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Cytotoxicity of three naturally occurring flavonoid derived compounds (artocarpesin, cycloartocarpesin and isobavachalcone) towards multi-factorial drug-resistant cancer cells

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ABSTRACT

Introduction: Cancer remains an aggressive deadly disease, if drug resistance develops. This problem is aggravated by the fact that multiple rather than single mechanisms are involved in resistance and that multidrug resistance (MDR) phenomena cause inefficacy of many clinical established anticancer drugs. We are seeking for novel cytotoxic phytochemicals to combat drug-resistant tumour cells.
Methods: In the present study, we investigated the cytotoxicity of three naturally occurring flavonoids including two flavones artocarpesin (**1**) and cycloartocarpesin (**2**) and one chalcone, isobavachalcone (**3**) against 9 drug-sensitive and MDR cancer cell lines. The resazurin reduction assay was used to evaluate the cytotoxicity of these compounds, whilst caspase-Glo assay was used to detect caspase activation. Cell cycle, mitochondrial membrane potential (MMP) and levels of reactive oxygen species (ROS) were all analysed *via* flow cytometry.
Results: Flavones **1** and **2** as well as chalcone **3** displayed cytotoxic effects at various extent on all the 9 tested cancer cell lines with IC_{50} values respectively below 106 μM, 50 μM and 25 μM. The IC_{50} values for the three investigational flavonoids ranged from 23.95 μM (towards hepatocarcinoma HepG2 cells) to 105 μM [towards colon carcinoma HCT116 ($p53^{-/-}$) cells] for **1**, from 15.51 μM (towards leukemia CCRF-CEM cells) to 49.83 μM [towards glioblastoma U87MG.Δ*EGFR* cells] for **2** and from 2.30 μM (towards CCRF-CEM cells) to 23.80 μM [towards colon carcinoma HCT116 ($p53^{+/+}$) cells] for **3** and from 0.20 μM (towards CCRF-CEM cells) to 195.12 μM (towards leukemia CEM/ADR5000 cells) for doxorubicin. Compounds **2** and **3** induced apoptosis in CCRF-CEM leukemia cells, mediated by caspase activation and the disruption of MMP.
Conclusions: The three tested flavonoids and mostly chalcone **3** are potential cytotoxic natural products that deserve more investigations to develop novel antineoplastic drugs against multifactorial drug-resistant cancers.

Introduction

Cancer is the second leading cause of death after cardiovascular disease worldwide with an estimated number of 11.5 million victims by 2030 (Mathers and Loncar, 2006). Despite considerable efforts to improve treatment, this alarming estimation could even exceed, as cancer still remains an aggressive killer, mainly because of the development of drug resistance (Kuete et al., 2013e; Solowey et al., 2014). In fact, the over-expression of ABC transporters (Shen et al., 2011), the epidermal growth factor receptor (EGFR) (Biedler and Spengler, 1994; Efferth et al., 2003a, 2003b) or the tumour suppressor p53 gene (el-Deiry, 1997) have been frequently reported in cancer cells with typical or atypical multidrug-resistance (MDR) phenomena. Medicinal plants are an important source of natural compounds used in cancer chemotherapy (Desai et al., 2008). Several clinically established anticancer drugs are derived from plants, such

Abbreviations: **1**, artocarpesin; **2**, cycloartocarpesin; **3**, isobavachalcone; ABC, ATP-binding cassette; DCF, dichlorofluorescein; EGFR, epidermal growth factor receptor; H_2DCF, 2′,7′-dichlorodihydrofluorescein; H_2DCFH-DA, 2′,7′-dichlorodihydrofluorescein diacetate; MDR, multidrug resistance; MMP, mitochondrial membrane potential; ROS, reactive oxygen species.

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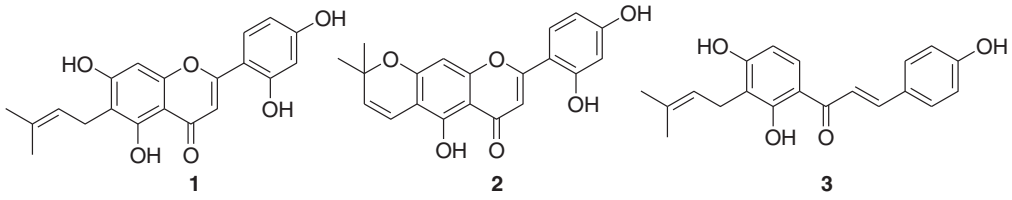

Fig. 1. Chemical structures of artocarpesin (**1**), cycloartocarpesin (**2**) and isobavachalcone (**3**).

as vincristine, vinblastine, paclitaxel, podophyllotoxin, camptothecin and combretastatin (Desai et al., 2008). Plant secondary metabolites and their derivatives constitute an arsenal against various types of cancers (Desai et al., 2008; Kuete and Efferth, 2015). Amongst phytochemicals, flavonoids derive from the phenyl propanoid pathway and many of previously displayed anticancer activities *in vitro* and also *in vivo* (Chen et al., 2015; Kuete et al., 2013e; Ngameni et al., 2013). In our continuing search for naturally occurring products to fight neoplastic diseases, several cytotoxic flavonoids were detected in African medicinal plants in the past 5 years. Some of these include flavones, such as gancaonin Q, 6-prenylapigenin, 6,8-diprenyleriodictyol, apigenin and dorsmanin F (Kuete et al., 2011, 2015; Saeed et al., 2015) and chalcones, namely 4-hydroxylonchocarpin, 4′-hydroxy-2′,6′-dimethoxychalcone, cardamomin and poinsettifolin B (Dzoyem et al., 2012; Kuete et al., 2011, 2014a, 2015). The present study was designed to evaluate the cytotoxic potential of three naturally occurring flavonoids, artocarpesin (**1**) and cycloartocarpesin (**2**) (flavones) and isobavachalcone (**3**) (a chalcone) against a panel of drug-sensitive and MDR cancer cell lines. We further investigated the mode of action of cycloartocarpesin and isobavachalcone in terms of induction of apoptosis, disruption of mitochondrial membrane potential (MMP) and generation of reactive oxygen species (ROS). Compound **3** previously demonstrated cytotoxic effects of cancer cells lines, such as ovarian carcinoma cell OVCAR-8 cells, prostate carcinoma PC-3 cells, breast carcinoma MCF-7 cells and lung carcinoma A5491 cells (Jing et al., 2010; Kuete and Sandjo, 2012). To the best of our knowledge, the cytotoxicity of the three compounds towards MDR cancer cells is being reported here for the first time.

Materials and methods

Chemicals

Artocarpesin (**1**), cycloartocarpesin (**2**) and isobavachalcone (**3**) (Fig. 1) were obtained from our Chemical Bank (Department of Chemistry, University of Yaoundé I, Cameroon). The isolation and identification of artocarpesin and cycloartocarpesin from *Morus mesozygia* Stapf (Moraceae) (Kuete et al., 2009) and isobavachalcone from *Dorstenia barteri* Bureau *var. multiradiata* (Moraceae) (Mbaveng et al., 2008) was previously reported. Doxorubicin 98.0% and vinblastine ≥96% from Sigma-Aldrich (Munich, Germany) were provided by the Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry of the Johannes Gutenberg University (Mainz, Germany) and dissolved in PBS (Invitrogen, Eggenstein, Germany) at a concentration of 10 mM. Geneticin >98% was purchased from Sigma-Aldrich and stored at a stock concentration of 72.18 mM. Dimethylsuphoxide from Sigma-Aldrich was used to dissolve the three flavonoids. The concentration of DMSO was not greater than 0.1% in all experiments.

Cell cultures

The cell lines used in the present work, their origins and their treatments were previously reported. They include drug-sensitive CCRF-CEM and multidrug-resistant P-glycoprotein-over-expressing CEM/ADR5000 leukemia cells (Efferth et al., 2003b; Gillet et al., 2004; Kimmig et al., 1990), MDA-MB-231-pcDNA3 breast cancer cells and its resistant subline MDA-MB-231-*BCRP* clone 23 (Doyle et al., 1998), HCT116 ($p53^{+/+}$) colon cancer cells and its knockout clone HCT116 ($p53^{-/-}$), U87.MG glioblastoma cells and its resistant subline U87.MGΔ*EGFR* (Kuete et al., 2013a, 2013b, 2013d).

Resazurin reduction assay

The cytotoxicity testing was performed by using the resazurin reduction assay as previously described (O'Brien et al., 2000; Kuete et al., 2013a, 2013c, 2013d).

Flow cytometry for cell cycle analysis and detection of apoptotic cells

All reagents, experimental conditions and apparatus were identical to those previously reported (Kuete et al., 2014b). Briefly, cell cycle analysis was performed by flow cytometry using Vybrant® DyeCycle™ (Invitrogen, Darmstadt, Germany). CCRF-CEM leukemia cells (1×10^6) were used as cell model, treated with compounds **2** and **3**. Cells were measured after Vybrant® DyeCycle™ Violet staining (30 min at 37 °C) on a LSR-Fortessa FACS analyser (Becton-Dickinson, Heidelberg, Germany) using the violet laser. Vybrant® DyeCycle™ Violet stain was measured with 440 nm excitation. Cytographs were analysed using FlowJo software (Celeza, Switzerland). All experiments were performed at least in triplicate.

Caspase-Glo 3/7, caspase-Glo 8 and caspase-Glo 9 assay

Caspase activity in CCRF-CEM leukemia cells was detected using Caspase-Glo 3/7, Caspase-Glo 8 and Caspase-Glo 9 Assay kits (Promega, Mannheim, Germany) as previously reported (Kuete et al., 2014b). Cells were treated with compounds **2** and **3** at their $2 \times IC_{50}$ and IC_{50} values with DMSO as solvent control for 6 h. Luminescence was measured using an Infinite M2000 Pro™ instrument (Tecan). Caspase activity was expressed as percentage of the untreated control.

Analysis of mitochondrial membrane potential (MMP)

The MMP was analysed in CCRF-CEM cells by 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1; Biomol, Hamburg, Germany) staining as previously reported (Kuete et al., 2014b). Cells (1×10^6) treated for 24 h with different concentrations of compounds **2** and **3** as well as vinblastine (drug control) or DMSO (solvent control) were incubated with JC-1 staining solution for 30 min according to the manufacturer's protocol. Subsequently, cells were measured in a LSR-Fortessa FACS analyser (Becton-Dickinson). The JC-1 signal was measured at an excitation of 561 nm (150 mW) and detected using a 586/15 nm band-pass filter. The signal was analysed at 640 nm excitation (40 mW) and detected using a 730/45 nm bandpass filter. Cytographs were analysed using FlowJo software (Celeza, Olten, Switzerland). All experiments were performed at least in triplicate.