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Cytotoxicity and structure activity relationship studies of maplexins A–I, gallotannins from red maple (*Acer rubrum*)

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ABSTRACT

Maplexins A–I are a series of structurally related gallotannins recently isolated from the red maple (*Acer rubrum*) species. They differ in number and location of galloyl derivatives attached to 1,5-anhydro-glucitol. Here, maplexins A–I were evaluated for anticancer effects against human tumorigenic (colon, HCT-116; breast, MCF-7) and non-tumorigenic (colon, CCD-18Co) cell lines. The maplexins which contained two (maplexins C–D) or three (maplexins E–I) galloyl derivatives each, inhibited cancer cell growth while those with only one galloyl group (maplexins A-B) did not. Moreover, maplexins C–D showed greater antiproliferative effects than maplexins E–I (IC_{50} = 59.8–67.9 and 95.5–108.5 μM vs. 73.7–165.2 and 115.5–182.5 μM against HCT-116 and MCF-7 cells, respectively). Notably, the cancer cells were up to 2.5-fold more sensitive to the maplexins than the normal cells. In further mechanistic studies, maplexins C–D (at 75 μM concentrations) induced apoptosis and arrested cell cycle (in the S-phase) of the cancer cells. These results suggest that the number of galloyl groups attached to the 1,5-anhydro-glucitol moiety in these gallotannins are important for antiproliferative activity. Also, this is the first *in vitro* anticancer study of maplexins.

1. Introduction

Cancer is the second leading cause of deaths in developed countries. Breast cancer is the leading cause of cancer related deaths among females, while colon cancer is the third most common cancer for both sexes, and their incidence continues to rise every year (DeSantis et al., 2011; Jemal et al., 2011). Extensive research has focused on the investigation of plant natural products, in particular polyphenols, for their anticancer potential including colon cancer chemoprevention (Rudolf et al., 2007). Furthermore, polyphenolic-enriched plant extracts, as well as their purified constituents, have attracted significant research attention as potential cancer chemopreventive agents (Hadi et al., 2000; Singh et al., 2003).

Tannins constitute a large sub-class of polyphenols which have been implicated with numerous biological properties including anticarcinogenic effects (Nepka et al., 1999; Crozier et al., 2009). Gallotannins are hydrolyzable tannins composed of a glucose core esterified to gallic acid residues. While gallotannins are common in the plant kingdom, those containing a 1,5-anhydro-glucitol moiety have only been previously isolated from members of the maple (*Acer*) genus (Hatano et al., 1990; Honma et al., 2010, 2011; Ogawa et al., 2011; Wan et al., 2011; Yuan et al., 2011a). Notably, these gallotannins have been extensively investigated for their antidiabetic properties in both *in vitro* and animal studies but knowledge of their anticancer effects is scarce.

The red maple (*A. rubrum* L.) species is native to eastern North America and has been used for medicinal purposes by Native Americans (Arnason et al., 1981). Along with the sugar maple (*Acer saccharum* Marsh) species, this plant is well known for yielding maple syrup, a natural sweetener which is obtained by concentrating the tree sap. While maple syrup has been previously investigated by our group and others for its phytochemical constituents (Li and Seeram, 2011, and references cited therein), there is paucity of data on compounds present in other parts of these plants. Recently, various plant part extracts of the red maple species have been investigated for their antioxidant effects (Royer et al., 2011) but the active compounds were not identified.

Our laboratory is involved in a comprehensive program of phytochemical and biological studies of maple syrup and the maple species primarily used for its production, namely the sugar and red maple species (Li and Seeram, 2010, 2011; Apostolidis et al., 2011; Yuan et al., 2011a,b; Wan et al., 2011; González-Sarrías et al., 2011a,b). To that end, we had previously investigated the cytotoxic effects of various plant part extracts of both maple species against a panel of human colon cancer and normal cells (González-Sarrías et al., 2011a). While these data suggested that

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gallotannins, namely the previously reported compounds, ginnalins A, B, and C, were indeed among the active compounds, we did not comprehensively identify the cytotoxic constituents in that initial study (González-Sarrías et al., 2011a).

Maplexins A–I are a series of nine new gallotannins recently isolated by our group from the stems and bark of the red maple species (Yuan et al., 2011a; Wan et al., 2011). These gallotannins contain one, two, or three galloyl derivatives attached to different positions of a 1,5-anhydro-glucitol core (Fig. 1). Interestingly, the maplexins differ remarkably in their ability to inhibit the activity of the α-glucosidase enzyme (relevant to type II diabetes management) which was dependent on their number of galloyl groups (Yuan et al., 2011a; Wan et al., 2011). Given our previous observations of the cytotoxic effects of maple plant part extracts (González-Sarrías et al., 2011a), and since maplexins are yet to be investigated for such effects, we initiated the current study.

The objectives of this research project were to: (1) evaluate maplexins A–I for cytotoxic effects against human colon and breast tumorigenic cells (HCT-116 and MCF-7, respectively) as well as a non-tumorigenic colon cell line (CCD-18Co), (2) probe whether the anticancer effects were mediated through cell cycle arrest and/or apoptosis and, (3) conduct structure activity relationship (SAR) studies of the maplexins with regard to their observed anticancer properties.

2. Material and methods

2.1. Maplexins A–I

Maplexins A–I are new gallotannins which were recently isolated by our laboratory from the stems and bark of the red maple (*A. rubrum*) species (Yuan et al., 2011a; Wan et al., 2011). Briefly a combination of chromatographic, ^{1}H- and ^{13}C-nuclear magnetic resonance, and mass spectroscopic methods were used for the isolation and identification of the pure compounds. The chemical structures of the maplexins are shown in Fig. 1 and their molecular weights are as follows: maplexins A and B = 316 g/moL; maplexins C and D = 468 g/moL; maplexins E and F = 620 g/moL; maplexins G and I = 634 g/moL; maplexin H = 618 g/moL.

2.2. Cell lines and culture conditions

Cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, USA) and cultured as recommended by the ATCC. Human colon carcinoma cells (HCT-116) were grown in McCoy's 5A medium supplemented with 10% v/v fetal bovine serum, 1% v/v nonessential amino acids, 2% v/v HEPES and 1% v/v antibiotic solution. Human breast cancer cells (MCF-7) were grown in Eagle's minimal essential medium (EMEM) containing 10% v/v fetal bovine serum, 1% v/v nonessential amino acids, 2% v/v HEPES, 1 mM sodium pyruvate and 1% v/v antibiotic solution. The normal colon cells (CCD-18Co) were grown in EMEM medium supplemented with 10% v/v fetal bovine serum, 1 mM sodium pyruvate, 1% v/v nonessential amino acids, 1% v/v L-glutamine and 1% v/v antibiotic solution. All cells were used from a population doubling level of 26–35 for all experiments. Cells were maintained at 37 °C in an incubator under a 5% CO_2/95% air atmosphere at constant humidity. The pH of the culture medium was determined using pH indicator paper (pHydrion™ Brilliant, pH 5.5–9.0, Micro Essential Laboratory, NY, USA) inside the incubator. Cells were counted using a hemacytometer and were plated at

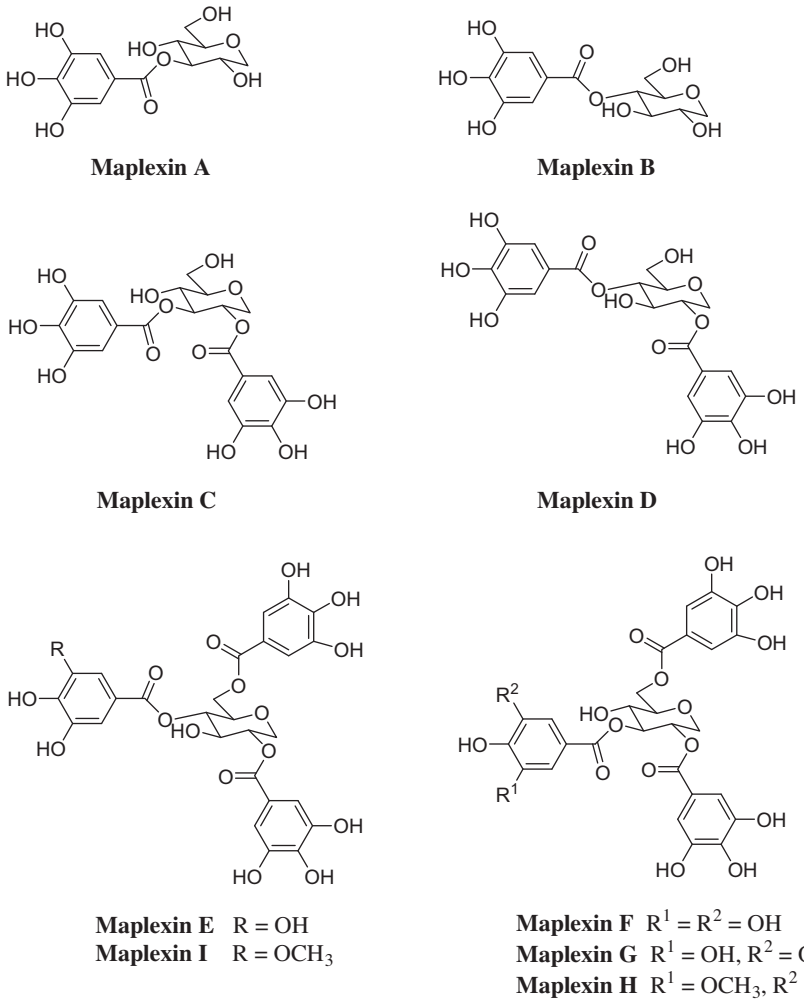

Fig. 1. Chemical structures of maplexins A–I isolated from red maple (*Acer rubrum*) species. The molecular weights of the compounds are as follows: maplexins A and B = 316 g/moL; maplexins C and D = 468 g/moL; maplexins E and F = 620 g/moL; maplexins G and I = 634 g/moL; maplexin H = 618 g/moL.