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Synthesis and structure–activity relationship studies on tryprostatin A, an inhibitor of breast cancer resistance protein

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Abstract—Tryprostatin A is an inhibitor of breast cancer resistance protein, consequently a series of structure–activity studies on the cell cycle inhibitory effects of tryprostatin A analogues as potential antitumor antimitotic agents have been carried out. These analogues were assayed for their growth inhibition properties and their ability to perturb the cell cycle in tsFT210 cells. SAR studies resulted in the identification of the essential structural features required for cytotoxic activity. The absolute configuration L-Tyr-L-pro in the diketopiperazine ring along with the presence of the 6-methoxy substituent on the indole moiety of **1** was shown to be essential for dual inhibition of topoisomerase II and tubulin polymerization. Biological evaluation also indicated the presence of the 2-isoprenyl moiety on the indole scaffold of **1** was essential for potent inhibition of cell proliferation. Substitution of the indole N_a–H in **1** with various alkyl or aryl groups, incorporation of various L-amino acids into the diketopiperazine ring in place of L-proline, and substitution of the 6-methoxy group in **1** with other functionality provided active analogues. The nature of the substituents present on the indole N_a–H or the indole C-2 position influenced the mechanism of action of these analogues. Analogues **68** (IC_{50} = 10 μM) and **67** (IC_{50} = 19 μM) were 7-fold and 3.5-fold more potent, respectively, than **1** (IC_{50} = 68 μM) in the inhibition of the growth of tsFT210 cells. Diastereomer-2 of tryprostatin B **8** was a potent inhibitor of the growth of three human carcinoma cell lines: H520 (IC_{50} = 11.9 μM), MCF-7 (IC_{50} = 17.0 μM) and PC-3 (IC_{50} = 11.1 μM) and was equipotent with etoposide, a clinically used anticancer agent. Isothiocyanate analogue **71** and 6-azido analogue **72** were as potent as **1** in the tsFT210 cell proliferation and may be useful tools in labeling BCRP.

1. Introduction

The cell cycle coordinates a variety of cellular functions involved in the accurate replication of the genome and cell division.[1] These processes are tightly regulated primarily at the G_1/S and G_2/M phase transitions by a series of checkpoints. It has become clear that checkpoint control defects in cancer cells contribute to tumorigenesis and are a significant reason for the increased selectivity of tumors over normal cells towards chemotherapy.[2,3] Cell cycle inhibitors or modulators are highly promising new therapeutic agents against human cancers.

With an increased understanding of the molecular biology of cell cycle control it has become possible to develop bioassays and screen for agents that specifically interfere with these processes. One such method was developed by Osada et al.[4,5] which utilizes the synchronous culture of the murine temperature-sensitive mutant cell line, tsFT210, defective in the $p34^{cdc2}$ gene. With this assay a family of 2-isoprenylated diketopiperazine indole alkaloids which effect cell cycle arrest at the G2/M phase was isolated from the fermentation broth of a marine fungal strain of *Aspergillus fumigatus* BM939. It was found that tryprostatin A **1** (Chart 1) and tryprostatin B **2** (Chart 1) completely inhibited cell cycle

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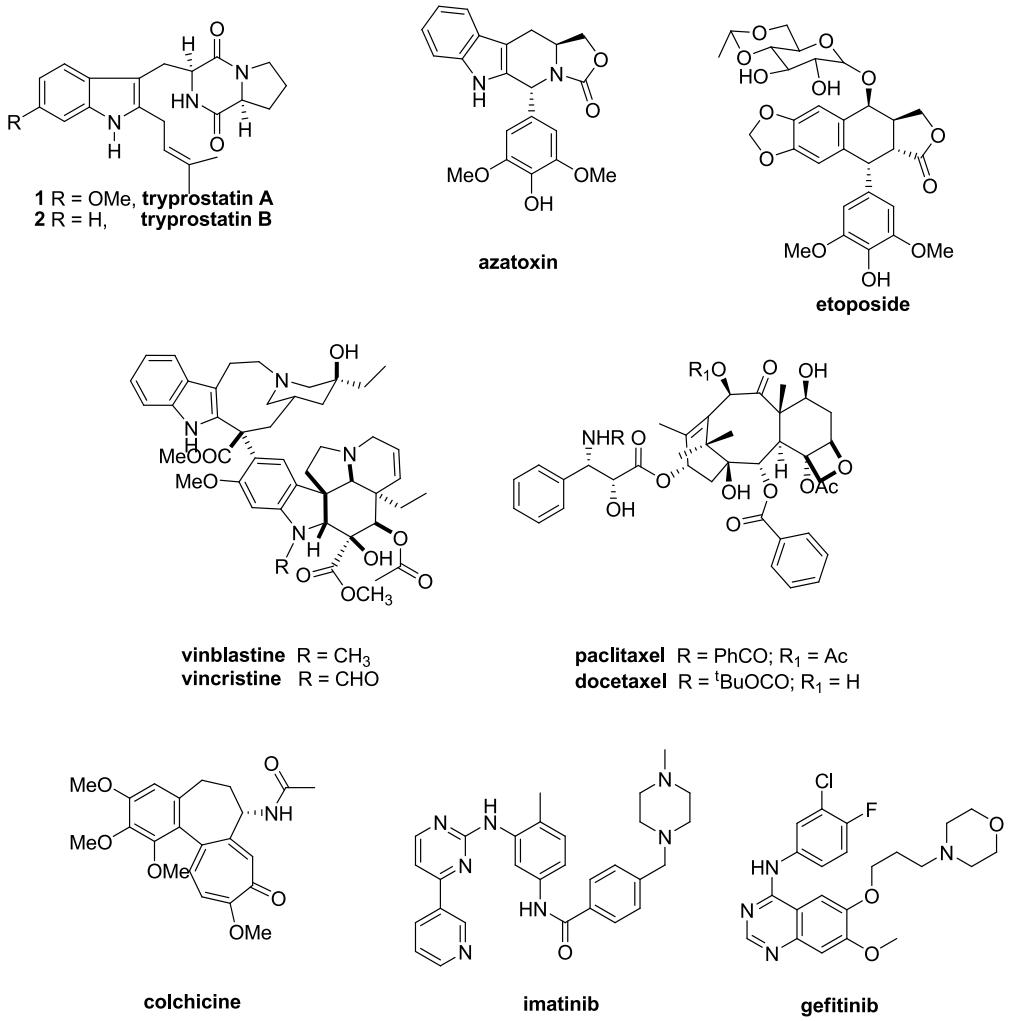

Chart 1.

progression of tsFT210 cells in the G2/M phase at a final concentration of 50 μg/mL of **1** and 12.5 μg/mL of **2**, respectively.[6–8] Since these indole alkaloids were isolated only in small amounts, studies on the mechanism and SAR were not reported earlier.

Tryprostatin A and B have previously been synthesized,[9–11] the aim of which was to study their mechanism of action. The similarities in the structures of the tryprostatins with etoposide (Chart 1) and azatoxin (Chart 1), a dual inhibitor of topoisomerase II (G2)/tubulin polymerization (M), led to the investigation of the ability of the two tryprostatins to inhibit topoisomerase II and tubulin polymerization. Biological evaluations[12] of **1** and **2** indicated that both alkaloids were very weak inhibitors of topoisomerase II in the topoisomerase II assay; while only **1** had marginal activity in the tubulin polymerization assay. This latter result was in agreement with the data reported for **1** by Osada et al.[13] Osada et al.[13] also reported **1** inhibited cell cycle progression of rat normal fibroblast 3Y1 cells specifically in the M phase. The concentration of **1** that arrested cell cycle progression in the M phase corresponded to that which induced a marked depolymerization *in situ* of the microtubules containing both cytoplasmic network and spindle apparatus. Although tryprostatin B **2** arrested cell cycle progression at a lower concentration than **1**, the inhibition was not due to inhibition of the M phase. It was shown that **1** inhibited microtubule assembly through a different type of mechanism than colchicine (Chart 1), vinblastine (Chart 1), or maytansine–rhizoxin.[13] Tryprostatin A **1** inhibited microtubule assembly by interfering with the interaction between microtubule-associated proteins (MAPs) and the C-terminal domain of tubulin. Since **1** operated by an entirely novel mechanism this may be important in cancer chemotherapy, especially in multiple drug resistance (MDR) cancers.

Microtubules are hollow cylindrical tubes found in almost all eukaryotic cell types. They play an important role in a variety of cellular functions, such as cell division, cell movement, cell shape, and transport of organelles inside the cell.[14] Tubulin exists as a heterodimer of α- and β-tubulin and is the major building block of microtubules. Proteins such as the MAPs bind to and modify microtubule properties.[14,15] In the absence of MAPs, α/β-tubulin heterodimers polymerize only by treatment with high concentrations of glycerol or organic acids such as glutamate.[16]