

Acknowledgments

Our research was supported by grants from FINEP, Programa de Oncobiologia FAF/FECD, PRONEX, FAPERJ, CNPq and CAPES. C.D.N. and P.R.R.C. are supported by CNPq fellowship and E.S.J.S. was partly supported by FAF/FECD fellowship and now by CAPES. We are grateful to Dr. Ottilia R. Affonso-Mitidieri for useful suggestions and Central Analítica NPPN-UFRJ for the analytical data.

References

[1] J.B. Harbone, J.J. Mabry, H. Mabry, The Flavonoids, Chapman and Hall, London, 1975.
[2] C.J.W. Brooks, D.G. Watson, Nat. Prod. Rep. 2 (1985) 427–459.
[3] V.S. Kamat, F.Y. Chuo, I. Kubo, K. Nakanishi, Heterocycles 15 (1981) 1163–1170.
[4] L.A. Mitscher, S. Drake, J. Nat. Prod. 50 (1987) 1025.
[5] K. Nakanish, L.L. Darko, M. Nakagawa, J.A. Vick, Tetrahedron Lett. 23 (1982) 3855–3858.
[6] R.R. Chilpa, M.J. Estrada, Interciencia 20 (1995) 257–258.

[7] A.J.M. Silva, A.L. Coelho, A.B.C. Simas, R.A.M. Moraes, D.A. Pinheiro, F.F.A. Fernandes, E.Z. Arruda, P.R.R. Costa, P.A. Melo, Biorg. Med. Chem. Lett. 14 (2004) 431–435.
[8] P.A. Melo, M.C. Do Nascimento, W.D. Mors, G. Suarez-Kurtz, Toxicon 32 (1994) 595–603.
[9] S.K. Chaudhuri, L. Huang, F. Fullas, D.M. Brown, M.C. Wani, M.E. Wall, J. Nat. Prod. 58 (1995) 1966–1969.
[10] O.B. Gottlieb, J.T. Cook, W.D. Ollis, I.O. Sutherland, Phytochemistry 17 (1978) 1419–1422.
[11] M. Mizuno, T. Tanaka, M. Katsuragawa, H. Saito, M. Iinuma, J. Nat. Prod. 53 (1990) 498–499.
[12] T.L. Meragelman, K.D. Tucker, T.G. McCloud, J.H. Cardellina II, R.H. Shoemaker, J. Nat. Prod. 68 (2005) 1790–1792.
[13] G.C.G. Militão, I.N.F. Dantas, C. Pessoa, M.J.C. Falcão, E.R. Silveira, M.A.S. Lima, R. Curi, T. Lima, M.O. Moraes, L.V. Costa-Lotufo, Life Sci. 78 (2006) 2409–2417.
[14] A.J.M. da Silva, C.D. Netto, P.R.R. Costa, J. Braz. Chem. Soc. 15 (2004) 979–981.
[15] A.B.C. Simas, A.J.M. da Silva, A.L. Coelho, P.R.R. Costa, Tetrahedron Lett. 42 (2001) 4111–4113.
[16] Y. Chau, S. Shiah, M. Don, M. Kuo, Free Radical Biol. Med. 24 (1998) 660–670.
[17] F. Nagai, E. Kato, H. Tamura, Biol. Pharm. Bull. 27 (2004) 492–495.
[18] V.M. Rumjanek, G.S. Trindade, K. Wagner-Souza, M.C. de-Oliveira, L.F. Marques-Santos, R.C. Maia, M.A. Capella, An Acad Bras Ciênc. 73 (2001) 57–69.
[19] G.S. Trindade, M.A. Capella, L.S. Capella, O.R. Affonso-Mitidieri, V.M. Rumjanek, Photochem. Photobiol. 69 (1999) 694–699.
[20] M.P. Barroso, C. Gómez-Díaz, G. López-Lluch, M.M. Malagón, F.L. Crane, P. Navas, Arch. Biochem. Biophys. 343 (1997) 243–248.
[21] M.P.M. Portela, A.O.M. Stoppani, Biochem. Pharmacol. 51 (1996) 275–283.
[22] M.D. Esposti, I. Hatzinisiriou, H. McLennan, S. Ralph, J. Biol. Chem. 274 (1999) 29831–29837.
[23] A. Kawahara, T. Kobayashi, S. Nagata, Oncogene 17 (1998) 2549–2554.
[24] J.W. Baty, M.B. Hampton, C.C. Winterbourn, Biochem. J. 389 (2005) 785–795.
[25] H. Horino, N. Inoue, J. Chem. Soc. Chem. Commun. (1976) 500–501.
[26] P.R.R. Costa, A.J.M. da Silva, P.A. Melo, N.M.V. Silva, F.V. Brito, C.D. Buarque, D.V. de Souza, V.P. Rodrigues, E.S.C. Poças, F. Noël, E.X. Albuquerque, Bioorg. Med. Lett. 11 (2001) 283–286.
[27] E.S.C. Pôças, D.V.S. Lopes, A.J.M. da Silva, P.H.C. Pimenta, F.B. Leitão, C.D. Netto, C.D. Buarque, F.V. Brito, P.R.R. Costa, F. Noel, Bioorg. Med. Chem. 14 (2006) 7962–7966.
[28] E.L. Cavalieri, E.G. Rogan, D. Chakravarti, Cell Mol. Life Sci. 59 (2002) 665–681.
[29] E.L. Cavalieri, K.-M. Li, N. Balu, M. Saeed, P. Davanesan, S. Higginbotham, J. Zhao, M.L. Gross, E.G. Rogan, Carcinogenesis 23 (2002) 1071–1077.
[30] M. Saeed, E. Rogan, E. Cavalieri, Tetrahedron Lett. 46 (2005) 4449–4451.
[31] A. Pezzella, M. d'Ischia, A. Napolitano, G. Misuraca, G. Prota, J. Med. Chem. 40 (1997) 2211–2216.
[32] T.G. Gantchev, D.J. Hunting, Mol. Pharmacol. 53 (1998) 422–428.
[33] C.C. Lindsey, C. Gómez-Díaz, J.M. Villalba, T.R.R. Pettus, Tetrahedron 58 (2002) 4559–4565.
[34] W.A. Denny, Eur. J. Med. Chem. 36 (2001) 577–595.
[35] P.J. O'Brien, Chem. Biol. Interactions 80 (1991) 1–41.
[36] M.N. da Silva, V.F. Ferreira, M.C.B.V. de Souza, Quim. Nova 26 (2003) 407–416.