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A new acridine derivative induces cell cycle arrest and antiangiogenic effect on Ehrlich ascites carcinoma model

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ABSTRACT

Background: Acridine derivatives, including amsacrine, have antitumor activity. However, side effects, development of resistance and their low bioavailability, have limited their use. Herein, we described the synthesis, and evaluated the toxicity and antitumor activity of a new amsacrine analogous, the *N′*-(2-chloro-6-methoxy-acridin-9-yl)-2-cyano-3-(4-dimethylaminophenyl)-acrilohidrazida (ACS-AZ10).
Methods: The compound was obtained in a linear pathway where the ASC-Az intermediate was obtained by coupling of 6,9-dichloro-3-methoxy-acridine and 2-ciany-acethohidrazide followed by condensation with the corresponding aldehyde. The toxicity of ACS-AZ10 was evaluated in mice using acute toxicity and micronucleus assays. Ehrlich ascites carcinoma model was used to investigate the antitumor activity and toxicity of ACS-AZ10 (7.5, 15 or 30 mg/kg, i.p.), after nine days of treatment. Cell cycle and angiogenesis were also evaluated.
Results: The ASC-AZ10 was obtained with satisfactory yields and its structure was confirmed by spectroscopic and spectrometric techniques. On acute toxicity study, ACS-AZ10 (2000 mg/kg, i.p.) induced transient depressant effects on central nervous system. The LD_{50} was approximately 2500 mg/kg. ACS-AZ10 (15 or 30 mg/kg) displayed significant antitumor activity considering the tumor weight and volume, cell viability, and total Ehrlich cell count. ACS-AZ10 (7.5 mg/kg) induced an increase in sub-G1 peak, suggesting apoptosis. At 15 mg/kg ACS-AZ10 induced cell cycle arrest in G2/M phase and a reduction in the percentage of cells in G0/G1 and S phases, suggesting a pre-mitotic blockade. ACS-AZ10 reduced the microvessel density, indicating an antiangiogenic effect. Weak hematological, biochemical and histopathological toxicity were observed. The compound doesn't show genotoxicity in micronucleus assay.
Conclusions: ACS-AZ10 has potent antitumor activity *in vivo* along with low toxicity.

1. Introduction

Cancer is a group of diseases characterized by uncontrolled growth and multiplication of transformed cells that can invade various tissues [1]. In 2016, 1,685,210 new cancer cases and 595,690 cancer deaths are projected to occur in the United States [2]. There will be 22.2 million new cancer cases and about 13.2 million deaths worldwide per year, by 2030 [3].

Various hallmarks are described for cancer, including the cell cycle deregulation and angiogenesis [4]. Cell cycle deregulation is

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related to the avoidance of cytostatic controls, sustaining proliferative signaling, and resistance to apoptosis [5]. The formation of new blood vessels (angiogenesis) is a multistep process that is essential in the development of solid and hematological tumors, by maintaining adequate supply of nutrients and oxygen to the rapid tumor growth [6]. To date, it has been reported about 300 antiangiogenic agents and more than 80 of them are drugs used in different stages of clinical trials [7,8].

For over a century, acridine derivatives are used for commercial purposes. The acridines are heterocyclic compounds formed by two benzene rings fused to a pyridine ring in the center [9]. Acridine derivatives have several biological activities, and are clinically used as antiviral [10], anti-inflammatory [11], analgesics [12] and anticancer [13,14].

Amsacrine (m-AMSA), the acridine derivative better known, was the first synthetic topoisomerase II inhibitor approved for clinical use. It is still used in the treatment of acute leukemia, and Hodgkin's and non-Hodgkin's lymphoma, but is ineffective in solid tumors [13,14]. However, the side effects, the development of resistance and its low bioavailability, have limited their use. These factors encouraged synthetic chemists to structurally modify this compound and produce different derivatives that have shown significant antitumor activity [14,15].

Herein, we described the synthesis of a new amsacrine analogous, the *N*′-(2-chloro-6-methoxy-acridin-9-yl) -2-cyano-3-(4-dimethylaminophenyl) -acrilohidrazida (ACS-AZ10, Fig. 1). In addition, we attempted to evaluate the effects of ACS-AZ10 on toxicity and *in vivo* antitumor activity on Ehrlich ascites carcinoma model.

2. Methods

2.1. Drugs and reagents

Propidium iodide, 5-fluorouracil (5-FU), Triton X-100, Tween 80, and cyclophosphamide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethylsulfoxide (DMSO) was purchased from Mallinckrodt CHEMICALS® (Phillipsburg, NJ, USA). Sodium thiopental (Thiopentax®) was purchased from Cristália (Itapira, SP, Brazil), and heparin (Parinex®) from Hipolabor (Sabará, MG, Brazil). Kits for biochemical and hematological analysis were purchased from LABTEST® (Lagoa Santa, MG, Brazil). The drugs and reagent solutions were prepared immediately before using. All reagents used in the syntesis of ACS-AZ10 are commercially available (Sigma-Aldrich, Acros Organics, Vetec).

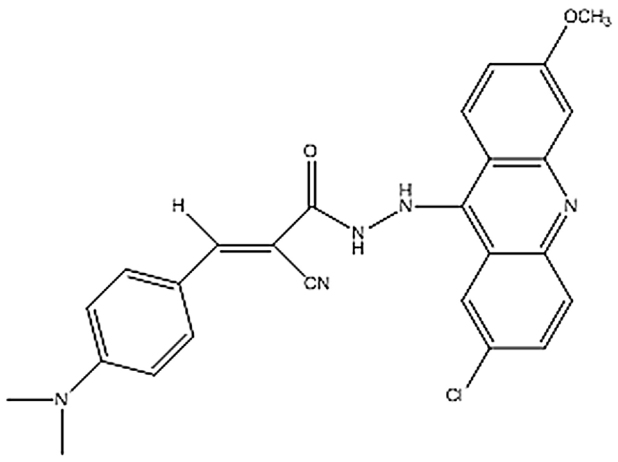

Fig. 1. Structure of derivative *N*′- (2-chloro-6-methoxy-acridin-9-yl) -2-cyano-3-(4-dimethylaminophenyl) -acrilohidrazida (ACS-AZ10).

2.2. Chemistry

The reactions progress was followed by thin-layer chromatography (TLC) analysis (Merck, silica gel 60 F254 in aluminium foil). Melting points were determined on a Quimis 340 (Quimis, Brazil) capillary melting point apparatus and were uncorrected. IR spectra were recorded with a Bruker model IFS66 FT-IR spectrophotometer (Bruker, Germany) using KBr pellets. NMR spectra were measured on either a Bruker AMX-300 MHz (300 MHz for 1H and 75.5 MHz for 13C) instruments. DMSO-*d*6 was purchased from Sigma-Aldrich. Chemical shifts are reported in ppm and multiplicities are given as *s* (singlet), *d* (doublet), *t* (triplet), *m* (multiplet), *dd* (double doublet), and coupling constants (J) in hertz. Mass spectrometry experiments were performed on a MALDI-TOF Autoflex III (Bruker Daltonics, Billerica, MA, USA). All organic solution were dried over anhydrous sodium sulfate and all organic solvents were removed under reduced pressure in rotator evaporate.

2.2.1. General procedure for the preparation of N′-(6-chloro-2-metoxyacridin-9-yl)-2-cyanoacetohydrazide (ACS-AZ)

In a round flask containing 1 g 2-ciany-acethohidrazide (0.01 mol) and 1Eq mole of the commercial compound 6,9-dichloro-3-methoxiacridin (CAS 86-38-4) from Sigma Aldrich (97%) was added 50 mL of the EtOH anhydrous followed by stirring and heating at 70 °C for 4 h. The mixture was cooled, filtered, washed with water and recrystallized from EtOH.

2.2.2. General procedure for obtaining of N′-(6-chloro-2-metoxyacridin-9-yl)-2-cyano-3-(4-dimethyaminephenyl)-acrylohydrazide (ACS-AZ10)

In a round flask (50 mL) it was added 0.1 g (0.0029 mol) of ACS-AZ and 1eq mole of the corresponding aromatic aldehyde in toluene and basic medium (10% morpholine mmol). The mixture was stirred and refluxed at 110 °C. The reaction was monitored by Thin Layer Chromatography (system *n*-hexane/AcOEt 7:3). The material was filtered and washed with EtOH (20 mL) after recrystallized from EtOH.

2.3. Animals

Swiss albino mice (*Mus musculus*), females (30–35 g), obtained from the Dr. Thomas George Bioterium (Research Institute in Drugs and Medicines/Federal University of Paraíba, Brazil), were used. The animals were randomly housed in cages containing six animals with free access to food and water. All animals were kept on a 12 h/12 h off light-dark cycle (lights on at 6:00 a.m.). All procedures were previously approved by the Animal Studies Committee of the Federal University of Paraíba (CEUA-UFPB, no. 0801/14).

2.4. Tumor cell line

Ehrlich carcinoma cell line was generously provided by Pharmacology and Toxicology Division, CPQBA, UNICAMP (Paulínia, SP, Brazil). The cells were maintained in the peritoneal cavities of Swiss mice in the Dr. Thomas George Bioterium (Research Institute in Drugs and Medicines/Federal University of Paraíba, Brazil).

2.5. Pharmacological assays

2.5.1. Evaluation of acute preclinical toxicity

The evaluation of acute preclinical toxicity for ACS-AZ10 was performed based on the "Guidelines for Testing of Chemicals" n°