Natural Product Research: Formerly Natural Product Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

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Available online: 18 Jun 2012

Natural Product Research
2012, 1–5, iFirst

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(*Received 17 November 2011; final version received 2 May 2012*)

Garcinielliptone FC (GFC), a natural prenylated benzophenone, was extracted from *Platonia insignis* Mart. (Clusiaceae), a native plant commonly known as bacuri and used in traditional Brazilian medicine for the treatment of skin diseases. The aim of this study was to evaluate the cytotoxic and leishmanicidal effects of GFC using *in vitro* models. The experimental data demonstrated that the polyisoprenylated benzophenone GFC possesses cytotoxic and leishmanicidal activities.

Keywords: cytotoxic; garcinielliptone FC; leishmanicidal; *Platonia insignis*

1. Introduction

An investigation on cytotoxic constituents with DNA strand-scission activity of various natural compounds from plants of the Clusiaceae family led to isolation of five new prenylated phloroglucinols, garcinielliptones HA, HB, HC, HD and HE from the heartwoods of *Garcinia subelliptica* (Lu, Wei, Ko, & Lin, 2008). A new benzoylphloroglucinol, garcinielliptone FB, was isolated from the pericarp of *G. subelliptica* and it exhibited cytotoxic activity against several human cancer cell lines (Wu, Weng, Won, & Lin, 2005). Six polyisoprenyl benzophenonoids isolated from the same plant exhibited cytotoxic activity against a small panel of human tumour cell lines (Zhang et al., 2010). Based on the investigations on the cytotoxicity of polyisoprenylated benzophenones, guttiferones Q, S and I, towards three human cancer cell lines, MCF-7, Hela and NCI-H-460, guttiferone Q showed potent cytotoxicity (H.D. Nguyen, Trinh, & L.-H.D. Nguyen, 2011).

The PPAPs, garcinielliptone FC (GFC; Figure 1), was isolated from the hexane extract of the seeds of the *Platonia insignis* Mart., a tree of the Clusiaceae family (Costa Júnior,

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ISSN 1478–6419 print/ISSN 1478–6427 online

http://dx.doi.org/10.1080/14786419.2012.695363
http://www.tandfonline.com

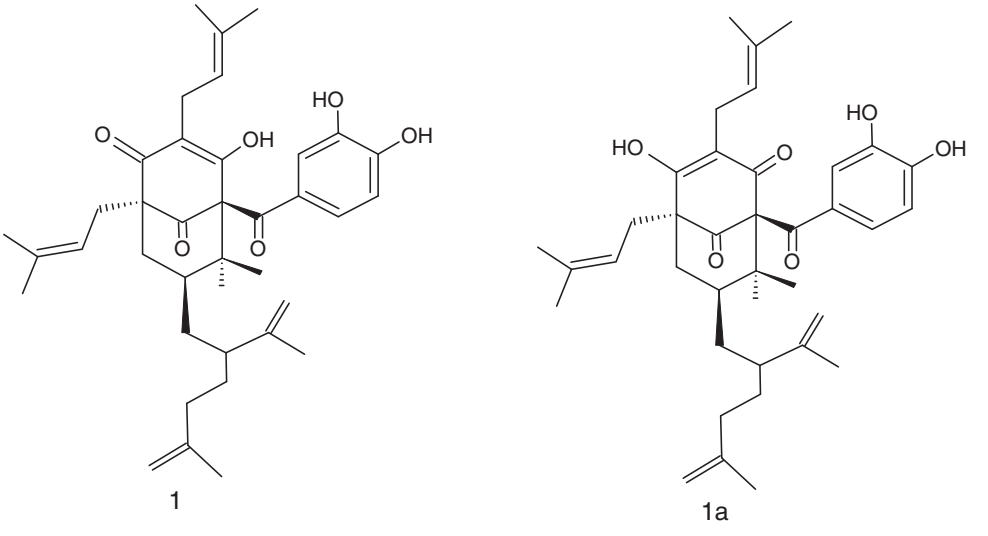

Figure 1. Chemical structure of GFC (tautomeric pair, 1/1a).

Ferraz, Feitosa et al., 2011). This native plant, commonly known as bacuri, has been used in traditional Brazilian medicine for the treatment of skin diseases and diarrhoea (Costa Júnior, Ferraz, & Feitosa et al., 2011). GFC exhibits activity pro-oxidant effect on DNA and induces cell death *in vitro* (Wu et al., 2008). Significant anti-HIV (Garnsey, Matous, Kwiek, & Coltart, 2011), anti-inflammatory (Weng, Tsao, Wang, Wu, & Lin, 2004) and antiplasmodial activities (Marti et al., 2010) have also been attributed to this polyprenylated benzophenones.

While many studies are underway to determine the chemical composition and antioxidant activity of the seeds of *P. insignis* (Costa Júnior, Ferraz, Feitosa et al., 2011), the aim of this study was to evaluate the cytotoxic and leishmanicidal properties of GFC.

2. Results and discussion

The cytotoxicity of GFC ($25\,\mu g\,mL^{-1}$) on two tumour cell lines was evaluated by the tetrazolium salt colourimetric MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-*2H*-tetrazolium bromide) assay, a rapid, sensitive and inexpensive method described by Mosman (1983) that analyses the viability and metabolic state of the cell. GFC showed cell growth inhibition on HEP-2 and NCI-H-292 corresponding to $77.8 \pm 6.9\%$ and $88.2 \pm 0.7\%$, respectively. Analysis of cytotoxicity by MTT method has been used in the *National Cancer Institute of United States* screening programme for many years, with tests more than 10,000 samples each year (Skehan et al., 1990). Further, HEP-2, NCI-H-292 and HL-60 cell lines were incubated with increasing concentrations of the GFC for 72 h at 37°C and analysed using MTT. Significant dose-dependent suppression of cell growth was observed. Table 1 presents the IC_{50} values, which ranged from 1.4 to $3.0\,\mu g\,mL^{-1}$, HL-60 being the most sensitive cell line (IC_{50} $1.4\,\mu g\,mL^{-1}$). In fact, these results agree with previous reports that GFC induced cell death by apoptosis on MCF-7 cells (breast cancer) and also showed a pro-oxidant effect on DNA at a high concentration ($300\,\mu M$) *in vitro* (Wu et al., 2008).

Table 1. Cytotoxic and leishmanicidal activities of GFC isolated from *P. insignis*.

	IC_{50} ($\mu g\,mL^{-1}$)			
Compounds	HL-60	HEP-2	NCI-H-292	*Leishmania amazonensis*
GFC	1.4	3.0	3.0	25.78
Doxorubicin	0.02	0.6	0.01	–
Amphotericin B	–	–	–	0.04

Data are presented as IC_{50} values and 95% confidence intervals (95% CI) for HL-60, HEP-2, NCI-H-292 and *L. amazonensis*.
[a]Positive control: doxorubicin (reference compounds) and [b]positive control: amphotericin B (reference compounds).

The evaluation of the leishmanicidal activity of GFC proved to be quite significant for 72 h with an IC_{50} value of 25.78 $mgmL^{-1}$, as presented in Table 1. Several compounds isolated from plants have proven to have leishmanicidal activity on promastigote and amastigotes of *Leishmania in vitro* assays. The leishmanicidal activity of xanthones (Lenta et al., 2007) and prenylated benzophenones (Pereira et al., 2010) has been reported. Recent studies have demonstrated the efficacy of some medicinal plants in the treatment of ulcerative lesions of cutaneous leishmaniasis and *in vitro* models. In this context, natural products have a great potential in the search for new and selective agents for the treatment of major diseases caused by protozoa (Dutra, Braga, Coimbra, Silva, & Barbosa, 2009). The results presented here suggest a leishmanicidal activity of GFC compared with amphotericin B ($IC_{50} = 0.04\,mgmL^{-1}$) used as a reference drug.

Compounds with more prenyl groups and free phenolic hydroxyl groups have the highest antiproliferative activity in relation to other compounds with a similar structure (Aga, Shibuya, Sugimoto, Kurimoto, & Nakajima, 1994). For this reason, it is believed that the leishmanicidal and cytotoxic activities demonstrated by the GFC may be related to the presence of these groups.

In this study, we have demonstrated that GFC isolated from the seeds of *P. insignis* possesses interesting leishmanicidal activity, showing strong activity on promastigote forms of *L. amazonensis* and strong cytotoxicity on mammal cells. Previous study indicated that GFC presented antioxidant activity *in vitro* (Costa Júnior, Ferraz, & Filho et al., 2011). GFC showed significant activity on promastigote forms of *L. amazonensis* when compared with IC_{50} values of 3.33, 5.04 and 18.12 $\mu g\,mL^{-1}$ of 7-epiclusianone, garciniaphenone and guttiferone-a, respectively (Pereira et al., 2010). These important results make GFC a potential new compound for the development of new drugs against leishmaniasis, but a further detailed evaluation about their mechanism of action is still needed. These results identify GFC as a potential lead compound for anticancer drug discovery.

The GFC effects were compared with doxorubicin since this drug is commonly used in the treatment of a wide range of cancers, including haematological malignancies, many types of carcinoma and soft tissue sarcomas. However, doxorubicin's most serious adverse effect is life-threatening heart damage. Thus, the search for new chemotherapeutic agents with a lower incidence of adverse reactions is extremely necessary. In addition, the effects of GFC were compared with amphotericin B (polyene antibiotic) *in vitro* leishmaniasis models, since this antibiotic is currently a second-line GFC presents leishmanicidal and cytotoxic activities, suggesting their possible use in pre-clinical studies to evaluate initially their adverse effects in rodents.

3. Experimental

3.1. *Plant material, extraction and isolation*

The fruits of *P. insignis* were collected at Barras, Piauí State, Brazil, in March 2009. A voucher specimen has been identified and deposited at the 'Graziela Barroso', Herbarium of Biology Department of Federal University of Piauí, Brazil (voucher no.: ICN TEPB27164).

The seeds were dried at 55°C and powdered and 848.2 g of this was extracted with hexane (63%, w/w). The hexane extract was subjected to silica gel (open column, 400 g, 4×60 cm, 1 mLmin^{-1}) column chromatography and eluted with *n*-hexane containing increased amounts of EtOAc and washed with methanol at end of the process. The resultant hexane extract yields 51 subfractions. Fraction 33 was further purified on TLC plates and eluted with $CHCl_3$–MeOH (9:1) to yield 1/1a (22 mg) which was identified by spectroscopic methods. Its structure was determined using spectroscopic techniques (IR, UV, MS and ^{1}H- and ^{13}C-NMR). The data were compared to those verified in a previous study investigating the chemical structure of this compound (Costa Júnior, Ferraz, & Feitosa et al., 2011; Wu et al., 2008).

3.2. *Cytotoxicity against tumour cell lines and* in vitro *leishmanicidal activity*

The antiproliferative activity of GFC was evaluated in the following human cancer cells: HL-60 (pro-myelocytic leukaemia), K562 (chronic myelogenous leukaemia), HT-29 (colon carcinoma), NCI-H-292 (lung carcinoma) and MCF-7 (breast carcinoma) obtained from Rio de Janeiro Cell Bank (RJ-Brazil). All cancer cells were maintained in RPMI 1640 medium supplemented with 10% foetal bovine serum (FBS), 2 mM glutamine, 100 U mL^{-1} penicillin, 100 μgmL^{-1} streptomycin at 37°C with 5% CO_2. The cytotoxicity of all compounds was tested using the MTT (Sigma Aldrich Co., St. Louis, MO, USA) reduction assay. For all experiments, tumour cells were plated in 96-well plates (10^5 cells mL^{-1} for adherent cells or 3×10^5 cells mL^{-1} for leukaemia). Tested Compounds (0.1–25 μg mL^{-1}) dissolved in 1% DMSO were added to each well and incubated for 72 h. Control groups received the same amount of DMSO (Mosmann, 1983). After 69 h of treatment, 25 μL of MTT (5 mg mL^{-1}) was added; 3 h later, the MTT–formazan product was dissolved in 100 μL of DMSO, and absorbance was measured at 595 nm in plate spectrophotometer. Doxorubicin (0.01–5 μg mL^{-1}) was used as a positive control. Data are presented as IC_{50} values with their CI 95% obtained by nonlinear regression.

Promastigote forms of *L. amazonensis* (IFLA/BR/67/PH-8) in the log phase of growth (1×10^6 parasites mL^{-1}) were incubated with different concentrations of the GFC (400 to 3.12 μg mL^{-1}) at 26°C in Schneider's medium (Sigma, USA) supplemented with 10% of FBS. Amphotericin B was used as a control. After 48 h, parasites were collected, fixed and examined using light microscopy. The inhibitory effect of the fraction on cellular growth was estimated by cell counting using a Neubauer chamber. The concentration that inhibited 50% of the growth (IC_{50}) was determined by regression analysis (Oliveira-Silva, Morais-Teixeira, & Rabello, 2008).

3.3. *Statistical analysis*

Data obtained are reported as the mean ± SEM and were evaluated by one-way analysis of variance followed by *t*-Student– Newman–Keuls as *post hoc* test. Differences were considered to be statistically significant when $p < 0.05$.

Acknowledgements

This study was supported by CNPq, IFPI, and FAPEPI through grants and fellowships.

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