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Anticancer activity of koningic acid and semisynthetic derivatives

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ABSTRACT

A screening program aimed at discovering novel anticancer agents based on natural products led to the selection of koningic acid (KA), known as a potent inhibitor of glycolysis. A method was set up to produce this fungal sesquiterpene lactone in large quantities by fermentation, thus allowing (i) an extensive analysis of its anticancer potential in vitro and in vivo and (ii) the semi-synthesis of analogues to delineate structure–activity relationships. KA was characterized as a potent, but non-selective cytotoxic agent, active under both normoxic and hypoxic conditions and inactive in the A549 lung cancer xenograft model. According to our SAR, the acidic group could be replaced to keep bioactivity but an intact epoxide is essential.

1. Introduction

Cancer diseases affect millions of people worldwide and the incidences increase as the population ages. Target-based drug discovery for tumor selective agents has been ongoing for many years, with important successes, thanks to the understanding of the molecular mechanisms underlying tumorigenesis and tumor progression. But despite major successes for some specific sub-types of malignancies, a large number of cancers, especially advanced carcinoma and metastatic diseases, remain devastating. Therefore, the use of phenotypic screening, starting with a cancer-relevant phenotype to screen for compounds that change the outcome of biological pathways rather than activities at certain specific targets, offers an alternative approach to find small molecules or targets that modulate the key characteristics of aggressive tumors. It is in this context that we continue to search for whole cell active compounds using libraries of natural products. In the course of a screening program for natural products anticancer agents isolated from soil microorganisms, we have identified koningic acid and here we report the work performed with this compound.

Koningic acid (designated KA, Fig. 1), also known as heptelidic acid, has been first isolated in 1980 from the culture filtrate of three strains of fungi: *Gliocladium virens*, *Chaetomium globosum* and *Trichoderma viride*.[1,2] The structure of the compound has been fully characterized by X-ray crystallography[3] and two total syntheses have also been described.[4–6] It is a sesquiterpene lactone initially identified as an antimicrobial agent, active against anaerobic bacteria and also displays antiparasitic properties.[7,8] Early on, it was demonstrated that KA is a blocker of ATP synthesis in the glycolytic pathway, acting as a potent and specific inhibitor of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) that catalyzes the conversion of glyceraldehyde 3-phosphate to 3-phosphoglycerate.[9] KA irreversibly inactivates GAPDH via covalent binding to a cysteine residue in the active site of the enzyme.[10–14] This observation led to the design of epoxide-containing agents with antiparasitic activities.[15] Interestingly, the chlorohydrin derivative of KA was shown to be cytotoxic to tumor cells.[16] KA itself induces DNA fragmentation in neuronal cells[17] but is also capable of inhibiting etoposide-induced apoptosis via

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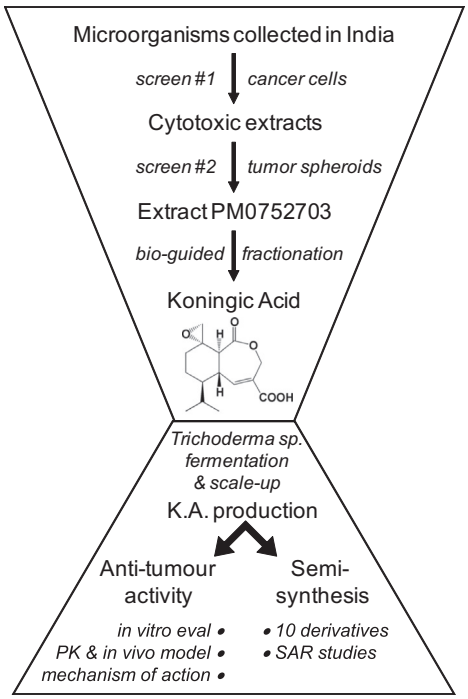

Figure 1. Schematic of the research program around KA.

downregulation of caspases[18]. A key property of this secondary metabolite is its capacity to selectively kill high-glycolytic cells through glucose-dependent active ATP deprivation.[19] In general, tumor cells are dependent upon glycolysis and the inhibition of GAPDH activity in the presence of KA in these high-glycolytic cells causes depletion in ATP stores, leading to apoptosis via caspase 3 activation. It has been reported that KA is only effective in cells with a supply of glucose otherwise alternate starvation-induced metabolic pathways may be initiated to prolong cell life and reestablish the tumor's dominance.[19]

Accelerated glucose metabolism is a common feature of cancer cells. Some of the specific enzymes driving glycolysis are considered as key cancer targets. This is the case of hexokinase 2 which catalyzes the first step of glucose metabolism and is expressed at high level in cancer cells.[20] Another enzyme regulator of the glycolytic flux, phosphofructokinase-2 (PFK-2), has also recently emerged as a promising anticancer target.[21] GAPDH is another key enzyme of the glycolysis metabolic processes which may be considered as a target, despite its ubiquitous nature. While most glycolytic enzymes are over-expressed in tumors, GAPDH is of particular interest as it can protect cells from caspase-independent cell death-induced chemotherapy through preservation of intact mitochondria that may facilitate tumor survival and chemotherapeutic resistance.[22] Thus, inhibiting GAPDH in tumor cells might be useful. Moreover, recent studies have also highlighted the non-glycolytic roles of GAPDH in cell death, survival mechanisms and diseases. Authors suggested that GAPDH might be a promising target for the therapy of some carcinomas.[23,24] Because cancer cells rely on aerobic glycolysis rather than oxidative phosphorylation, GAPDH-depleting agents have a therapeutic potential to impede cancer cell proliferation. GAPDH-targeted combination therapy is a novel strategy to control the proliferation of tumor cells.[25] A few compounds are known to target GAPDH, such as 3-bromopyruvate which presents anticancer efficacy in multiple tumor models.[26] These considerations prompted us to select KA from a pool of cytotoxic natural products and to evaluate its anticancer potential using different molecular and cellular assays, and the design of semisynthetic analogues (Fig. 1).

2. Results

2.1. Screening of extracts, bioguided fractionation, characterization of KA

A primary pharmacological screening of a large bank of microorganism samples was performed at Piramal Enterprises Limited Laboratories (PEL, Mumbai, India), leading to the selection of 40 culture extracts endowed with a potent cytotoxic activity on different cancer cell lines. Actinomycetes and fungi strains, collected from various environmental sources in India, represented a major part of the natural products collection. The 40 active samples were then the subject of a new evaluation at Pierre Fabre Laboratories (IRPF, Toulouse, France), aimed at identifying new antitumor agents. In this program, three phenotypic biological assays were implemented to screen these active extracts, including a model for the selection of potent cytotoxic agents from natural sources, based on the use of tumor spheroid of WM266 melanoma cells. Among the hits of interest, a fungus sample (PM0752703) presented a complete inhibition of cell growth at 10 μg/mL. The 100% methanolic fraction of PM0752703 was fractionated by automated preparative HPLC. Identification of the active components was accomplished by High-Resolution MS and NMR spectroscopy. The main pure compound isolated from the extract through bioguided fractionation being koningic acid (KA) (Fig. 1), it was then decided to investigate further the pharmacological profile of this natural product.

2.2. Production of KA by fermentation

Following the screenings and identification of KA, a fermentation procedure was adapted to produce large quantities of the compound, for subsequent pharmacological evaluation and the semisynthesis of derivatives, as described below (Fig. 1). The fungal strain *Trichoderma virens* (PM0752703) was grown on agar plates to obtain colonies and an initial laboratory protocol was implemented using HP20 column to purify the sample. Then a scale-up protocol was set up, based on 9 fermentation batches (100 L each), representing a total culture filtrate extract of 800 L, from which an ethyl acetate crude extract of 514 g was obtained (after acidification of the whole broth). The resulting crude product was subjected to open column chromatography over silica gel using a solvent system (petroleum ether to ethyl acetate). Fractions were combined after analysis by TLC and HPLC affording 8 final fractions. The mid-polar to polar range of these fractions were further purified by flash chromatography leading to the isolation of KA. The fractions containing the compound of interest were dried under vacuum and further suspended in water/acetonitrile prior to lyophilisation to afford an off-white solid powder. HPLC purity of the isolated compound was greater than 98% when analyzed in two different HPLC systems with PDA and ELSD detectors. ^{1}H and ^{13}C NMR chemical shifts were exactly matching with respective chemical shifts of KA reported in literature.[27] Globally, the final purification of KA has provided more than 15 g of the pure natural product, fully characterized by HPLC analysis, NMR and mass spectrometry.

2.3. Anti-proliferative activities of KA

To begin with, we investigated the anti-proliferative properties of KA using four tumor cell lines: A549 (lung cancer), HCT116 (colorectal cancer), KG1 (leukemia) and A375 (melanoma). Exponentially growing cells were treated with the natural product for 72 h and cell viability was evaluated using a conventional ATP quantification method. All four cell lines were sensitive to the drug