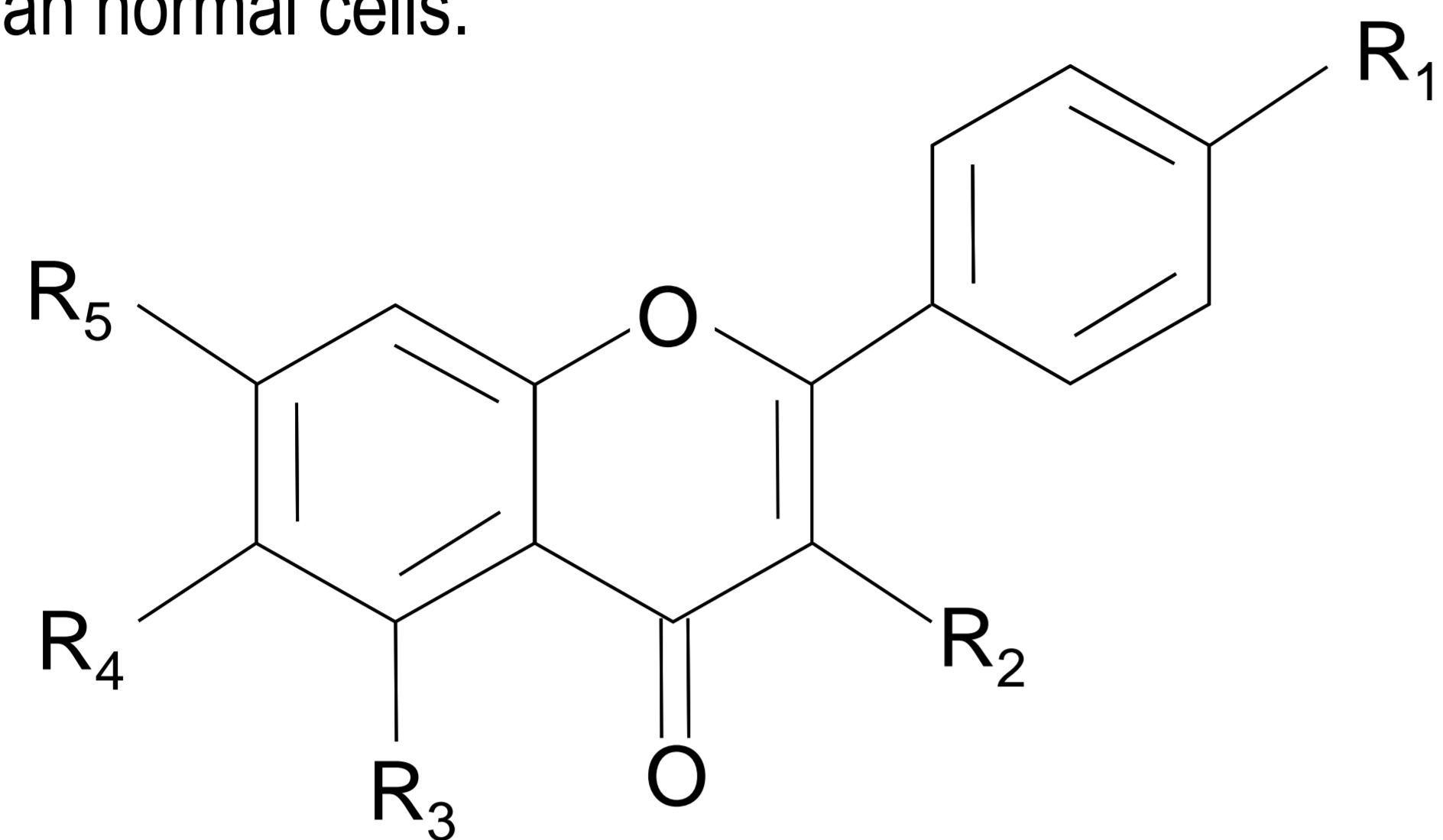


## INTRODUCTION

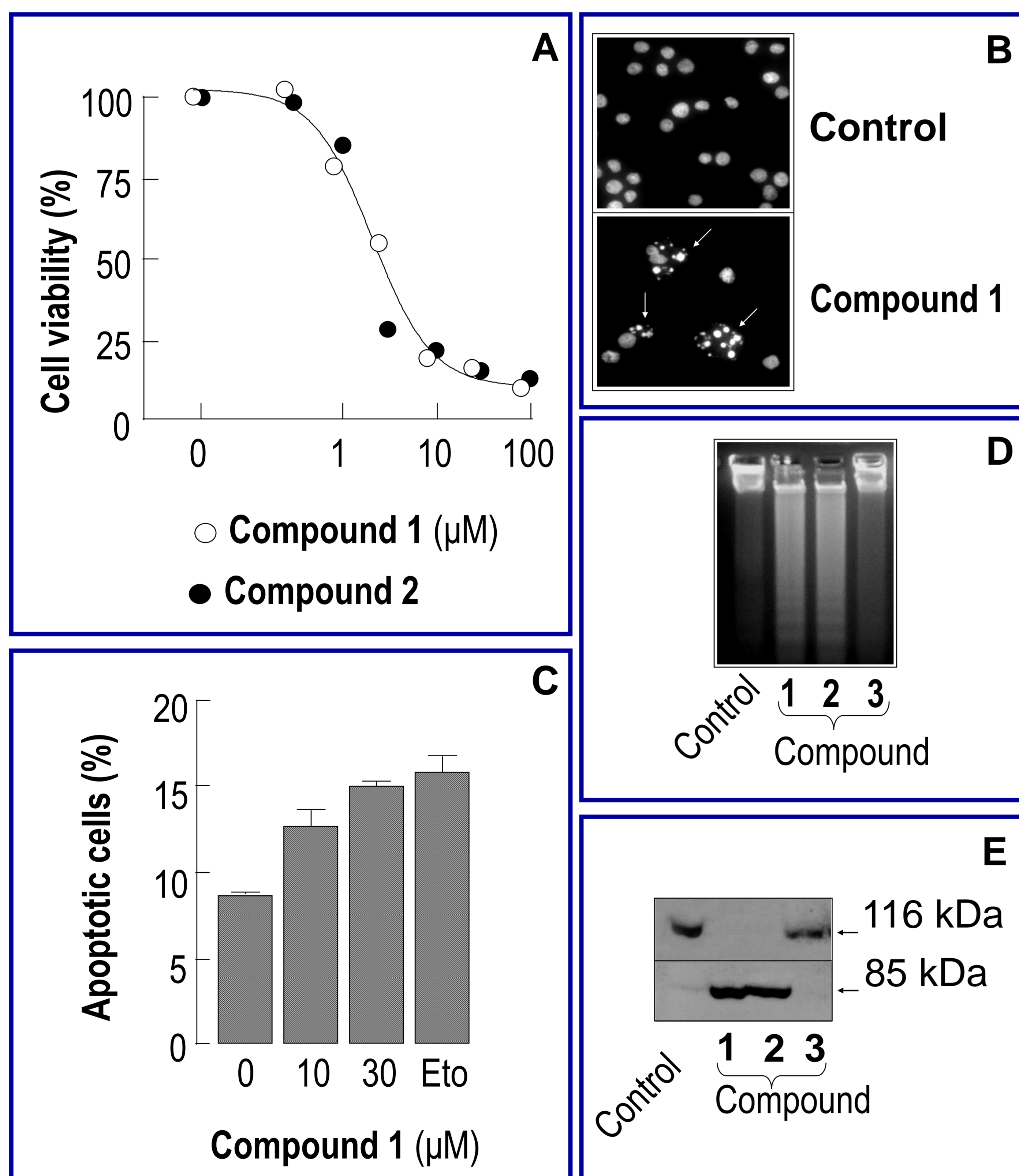
The aim of this study was to investigate the cytotoxicity of several betuletol derivatives on human U937 myeloid leukaemia cells and to compare their effects on human normal cells.



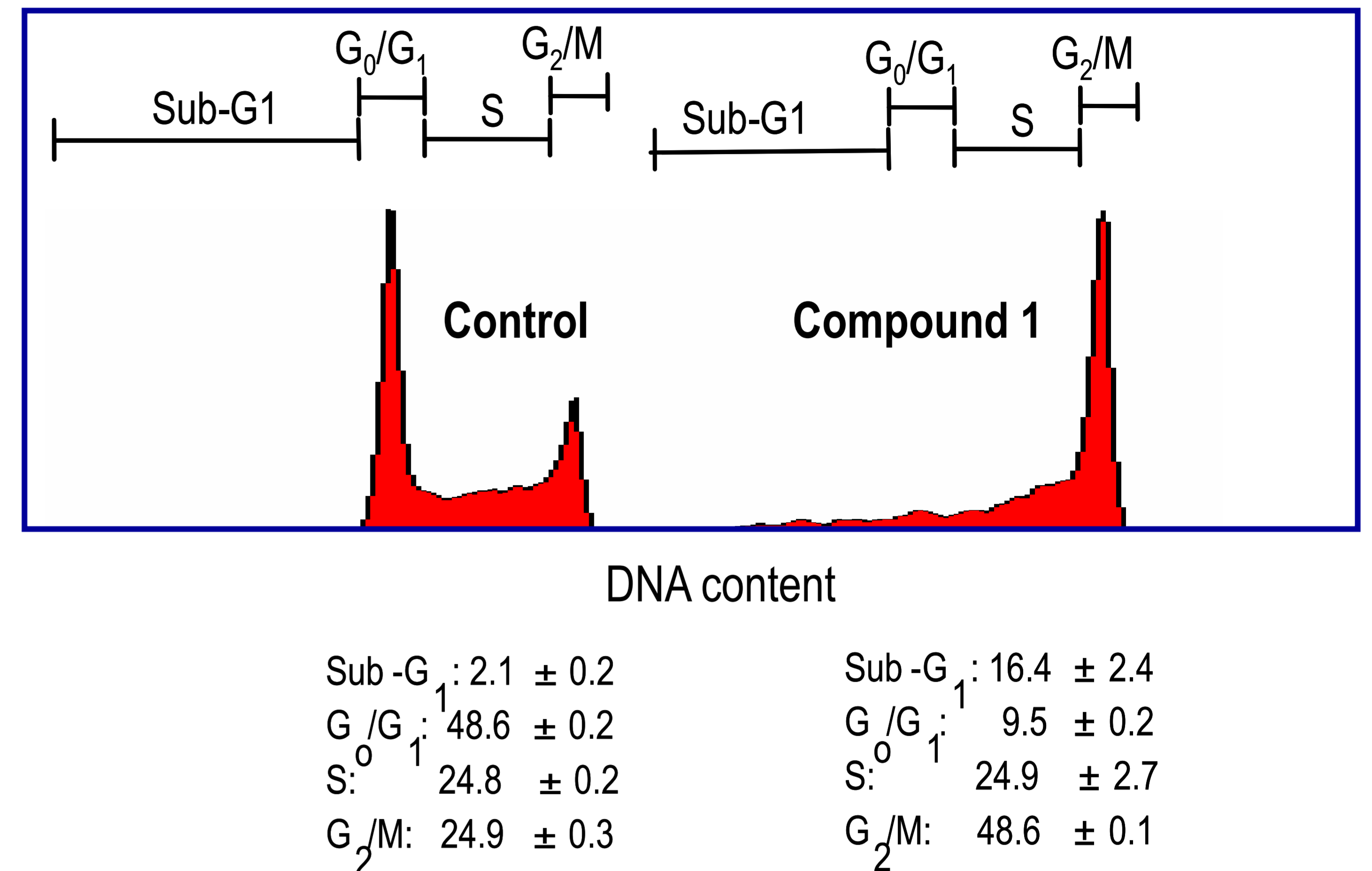
Compound	R <sub>5</sub>	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
1. Betuletol 3-methyl ether		OCH <sub>3</sub>	OCH <sub>3</sub>	OH	OCH <sub>3</sub>
2. Betuletol 3-methyl ether diacetate		OCH <sub>3</sub>	OCH <sub>3</sub>	OAc	OCH <sub>3</sub>
3. Betuletol 3,5,7-trimethyl ether		OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>
4. 4',5,7-trihydroxy-3,6-dimethoxyflavone	OH	OCH <sub>3</sub>	OH	OCH <sub>3</sub>	OH

**Figure 1.** Chemical structures of the phenylbenzo- $\gamma$ -pyrones investigated.

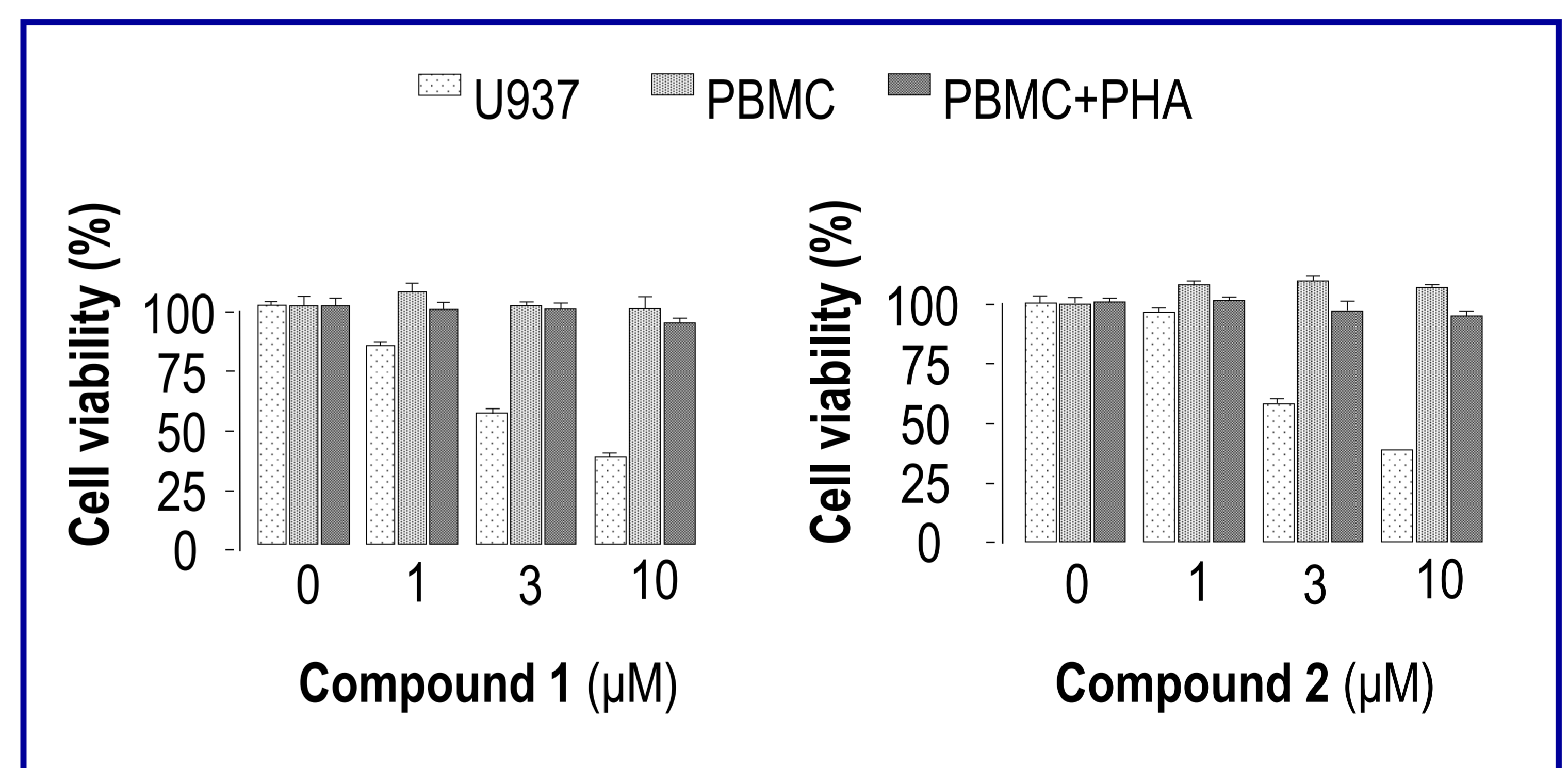
## RESULTS



**Figure 2.** (A) Effects on the growth of human tumor cell line U937. (B) Photomicrographs of representative fields of cells stained with Hoechst 33258. (C) Quantification of hypodiploid cells. Eto = etoposide. (D) DNA fragmentation. (E) Poly(ADP-ribose) polymerase (PARP) cleavage.



**Figure 3.** FACS cell cycle analysis of human myeloid leukaemia cells treated for 16 h with 0.1% DMSO (control) or 10 μM compound 1.



**Figure 4.** Differential effect of betuletol derivatives on proliferation of normal peripheral blood mononuclear cells (PBMC) vs. U937 cells. Proliferation of U937 cells, quiescent PBMC and phytohemagglutinine (PHA)-activated healthy human PBMC cultured in presence of indicated concentrations of compounds.

## CONCLUSIONS

1. The methylether- and acetyl- derivatives of betuletol (compounds 1 and 2) displayed high cytotoxic activities on human U937 and HL-60 myeloid leukaemia cells.
2. The cytotoxic effects of these compounds were accompanied by the dose- and time-dependent appearance of apoptosis on human myeloid leukaemia cells, including DNA fragmentation, sub-G<sub>1</sub> ratio and cleavage of the DNA-repair enzyme poly(ADP-ribose) polymerase.
3. Both compounds induce G<sub>2</sub>/M phase cell cycle arrest in a time dependent manner.
4. Dose-response studies revealed that quiescent peripheral blood mononuclear cells (PBMC) and phytohemagglutinine-activated healthy human peripheral blood mononuclear cells were resistant toward the natural and the semi-synthetic derivative of betuletol.