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4-PHENYLTHIAZOLE DERIVATIVES INHIBIT IL-6 SECRETION IN OSTEOBLASTIC CELLS AND SUPPRESS BONE WEIGHT LOSS IN OVARIECTOMIZED MICE

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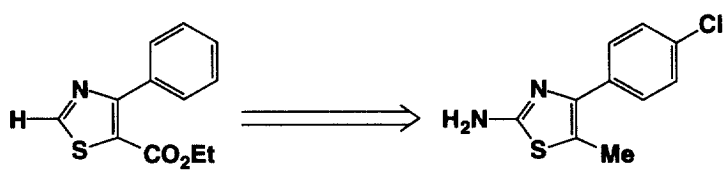
Abstract: A series of 4-phenylthiazole derivatives were synthesized and tested their inhibitory effect on the interleukin-6 secretion stimulated by PTH in osteoblastic cells. SCRC2941-18, 2-amino-4-(4-chlorophenyl)-5-methylthiazole, was found to be the most potent inhibitor in the derivatives. Furthermore, SCRC2941-18 significantly suppressed the bone weight loss in the ovariectomized mice, an osteoporosis model.

Interleukin-6 (IL-6) is a cytokine mainly works in the regulation of inflammatory and immune responses. It induces growth and differentiation of B-cells and T-cells to antibody-producing cells and cytotoxic T-cells, respectively. It regulates the biosynthesis of acute phase proteins by liver cells. An abnormal expression of IL-6 may be involved in the pathogenesis of various diseases. Some of these are multiple myeloma, postmenopausal osteoporosis, chronic autoimmune diseases and AIDS.[1] Recently, small molecule inhibitors of IL-6 synthesis or secretion were reported.[2]

Postmenopausal osteoporosis is caused by an imbalance between bone resorption and bone formation, which results in bone loss and fractures after mineral flux. The frequency of fracture is significantly increased in osteoporosis, and hip fracture in senile patients is a very serious problem because it often limits their quality of life. Since IL-6 is a mediator of the bone resorption in osteoporosis, the inhibitor of its secretion will be expected to be the bone resorption suppresser.[3-5]

During the screening of IL-6 secretion inhibitors, a series of 4-phenylthiazole derivatives was found to inhibit the IL-6 induction stimulated by parathyroid hormone (PTH) in osteoblastic MC3T3-E1 cells. Then 2-amino-4-(4-chlorophenyl)-5-methylthiazole **13** (SCRC2941-18) was found to be the most potent inhibitor. This derivative significantly suppressed the bone weight loss in the ovariectomized mice, a postmenopausal osteoporosis model.

In this paper, we report the preliminary optimization of 4-phenylthiazole for inhibition of IL-6 secretion and their effect on the bone weight loss in the ovariectomized mice.



1
(IC_{50}=30 μg/ml)

SCRC2941-18 (13)
(IC_{50}=1.0 μg/ml)

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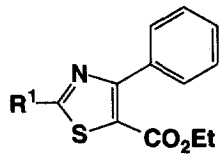
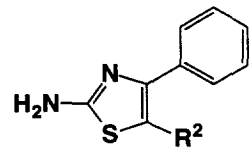
Table I. R^1 substituents



Compound No.	R^1	IC_{50} (μg/ml)
1	H	30
2	NH_2	6.0
3	MeNH	40
4	Me	40
5	4-Pyridyl	90
6	HOCNH	>100

Table II. R^2 substituents



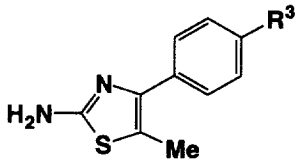
Compound No.	R^2	IC_{50} (μg/ml)
7	CO_2H	>100
8	H	60
9	Me	10
10	Et	>100
11	MeNH	40
12	PrNHCO	40

The lead compound, 4-phenylthiazole derivative **1**, was found to inhibit the IL-6 secretion with IC_{50}=30 μg/ml. A series of 4-phenylthiazole derivatives were then synthesized.[6,7] The derivatives were tested for their inhibitory effects on the IL-6 secretion stimulated by 10 ng/ml of PTH in osteoblastic MC3T3-E1 cells.[8,9] IL-6 secreted in the medium was measured by bioassay using MH60 cells.[9-11] As shown in Table I, amino analog **2** was improved in the inhibitory activity. Therefore, amino group was fixed as R^1 and R^2 was surveyed. As shown in Table II, 5-methyl derivative **9** showed potent activity. Although 5-CO_2Et analog **2** was more potent than **9** in this stage, methyl moiety was suitable for R^2 part in 4-chlorophenyl analog as described below (Table IV). Next, various substituents were introduced into 4 position of the phenyl group of 5-methyl derivative **9**. As shown in Table III, 4-Cl, 4-OH and 4-CF_3 derivatives (**13**, **14**, **15**) were more active than phenyl analog **9**. Finally, the position of the chloro group and substituent at 5 position were optimized. As shown in Table IV, 4-(4-chlorophenyl)-5-methyl analog **13** (SCRC2941-18) was most active.

Three 4-phenylthiazoles (**13**, **14**, **22**) were then selected and tested using the ovariectomized mice.[12] The ovariectomy significantly caused the decrease in the bone weight.[13,14] SCRC2941-18 (**13**) significantly suppressed the bone weight loss at the dose of 2 mg/kg/day, ip (Table V). This derivative was the potent inhibitor of IL-6 secretion *in vitro* as described above. 4-Hydroxyphenyl analog **14** did not significantly suppress the bone loss, but tended to increase the bone weight. Furthermore, these compounds did not induce the uterine hypertrophy (data not shown). Since the hypertrophy indicated the estrogen-like effect on reproductive organs[15], the 4-phenylthiazoles would not be an estrogen agonist. These 4-phenylthiazoles could be a candidate for osteoporotic drug. Further detailed effects of the 4-phenylthiazole derivatives, especially SCRC2941-18, on the osteoporosis model mice are currently under investigation.

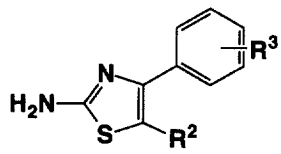
Furthermore, 4-phenylthiazole derivatives will be expected to apply not only for osteoporosis but for the other diseases, i.e. multiple myeloma, chronic autoimmune diseases and AIDS. Control of cytokines involved in various diseases, for example, IL-1, IL-6 and TNF, will become an important target for medicine.

Table III. R^3 substitutents



Compound No.	R^3	IC_{50} (μg/ml)
9	H	10
13	4-Cl	1.0
14	4-OH	3.0
15	4-CF_3	7.0
16	4-MeO	24
17	4-Me	27
18	4-F	39
19	4-Ph	50

Table IV. chlorophenyl analogs



Compound No.	R^2	R_3	IC_{50} (μg/ml)
20	Me	2-Cl	2.5
21	Me	3-Cl	20
13	Me	4-Cl	1.0
22	CO_2H	4-Cl	5.0
23	CO_2Et	4-Cl	6.0
24	Me	2,4-$(Cl)_2$	8.0
25	Me	3,4-$(Cl)_2$	35

Table V. Suppressive effect of 4-phenylthiazole derivatives on femoral weight loss in ovariectomized mice.[12]
*: $p<0.05$ vs. ovariectomized control, calculated by Student's t-test.

Compound				Recovery (%)	
No.	R^1	R^2	R^3	(0.4mg/kg/day, ip)	(2.0 mg/kg/day, ip)
13	NH_2	Me	4-Cl	22.8	57.8*
14	NH_2	Me	4-OH	13.9	35.5
22	NH_2	CO_2H	4-Cl	7.1	8.6

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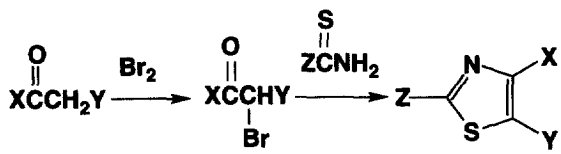
6. In the case of most, the series of 4-phenylthiazole derivatives were synthesized by the reaction of various bromoketones with thiourea derivatives.[7]



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12. Osteoporosis model using ovariectomized mice.[13,14] Four-week-old female ddY mice were obtained from Japan SLC, Inc. The mice were allowed free access to commercial chow and tap water, and were maintained at 25 ± 0.5 °C. Ovariectomies and sham operations were carried out under ketamine hydrochloride anesthesia. The mice were divided into some groups: sham-operated, ovariectomized control groups and ovariectomized groups treated by test sample. Each group contained 5 mice. The administration of vehicle or test sample dissolved in sterilized water containing 5% DMSO was started from the day after the operations and continued 5 days a week. At 4 weeks after the operations, all the mice had their body weight measured and then killed to retrieve femurs and uterus for measurements. The right femurs were used for the measurement of dry weight (dried at 60 °C for 24 h).

    Recovery (%) = [(femoral weight of ovariectomized mouse treated by test sample) - (femoral weight of ovariectomized control)] / [(femoral weight of sham-operated control) - (femoral weight of ovariectomized control)] X 100
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