BIOLOGICAL ACTIVITY:
Chlorambucil is an alkylating agent with antitumor activity. IC50 & Target: DNA Alkylator[1]

In Vitro: Chlorambucil can deprive the function of complementary strands of DNA molecules via alkalization-induced cross interaction, and then inhibits tumor cell proliferation. Chlorambucil (0, 2.5, 5, 10 μM) exhibits slight inhibitory effect on Raji cell apoptosis, but potently increases DR4 and DR5 mRNA expression in Raji cells. Chlorambucil (10 μM) in combination with Tumor necrosis factor (TNF) related apoptosis inducing ligand (TRAIL, 80 ng/ml) has synergistic effect on Raji cell apoptosis and inhibition on proliferation[1].

In Vivo: Chlorambucil (0.2 mg/kg, p.o.) in combination with levamisole (5 mg/kg) has enhanced anti-cancer effect on Ehrlich ascites carcinoma which elevates apoptosis of Ehrlich ascites carcinoma and the survival rate of the mice. However, Chlorambucil exhibits adverse effects on the liver and kidneys of mice[2].

PROTOCOL (Extracted from published papers and Only for reference)

Cell Assay: [1] Cultured cells at log-growth phase are digested by trypsin into single cell suspension and are seeded into 96-well plate at 1000 per well density. The plate is placed in a 37°C chamber with 5% CO2. After attached growth for 24 h, cells are treated with TRAIL at 0, 20, 40 and 80 ng/ml or Chlorambucil at 0, 2.5, 5 and 10 μM for 48 h. 10 μL CCK-8 reagent is added to each well, followed by incubation at 37°C for 4 h. Absorbance values at 450 nm are then measured by a micro-plate reader. Six parallel samples are performed in each treatment group. Cell proliferation rate (%) = mean value of experimental group/mean value of control group × 100%[1].

Female Swiss mice are divided randomly into five group (20 mice per group). Group 1 is kept as the control group, Group 2 receives intraperitoneal injection of by 2.5 × 10⁶ Ehrlich ascites carcinoma cell. Group 3 is treated orally with Chlorambucil 0.2 mg/kg body weight, Group 4 is treated orally with levamisole (5 mg/kg body weight) and Group 5 is treated orally with a combination of Chlorambucil and levamisole each day, using a bent stainless steel stomach tube[2].

References: