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Short communication

Cytotoxic activities of Amaryllidaceae alkaloids against gastrointestinal cancer cells

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ABSTRACT

The treatment of many diseases is highly dependent on natural products and natural products can also be used as design templates for future anticancer drugs. Thirteen Amaryllidaceae alkaloids possessing α-crinane, β-crinane, galantamine, lycorine and tazettine-type skeleton have been isolated in our laboratory, and their cytotoxicity against p53-mutated gastrointestinal cancer cells were evaluated. At the same time, healthy small intestine cells were used to determine overall toxicity against noncancerous cells. In this study, we demonstrated that haemanthamine, haemanthidine and lycorine showed strong cytotoxicity against p53-mutated Caco-2 and HT-29 colorectal adenocarcinoma cells as quantified in terms of IC_{50} values. We for the first time observed approximately 20 times higher IC_{50}values against normal intestine epithelial cells FHs-74 Int after haemanthamine and lycorine treatment when compared with Caco-2 and HT-29 cancer cells. In conclusion, our data indicate that α-C2 bridged haemanthamine may be perspective anticancer drug candidate for further semisynthetic modification and structure-activity relationship study.

1. Introduction

Oncological diseases are one of the leading causes of death in the developed countries and the increase of its prevalence seems to be inevitable. According to World Health Organization's International Agency for Research on Cancer (IARC) in Lyon, France, the incidence of cancer is expected to increase by more than 75% by the year 2030 in developed countries. Since the last decades have witnessed tremendous advances in the understanding and treatment of cancers, oncological patients still die due to resistance of cancer cells to therapy. These resistant cells drive grow of tumors and contribute to the dissemination of malignant cells into vital organs (Mellor and Callaghan, 2008; Havelek et al., 2014). Common mechanisms responsible for cancer cells resistance represent the upregulation of DNA damage sensing and repair capacity of cells (Beskow et al., 2009; Ader et al., 2002) induction of cell cycle arrest allowing time for DNA damage repair (Gogineni et al., 2011; Vávrová et al., 2004) and defects in the apoptosis signalling machinery (Snyder and Morgan, 2004).

Colorectal cancer (CRC) is the third most diagnosed cancer worldwide and has mortality rate of nearly one in every two of patients. Due to the late onset of symptoms, the majority of cases are diagnosed in Duke's stages C or D. Most patients with CRC undergo surgical resection and then commence adjuvant chemotherapy. Standard chemotherapy for CRC covers DNA replication-targeting drugs irinotecan, oxaliplatin and fluorouracil commonly modulated by calcium folinate. Unfortunately survival time in these patients is typically short and quality of life in patients receiving this treatment is generally poor (Weekes et al., 2009).

It is well known that many proteins control cell death machinery. Example includes protein p53, as a transcription factor regulating downstream genes important in cell cycle arrest, DNA damage repair, and cell death. Mutations of tumor suppressor p53 can be frequently observed in malignant colorectal tumors.

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Mutation or deficiency of p53 is thought to reflect tumor aggressiveness, resistance and to be indicative of a lower survival rate. Activation of wild-type (functional) p53 stimulates transcription of proapoptotic molecules and there is also evidence that wild-type p53 can directly enter mitochondria and stimulate cell death. For this detection of mutant (defective) p53 has a negative impact on both disease-free survival and overall survival. Because p53 mutants are highly expressed in cancer cells (in over 50% of human cancers) and not in normal cells, target therapy against p53 deficient cells may eliminate these cancer cells by cell death. Therefore, substantial efforts are being invested into identifying and developing compounds that would be able target resistant forms of tumor cells while not damage healthy cells (Havelek et al., 2014; Ghobrial et al., 2005).

Among various natural sources that have been investigated for constituents with potential use in cancer treatment, plants of the Amaryllidaceae family have been particularly promising and fruitful. These compounds exhibit a diversity of biological activities including antitumor, antiviral, antibacterial, antifungal, antimalarial, acetylcholinesterase (AChE) inhibitory and cytotoxic activities (He et al., 2015). The medical properties of these plants were already known in the fourth century B.C., when Hippocrates of Cos used oil from the daffodil *Narcissus poeticus* L. for the treatment of uterine tumors. Since the isolation of the first alkaloid lycorine from *Narcissus pseudonarcissus* in 1877, more than 500 Amaryllidaceae alkaloids representing 18 skeletal types have been isolated (Dalecká et al., 2013). Among them, galantamine is one of the most therapeutically significant Amaryllidaceae alkaloids, which has been approved as a long acting AChE drug in the treatment of Alzheimer's disease (Evidente and Kornienko, 2009). Currently many Amaryllidaceae alkaloids have been reported to exhibit promising antitumor properties (Dalecká et al., 2013; Evidente and Kornienko, 2009). Of these, the frequently described and cytotoxic active at micromolar concentrations are lycorine (Cao et al., 2013), narciclasine (Dumont et al., 2007) and pancratistatin (Pettit et al., 1986, 1995, 2004; McNulty et al., 2008). In light of this perspective anticancer potential, recent works are designed to investigate whether the effect of Amaryllidaceae alkaloids on viability and cell proliferation are cancer cell-specific. Based on perspective observations when evaluating different Amaryllidaceae skeletal types-alkaloids, the selective cytotoxic effect on tumour cells is currently published for limited amount of congeners and cell types (McLachlan et al., 2005; Dumont et al., 2007; McNulty et al., 2007; Lamoral-Theys et al., 2009; Griffin et al., 2010). However, since the pioneer works in the selection of promising drug candidates indicates their selectivity to diverse types of tumor cells and relatively low toxicity to healthy and quiescent cells, this is a topic in need of further study (Nair et al., 2012; Weniger et al., 1995). In these work, a series of Amaryllidaceae alkaloids was evaluated for their cytotoxic capacity against gastrointestinal p53-mutated Caco-2 and HT-29 cancer cells in comparison to the effect on healthy small intestine epithelial cells FHs 74 Int.

2. Results and discussion

In the current study we screened and determined *in vitro* IC_{50} half-maximal growth inhibitory values of 13 Amaryllidaceae alkaloids against two p53-mutated human gastrointestinal cancer cell lines. The cytotoxic activity was in parallel tested against epithelial-like colorectal adenocarcinoma cells Caco-2 and epithelial colorectal adenocarcinoma cells HT-29 with increasing concentrations up to 100 μM for 72 h and analysed by the MTT assay (Mosmann, 1983). Human normal intestine epithelial cells FHs 74 Int were used as a control for the overall toxicity. All tested alkaloids have been previously isolated in our laboratory from two plant species *Zephyranthes robusta* (Kulhánková et al., 2013; Šafratová et al., 2014) and *Chlidanthus fragrans* (Cahlíková et al., 2013) and belong to five structural types of Amaryllidaceae alkaloids: α-crinane (**1**-**3**), β-crinane (**4**-**7**), galantamine (**8**, **9**), lycorine (**10**-**12**), and tazettine (**13**) (Fig. 1). Vinorelbine, a semisynthetic vinca alkaloid was used as positive controls in our experiments. The IC_{50} values as determined by MTT-based

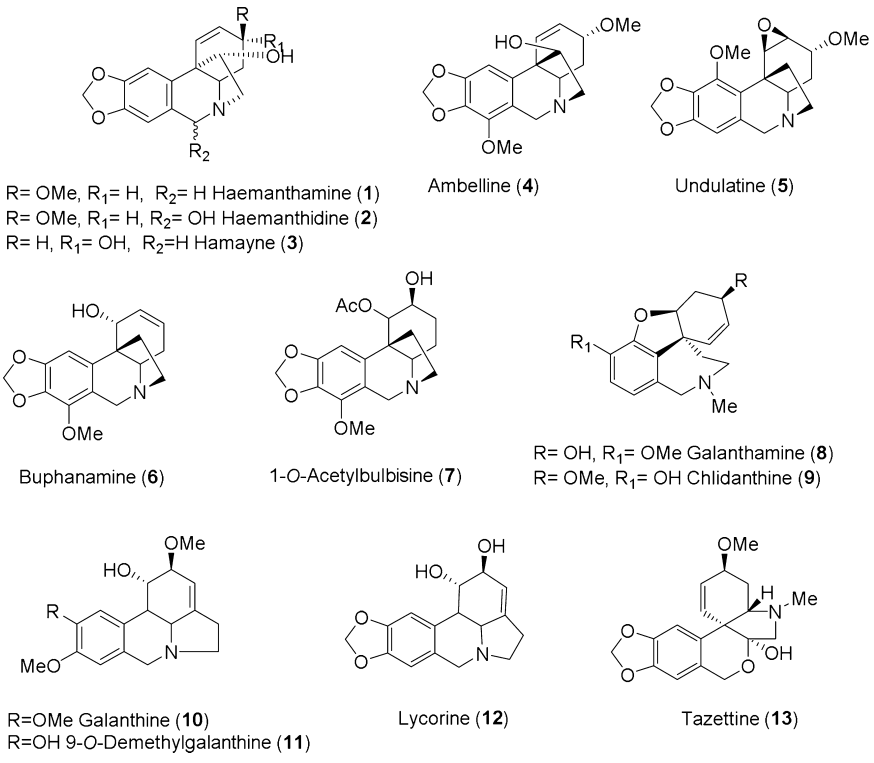

Fig. 1. Chemical structures of assayed Amaryllidaceae alkaloids.