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# Raman micro-spectroscopic analysis of cultured HCT116 colon cancer cells in the presence of roscovitine

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ABSTRACT

Raman micro-spectroscopic analysis of cultured HCT116 colon cancer cells in the presence of roscovitine, [seliciclib, 2-(1-ethyl-2-hydroxy-ethylamino)-6-benzylamino-9-isopropylpurine], a promising drug candidate in cancer therapy, has been performed for the first time. The aim of this study was to investigate modulations in colon cancer cells induced by roscovitine. Raman spectra of the cultured HCT116 colon cancer cells treated with roscovitine at different concentrations (0, 5, 10, 25 and 50 μM) were recorded in the range 400–1850 $cm^{-1}$. It was shown that the second derivative profile of the experimental spectrum gives valuable information about the wavenumbers and band widths of the vibrational modes of cell components, and it eliminates the appearance of false peaks arising from incorrect baseline corrections. In samples containing roscovitine, significant spectral changes were observed in the intensities of characteristic protein and DNA bands, which indicate roscovitine-induced apoptosis. Roscovitine-induced apoptosis was also assessed by flow cytometry analysis, and analysis of propidium iodide staining. We observed some modifications in amide I and III bands, which arise from alterations in the secondary structure of cell proteins caused by the presence of roscovitine.



## 1. Introduction

Raman spectroscopy is a well-established analytical method that can provide valuable information about biological materials [1,2]. Alterations in cell behaviour involve changes in cellular biochemistry that are reflected in the Raman spectra. Several investigations have shown that Raman spectroscopy can successfully differentiate dead cells from live cells [3–7]. Furthermore, Raman spectra provide information on molecular interactions within a cell, allowing for the investigation of metabolic changes involved in specific cell–drug interactions. Recently, a Raman spectroscopic investigation of the interaction of malignant hepatocytes with the antitumor drug doxorubicin has been reported [8], and it was concluded that doxorubicin affected the secondary structure and the environment of the proteins. The effects of the anticancer drug 5-fluorouracil on gastric carcinoma cells were investigated by Yao et al., and reductions in the intensity of vibrational bands generated by cellular lipids, proteins and, nucleic acids as a result of apoptosis were observed [9].

Roscovitine (CYC202) (Seliciclib, $C_{19}H_{26}N_6O$; 2-(1-ethyl-2-hydroxy-ethylamino)-6-benzylamino-9-isopropylpurine) is a cyclin-dependent kinase (CDK) inhibitor that competes for the ATP binding site on the kinase [10]. Thus, it is a promising drug candidate in cancer therapy which acts by blocking cell cycle transitions in cancerous cells by CDK1, and CDK2 inhibitions [10–12]. MacCallum et al. reported the mechanism of cell death in multiple myeloma cells treated with roscovitine [13]. It has been shown that roscovitine inhibits the kinases, which phosphorylate the carboxyl-terminal domain of RNA polymerase II, resulting in inhibition of transcription, down-regulation of the levels of Mcl-1 mRNA and protein, and rapid induction of apoptosis [13]. Moreover, previous studies also showed that roscovitine is a successful apoptotic inducer in various cell lines [14,15]. To the best of our knowledge no Raman spectroscopic study has been reported of effects in cell lines induced by roscovitine. In our previous study the conformational preferences, and theoretical and experimental vibrational spectra of roscovitine, were investigated [16]. In this study, Raman spectra of cultured HCT116 colon cancer cells containing different roscovitine concentrations (0, 5, 10, 25 and 50 μM) are recorded for the first time in the range of 400–1850 $cm^{-1}$. The aim of this study was to investigate roscovitine-induced modulations in colon cancer cells.

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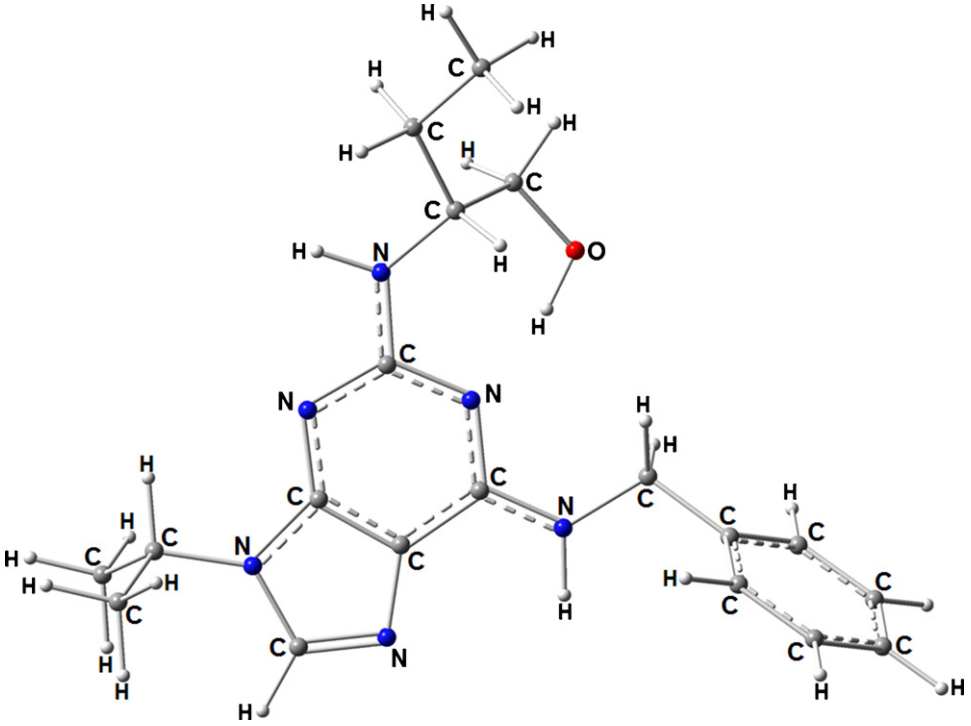

**Fig. 1.** The molecular model of roscovitine ($C_{19}H_{26}N_6O$).

## 2. Materials and methods

Roscovitine was purchased from Calbiochem (Cat No. 557360). Solutions in DMSO of different concentrations were prepared and aliquots were kept at −20 °C. HCT116 colon carcinoma cells were obtained from American cell collections (ATCC, LGCpromochem, UK). Cells were seeded overnight in 6 well plates ($5 \times 10^5$ cells/well). After attachment, HCT116 colon carcinoma cells were treated with roscovitine (0, 5, 10, 25 and 50 μM) for 24 h. As mentioned in Guo et al. [8] and Yao et al. [9], HCT116 colon carcinoma cells were carefully trypsinized and then solubilized in PBS, a friendly buffer system for cells. Following trypsinization, a fresh medium was added to finish trypsin activity, and samples were centrifuged for 5 min at 1200 rpm.

The effect of roscovitine on cell cycle distribution was determined by flow cytometric (FACS flow) analysis following propidium iodide staining. $1 \times 10^5$ MCF-7 cells were seeded into 6-well plates. Following drug treatment (a moderate cytotoxic dosage 20 μM), cells were fixed with ice cold 95% ethanol. Cells then were incubated with propidium iodide staining solution (50 μg/ml PI, 10 mM Tris pH 7.5, 5 mM $MgCl_2$, 10 μg/ml RNase) for 15 min, and analyzed using FACS flow (BD Biosciences). Moreover, in order to evaluate the roscovitine-induced apoptotic cell death, cells were stained with propidium iodide for 15 min following drug treatment (0–100 μM) for 24 h. Fluorescence microscopy observations were utilized in Olympus microscopy (IX71, Japan).

Cells were dispersed in 1× PBS prior to Raman spectroscopic analysis. A 50 μl sample drop was placed onto an alumina support for Raman analysis. The spectra of the alumina substrate and PBS were subsequently subtracted.

Raman spectra were recorded using a Jasco NRS 3100 Raman micro-spectrometer (1200 lines/mm grating, and high sensitivity cooled CCD) equipped with a 532 nm diode laser. The spectrometer was calibrated by using both the silicon phonon mode at 520 $cm^{-1}$ and toluene vibrational wavenumbers. A 20× microscope objective (Olympus) was used to focus the laser and collect Raman scattering of the sample. The laser power during signal acquisition was 42.5 mW, and 50 spectra were accumulated. Spectral resolution was 3.9 $cm^{-1}$. The reproducibility of data for the samples used has been examined carefully to avoid photodecomposition caused by the laser beam. Therefore, three different positions per sample were analyzed and, for each concentration, experiments were repeated three times with freshly prepared samples. All the analyzed spectra were obtained by averaging 9 individual spectra that were treated in exactly the same way.

Spectral manipulations, such as baseline adjustment, band fitting, and obtaining the second derivative, were performed using the GRAMS/AI 7.02 (Thermo Electron Corporation) software package. The area under the individual Raman bands of interest was calculated using the band fitting results. Band fitting was done using a Gaussian function, and fitting was undertaken until reproducible and converged results were obtained with squared correlations better than $r^2 \sim 0.9998$. The second derivatives of the spectra were obtained by using the Savitzky-Golay function (two polynomial degrees, 19 points).

## 3. Results and discussion

The chemical structure of roscovitine is given in Fig. 1. In order to evaluate the cell cycle distribution following roscovitine treatment at a moderate cytotoxic concentration (20 μM), HCT116 cells were analyzed by flow cytometry (FACS flow) analysis following propidium iodide staining. In Fig. 2 we showed the alteration of cell cycle distribution following drug treatment in HCT116 colon cancer cells (Fig. 2a–c). Roscovitine increased Gsub/G1 cell population compared to control. This data verifies that roscovitine induced apoptosis in HCT116 colon cancer cells [17]. In addition, roscovitine treatment caused an arrest at G0/G1 and therefore G2/M population was decreased following drug treatment. In order to confirm that roscovitine induced cell death, $1 \times 10^5$ cells were treated with roscovitine at various concentrations (0–100 μM). Then cells were stained with propidium iodide for 15 min. Fluorescence microscopy