INTRODUCTION
Osteoporosis is a significant disease in developed countries. Not only the expenditure of medical care for osteoporosis is a socioeconomic burden for a society but also altering the patients life quality. A nutritional approach to prevent bone loss would be a future goal to achieve an inexpensive way for managing osteoporosis. Ipriflavone (Fig. 1) is a synthetic isoflavone derivative, which has been suggested to be an inhibitor of bone resorption in vitro and in vivo.1,2 Several clinical studies suggested that ipriflavone is able to prevent bone loss. We have screened over 150 ipriflavone derivatives by the tartrate resistant acid phosphatase (TRAP) activity of RAW 264.7 cells after RANKL induction for osteoclastogenesis. The results indicated that 3-(3,4-dimethoxyphenyl)-7-(oxiran-2-ylmethoxy)-4H-chromen-4-one (4) and 3-[[3-(cyclohexylamino)-2-hydroxypropoxy]phenyl]-7-methoxy-4H-chromen-4-one (5a) exhibited significant inhibitory effects on osteoclast activity (TRAP activity in RAW 264.7 with an IC50 of 0.56 and 2.28 μM respectively). The results imply that these two compounds can be candidates for the discovery of anti-osteoporotic drug.

METHODS
The murine macrophage cell line RAW 264.7 (ATCC, Rockville, MD) was maintained in Modified Eagle Medium (MEM) containing 10% FBS. Cells were induced to differentiate into osteoclasts by supplement of 100 ng/ml RANKL. Cultures were treated with isoflavone derivative for 5 days. Osteoclastogenesis was examined by tartrate resistant acid phosphatase (TRAP) solution assay. Cytotoxicity of EGCG on RAW 264.7 cells was measured by MTT assay according to a previous established protocol.

RESULTS:
All the synthesized 3-amino-2-hydroxypropoxyisoflavones and their epoxide precursors were evaluated the TRAP activity of RAW 264.7 cells, the precursor cells of osteoclasts, after RANKL induction for osteoclastogenesis, and the results are summarized in (Table. 1) Among these compounds, (5a) exhibited significant inhibition of osteoclast activity. The inhibition of TRAP activity in RAW 264.7 with an IC50 of 2.28 μM which is approximately 2-fold more active than its cyclopropyl counterpart 5c, while the morpholinyl isomer 5b was not effective on TRAP activity. Compound (6a) inhibited the TRAP activity in RAW 264.7 with an IC50 of 4.17 μM while its morpholinyl counterpart 6b was not effective on TRAP activity. Both epoxide precursors 3 and 4 have also been found to possess significant inhibitory effect on osteoclast inhibitor activity with IC50s 6.39 and 0.56 μM respectively. With exception of 5b and 6b, all the isoflavone derivatives are more effective than the positive baicalein which exhibited an IC50 of 13.01 μM.

Compounds 4 and 5a are non-cytotoxic on RAW 264.7 as shown in Fig. 2.

DISCUSSION
In this study, using cell-based assay systems in RAW 264.7 murine macrophage cells, we found that ipriflavone derivatives, 4 and 5a significantly inhibited the receptor activator of RANKL induced tartrate-resistance acid phosphatase (TRAP) activity in a dose-dependent manner. Further structural optimization and mechanism studies are on-going. Peripheral substitution of isoflavone core plays an important role in the inhibition of osteoclast activity in which the TRAP activity decreased in an order of cyclohexyl > cyclopropyl > morpholinyl.

In summary, the present study demonstrated that 4 and 5a could inhibit both the osteoclast differentiation a of mature osteoclasts, and suggested that its inhibitory activity could result from its potential to block the RANKL-induced activations of signaling molecules.

REFERENCE