Hydantoin derivatives have various biochemical and pharmacological properties. Although hydantoin derivatives have been in use for a long time, the anticancer activity of these derivatives has received scant attention in the last decades.

**AIMS OF THE STUDY**

1. Characterization of the the ABCB1 efflux pump modulating activity of previously selected hydantoin derivatives on multidrug resistant human T-lymphoma cells and multidrug resistant colon adenocarcinoma cells (Colo 320)

2. Characterization of the apoptosis inducing activity of the selected hydantoin derivatives on multidrug resistant human T-lymphoma cells and multidrug resistant colon adenocarcinoma cells (Colo 320)

3. Determination of the structure-activity relationships (QSAR)

**MATERIALS AND METHODS**

**Cell lines:** LS178 (parental, PAR) mouse T-cell lymphoma cells and the human ABCB1 (MDR1)-transfected subline (MDR), human colon adenocarcinoma cell lines (Colo 205 doxorubicin sensitive parental and Colo 205 doxorubicin resistant to anti-cancer agents expressing ABCB1 (MDR1) LVP), ATCC-CCL-221 (Colo 320 and CCL-222 (Colo 205), were purchased from LLC Promchem, Teddington, England.

**Media:** McCoy’s 5A medium, supplemented with 10% heat-inactivated horse serum, L-glutamine and antibiotics; RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM NaHCO3 and 100 U/mL of penicillin and streptomycin.

**Compounds:** Ethidium bromide (Sigma), Vaminapril (Sigma), thirty hydantoin derivatives (SZ-2, SZ-7, LL-4, BS-1, JH-63, M3, T-D3, Q6-5, P3, P7, P10, P11, RW-16, AD-28, RW-13, AD-29, HJ-2, PDPH, HJ-3, F, KO-1, KO-2, M-1, JH, JF-2, HJ-1, F-29, M3-1, -4, Q3-6, Q3-16, Q1-14) were tested, kindly provided by Jolanda Handzlik and Prof. Dr. Katarzyna Kiec-Kononowicz, Cracow, Poland. The compounds were dissolved in DMSO, the structures are confidential.

**Exposure to anticancer and cytotoxic effects:**

The effects of increasing concentrations of the drugs alone on cell growth were tested in 96-well flat-bottomed microplates using MTT (3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide, Sigma, St Louis, MO, USA) assay. Cell viability was determined by measuring the optical density (OD) at 550 nm (ref. 630 nm) with Multiscan EX ELISA reader (Thermo Labsystems, Cheshire, WA, USA). All experiments were conducted in duplicate. The cell growth was determined by calculation of OD550/OD630, where OD630 is defined as the inhibition dose that reduces the growth of the compound-exposed cells by 50%.

**Real-time flow cytometry:**

The fluorescence uptake of the cell population was measured with FACStar Plus flow-cytometer (Becton Dickinson and Company, Franklin Lakes, NJ, USA). Vaminapril was used as a positive control in the rhodamine 123 exclusion experiments. The percentage mean fluorescence intensity was calculated for the treated MDR and parental cell lines as compared to untreated controls (2.3).

**Checkerboard microplate method:**

This microplate method was applied to study the drug interactions between resistance modifiers and anticancer drugs on cancer cells. The interaction of the anticancer drug doxorubicin and the resistance modifiers hydantoin was studied in combination on mouse T-lymphoma cells. The cell growth rate was determined after MTT staining and the intensity of the blue color was measured with Multiscan EX ELISA reader (Thermo Labsystems, Cheshire, WA, USA). Drug interactions were evaluated according to the following system: (D) inhibition dose, F(0) fraction inhibitory concentration, F(1) fraction inhibitory index, F(0)/F(1) combination 1, 2, 3 combination / 1 alone, 2 alone, 3 alone, 1+2 combination / 1 alone + F(0)/F(1) + F(2)/F(3).

**RESULTS**

**Table 1.** Effect of hydantoin derivatives on rhodamine 123 retention by multidrug resistant Colo 320 colon adenocarcinoma cells and multidrug resistant colon adenocarcinoma cells.

**Table 2.** Effect of selected hydantoin derivatives on rhodamine 123 retention by multidrug resistant Colo 320 colon adenocarcinoma cells and multidrug resistant colon adenocarcinoma cells.

**REFERENCES**