

Furanoditerpenes from *Pterodon pubescens* Benth with Selective *in vitro* Anticancer Activity for Prostate Cell Line

Humberto M. Spindola,^{a,b} João E. de Carvalho,^{a,b} Ana Lúcia T. G. Ruiz,^a Rodney A. F. Rodrigues,^a Carina Denny,^{a,b} Ilza M. de Oliveira Sousa,^a Jorge Y. Tamashiro^c and Mary Ann Foglio^{*,a,b}

^aCPQBA, Universidade Estadual de Campinas, CP 6171, 13083-970 Campinas-SP, Brazil

^bDepartamento de Farmacologia, Anestesiologia e Terapêutica, Faculdade de Odontologia, Universidade Estadual de Campinas, CP 52, 13414-903 Piracicaba-SP, Brazil

^cInstituto de Biologia, Universidade Estadual de Campinas, CP 6109, 13083-970 Campinas-SP, Brazil

O fracionamento biomonitorado do extrato diclorometânico das sementes de *Pterodon pubescens* Benth forneceu o 6 α -acetóxi-7 β -hidróxi-vouacapano **1** (inédito), além de quatro diterpenos furânicos (**2**, **3**, **4** e **5**). A atividade antiproliferativa dos compostos foi avaliada *in vitro* contra as linhagens de células tumorais humanas UACC-62 (melanoma), MCF-7 (mama), NCI-H460 (pulmão), OVCAR-03 (ovário), PC-3 (próstata), HT-29 (colon), 786-0 (rim), K562 (leucemia) e NCI-ADR/RES (ovário com fenótipo de resistência a múltiplos fármacos). Os resultados foram expressos em três concentrações efetivas GI₅₀ (concentração para que ocorra 50% de inibição de crescimento), TGI (concentração que resulta em inibição total de crescimento) e LC₅₀ (concentração que resulta em 50% de morte celular). A citotoxicidade *in vitro* foi avaliada também frente a uma linhagem de célula murina normal (3T3). Este é o primeiro relato de atividade anticâncer para os compostos **1**, **4** e **5**, que apresentaram grande seletividade, dependente da concentração, para PC-3. O composto **1** foi 26 vezes mais potente para inibir 50% do crescimento (GI₅₀) de PC-3, 15 vezes mais citostático (TGI) e 6 vezes menos tóxico (LC₅₀) quando comparado com Doxorubicina (controle).

Activity guided fractionation of *Pterodon pubescens* Benth. methylene chloride-soluble fraction afforded novel 6 α -acetoxi 7 β -hydroxy-vouacapan **1** and four known diterpene furans **2**, **3**, **4**, **5**. The compounds were evaluated for *in vitro* cytotoxic activities against human normal cells and tumour cell lines UACC-62 (melanoma), MCF-7 (breast), NCI-H460 (lung, non-small cells), OVCAR-03 (ovarian), PC-3 (prostate), HT-29 (colon), 786-0 (renal), K562 (leukemia) and NCI-ADR/RES (ovarian expressing phenotype multiple drugs resistance). Results were expressed by three concentration dependent parameters GI₅₀ (concentration that produces 50% growth inhibition), TGI (concentration that produces total growth inhibition or cytostatic effect) and LC₅₀ (concentration that produces -50% growth, a cytotoxicity parameter). Also, *in vitro* cytotoxicity was evaluated against 3T3 cell line (mouse embryonic fibroblasts). Antiproliferative properties of compounds **1**, **4** and **5** are herein reported for the first time. These compounds showed selectivity in a concentration-dependent way against human PC-3. Compound **1** demonstrated selectivity 26 fold more potent than the positive control, doxorubicin, for PC-3 (prostrate) cell line based on GI₅₀ values, causing cytostatic effect (TGI value) at a concentration fifteen times less than positive control. Moreover comparison of 50% lethal concentration (LC₅₀ value) with positive control (doxorubicin) suggested that compound **1** was less toxic.

Keywords: *Pterodon pubescens*, leguminosae, furanoditerpenes, *in vitro* assay, prostate cell line, cytotoxicity

Intoduction

Throughout history, natural products have afforded a rich source of compounds that have found many applications in

the fields of medicine, pharmacy and biology. Within the sphere of cancer, a number of important new commercialized drugs have been obtained from natural sources, by structural modification of natural compounds, or by the synthesis of new compounds, designed following a natural compound as model.

*e-mail: foglioma@cpqba.unicamp.br

Among the many compounds identified for cancer treatment Taxol, isolated from *Taxus brevifolia*, has proven to be an important chemotherapeutic agent.¹ Medicinal plants still play an important role as source of new targets for drug discovery. The huge structural diversity of natural compounds and their bioactivity potential have meant that several products isolated from plants, marine flora and microorganisms can serve as "lead" compounds for improvement of their therapeutic potential by molecular modification.^{2,3}

Pterodon genus comprises four species native to Brazil: *P. abruptus* Benth., *P. apparucuri* Pedersoli, *P. pubescens* Benth. (*P. emarginatus* Vog.) and *P. polygalaeflorus* Benth. Initially scientific studies of these plants were motivated by evidence that the seed's oil had cercaricidal⁴ and anti-microbial activity.⁵

Pterodon pubescens Benth. (Leguminosae) known, as Sucupira Branca is widespread throughout Goiás, Minas Gerais and São Paulo states in Brazil. The seeds are commercially available in Brazilian medicinal flora market. Plant's crude alcoholic extracts are used in folk medicine as anti-inflammatory, analgesic and anti-rheumatic preparations.⁵⁻⁷

Phytochemical studies of *Pterodon* genus have revealed the presence of alkaloids, isoflavones and diterpenes. Furan-diterpenes were identified and isolated from *Pterodon* fruits.⁸⁻¹¹ Some authors have suggested that furan-diterpenes possessing vouacapan skeleton are involved with anti-inflammatory properties of *Pterodon pubescens* seeds' oil.^{5,12,13} Diterpenes 6 α -hydroxyvouacapan-7 β -17 β -lactone and 6 α , 7 β -dihydroxyvouacapan-17 β -oate methyl ester, present in *P. emarginatus* and *P. polygalaeflorus* seeds, respectively, were previously found to be associated with anti-inflammatory activity of these species.¹² Another compound, acid 6,7-dihydroxyvouacapan-17 β -oic, was suggested to be one of the possible compounds involved with anti-inflammatory activity, since this compound was identified in the active fraction that exhibited anti-edematogenic activity when tested in carrageenin-induced paw edema or in Croton oil-induced ear edema assays.¹³

Evidence of biogenic amines involved with antinociceptive effect of a vouacapan extracted from *P. polygalaeflorus* Benth was studied by Duarte *et al.*¹⁴ suggested that the pharmacological activity was triggered by catecholaminergic system.

Coelho *et al.*¹⁵ studied *Pterodon* seed extract's antinociceptive activity suggesting that both peripheral and central inhibitory mechanisms are involved.

In the present study we report the isolation by activity-guided fractionation, identification and *in vitro* anticancer activities of vouacapan from *Pterodon pubescens* Benth that are herein reported for the first time.

Results and Discussion

Compounds 6 α ,7 β -diacetoxyvouacapan **2**, 7 β -diacetoxyvouacapan **3**, 6 α ,7 β -dihydroxyvouacapan-17 β -oate methyl ester **4**, and 6 α ,7 β -dihydroxyvouacapan-17 β -methylene-ol **5** (Figure 1) were identified based on comparison of experimental ¹H and ¹³C-NMR with reported spectral data.^{9,10,16,17}

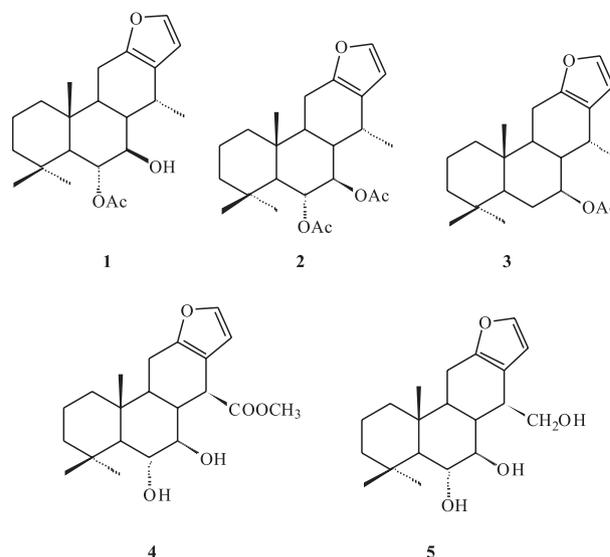


Figure 1. Chemical structures of vouacapan **1**, **2**, **3**, **4** and **5** isolated from *Pterodon pubescens* Benth. seeds.

Novel compound **1** was deduced as having an elemental formula C₂₂H₃₂O₄, by HREI-MS (observed M⁺ = 360.23556, required M⁺ = 360.23010), which indicated seven insaturation sites. Infrared absorptions at 3449 (OH) and 1713 (C=O) cm⁻¹ provided evidences for hydroxyl and carbonyl functionalities. The ¹H-NMR spectral data (Table 1) showed a signal at δ_{H} 3.48 (1H, dd, *J* 9.7; 9.3 Hz, H-7) that presented correlations with hydrogens H-8 (δ_{H} 1.88) and H-6 (δ_{H} 5.2) in H-H COSY experiment. This signal at δ_{H} 3.48 was attributed to a proton geminal to the hydroxyl group at C-7. Signal at δ_{H} 5.2 (1H, dd, *J* 11.7; 9.3Hz) was attributed hydrogen H-6, which was confirmed by correlations with hydrogens H-5 (δ_{H} 1.3) and H-7 (δ_{H} 3.48) in H-H COSY experiment. The coupling constant of hydrogens H-6 and H-7 was observed as 9.3 Hz indicating a *trans*-diaxial relationship. When compound **1** was acetylated with excess acetic anhydride /pyridine, this compound showed identical ¹H and ¹³C-NMR spectral data to compound 6 α ,7 β -diacetoxyvouacapan **2**⁹ (HREI-MS 402.2630), suggesting that compound **1** has the same relative configuration to 6 α ,7 β -diacetoxyvouacapan **2**

Table 1. ^1H and ^{13}C NMR (11 Tesla, CDCl_3/TMS) data for diterpene **1**^a

| Atom | C type | δ ^{13}C | δ ^1H | δ $^1\text{H} \times ^1\text{H}$ COSY |
|------|------------------|--------------------------|--|--|
| C-1 | CH_2 | 39.9 | 1.06; 1.76 | |
| C-2 | CH_2 | 18.3 | 1.38; 1.58 | |
| C-3 | CH_2 | 43.6 | 1.3; 1.55 | |
| C-4 | C° | 33.1 | - | |
| C-5 | CH | 54.6 | 1.3 (d, J 11.7 Hz) | 5.2 |
| C-6 | CH | 76.4 | 5.2 (dd, J 11.7; 9.3 Hz) | 3.48; 1.3 |
| C-7 | CH | 75.8 | 3.48 (dd, J 9.7; 9.3 Hz) | 5.2; 1.88 |
| C-8 | CH | 43.2 | 1.88 (td, J 9.7, 5.1 Hz) | 3.48; 3.13; 1.92 |
| C-9 | CH | 43.3 | 1.92 (ddd, J 12.3; 10.5; 5.1 Hz) | 1.88; 0.95 |
| C-10 | C° | 38.6 | - | |
| C-11 | CH_2 | 22.6 | 0.95 (dd, J 12.3 Hz); 2.15 (dd, J 10.5 Hz) | 1.92 |
| C-12 | C° | 148.7 | - | |
| C-13 | C° | 121.7 | - | |
| C-14 | CH | 27.4 | 3.13 (d, J 5.1 Hz) | 1.88 |
| C-15 | CH | 109.6 | 6.21 (d, J 3.5 Hz) | 7.25 |
| C-16 | CH | 140.6 | 7.25 (d, J 3.5 Hz) | 6.21 |
| C-17 | CH_3 | 16.9 | 0.99 | |
| C-18 | CH_3 | 36.4 | 1.08 | |
| C-19 | CH_3 | 22.7 | 0.95 | |
| C-20 | CH_3 | 15.8 | 0.99 | |

^aChemical shifts are in δ (ppm).

with a hydroxy group attached β to C-7 whereas the acetyl group positioned α at C-6 (Table 1).

The activity-guided fractionation of methylene chloride soluble fraction was monitored by *in vitro* anticancer activity assay in UACC-62 (melanoma), MCF-7 (breast), NCI-H460 (lung, non-small cells), OVCAR-3 (ovarian), PC-3 (prostate), HT-29 (colon), 786-0 (renal), K562 (leukemia) and NCI-ADR/RES (ovarian expressing phenotype multiple drugs resistance) cancer cell lines. A 48 h SRB (Sulforhodamine B) cell viability assay was performed to determine growth inhibition and cytotoxic properties of fractions and compounds. Cells were treated with at least four different concentrations levels (0.25 to 250 $\mu\text{g mL}^{-1}$) with determination of three endpoints, concentration inhibiting the growth of 50% of the cells (GI_{50}), concentration for total growth inhibition (TGI) and concentration needed to kill 50% of the cells (Table 2).¹⁸ Compounds **2** and **3** were equally not potent based on GI_{50} , TGI and LC_{50} values.

A mean graph for compounds **1-5** corroborated the selectivity of compounds **1**, **4** and **5** for PC-3 human prostate cancer cell lines (Figure 2). The mean graph was developed by NCI emphasize differential effects of test compounds on various human tumor cell lines. This graph is generated from a set of GI_{50} , TGI, or LC_{50} values.

Positive values project to the right of the vertical line and represent cellular sensitivities to the test agent that exceed the mean. Negative values project to the left and represent cell line sensitivities to the test agent that are less than the average value.¹⁹ Based on the three graphics, PC-3 cell line was high sensible to compounds **1**, **4** and **5**. The interesting thing to notice is that compound **1** was more potent than **4** and **5** to inhibit cellular growth in 50% (GI_{50} , Figure 2A), whereas compounds **1** and **5** showed almost same potency in causing cytostatic (TGI, Figure 2B). On the other hand, when cytotoxicity parameter (LC_{50} , Figure 2C) was evaluated, compound **5** was more toxic whereas **1** and **4** were similarly toxics to PC-3 cell line. This high selectivity to PC-3 cell line suggests that furanoditerpenes **1**, **4** and **5** may share a similar action mechanism, probably evolving androgenic receptors.

Compound **4** was able to reduce in 50% cellular growth of MCF-7 and NCI-H460 cell lines and also presented activity against NCI/ADR-RES cell line measured by all three parameters.

Compound **1** demonstrated selectivity 26 fold more potent than the positive control (doxorubicin) for PC-3 (prostate) cell line based on GI_{50} values, causing cytostatic effect (TGI value) at a concentration fifteen times inferior than positive control (doxorubicin). Moreover comparison

Table 2. Cytotoxic activity of compounds **1**, **2**, **3**, **4** and **5**

| Cell lines | | 1 | 2 | 3 | 4 | 5 | Doxorubicin |
|-------------|------------------|----------|----------|----------|----------|----------|-------------|
| UACC-62 | GI ₅₀ | 27.8 | 71.95 | >250 | 12.7 | 6.23 | 0.02 |
| | TGI | 64.47 | >250 | >250 | 39.58 | 118.3 | 0.19 |
| | LC ₅₀ | 198.3 | >250 | >250 | 241.9 | 250 | 1.36 |
| MCF-7 | GI ₅₀ | 69.7 | 148.1 | 212.6 | 3.2 | 5.18 | 0.20 |
| | TGI | 172.88 | >250 | >250 | 20.97 | 105.3 | 2.69 |
| | LC ₅₀ | 179.6 | >250 | >250 | 185.3 | 250 | 40.40 |
| NCI-ADR/RES | GI ₅₀ | 30.5 | >250 | >250 | 3.3 | 3.02 | 0.09 |
| | TGI | 96.72 | >250 | >250 | 11.56 | 21.43 | 5.31 |
| | LC ₅₀ | 230.1 | >250 | >250 | 23.47 | 223.2 | 36.23 |
| 786-0 | GI ₅₀ | 28.5 | 86.16 | 196.6 | 5.2 | 2.86 | 0.03 |
| | TGI | 59.30 | >250 | >250 | 24.29 | 14.41 | 0.26 |
| | LC ₅₀ | 173.8 | >250 | >250 | 32.85 | 30.85 | 12.92 |
| NCI-H460 | GI ₅₀ | 3.50 | 122.3 | 21.43 | 2.0 | 2.98 | 0.02 |
| | TGI | 48.34 | >250 | >250 | 47.08 | 77.48 | 0.11 |
| | LC ₅₀ | 228.8 | >250 | >250 | 250 | 250 | 2.14 |
| PC-3 | GI ₅₀ | 0.0053 | 0.006 | 0.50 | 0.37 | 0.0085 | 0.14 |
| | TGI | 0.38 | >250 | >250 | 0.27 | 0.17 | 4.53 |
| | LC ₅₀ | 28.02 | >250 | 214.4 | 15.80 | 4.85 | 5.90 |
| Ovcar-03 | GI ₅₀ | 29.3 | >250 | >250 | 13.05 | 3.14 | 0.20 |
| | TGI | 87.52 | >250 | >250 | 49.66 | 24.02 | 12.91 |
| | LC ₅₀ | 243.4 | >250 | >250 | 240.0 | 178.6 | 250 |
| HT-29 | GI ₅₀ | 60.9 | >250 | >250 | 10.8 | 19.04 | 0.25 |
| | TGI | 200.7 | >250 | >250 | 37.97 | 35.83 | 2.26 |
| | LC ₅₀ | 248.7 | >250 | >250 | 245.9 | 218.3 | 37.59 |
| K562 | GI ₅₀ | 27.7 | >250 | 132.8 | 4.1 | 3.37 | 0.22 |
| | TGI | 80.97 | >250 | >250 | 26.29 | 36.16 | 2.02 |
| | LC ₅₀ | 232.1 | >250 | >250 | 213.1 | 250 | 25.82 |

UACC-62 (melanoma), MCF-7 (breast), NCI-H460 (lung non-small cells), OVCAR-3 (ovarian), PC-3 (prostate), HT29 (colon), 786-0 (renal), K562 (leukemia) and NCI-ADR/RES (ovarian expressing phenotype multiple drugs resistance). GI₅₀: concentration ($\mu\text{g mL}^{-1}$) inhibiting the growth of 50% of the cells; TGI: concentration ($\mu\text{g mL}^{-1}$) total growth inhibition; LC₅₀: concentration ($\mu\text{g mL}^{-1}$) need to kill 50% of the cells.¹⁸

of 50% lethal concentration (LC₅₀ value) with positive control (doxorubicin) demonstrated compound **1** to be less toxic.

Cytotoxicity in normal cell lines of compounds **1**, **4**, **5** were evaluated against 3T3 cell line (mouse embryonic fibroblasts) assessing mitochondrial functions by MTT reduction with succinate dehydrogenase in order to obtain cell viability. Compound **1** (IC₅₀ = 34.33 $\mu\text{g mL}^{-1}$) demonstrated to be slightly less cytotoxic than compounds **4** (IC₅₀ = 22.83 $\mu\text{g mL}^{-1}$) and **5** (IC₅₀ = 23.55 $\mu\text{g mL}^{-1}$). All these values were higher than almost all GI₅₀ and TGI obtained for promissory compounds.

Cyproterone, a steroid formed by the mevalonate pathway is a known drug used as anti-androgen for prostate

cancer treatment.²⁰ Cyproterone is a steroidal antiandrogen agent that inhibits the action of adrenal and testicular androgens on prostate cells, seminal vesicles, testes, and the vas deferens. Additionally causes a centrally mediated reduction in testicular secretion of androgens. This drug is indicated for treatment of prostate cancer, androgen induced disorders of the skin (acne, seborrhoea, hirsutism, alopecia), precocious puberty and sexual disorders in men.^{21,22}

Male rats treated during two weeks with 100 and 300 mg kg⁻¹ doses of *Pterodon pubescens* dichloromethane crude extract decreased body weight gain by 57 and 75% respectively.²³ That difference in body weight gain may have a relationship with antiandrogen activity of vouacapan type compounds found in the crude dichloromethane

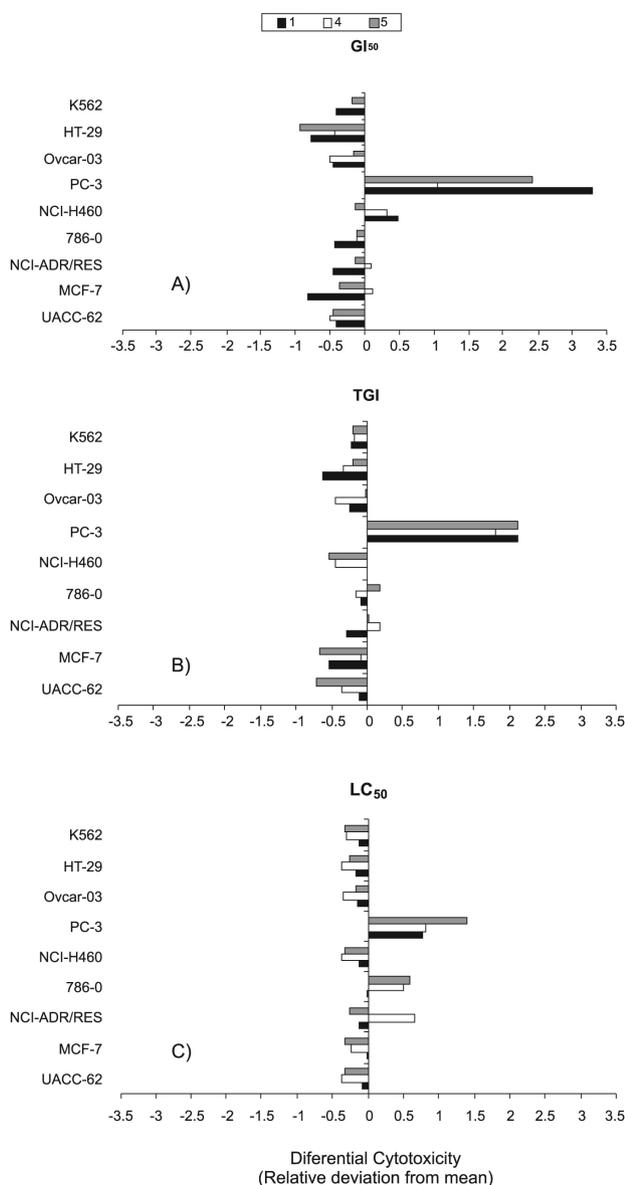


Figure 2. Patterns of differential cytotoxicity toward human tumor cell lines. A mean graph for compounds **1**, **4** and **5** is shown. The midline of each portion represents the mean for GI₅₀ (A), TGI (B) and LC₅₀ (C) endpoint, calculated across nine cell lines: UACC-62 (melanoma), MCF-7 (breast), NCI-ADR/RES (ovarian expressing phenotype multiple drugs resistance), 786-0 (renal), NCI-H460 (lung, non-small cells), PC-3 (prostate), OVCAR-3 (ovarian), HT29 (colon), and K562 (leukemia). This mean value is then subtracted from the value for each individual cell line and plotted. Cell lines more sensitive are visualized as bars deflecting to the right, whereas more resistant cell lines have bars extending to the left of the mean.¹⁹

extract. Decrease of mean final body weight was also observed with cyproterone after 15 days treatment.²⁴ This data corroborates with the hypothesis that vouacapan type compounds interact with testosterone receptors. Further animal studies shall evaluate these findings.

Considering that compounds **1**, **4** and **5**, furan-diterpenoid, also originates from the mevalonate biosynthetic

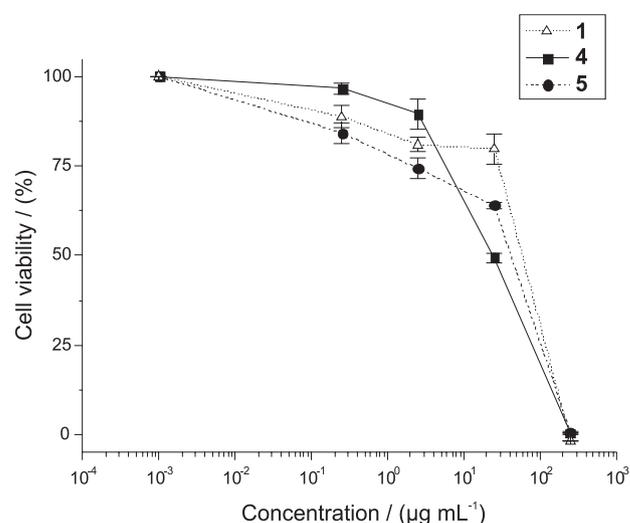


Figure 3. Cell viability test with compounds **1**, **4**, and **5** on 3T3 cell lines (mouse fibroblast) after 48 hours exposure.

pathway,²⁰ PC-3 human tumor cell line inhibition observed might arise by similar pharmacological mechanism such as Cyproterone. Therefore compounds **1**, **4**, **5** are interesting pharmacophores capable of providing new insights to the understanding of agonist *versus* antagonist properties of androgens, leading to the development of new anticancer chemotherapeutics agents.

Experimental

General experimental procedures

IR spectra: JASCO-FT/IR-410 spectrometer. ¹H, ¹³C NMR and 2D experiments: Varian Inova-500 spectrometer (11 Tesla). Chemical shifts were recorded in CDCl₃ solutions and quoted relative to TMS (δ 0.0, ¹H NMR) and CHCl₃ (δ 77.0, ¹³C NMR). High-resolution electron ionization mass spectroscopy (HREIMS) was recorded on a VG-AutoSpec High Resolution Mass Spectrometer (70 eV) using direct probe. Column chromatography (CC): silica gel (0.063 \times 0.200 mm, Merck®). TLC (thin layer chromatography): precoated plates (775554 Merck®), UV detection and anisaldehyde solution.

Plant material

P. pubescens Benth. seeds were collected in Pedregulho (SP) and São Carlos (SP) cities, in march 2004. Prof. Dr. Jorge Yoshio Tamashiro from IB-UNICAMP (Department of Botany) identified the plant species. A voucher specimen was deposited at Universidade Estadual de Campinas (UEC) Herbarium, under numbers 1398 and 1402.

Preparation of plant extract (EB) and fraction purification

Freeze-dried seeds (100g) were grinded prior to use on a Stephen mill (model UM 40) and extracted with dichloromethane three times during two hour periods, with 5:1 solvent/plant ratio, at room temperature. The extract was dried over anhydride Na_2SO_4 filtered and concentrated under vacuum (Buchi RE 120), with 32% yield of crude seed oil extract (EB).

This crude oil (18.2 g) was purified on pre-column chromatography using silica gel (Merck 7734) (5×60 cm) with hexane [FR1] (0-450 mL); hexane/ethyl acetate (95:5), [FR2] (500-900 mL); hexane/ethyl acetate 1(80:20) [FR3-4] (1000 x 1350 mL); hexane/ethyl acetate (60:40) (1400 x 1800 mL) [FR5-6]; hexane/ethyl acetate (40:60) (1900 x 2300 mL) [FR7]; rest flushed with methanol. The resulting fractions were monitored by thin layer chromatography (TLC), exposed with anisaldehyde reagent (50 mL acetic acid, 0.5 mL sulfuric acid and 0.5 mL anisaldehyde) followed by heating at 110 °C. According to TLC profile the fractions were group and submitted to biological assay. The *in vitro* anticancer model on nine human cell lines determined the fractions that were further purified. Among the fractions isolated by column chromatography, Fraction 7 presented the best anticancer *in vitro* activity (data not shown).

Fraction 7 (10 g) was successively chromatographed by CC on silica-gel (70-230 mesh) (5×60 cm) and eluted with hexane/dichloromethane (7:3) (900-1800 mL) yielded **3** (333 mg, 3% yield); R_f **3** 0.75; hexane/dichloromethane (6:4) (1900-2600 mL) yielded **2** (990 mg, 9.9% yield), R_f **2** 0.56; hexane/dichloromethane (2:8) (2650-3100 mL) yielded **1** (963 mg, 9.63% yield), R_f **1** 0.29; (3350-4100 mL) yielded **4** (1.2 g, 12% yield) R_f **4** 0.17 and **5** (0.3g, 3% yield) R_f **5** 0.14.

*6*α-*acetox*y-*7*β-*hydroxy*-*vouacapan* (**1**)

White crystal; mp 168-171 °C, $[\alpha]_D^{20}$: +39.4° (CHCl_3 ; c 0.0094); FTIR ν_{max} /cm⁻¹: 3449, 1713; ¹H and ¹³C NMR (11 Tesla, CDCl_3/TMS): see Table 1.

*6*α,*7*β-*diacetox*y*vouacapan* (**2**)

White crystal; mp 167.3-168.0 °C; FTIR, ¹H and ¹³C NMR data are in agreement with those reported in the literature.^{9,10,16,17}

*7*β-*acetox*y*vouacapan* (**3**)

White crystal; mp 125.6-127.2 °C; FTIR, ¹H and ¹³C NMR data are in agreement with those reported in the literature.^{9,10,16,17}

*6*α,*7*β-*dihydroxyvouacapan-17*β-*oate methyl ester* (**4**)

Colorless oil; FTIR, ¹H and ¹³C NMR data are in agreement with those reported in the literature.^{9,10,16,17}

*6*α,*7*β-*dihydroxyvouacapan-17*β-*methylene-ol* (**5**)

Colorless oil; FTIR, ¹H and ¹³C NMR data are in agreement with those reported in the literature.^{9,10,16,17}

Chromatographic analysis

The GC/MS analysis were carried out using a HP-6890/5975 system equipped with a J&W Scientific DB-5 fused capillary column (25 m x 0.2 mm x 0.33 m). Temperature program: 60 °C (5 °C min⁻¹)-300 °C (10 min), injector 250 °C, detector 300 °C. Helium was used as carrier gas (0.7 bar, 1 mL min⁻¹). The MS were taken at 70 eV. Scanning speed was 0.84 scans s⁻¹, from 40 to 550. Sample volume was 1 μL. Split: 1:40.

In vitro anticancer activity assay

Human tumour cell lines UACC-62 (melanoma), MCF-7 (breast), NCI-H460 (lung, non-small cells), OVCAR-03 (ovarian), PC-3 (prostate), HT-29 (colon), 786-0 (renal), K562 (leukemia) and NCI-ADR/RES (ovarian expressing phenotype multiple drugs resistance) were kindly provided by National Cancer Institute (NCI). Stock cultures were grown in medium containing 5 mL RPMI 1640 (GIBCO BRL) supplemented with 5% fetal bovine serum. Gentamicine (50 μg mL⁻¹) was added to experimental cultures. Cells in 96 well plates (100 μL cells well⁻¹) were exposed to sample concentrations in DMSO/RPMI (0.25, 2.5, 25 and 250 μg mL⁻¹) at 37 °C, 5% of CO₂ in air for 48 h. Final DMSO concentration did not affect cell viability. Afterwards cells were fixed with 50% trichloroacetic acid and cell proliferation determined by spectrophotometric quantification (540 nm) of cellular protein content using sulforhodamine B assay. Using the concentration-response curve for each cell line, GI₅₀ (concentration that produces 50% growth inhibition), TGI (concentration that produces total growth inhibition or cytostatic effect) and LC₅₀ (concentration that produces -50% growth, a cytotoxicity parameter) were determined through non-linear regression analysis (Table 2) using software ORIGIN 7.5 (OriginLab Corporation).¹⁸

In vitro cytotoxicity assay

Cell line 3T3 (mouse embryonic fibroblasts) was grown in medium containing 5 mL DMEM (glucose 4.5g L⁻¹, glutamine 4 mmol L⁻¹) (LGC Biotecnologia) supplemented

with 10% fetal bovine serum. Gentamicine (50 $\mu\text{g mL}^{-1}$) was added to experimental cultures. Cells in 96 well plates (100 μL cells well^{-1} , 1×10^4 cell mL^{-1}) were exposed to sample concentrations in DMSO/RPMI (0.25, 2.5, 25 and 250 $\mu\text{g mL}^{-1}$) at 37 °C, 5% of CO_2 in air for 48 h before MTT assay to access cell viability.²⁵ Cells not exposed to samples were used as control. Final DMSO concentration did not affect cell viability. IC_{50} (concentration reducing cell viability in 50%) was determined through non-linear regression analysis using software ORIGIN 7.5 (OriginLab Corporation).

Conclusions

Considering the data presented herein the chemotherapeutic potential of compounds **1**, **4** and **5** were determined as possible candidates of new agents with high selectivity for prostate cancer. Further *in vivo* studies and *in vitro* assays are needed to establish pharmacological mechanism, toxicity and production viability.

Acknowledgments

The authors are grateful to Fundação de Amparo a Pesquisa do Estado de São Paulo, FAPESP, for financial support (02/10226-1, 05/54582-4 and 06/52454-1). C.D., M.E.Z., M.C.E.A., J.E.C., and M.A.F. wish to thanks CAPES, FAPESP and CNPq respectively for research fellowship.

Supplementary Information

NMR spectral data of compounds **1-5** are available free of charge at <http://jbcbs.sbq.org.br>, as PDF file.

References

- Gordaliza, M.; *Clin. Transl. Oncol.* **2007**, *9*, 767.
- Newman, D. J.; *J. Med. Chem.* **2008**, *51*, 2589.
- Salatino, A.; Salatino, M. L. F.; Negri, G.; *J. Braz. Chem. Soc.* **2007**, *18*, 11.
- Mors, W. B.; Pellegrino, J.; Santos Filho, M. F.; *An. Acad. Bras. Cienc.* **1966**, *38*, 325.
- Carvalho, J. C. T.; Sertié, J. A. A.; Barbosa, M. V. J.; Patrício, K. C. M.; Caputo, L. R. G.; Sarti, S. J.; Ferreira, L. P.; Bastos, J. K.; *J. Ethnopharmacol.* **1999**, *64*, 127.
- Lorenzi, H.; *Árvores Brasileiras. Manual de Identificação e Cultivo de Plantas Arbóreas Nativas do Brasil*, vol. 1, 2nd ed., São Paulo, 1998.
- Pio Correa, M.; *Dicionário das plantas úteis do Brasil e das exóticas cultivadas*, vol. III, Ministério da Agricultura, Instituto Brasileiro de Desenvolvimento Florestal: Rio de Janeiro, 1984.
- Mahajan, J. R.; Monteiro, M. B.; Ferreira, G.; Pellegrino J.; *An. Acad. Bras. Cienc.* **1972**, *44*, 429.
- Fascio, M.; Mors, W. B.; Gilbert, B.; Mahajan, J. R.; Monteiro, M. B.; Dos Santos Filho, D.; Vichnewski, W.; *Phytochemistry* **1976**, *15*, 201.
- Campos, A. M.; Silveira, E. R.; Braz-Filho, R.; Teixeira, T. C.; *Phytochemistry* **1994**, *36*, 403.
- Arriaga, A. M. C.; Castro, M. A. B.; Silveira, E. R.; Braz-Filho, R.; *J. Braz. Chem. Soc.* **2000**, *11*, 187.
- Nunan, E. A.; Carvalho, M. G.; Pilo-Veloso, D.; Turchetti-Maia, R. M. M.; Ferreira-Alves, D. L.; *Braz. J. Med. Biol. Res.* **1982**, *15*, 450.
- Silva, M. C. C.; Gayer, C. R. M.; Lopes, C. S.; Calixto, N. O.; Reis, P. A.; Passeas, C. P. B.; Paes, M. C.; Dalmau, S. R.; Sabino, K. C. C.; Todeschini, A. R.; Coelho, M. G. P.; *J. Pharm. Pharmacol.* **2004**, *55*, 135.
- Duarte, I. D. G.; Ferreira-Alves, D. L.; Veloso, D. P.; Nakamura-Craig, M.; *J. Ethnopharmacol.* **1996**, *55*, 13.
- Coelho, L. P.; Reis, P. A.; Castro, F. L.; Gayer, C. R. M.; Lopes, C. S.; Silva, M. C. C.; Sabino, K. C. C.; Todeschini, A. R.; Coelho, M. G. P.; *J. Ethnopharmacol.* **2005**, *98*, 109.
- Fascio, M.; Gilbert, B.; Mors, W. B.; Nishida, T.; *An. Acad. Bras. Cienc.* **1970**, *42*, 97.
- Ortalo-Magne, A.; Culioli, G.; Valls, R.; Pucci, B.; Piovetti, L.; *Phytochemistry* **2005**, *66*, 2316.
- Shoemaker, R. H.; *Nat. Rev. Cancer* **2006**, *6*, 813.
- Compare methodology, http://dtp.nci.nih.gov/docs/compare/compare_methodology.html, accessed in August, 2008.
- Dewick, P. M.; *Medicinal Natural Products: A Biosynthetic Approach*, 2nd ed., Wiley: New York, 2001, 167.
- Neumann, F.; Kalmus, J.; *Exp. Clin. Endocrinol.* **1991**, *98*, 71.
- Barradell, L. B.; Faulds, D.; *Drug Aging* **1994**, *5*, 59.
- Denny, C.; *M. Sc. Dissertation*, Universidade Estadual de Campinas, Brazil, 2002.
- O'Connor, J. C.; Frame, S. R.; Ladies, G. S.; *Toxicol. Sci.* **2002**, *69*, 92.
- Mosmann, T.; *J. Immunol. Methods* **1983**, *65*, 55.

Received: January 30, 2008

Web Release Date: March 6, 2009

FAPESP helped in meeting the publication costs of this article.