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1. Introduction

An important tool in cancer chemotherapy is quantitative monitoring of the response of tumor to treatment. Currently, tumor response is monitored by detection of changes in tumor morphology, mostly its size. There is a potential in using Magnetic Resonance Imaging (MRI) for monitoring of the therapy as MRI detects an early response to treatment at morphological level. Therefore, we used MRI to investigate the visualization of human tumor cultured in three-dimensional (3-D) form treated with thiocolchicine derivatives by monitoring cell changes.

Inhibitory effects of cancer cells growth are principal targets in the development of novel therapeutics. Among cellular structures, required to maintain the growth of normal and malignant cells, the microtubules play a pivotal role as the template for transcription and transactivation of cells. Drugs binding to tubulin are known to disrupt the dynamic of cancer cells growth. Advances in synthetic methods, coupled with continued effort aimed at identification of cell surface receptors contribute to the growing library of small-molecule inhibitors of cancer cell growth. Well-known examples include paclitaxel [1], an antimitotic agent known to stabilize microtubules by binding to β-tubulin [2] and colchicine [3], an antimitotic agent that binds to a different site of β-tubulin and inhibits its assembly into microtubules. Colchicine, a major alkaloid of *Colchicum autumnale* and *Gloriosa superba*, is a drug interfering with microtubule structure both *in vitro* and *in vivo*, thereby causing cells to accumulate in mitotic arrest during the cell cycle [4]. Although the antitumor properties of many colchicinoids have been well-known [5], only one compound, N-deacetyl-N-methylcolchicine (Colcemid), has been used for the treatment of Hodgkin's lymphoma and chronic granulocitic leukemia [6]. Analogues of colchicine are currently used in therapy of Mediterranean fever [7], Behcet's disease [8], progressive scleroderma [9] and gout [10].

The main aim of the present study was to determine the impact of structural properties of selected thiocolchicine derivatives on CEM cells' growth. We applied Hollow Fiber Bioreactor (HFB) to grow 3-D culture and produce high density cell culture suitable for MRI study. For the visualization of the interactions between thiocolchicine derivatives and cells we used ^{1}H and ^{19}F MRI as well as ^{19}F Magnetic Resonance Spectroscopy (MRS). Our studies were aimed at the measurements of the effects of the drug action in cell culture *ex vivo*.

2. Materials and methods

2.1. Compounds and reagents

Colchicine (**1**), N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[α]heptalen-7-yl]acetamide, and other compounds used to synthesis and cell culture were purchased from

Sigma–Aldrich (Oakville, ON). Major histocompatibility complex (MHC) class I antibody was purchased from Abcam Inc. (Cambridge, MA).

2.2. Synthesis of the compounds

2.2.1. Preparation of thiocolchicine, N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydrobenzo[α]heptalen-7-yl]acetamide (2)

Colchicine **(1)** (1 mmol) was dissolved in 10 mL of methanol/dimethylformamide (1:1) at 70–80 °C. The solution was cooled to room temperature and sodium methanethiolate (2 mmol) was added. The mixture solution was stirred overnight. Water (20 mL) was added, and the reaction mixture was extracted with CH_2Cl_2 (10 mL), dried over Na_2SO_4 and concentrated. Crystallization of the residue from ethyl ether/acetone (1:1) gave product **(2)** with 71% yield.

2.2.2. Preparation of N-[(7S)-3-hydroxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydrobenzo[α]heptalen-7-yl]acetamide (3a)

Ten millimoles of methanol was used to dissolve 1 mmol of thiocolchicine **(2)** and 30 mL of 0.2 M of hydrochloric acid was added. The methanol was evaporated, cooled and sodium hydroxide solution was added until pH value was 11. The resulting alkaline solution was extracted with chloroform in order to free it from non-phenolic substances. The sodium hydroxide solution, (color red), was acidified with hydrochloric acid and was extracted with chloroform. After drying and evaporation, the yield of **(3a)** was 58%.

2.2.3. Preparation of N-[(7S)-1,2-dimethoxy-10-methylsulfanyl-9-oxo-3-(prop(2-en)oxy)-5,6,7,9-tetrahydrobenzo[α]heptalen-7-yl]acetamide (3b) and N-[(7S)-3-ethoxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydrobenzo[α]heptalen-7-yl]acetamide (3c)

One millimole of **(3a)** compound was dissolved in 2.5 mL of 1 M sodium hydroxide solution. The resulting solution was cooled to 0 °C and 3-bromoprop-1-ene (1 mmol) for obtained compound **(3b)** or 1-bromoethane (1 mmol) for obtained **(3c)** was dissolved in 3.5 mL acetone and added thereto. The solution was allowed to stand for 15 h and then 25 mL of alkaline water was added. Chloroform was used to extract the resulting product and drying over magnesium sulfate. The yield of **(3b)** was 68% and **(3c)** was 71%.

2.2.4. Preparation of the N-deacetyl-N-(N-trifluoroacetylaminoacyl) thiocolchicine

N-[(7S)-3-hydroxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydrobenzo[α]heptalen-7-yl]amine **(4a)**;

N-[(7S)-1,2-dimethoxy-10-methylsulfanyl-9-oxo-3-(prop(2-en)oxy)-5,6,7,9-tetrahydrobenzo[α]heptalen-7-yl]amine **(4b)**;

N-[(7S)-3-ethoxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydrobenzo[α]heptalen-7-yl]amine **(4c)**;

N-[(7S)-3-hydroxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydrobenzo[α]heptalen-7-yl]-N-[(trifluoroacetyl)glycyl]acetamide **(5a)**;

N-[(7S)-1,2-dimethoxy-10-methylsulfanyl-9-oxo-3-(prop-2-enoxy)-5,6,7,9-tetrahydrobenzo[α]heptalen-7-yl]-N-[(trifluoroacetyl)glycyl]acetamide **(5b)**;

N-[(7S)-3-ethoxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydrobenzo[α]heptalen-7-yl]-N-[(trifluoroacetyl)glycyl]acetamide **(5c)**.

One millimole of appropriate derivative **(3a–c)** was dissolved in methanol (20 mL) with 2 M HCl (10 mL), heated at 90 °C and stirred for 24 h. The reaction mixture was cooled, neutralized with $NaHCO_3$ and extracted with CH_2Cl_2. The obtained extract was dried over Na_2SO_4 and evaporated. The crystallization was from (1:1) CH_2Cl_2/CH_3OH. The yield of deacetylated compound **(4a–c)** was 58%, 63% and 71%, respectively.

One millimole of deacetylated compound of **(4a–c)** and N-trifluoroacetylglycine (1 mmol) were dissolved at room temperature and dichloromethane (6 mL) was added with stirring. N-trifluoroacetylglycine prepared accordingly to previously published reference [11]. Dicyclohexylcarbodiimide (1 mmol) was added to the suspension. After 2 h the mixture was cooled to 0 °C and filtrated. Each compound **(5a–c)** was crystallized from dichloromethane: ethyl ether (1:1). The yield of **(5a–c)** was 64%, 67% and 75%, respectively.

2.3. Cell cultures

CEM cells, American Type Culture Collection (Rockville, MD), were maintained in tissue culture flasks and cultured as monolayer in 20 mL of RPMI media containing 10% Fetal Bovine Serum (FBS). The cells were divided from 5×10^5 cells/mL to 2.5×10^4 cells/mL two times per week. When the number of cells in the culture flask reached $5–6 \times 10^6$ cells/mL the culture was harvested and then inoculated into six Hollow Fiber Bioreactors (HFB, FiberCells System Inc., Frederick, MD) and then continuously cultured in 37 °C and 5% CO_2. The HFB consists of a single, hydrophylic and polysulfone fiber with 0.1 μm diameter pores. The media circulate within the HFB cartridge and polysulphone tubing, at a flow rate of 14 mL/min, bringing oxygen and nutrients to cells and removing CO_2 and other waste. We used collagen solution to create an extracellular matrix between cells and fiber. The polysulfone fiber was coated with protein by flushing with 10 mL of coating solution containing 1 mg collagen per 1 mL Phosphate Buffered Saline (PBS). In this manner CEM cells growing originally in suspension build up a 3-D solid tumor. During 4 weeks of culturing, the media were replaced each week.

2.4. Preparation of media with colchicine derivatives (3a–c) and 4(a–c)

The colchicine derivatives treated media were prepared using 1 nM, 10 nM, 20 nM, 100 nM, 500 nM and 1000 nM of **(3a–c)** and **(4a–c)** placed in a 1.5 mL glass vial and dissolved in 10 μL of dimethyl sulfoxide. Dimethyl sulfoxide is a solvent of the **(3a–c)** and **(4a–c)** derivatives. Once dissolved, the dimethyl sulfoxid/**(3a–c)** mixture or dimethyl sulfoxid/**(4a–c)** was added to media and incubated overnight in 37 °C.

2.5. Preparation of media with colchicine derivatives (5a–c)

The media were supplemented with 1 nM, 10 nM, 20 nM, 100 nM, 500 nM and 1000 nM of **(5a–c)** derivatives dissolved in 10 µL of dimethyl sulfoxide. We observed that **(5a–c)** derivatives were dissolved in media solution. However, we used dimethyl sulfoxide regime for the same media condition as for **(3a–c)** and **(4a–c)** derivatives.

2.6. Treatment of cells

To establish IC_{50}, 4×10^4 CEM cells/mL were treated on culture plates and placed for the 72 h incubation with **(3a–c)**, **(4a–c)** and **(5a–c)** derivatives. After 72 h, the growth was inhibited more than 50% for 20 nM of **(3a)**, 10 nM of **(3b)** and **(3c)**, 5 nM of **(5a)**, **(5b)** and **(5c)**. Compounds **(4a–c)** were less active and we did not perform studies with **(4a–c)** in HFB device. Six synthesized compounds **(3a–c)** and **(5a–c)** were tested in HFB device. Therefore, we selected these compounds for 10^9 CEM cells/mL concentrations for cells' treatment in HFB, after 4 weeks in culture.

2.7. Viability

The number of cells was determined manually with a hemacytometer chamber (Hausser Scientific, Horsham, PA) using Trypan blue (Sigma–Aldrich, Oakville, ON) exclusion method [12].

2.8. Cell preparation for ^{1}H and ^{19}F Magnetic Resonance experiments

^{1}H and ^{19}F MRI measurements of cells in the HFB were performed in control (n = 2, HFB) and treated cells (n = 6, HFB) using 1 nM, 5 nM, 10 nM, 20 nM, 100 nM, 500 nM and 1000 nM of **(3a)**, **(3b)**, **(3c)**, **(5a)**, **(5b)** and **(5c)** derivatives, respectively. Throughout the MRI experiments, the HFBs were maintained under incubator-like conditions (37 °C, 5% CO_2 and 95% air). All MR images were collected with 9.4 T (T)/21 cm magnets (Magnex, UK) and TMX console (NRC-IBD, Canada). The HFBs with cell cultures were placed in double tuned transmit/receive radio frequency (RF) volume coil operating at 376 MHz and 400 MHz corresponding to ^{19}F and ^{1}H Larmour frequency at 9.4 T, respectively. All ^{1}H and ^{19}F imaging parameters were the same for each HFB treated with **(3a)**, **(3b)**, **(3c)**, **(5a)**, **(5b)** and **(5c)** derivatives, respectively. The MRI experiments were performed after 72 h of cell treatment in HFB device. For ^{1}H MR imaging, a spin-echo pulse sequence was used with Echo Time (TE)/Repetition Time (TR) = 16.5/5000 ms. For ^{19}F MR imaging, Inversion Recovery (IR) spin-echo method with Inversion Time (IT) equal to 400 ms and TE/TR = 16.5/5000 ms were used. A single slice of 1 mm thickness was acquired with matrix size of 256 × 256 and field of view 3 cm × 3 cm.

^{19}F spectra of pure **(5a–c)** solved in media were acquired using a one-pulse sequence (flip angle 60°; Repetition Time (TR) = 800 ms; number of average 2; Echo Time (TE) = 6 ms). The concentration of each fluorinated compound in media was the same as the one used to treat the cell culture. Moreover, ^{19}F MRS of culture cells treated with **(5a–c)** was performed using single pulse measurements with parameters used to pure **(5a–c)** compounds. Chemical shifts were referenced to an external standard containing 1 µM CF_3COOH.

2.9. High Performance Liquid Chromatography-Ultra Violet (HPLC-UV) analysis

Digested cell samples were fractionated with Gold HPLC chromatograph system equipped with Gold 166 Ultra Violet (UV) Detector and 32-Karat software (Beckman-Coulter, Mississauga, ON). For reversed-phase HPLC, a Vydac 218 TP54 Protein & Peptide C18 analytical column, 300 Å pore size, 0.46 cm × 25 cm (Separation Group, Hesperia, CA) was used. The chromatograph was equipped with a Rheodyne injector (5 μL). UV detection was performed at 245 nm. Eluent A consisted of 5% acetonitrile (ACN) water solution and eluent B of 0.01% trifluoroacetic acid in 95% ACN water solution. A linear gradient from 5% to 70% ACN was applied over 60 min.

2.10. Antibody targeting of MHC (class I) receptor

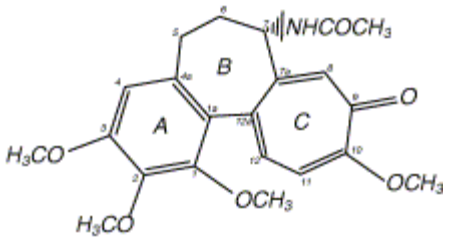
The stock solution of 0.2 mg/mL antibodies in PBS pH 7.2 with 10 mg/mL bovine serum albumin (BSA) was used to treat 10^9 cell/mL.

2.11. Statistical analysis

All results were expressed as a mean ± SD. Error bars in all graphs represent the standard error of the mean. Differences between groups at each time-point were identified by one-way Anova and the comparison between two independent variables was determined by two-way Anova with Dunnet's correction performed post hoc to correct multiple comparisons. The *p*-values <0.05 were considered statistically significant. All data reported here are from sets of six separate experiments. The data was analyzed using the Sigma Stat Soft (Chicago, IL) software.

3. Results

Colchicine molecule (Fig. 1) is composed of three rings, a trimethoxy benzene ring, (ring **A**), a methoxy tropone ring (ring **C**), and a seven-membered ring (ring **B**) carrying an acetamido group at its **C-7** position which anchors the **A** and **C** ring. Thiocolchicine **(2)** was the starting compound for the preparation of a series of thiocolchicine derivatives **(3a–c)**, **(4a–c)** and **(5a–c)** (Fig. 2). Hydrolysis of acetamides **(3a–c)** with 20% of methanolic HCl gave the amines **(4a–c)**, respectively. The choice of introduction of the trifluoroacetyl group at the **C-7** resulted in **(5a–c)** compounds. These functionalized N-fluoroacethylthiocolchicines were prepared from **(4a–c)** compounds, respectively.



Full-size image (11K)

Fig. 1.

Structure of colchicine.

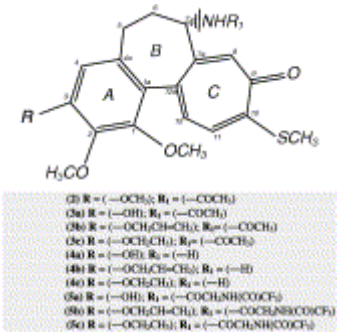


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Fig. 2.

Derivatives of thiocolchicine.

The control CEM cells cultured in the HFB reached density of 10^9 cells/mL with the viability greater than 95% within 4 weeks. Specifically, we used conventional culture CEM cells to establish IC_{50} values. The growth inhibitory activity of thiocolchicine with IC_{50} = 8.5 nM was about 5-fold lower than IC_{50} of colchicine (40 nM). Considering thiocolchicine as the model compound, we evaluated the effect of the substitution of 3-methyloxy or 7-acetamido group on the ring **A** or **B** in the thicolchicine derivatives series on the CEM cell lines' growth. Therefore, we determined the IC_{50} values for all synthesized compounds. All thicolchicine derivatives demonstrated strong cytotoxicity with mean IC_{50} values of 6.8 ± 3 nM. Compound **(3c)** with substitution at the **C-3** position, showed IC_{50} lower than IC_{50} of **(3a–b)**. These results provided motivation to use novel fluorine derivatives in CEM cell cultures. We examined *in vitro* structures **(5a–c)** in the presence of fluorine nuclei at the **C-7** in the form of CF_3-group. What is interesting, the IC_{50} values of **(5a–c)** were about 8-fold lower than IC_{50} of **(2)**. Thicolchicine derivatives with substitution at **C-7** and **C-3** showed about 5-fold lower IC_{50} than thiocolchicine derivatives with substitution at **C-3**. The IC_{50} for all of the nine compounds **(3a–c)**, **(4a–c)** and **(5a–c)** are presented in Fig. 3A–C. The data show that compounds **(3a–c)** (1000 nM) and **(5a–c)** (1000 nM) induced about 90% growth inhibition of CEM cells at 4×10^4 cells/mL. Preferably, the best kill effects in cell culture were provided by **(3c)** and **(5b)**. Compounds **(4a–c)** were less effective by inhibiting the growth of 70% of the same cell line at the same concentration. We did not observe any decrease in cells' growth for cells placed in 10 μL of dimethyl sulfoxide only.

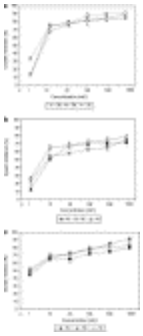
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Fig. 3.

Effects of various doses of derivatives on CEM cells. Data points indicated the inhibitory percentage of cells ± SD in each treatment group: **(3a–c)** (A), **(4a–c)** (B) and **(5a–c)** (C), as compared with controls.

The results of the HPLC analysis of untreated and treated CEM cells are shown in Fig. 4. The chromatograms showed expression of MHC (class I) receptor eluted at 50–55 min in samples treated with **(3b)** and **(3c)**. Treatment with monoclonal antibody directed to MHC (class I) receptor resulted in more than 90% killing effect. Compounds **(3b)** and **(3c)** were more active in growth inhibition. The HPLC fraction of the untreated cells contains only a major peak at 5 min. Thus, the changes in profiles of treated cells correspond to the changes in cells' viability and cellular pathogenesis.

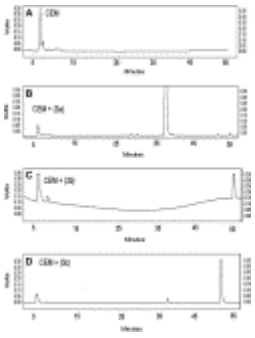
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Fig. 4.

HPLC-UV chromatograms of untreated CEM cells (A), CEM cells treated with **(3a)** (B), CEM cells treated with **(3b)** (C), and CEM cells treated with **(3c)** (D).

The use of 3-D cultured cells proved that CEM cells originally cultured in suspension can form high density structure suitable for MRI experiments. An important aspect of the current study is that thiocolchicine derivatives suppressed cells number during 72 h of treatment and these changes are visible at Fig. 5B as compared to initial tumor size at Fig. 5A. ^{1}H MR image shows cells' aggregation in HFB before and after 72 h treatment. Because synthesized compounds **(5a–c)** have fluorine nuclei, we used ^{19}F MRI to show changes in 3-D cells formation after 72 h. The ^{19}F MR images (Fig. 5D) showed regions of cells with fluorine derivative uptake. No ^{19}F signal was observed in cell culture before treatment (Fig. 5C). The ^{19}F MR image (Fig. 5D) showed regions of cells with fluorine derivative uptake. Moreover, no ^{19}F signal was observed from extracellular media in HFB device, because fresh media were flushed right after treatment. Compounds **(3a–c)** and **(5a–c)** inhibited CEM cells growth in HFB device with 90%. However, the IC_{50} were lower for **(5a–c)** as presented in Table 1.

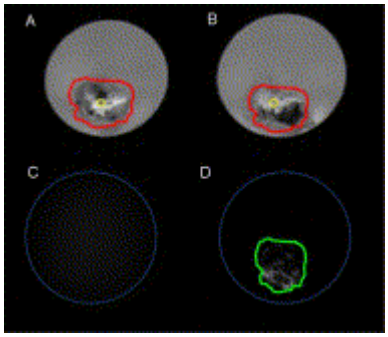

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Fig. 5.

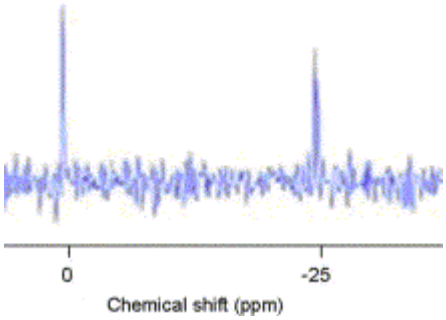
An MR images of the CEM cells in the hollow fiber bioreactor at 9.4 T. The solid red line indicates the area of cells and yellow solid line indicated the fiber. The images: ^{1}H MRI of cells before treatment with **(5c)** (A) and after 72 h treatment with **(5c)** derivative (B). Spin echo (SE) pulse sequence (TR/TE = 5000/12.8 ms, FOV = 3 cm × 3 cm, slice thickness 1 mm and matrix 256 × 256) was used. ^{19}F MRI of cells before treatment with **(5c)** (C) and after 72 h treatment with **(5c)** derivative (D). The blue line at ^{19}F images indicates contour of HFB device and the green solid line indicated treated cells. Inversion Recovery (IR) spin-echo method with Inversion Time (IT) equal to 400 ms and TE/TR = 16.5/5000 ms, slice thickness 1 mm and matrix 256 × 256 was used.

Table 1. IC_{50} values of the thiocolchicine derivatives in the HFB device measured with 10^{9} CEM cells/mL.

Derivatives	**IC_{50} (nM)**
(3a)	5.0 ± 0.7
(3b)	4.1 ± 1.1
(3c)	4.2 ± 0.5

Derivatives	IC_{50} (nM)
(5a)	2.0 ± 0.8
(5b)	1.5 ± 0.3
(5c)	1.1 ± 0.6

The ^{19}F MR spectra did not show any fluorine signal in cell culture before treatment. After treatment with **(5a–c)** fluorine signal was detectable by ^{19}F MRS measurement in the treated cell cultures. The chemical shifts of CF_3- in cell cultures were different from pure **(5a–c)** compounds. The pure compounds **(5a–c)** have the chemical shift −10 ppm, −12 pm and −15 ppm, respectively. The structure of each **(5a–c)** is based on colchicine, thus the chemical shifts did not show differences larger than 5 ppm. In particular, Fig. 6 shows the signal (−25 ppm) of the cells treated with **(5c)**. As expected, the loss of pure **5c** signal was visible on ^{19}F MRS. The values of chemical shift of CF_3-group of **(5a)** and **(5b)** after treatment were −20 ppm and −23 ppm. Each ^{19}F MRS was repeated three times.



Full-size image (19K)

Fig. 6.

^{19}F MRS of CEM cells treated with **(5c)**. CF_3COOH was used as chemical shift reference at 0 ppm.

3.1. Analytical analysis

(2) $C_{22}H_{25}N_1O_5S_1$; Calcd. for $C_{22}H_{25}N_1O_5S_1$: C, 63.60; H, 6.06; N, 3.37; S, 7.72. Found: C, 63.71; H, 6.15; N, 3.42; S, 7.79. **(3a)** $C_{21}H_{23}O_5N_1S_1$; requires M, 401.1. Found: EIMS m/e 401.1 (M^+); Calcd. for $C_{21}H_{23}O_5N_1S_1$: C, 62.8; H, 5.8; N, 3.5; S, 8.0. Found: C, 62.9; H, 5.8; N, 3.3; S, 7.5. **(3b)** $C_{24}H_{27}O_5N_1S_1$; requires M, 454.5. Found: EIMS 454.5 (M^+Na^+); Calcd. for $C_{24}H_{27}O_5N_1S_1$: C, 65.3; H, 6.12; N, 3.17; S, 7.24. Found: C, 65.07; H, 6.59; N, 3.21; S, 7.28. **(3c)** $C_{23}H_{27}O_5N_1S_1$; requires M, 452.6. Found: EIMS 452.6 (M^+Na^+); Calcd. for C, 64.33; H, 18.64; N, 3.26; S, 7.45. Found: C, 64.4; H, 18.9; N, 3.27, S% 7.61. **(4a)** $C_{19}H_{21}O_4N_1S_1$; Calcd. for

$C_{19}H_{21}O_4N_1S_1$: C, 63.51; H, 5.91; N, 3.88; S, 8.92. Found: C, 63.55; H, 5.83; N, 3.75; S, 8.93. **(4b)** $C_{22}H_{25}O_4N_1S_1$; Calcd. for $C_{22}H_{25}O_4N_1S_1$: C, 65.8; H, 6.77; N, 3.52; S, 7.99. Found: C, 65.83; H, 6.49; N, 3.63; S, 8.31. **(4c)** $C_{21}H_{25}O_4N_1S_1$; C, 65.81; H, 6.50; N, 3.6; S, 8.24. Found: C, 65.12; H, 6.54; N, 3.57; S, 8.27. **(5a)** $C_{23}H_{23}O_6N_2S_1F_3$; Calcd. for $C_{23}H_{23}O_6N_2S_1F_3$: C, 55.42; H, 4.61; N, 2.2; S, 6.42; F, 11.44. Found: C, 55.43; H, 4.62; N, 2.91; S, 6.42; F, 11.44. **(5b)** $C_{26}H_{27}O_6N_2S_1F_3$; Calcd. for $C_{26}H_{27}O_6N_2S_1F_3$: C, 56.52; H, 4.89; N, 5.07; S, 5.79; F, 10.32. Found: C, 56.52; H, 4.87; N, 7.01; S, 5.79; F, 10.32. **(5c)** $C_{25}H_{27}O_6N_2S_1F_3$; Calcd. for $C_{25}H_{27}O_6N_2S_1F_3$: C, 57.03; H, 5.13; N, 5.32; S, 6.08; F, 10.87. Found: C, 53.67; H, 4.5; N, 5.32; S, 6.05; F, 10.85.

4. Discussion

Colchicine inhibits cancer cell growth by affecting mitose. According to [13], the previous studies of large numbers of colchicine analogues showed that derivatives modified at the **B** ring at **C-7** position can significantly influence the binding kinetics, association rates, activation energy and the thermodynamics of the drug binding to cells' tubulin. Moreover, thiocolchicine available from colchicine after treatment with sodium methanethiolate, is a potent inhibitor of tubulin polymerization and cell growth, and binds to tubulin more rapidly than colchicines [14]. In the literature, there are examples of total suppression of animal tumors by colchicine in the Shope papilloma of the rabbit lymphosarcoma [15], the mouse sarcoma [16] and the rat sarcoma [17]. The lack of clinical interest in the colchicines seemingly arises from their extreme toxicity. However, cancer cells are significantly more vulnerable to colchicine poisoning than normal cells. Therefore, many attempts have been made to discover more effective and less toxic analogues of colchicine by modifying basic structure of colchicine. In our study, the effective concentrations of derivatives required to induce the growth block in CEM cells were relatively low, in nM range.

The studies [18] on fluorine derivatives have reported promising effect of the trifluoroacetyl analogue on P388 leukemia in mice. We found that the new fluorinated analogues have higher potency than their nonfluorinated counterparts and are more hydrophobic and have higher intracellular intake. Using noninvasive ^{19}F MR techniques, we demonstrate that *ex vivo* fluorine containing drug uptake and cancer cells suppression resulted within 72 h after drug administration. Furthermore, we determined expression of MHC (class I) receptor using HPLC-UV suggested in previous study. It is known that, the CEM cells expressed the MHC (class I) receptor and expression is very specific [19].

Because cancer cells responses to chemotherapeutic agents such as thiocolchicine depend upon cell architecture and tissue polarity, 3-D model of tumor is a very useful model for cell growth. In cell culture, the compound is in direct contact with the cells, and its concentration is constant during its time of action. The change in the concentration occurs only with labile compounds or by an interaction with the cells. Moreover, standard culture methods produce rather low cell concentrations, which are difficult or impossible to detect with MRI while 3-D provides concentration high enough. The results presented here show that MRI can identify suppressed regions of treated cells. Moreover, MRI can give a unique insight into the treatment effects within a tumor over the long course of treatment.

5. Conclusion

In summary, the present study may explain some of the effects of thiocolchicine derivatives on CEM cells. The following conclusion on the structural changes can be drawn: thiocolchicine is the active agent on the CEM cells' growth inhibition; functionalization of **C-7** side chain with fluorine enhanced the growth inhibitory activity of derivatives and the introduction of a trifluoromethyl group in side chain of ring **B** increased the inhibitory activity of derivatives *ex vivo*.

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