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Cytotoxicity of seven naturally occurring phenolic compounds towards multi-factorial drug-resistant cancer cells

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ABSTRACT

Introduction: In medical oncology, multi-drug resistance (MDR) of cancer cells continues to be a major impediment. We are in quest of novel anti-proliferative agents to overcome drug-resistant tumor cells.
Methods: In the present study, we investigated the cytotoxicity of 7 naturally occurring phenolic compounds including two isoflavonoids alpinumisoflavone (**1**) and laburnetin (**2**), one biflavonoid amentoflavone (**3**), three lignans pycnanthulignene A (**4**), pycnanthulignene B (**5**), and syringaresinol (**7**) and one xanthone, euxanthone (**6**) 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 analyzed *via* flow cytometry.
Results: The IC_{50} values for the investigational phenolics ranged from 5.91 μM (towards leukemia CEM/ADR5000 cells) to 65.65 μM (towards drug-resistant breast adenocarcinoma MDA-MB-231-*BCRP* cells) for **1**, 27.63 μM (towards leukemia CCRF-CEM cells) to 107.57 μM (towards MDA-MB-231-*pcDNA* cells) for **2**, from 5.84 μM (towards CEM/ADR5000 cells) to 65.32 μM (towards colon carcinoma HCT116 ($p53^{-/-}$) cells) for **4** and 0.20 μM (towards CCRF-CEM cells) to 195.12 μM (towards leukemia CEM/ADR5000) for doxorubicin. Phenolics **3, 5, 6** and **7** displayed selectivity cytotoxic effects on cancer cells lines. Compounds **1** and **4** induced apoptosis in CCRF-CEM cells, mediated by loss of MMP and increase ROS production.
Conclusions: The studied phenolics and mostly isoflavonoid **1** and lignan **4** are potential cytotoxic natural products that deserve more investigations to develop novel antineoplastic drugs against multifactorial drug-resistant cancers.

Introduction

Cancer represents a worldwide health concern, being the leading cause of death after cardiovascular disease worldwide with an estimated number of 11.5 million victims by 2030 (Mathers and Loncar, 2006). Medical oncology is seriously challenged by the ability of cancer cells to rapidly develop multi-drug resistance (MDR) (Solowey et al., 2014). In the past, several clinically established anticancer drugs such as vinblastine, paclitaxel, podophyllotoxin and camptothecin were isolated from plants (Desai et al., 2008). The low success of cancer chemotherapy as a result of the development of MDR phenotypes propels continuous efforts of scientists to search for new anti-neoplastic agents. In regards to the high diversity of plant secondary metabolites, the quest of novel cytotoxic molecules from botanicals remains an attractive strategy (Desai et al., 2008; Kuete and Efferth, 2015). In recent years, our cytotoxicity screenings of African medicinal plants and derived products have delivered several promising compounds (Kuete and Efferth, 2015). Many of these phytochemicals also displayed interesting anti-proliferative effects against MDR cancer cell lines. Amongst them were several phenolic compounds such as artocarpesin, cycloartocarpesin,

Abbreviations: **1**, alpinumisoflavone; **2**, laburnetin; **3**, amentoflavone; **4**, pycnanthulignene A; **5**, pycnanthulignene B; **6**, euxanthone; **7**, syringaresinol; ABC, adenosine triphosphate-binding cassette; BCRP, breast cancer resistance protein; DCF, dichlorofluorescein; DMSO, dimethylsufoxide; EGFR, epidermal growth factor receptor; H_2DCFH-DA, 2′,7′-Dichlorodihydrofluorescein diacetate; IC_{50}, inhibitory concentration 50%; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide; MDR, multi-drug resistance; MMP, mitochondrial membrane potential; PBS, phosphate buffer saline; ROS, reactive oxygen species.

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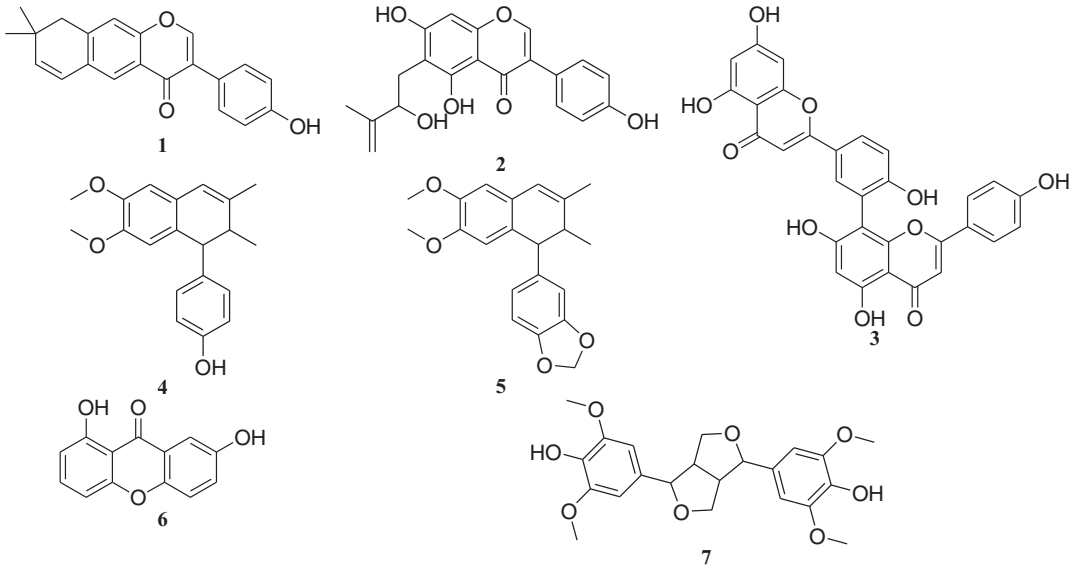

Fig. 1. Chemical structures of tested compounds alpinumisoflavone (**1**), laburnetin (**2**), amentoflavone (**3**), pycnanthulignene A (**4**), pycnanthulignene B (**5**), euxanthone (**6**) and syringaresinol (**7**).

isobavachalcone (Kuete et al., 2015b), dorsmanin F, poinsettifolin B (Kuete et al., 2015c), 4′-hydroxy-2′,6′-dimethoxychalcone (Kuete et al., 2014a), neobavaisoflavone, sigmoidin H, isoneorautenol (Kuete et al., 2014c), 3, 4′, 5-trihydroxy-6′′, 6′′-dimethylpyrano [2, 3 g]flavone (Kuete et al., 2015d), damnacanthal and damnacanthol (Kuete et al., 2015a). In our continuous search of anti-proliferative molecules to combat MDR, the present study was designed to evaluate the cytotoxicity of 7 phenolic compounds occurring in African medicinal plants, *i.e.* alpinumisoflavone (**1**), laburnetin (**2**), amentoflavone (**3**), pycnanthulignene A (**4**), pycnanthulignene B (**5**), euxanthone (**6**) and syringaresinol (**7**) against a panel of drug-sensitive and MDR cancer cell lines. The mode of action of alpinumisoflavone and pycnanthulignene A in terms of induction of apoptosis, alteration of mitochondrial membrane potential (MMP) and generation of reactive oxygen species (ROS) was further investigated. Compound **7** was previously found active against HL60 leukemia cells (Park et al., 2008), while **2** had antiproliferative effects against UMR106 rat osteogenic sarcoma (Xiaoli et al., 2006). Compound **6** also displayed cytotoxic effects against MDA-MB-231 cells, HeLa cervix cancer cells, CEM-SS leukemia cells and CaOV3 ovarian carcinoma cells (Ee et al., 2005). The cytotoxicity of **3** was reported against MCF-7 breast adenocarcinoma cells (Chen et al., 2015), B16F-10 melanoma cells (Guruvayoorappan and Kuttan, 2008) and SW480 colon carcinoma cells (Yang et al., 2014). To the best of our knowledge, the cytotoxicity of the studied compounds towards MDR cancer cells is being reported here for the first time.

Materials and methods

Chemicals

Alpinumisoflavone (**1**), laburnetin (**2**), amentoflavone (**3**), pycnanthulignene A (**4**), pycnanthulignene B (**5**), euxanthone or 1,7-dihydroxyxanthone (**6**) and syringaresinol (**7**) (Fig. 1) were obtained from our Chemical Bank (Department of Chemistry, University of Dschang, Cameroon). The isolation and identification of compounds **1** and **2** from *Ficus chlamydocarpa* (Moraceae) (Kuete et al., 2008), **3** from *Dorstenia barteri* Bureau (Moraceae) (Mbaveng et al., 2008; Kuete et al., 2010), **4** and **5** from *Pycnanthus angolensis* (Welw.) Ward (Myristicaceae) (Nono et al., 2010; Kuete et al., 2011b), **6** from *Oricia suaveolens* Engl. (Rutaceae) (Fouotsa et al., 2013) and **7** from *Allanblackia floribunda* (Guttiferae) (Kuete et al., 2011a) 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. Dimethylsulfoxide (DMSO) from Sigma-Aldrich was used to dissolve the compounds.

Cell cultures

The cell lines used in the present study, their origins and their treatments were previously reported. They include drug-sensitive CCRF-CEM leukemia and multidrug-resistant P-glycoprotein-overexpressing subline CEM/ADR5000 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 ($p53^{-/-}$), U87MG glioblastoma cells and its resistant subline U87MG.Δ*EGFR* (Kuete et al., 2013b; 2013c; 2013d). To compare tumor with normal cells, HepG2 liver cancer cells and AML12 normal hepatocytes were used (Kuete et al., 2013b).

Resazurin reduction assay

The cytotoxicity of the tested samples was performed by resazurin reduction assay as previously described (O'Brien et al., 2000; Kuete et al., 2013d). Doxorubicin was used as positive control, while dimethylsulfoxide (DMSO) used to dissolve the samples was used as negative control. The highest concentration of DMSO was less than 0.1%. Fluorescence was measured by an Infinite M2000 Pro™ plate reader (Tecan, Crailsheim, Germany) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. Each assay was done at least two times, with six replicate each. The viability was evaluated based on a comparison with