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Monoterpene bisindole alkaloids, from the African medicinal plant *Tabernaemontana elegans*, induce apoptosis in HCT116 human colon carcinoma cells

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ABSTRACT

Ethnopharmacological relevance: *Tabernaemontana elegans* is a medicinal plant used in African traditional medicine to treat several ailments including cancer. The aims of the present study were to identify anti-cancer compounds, namely apoptosis inducers, from *Tabernaemontana elegans*, and hence to validate its usage in traditional medicine.
Methods and materials: Six alkaloids, including four monomeric indole (**1**–**3**, and **6**) and two bisindole (**4** and **5**) alkaloids, were isolated from the methanolic extract of *Tabernaemontana elegans* roots. The structures of these compounds were characterized by 1D and 2D NMR spectroscopic and mass spectrometric data. Compounds **1**–**6** along with compound **7**, previously isolated from the leaves of the same species, were evaluated for in vitro cytotoxicity against HCT116 human colon carcinoma cells by the MTS metabolism assay. The cytotoxicity of the most promising compounds was corroborated by Guava-ViaCount flow cytometry assays. Selected compounds were next studied for apoptosis induction activity in HCT116 cells, by evaluation of nuclear morphology following Hoechst staining, and by caspase-3 like activity assays.
Results: Among the tested compounds (**1**–**7**), the bisindole alkaloids tabernaelegantine C (**4**) and tabernaelegantinine B (**5**) were found to be cytotoxic to HCT116 cells at 20 μM, with compound **5** being more cytotoxic than the positive control 5-Fluorouracil (5-FU), at a similar dose. In fact, even at 0.5 μM, compound **5** was more potent than 5-FU. Compounds **4** and **5** induced characteristic patterns of apoptosis in HCT116 cancer cells including, cell shrinkage, condensation, fragmentation of the nucleus, blebbing of the plasma membrane and chromatin condensation. Further, general caspase-3-like activity was increased in cells exposed to compounds **4** and **5**, corroborating the nuclear morphology evaluation assays.
Conclusions: Bisindole alkaloids tabernaelegantine C (**4**) and tabernaelegantinine B (**5**) were characterized as potent apoptosis inducers in HCT116 human colon carcinoma cells and as possible lead/scaffolds for the development of anti-cancer drugs. This study substantiates the usage of *Tabernaemontana elegans* in traditional medicine to treat cancer.

1. Introduction

Apoptosis, also referred as 'programmed cell death', is a vital component of various biological processes of the body to get rid of abnormal and cancerous cells. Cell survival is maintained in the body by a balance between pro-apoptotic and anti-apoptotic stimuli. Deregulation of apoptosis disrupts the equilibrium between cell growth and cell death, and is an important step in the development of cancer (Kinloch et al., 1999). This principle lies behind the search for novel molecules that can therapeutically activate apoptosis in cancer cells to eliminate them from body. Various classes of biologically active natural products, including several alkaloids, terpenoids, polyphenols, and saponins can induce apoptosis by interacting with important molecular targets such as DNA, microtubules, biomembranes and receptors (Taraphdar et al., 2001).

The genus *Tabernaemontana* (Apocynaceae) has a wide distribution and plants belonging to this genus have been used in African traditional medicine to treat tumors and cancer (Graham et al., 2000). *Tabernaemontana elegans* Stapf. (Syn. *Conopharyngia elegans*

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Stapf.) is an important member under this genus that occurs in tropical or subtropical regions including Indonesia, Malaysia, and Africa (Vanbeek et al., 1984). It is known in English as the "toad tree", due to the brown and wart like skin of its fruit. Folklore claims suggested that the root-bark is used for cancer treatment (Chhabra et al., 1987). Other usages include: root decoction is applied as a wash to wounds, and drunk for pulmonary diseases and chest pains (Watt and Breyer-Brandwijk, 1962; Arnold and Gulumian, 1984; Vanderheijden et al., 1986); treatment of heart diseases with the seeds, stem-bark and roots; and root decoction is also said to have aphrodisiac properties (Neuwinger, 1966). *Tabernaemontana* plants are characterized to produce various skeletal types of indole alkaloids, including iboga-type, aspidosperma-type, vobasinyl-ibogan-type and tetrakis-aspidosperma-type (Vanbeek et al., 1984, 1985). These alkaloids have shown a wide range of biological activities including cytotoxicity, reversal of vincristine-resistance, antimicrobial activity against Gram-positive bacteria, antifungal activity, and acetylcholinestrase inhibitory activity (Vanbeek, et al., 1985; Achenbach et al., 1997; Kam et al., 1998; Chaturvedula et al., 2003; Andrade et al., 2005).

In our search for apoptosis inducing compounds from medicinal plants (Duarte et al. 2007; Mansoor et al. 2009b, 2011, 2012; Wesołowska et al. 2012), we report the isolation and identification of six compounds (**1**–**6**) from the MeOH extract of *Tabernaemontana elegans* roots. Our previous phytochemical studies of *Tabernaemontana elegans* leaves yielded several alkaloids, including compound **7** (Mansoor et al., 2009a, 2009b). Compounds **1**–**7** were evaluated for cytotoxicity/cell viability in HCT116 colon carcinoma cells. The most active compounds in cell viability assays were further subjected to Guava-ViaCount flow cytometric assays and apoptosis induction activity by morphological evaluation of nuclear morphology following Hoechst staining and caspase-3 like activity assays.

2. Materials and methods

2.1. General experimental procedures

NMR spectra were recorded on a Bruker ARX-400 NMR spectrometer (^{1}H NMR 400 MHz; ^{13}C NMR 100.61 MHz), using $CDCl_3$ as solvent. Mass spectrometry was carried out on a Triple Quadrupole mass spectrometer (Micromass Quattro Micro API, Waters). Column chromatography was carried out on SiO_2 (Merck 9385). TLC were performed on precoated SiO_2 F_{254} plates (Merck 5554 and 5744), visualized under UV light and by spraying either with Dragendorff's reagent or a solution of H_2SO_4–MeOH (1:1), followed by heating.

2.2. Plant material

The roots of *Tabernaemontana elegans* were collected in Mozambique during February, 2011. Taxonomical identification was performed by the botanist Dr. Silva Mulhovo, Centro de Estudos Moçambicanos e de Etnociências, Universidade Pedagógica, Maputo, Mozambique. A voucher specimen (23/SM) has been deposited at the herbarium (LMA) of the Instituto de Investigação Agrária de Moçambique (IIAM), Maputo, Mozambique.

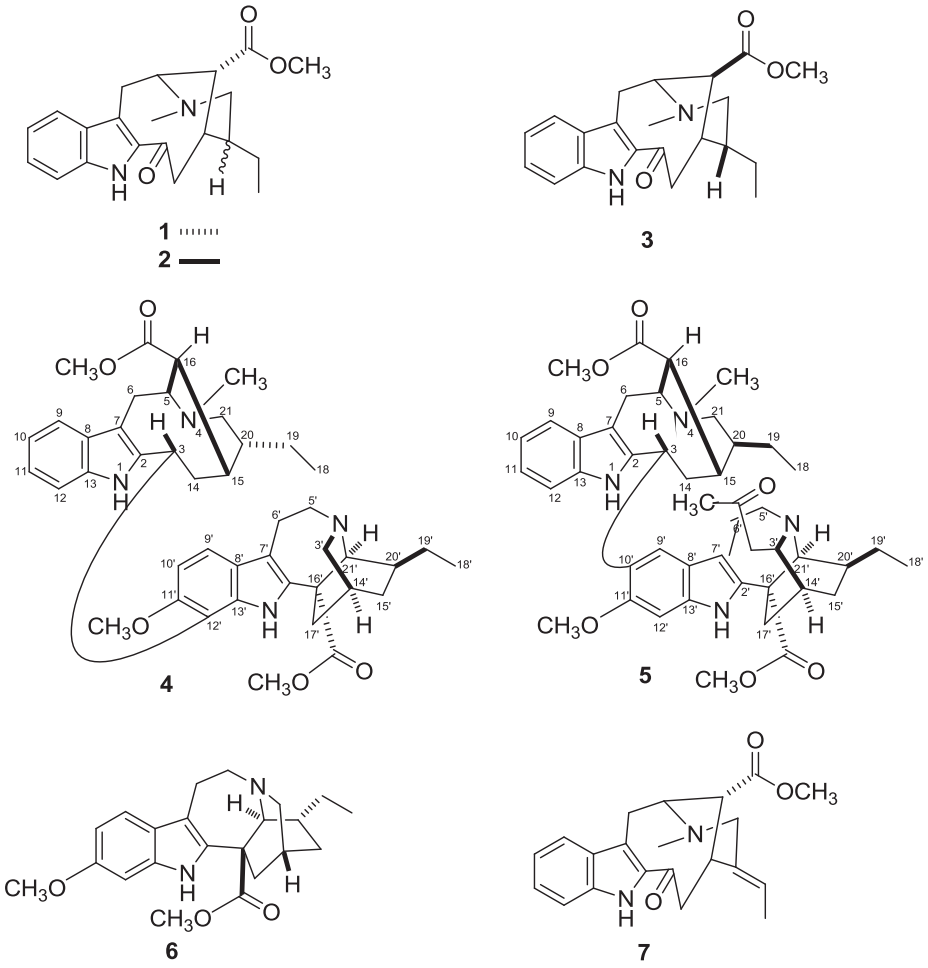

Fig. 1. Chemical structures of tabernaemontanine (**1**), dregamine (**2**), 16-epidregamine (**3**), tabernaelegantine C (**4**), tabernaelegantinine B (**5**), voacangine (**6**) and vobasine (**7**), isolated from *Tabernaemontana elegans*.