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BRIEF ARTICLE

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Discovery of Indeno[1,2-*c*]quinoline Derivatives as Inhibitors of Osteoclastogenesis Induced by Receptor Activator of NF-κB Ligand (RANKL)

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Supporting Information

ABSTRACT: Certain indeno[1,2-*c*]quinolines were synthesized and evaluated for antiosteoclastogenic activities. Among them, 6,9-dimethoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (**8a**) and 9-methoxy-6-(methylthio)-11*H*-indeno[1,2-*c*]quinolin-11-one (**16a**) inhibited RANKL-induced osteoclast formation in Raw 264.7 cells with an IC_{50} of 2.00 and 2.58 μM, respectively. Compound **8a** was only weakly active in the inhibition of the RANKL-induced NFAT activation, while **16a** was inactive. These results indicated that the antiosteoclastogenic effect of **8a** is only partly related while **16a** is not related to the suppression of NFAT.

INTRODUCTION

The equilibrium between bone formation mediated by osteoblasts and bone resorption mediated by osteoclasts is essential to the growth and maintenance of a healthy skeleton. A major bone disease in elderly persons and especially postmenopausal women is osteoporosis, which results from a reduction in bone mass. Therapeutical strategies are now used to prevent osteoporosis by inhibiting osteoclast activity (bisphosphonates and calcitonin) or by reducing osteoclast generation (estrogens).[1−3] Concerning the management of osteoporosis, hormone replacement therapy (HRT) by prescribing estrogen hormones has been widely accepted for relieving climacteric symptoms of postmenopausal women since the regulative role of some estrogen hormones in maintaining bone mass in women has been ascertained. Although HRT reduces the risks of osteoporosis, side effects such as resumption of menses, central nerveous system disturbances, increased risk of breast and uterine cancers have stimulated the search for novel compounds that can selectively regulate bone metabolism with negligible estrogenic activity on uterus even after long-term administration.[4,5] Recently, selective estrogen receptor modulators (SERMs) that fully antagonize the effect of estrogen on uterine and mammary tissue while mimicking the effects of estrogen on bone have been investigated as a possible alternative to HRT.[6] Tamoxifen (Chart 1), an anticancer drug that functions as an antagonist in breast tissue, is an agonist on bone in maintaining bone density in postmenopausal women but possesses some estrogenic effects in the uterus. Raloxifene, the antiosteoporosis drug, acts as an antagonist in breast and uterine but is agonist in bone. However, neuronal toxicity of raloxifene at higher concentrations could be its drawback, necessitating a continuous search for more reliable SERMs.[7] Genistein (a natural isoflavone) and especially some of its derivatives act as SERMs on osteoblastic cells and in ovariectomized mice highlight the possibility that isoflavone derivatives exert estrogen-like regulative action and bone marrow metabolism in some dose range that does not affect the uterus.[8−10]

Another synthetic isoflavone derivative, ipriflavone, is capable of reducing bone loss in various types of animal models of experimental osteoporosis, providing a rationale for its use in the prevention and treatment of postmenopausal and senile osteoporosis in human. This drug interferes with bone remodeling mainly by inhibiting bone resorption, although some evidence has also suggested a stimulatory effect on bone formation.[11−13] To improve antiosteoporotic potency of ipriflavone, we have synthesized certain 3-amino-2-hydroxypropoxyisoflavone derivatives and evaluated the effects of these compounds on cell viability, cytotoxicity, and osteogenesis functions. Results indicated that 3-(3,4-dimethoxyphenyl)-7-(oxiran-2-ylmethoxy)-4*H*-chromen-4-one (**1a**) and 3-{4-[3-(cyclohexylamino)-2-hydroxypropoxy]phenyl}-7-methoxy-4*H*-chromen-4-one (**1b**) exhibited significant inhibitory effects on osteoclast activity. Both compounds have also exhibited very strong osteogenic effects, a ∼10-fold effect of ipriflavone on mineralization of osteoblasts.[14] Certain tetracyclic benzopyran derivatives have also been discovered as inhibitors of osteoclastogenesis induced by receptor activator of NF-κB ligand (RANKL). Among them, **2** was identified as the best inhibitor of RANKL-induced osteoclastogenesis with an IC_{50} of 6.97 μM in RAW264.7 cell.[15]

Recently, a number of benzofuro[3,2-*c*]quinoline (**3a**) and benzofuro[3,2-*c*]coumarin (**3b**) derivatives have been reported as novel classes of antiosteoporotic agents.[16] We have also synthesized certain indeno[1,2-*c*]quinoline derivatives for evaluation of antiproliferative activity.[17−19] Results indicated that the introduction of various aminoalkyl side chains on the C-11 position led to the discovery of novel duel inhibitors of topoisomerases I/II.[19]

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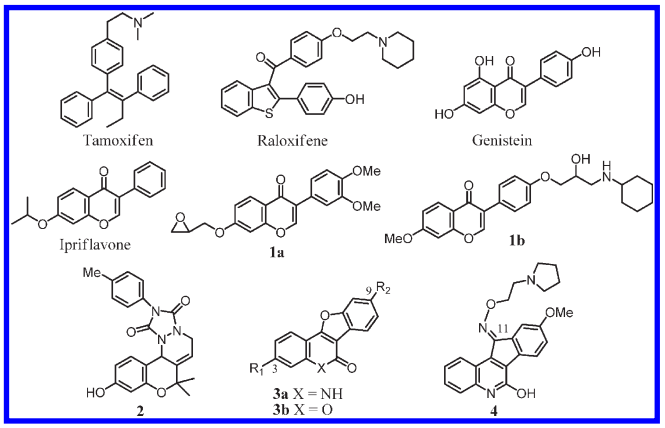
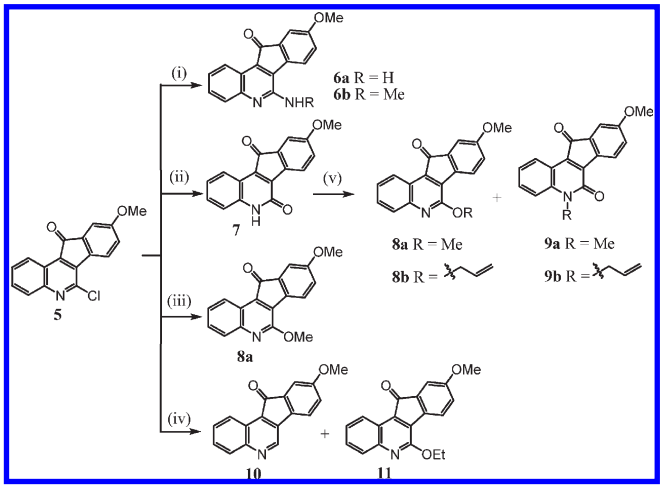
Chart 1. Structures of Tamoxifen, Raloxifene, Genistein, Ipriflavone, Isoflavoids 1a,b, Benzopyran-Fused Tetracyclic Compound 2, Benzofuro[3,2-c]quinoline 3a, Benzofuro[3,2-c]-coumarin 3b, and Indeno[1,2-c]quinolin-11-one Oxime 4



Scheme 1. Synthesis of 9-Methoxyindeno[1,2-c]quinoline Derivatives[a]



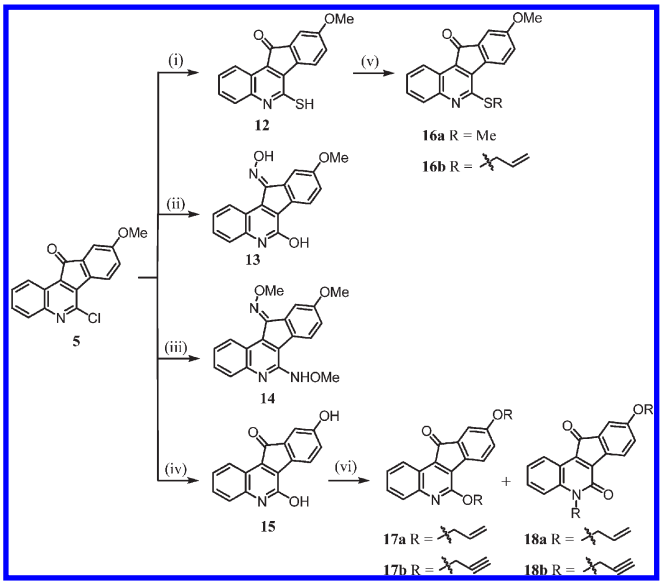
[a] Reagents and conditions: (i) NH_4OH or $MeNH_2$ in sealed tube, 200 °C (62% for **6a** and **6b**); (ii) HCl (cat.), DMF, 2 h (32%); (iii) Na, MeOH, 24 h (88%); (iv) Na, EtOH, 24 h (58% for **10**, 10% for **11**); (v) MeI or allyl bromide, K_2CO_3, acetone, room temp, 8 h (23% for **8a**, 27% for **8b**, 58% for **9a**, 59% for **9b**).

Among them, (*E*)-6-hydroxy-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-2-(pyrrolidin-1-yl)ethyloxime (**4**) was found to be one of the most cytotoxic agents with a GI_{50} of 0.84, 0.89, and 0.79 μM against SAS, A549, and BT483, respectively, which is more active than the positive camptothecin.[19] However, most of the C-11 carbonyl and C-11 oxime precursors were noncytotoxic.[17−19] These noncytotoxic tetracyclic indeno[1,2-*c*]quinolin-11-one derivatives were evaluated for their antiosteoporotic activities because of the structural similarity with **3a**. Our aim is to find a novel antiosteoporotic structural type that is noncytotoxic.

CHEMISTRY

Preparation of the desired 9-methoxyindeno[1,2-*c*]quinoline derivatives is outlined in Scheme 1. 6-Chloro-9-methoxyindeno[1,2-*c*]quinoline **5** was prepared according to known procedures.[17] Reaction of **5** with $MeNH_2$ gave 9-methoxy-6-(methylamino)-11*H*-indeno[1,2-*c*]quinolin-11-one (**6b**) in 62% yield. Preparation of **6a** was previously described.[17] 6-Hydroxy-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (**7**) was obtained by the hydrolysis of **5** with HCl.[19] Treatment of **7** with MeI gave a mixture of 6,9-dimethoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (**8a**) and its N-alkylated isomer **9a**, with the former one as a minor product. Accordingly, a mixture of O-allylated product **8b** and its N-allylated counterpart **9b** was obtained by the treatment of **7** with allyl bromide in K_2CO_3/acetone. The sole product of **8a** can also be synthesized by the reaction of **5** with sodium in MeOH. Reaction of **5** with sodium in EtOH afforded a mixture of 9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (**10**)[19] and 6-ethoxy-9-methoxy-11*H*-indeno[1,2-*c*]quinolin- 11-one (**11**), with the former one as a major product.

Scheme 2. Synthesis of 6-Hydoxy and 6-Mercapto Derivatives of the 9-Methoxyindeno[1,2-c]quinoline[a]



[a] Reagents and conditions: (i) Lawesson's reagent, toluene, room temp, 0.5 h (87%); (ii) NH_2OH, microwave, 0.5 h (85%); (iii) NH_2OMe, microwave, 0.5 h (89%); (iv) 48% HBr, AcOH, reflux, 48 h (78%); (v) MeI or allyl bromide, K_2CO_3, acetone, room temp, 8 h (45% for **16a**, 73% for **16b**); (vi) allyl bromide or propagyl bromide, K_2CO_3, acetone, room temp, 8 h (21% for **17a**, 22% for **17b**, 53% for **18a**, 57% for **18b**).

Scheme 2 depicted the preparation of 6-hydoxy and 6-mercapto derivatives of 9-methoxyindeno[1,2-*c*]quinoline. Reaction of **5** with Lawesson's reagent afforded 6-mercapto-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one (**12**) which was alkylated with MeI to give 9-methoxy-6-(methylthio)-11*H*-indeno[1,2-*c*]-quinolin-11-one (**16a**). Accordingly, **16b** was prepared by the treatment of **12** with allyl bromide under the same reaction conditions. (*E*)-6-Hydroxy-9-methoxy-11*H*-indeno[1,2-*c*]quinolin-11-one oxime (**13**)[19] and (*E*)-9-methoxy-6-(methoxyamino)-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-methyloxime (**14**) were obtained from **5** by the treatment with NH_2OH and NH_2OMe, respectively. Reflux of **5** with 48% HBr afforded 6,9-dihydroxy-11*H*-indeno[1,2-*c*]quinolin-11-one (**15**) which was alkylated with allyl bromide to give 6,9-bis(allyloxy)-11*H*-indeno[1,2-*c*]-quinolin-11-one (**17a**) and its N-allylated isomer **18a**, with the former one as a minor product. Accordingly, a mixture of the

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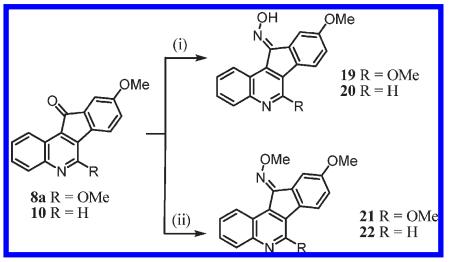
O-propargylated product **17b** and its N-propargylated counterpart **18b** was obtained by the treatment of **15** with propargyl bromide in K_2CO_3/acetone.

Preparation of the 6-methoxy and 6-unsubstituted derivatives is outlined in Scheme 3. Reaction of **8a** and **10**, respectively, with NH_2OH afforded (*E*)-6,9-dimethoxy-11-(hydroxylimino)-11*H*-indeno[1,2-*c*]quinoline (**19**) and its C-6 unsubstitutued counterpart **20**.[19] Accordingly, compounds **21** and **22** were obtained by the treatment of **8a** and **10** respectively with NH_2OMe.

Scheme 3. Synthesis of the 6-Methoxy and 6-Unsubstituted Derivatives of the 9-Methoxyindeno[1,2-*c*]quinoline[a]

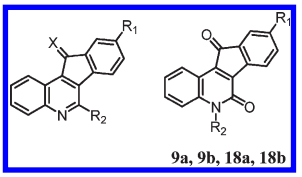


[a] Reagents and conditions: (i) NH_2OH, microwave, 0.5 h (84% for **19**, 85% for **20**); (ii) NH_2OMe, microwave, 0.5 h (83% for **21**, 85% for **22**).

PHARMACOLOGICAL RESULTS AND DISCUSSION

All the synthesized 9-methoxyindeno[1,2-*c*]quinoline derivatives were evaluated for their tartrate resistant acid phosphatase (TRAP) activity[20] in RAW 264.7 cells, the precursor cells of osteoclasts, after RANKL induction for osteoclastogenesis, and the results are summarized in Table 1. For the 9-methoxy-11-oxo (R_1 = OMe, X = O) derivatives, substitution at C-6 is unfavorable for osteoclast inhibitory activity, in which the inhibition on the TRAP activity of R_2 decreased in the order of **10** (H, 1.43 μM) > **8a** (OMe, 2.0 μM) > **16a** (SMe, 2.58 μM) > **6b** (NHMe, 5.58 μM) > **12** (SH, 13.79 μM) > **8b** (*O*-allyl, 15.38 μM), **16b** (*S*-allyl, 15.62 μM) > **7** (OH, >20 μM), **6a** (NH_2, >20 μM). Thus, the unsubstituted 9-methoxy-11-oxo-indeno[1,2-*c*]quinolinone (**10**) exhibited the most significant inhibition of osteoclast

Table 1. Inhibitory Effect of Indeno[1,2-c]quinoline Derivatives on RANKL-Induced Osteoclast Formation by Tartrate Resistant Acid Phosphatase (TRAP) Assay



compd	R_1	X	R_2	IC_{50} [a] (μM)	survival rate at 10 μM (%)[b]
6a	OCH_3	O	NH_2	>20	ND
6b	OCH_3	O	$NHCH_3$	5.58 ± 1.23	25.9
7	OCH_3	O	OH	>20	ND
8a	OCH_3	O	OCH_3	2.00 ± 0.01	100.3
8b	OCH_3	O	$OCH_2CH{=}CH_2$	15.38 ± 2.30	105.0
9a	OCH_3		CH_3	11.51 ± 1.21	66.6
9b	OCH_3		$CH_2CH{=}CH_2$	>20	ND
10	OCH_3	O	H	1.43 ± 0.35	25.3
11	OCH_3	O	OCH_2CH_3	>20	ND
12	OCH_3	O	SH	13.79 ± 2.78	63.0
13	OCH_3	NOH	OH	9.11 ± 0.20	85.2
14	OCH_3	$NOCH_3$	$NHOCH_3$	1.58 ± 0.01	105.2
15	OH	O	OH	15.29 ± 0.03	16.8
16a	OCH_3	O	SCH_3	2.58 ± 0.01	86.6
16b	OCH_3	O	$SCH_2CH{=}CH_2$	15.62 ± 3.70	105.8
17a	$OCH_2CH{=}CH_2$	O	$OCH_2CH{=}CH_2$	5.70 ± 0.87	75.1
17b	$OCH_2C{\equiv}CH$	O	$OCH_2C{\equiv}CH$	9.46 ± 3.63	30.9
18a	$OCH_2CH{=}CH_2$		$CH_2CH{=}CH_2$	>20	ND
18b	$OCH_2C{\equiv}CH$		$CH_2C{\equiv}CH$	>20	ND
19	OCH_3	NOH	OCH_3	8.44 ± 2.76	46.9
20	OCH_3	NOH	H	>20	ND
21	OCH_3	$NOCH_3$	OCH_3	7.97 ± 0.31	62.3
22	OCH_3	$NOCH_3$	H	4.79 ± 1.59	55.4
ipriflavone				31% at 10 μM	102.0
epigallocatechin gallate (EGCG)				29.8 ± 0.33	ND

[a] IC_{50} is the concentration (μM) of the test compound required to reduce to 50% intensity of the bioluminescence induced by 100 ng/mL RANKL. Values represent the mean ± SD from at least three experiments. [b] The label "ND" indicates that we did not collect data.

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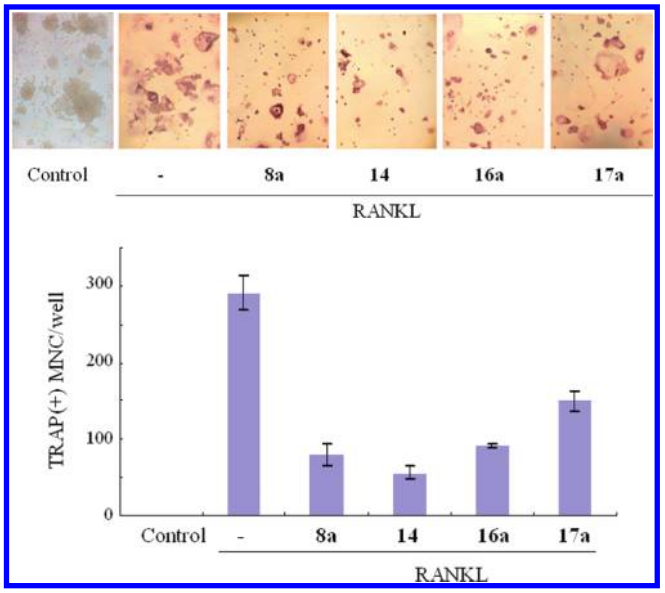

Figure 1. Effect of **8a**, **14**, **16a**, and **17a** on RANKL-induced osteoclast formation. Raw 264.7 cells were cultured with 10 μM **8a**, **14**, **16a**, **17a** and 100 ng/mL RANKL. After 5 days, cells were stained for TRAP activity, and the TRAP-positive multinucleated cells with three or more nuclei/cell were counted by light microscopy.

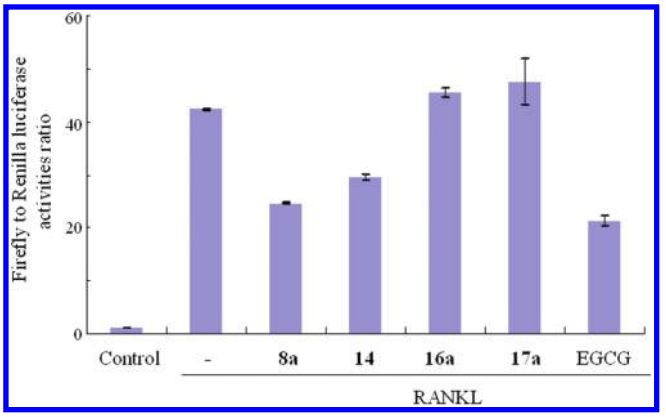

Figure 2. NFAT reporter assay using pNFAT/Luc-RAW264.7 cells were cultured with 10 μM **8a**, **14**, **16a**, **17a**, and EGCG and 100 ng/mL RANKL as described in Table 2 of the Supporting Information. The data shown in this result are the ratio of firefly to Renilla luminescence. The fold induction was calculated as follows: fold induction = average relative light units of induced cells/average relative light units of control cells.

activity in RAW 264.7 with an IC_{50} of 1.43 μM, which is ~20-fold more active than that of the positive epigallocatechin gallate (EGCG, 29.8 μM), while its allyloxy isomer **8b** was much less active with an IC_{50} of 15.38 μM. The inactivity of 6-hydroxy derivative **7** on TRAP activity indicated that the H-bonding donating group is unfavorable. The same trends were observed in which the osteoclast inhibitory activity of **6b** (NHMe, 5.58 μM) is more favorable than that of **6a** (NH_2, >20 μM), and that of **16a** (SMe, 2.58 μM) is more favorable than that of **12** (SH, 13.79 μM). For the methyloxime (X = NOMe) derivatives, **14**, with a substituent of NHOMe at C-6 position, exhibited the most significant inhibition of osteoclast activity with an IC_{50} of 1.58 μM and is more active than its C-6 unsubstituted isomer **22** (4.79 μM) and C-6 OMe counterpart **21** (7.97 μM). With the same substituents at C-6 and C-9, 11-oxo (X = O) derivatives are more favorable than methyloximes (X = NOMe) which in turn are more favorable than oximes (X = NOH). Thus, the inhibition on the TRAP activity decreased in the order of **10** (1.43 μM) > **22** (4.79 μM) > **20** (>20 μM), and **8a** (2.0 μM) > **21** (7.97 μM) > **19** (8.44 μM). In general, alkylation at oxygen is more favorable than at nitrogen in which compound **8a** (2.00 μM) is more active than **9a** (11.51 μM), **8b** (15.38 μM) is more active than **9b** (>20 μM), **17a** (5.70 μM) is more active than **18a** (>20 μM), and **17b** (9.46 μM) is more active than **18b** (>20 μM). Among these indeno[1,2-*c*]quinolinone derivatives, compounds **8a**, **14**, and **16a** not only exhibited significant inhibition of osteoclast activity in RAW 264.7 with an IC_{50} of <3.0 μM in each case but also proved to possess very low cytotoxicities, with cell survival rate of higher than 86% at 10 μM. Although **10** was the most active among these indeno[1,2-*c*]quinolinone derivatives, it was also highly cytotoxic on the RAW 264.7 cell line. Compounds **8a**, **14**, **16a**, and **17a**, which exhibited potent inhibitory activity of RANKL-induced osteoclast formation in Raw 264.7 cells (Figure 1), were further evaluated for their inhibitory effects on RANKL-induced nuclear factor of activated T-cells (NFAT) activation, since NFAT plays a critical role in osteoclast differentiation and activation.[20]

The NFAT reporter is designed to monitor the activity of NFAT-regulated signal transduction pathways in preosteoclast (Raw 264.7) cells. This NFAT vector allows more accurate and precise measurement of NFAT inhibition by bioactive compounds paralleled by an economically better consumption of the firefly luciferase vector. NFAT is one of the key transcription factors induced by RANKL in osteoclast. The natural product, epigallocatechin gallate (EGCG), has been found to inhibit RANKL-induced NFAT activation significantly as the previous report.[21] Results from Figure 2 indicated the activation with RANKL at 100 ng/mL is 42-fold in comparison to the control. The inhibitory activity of RANKL activation was decreased in the order of EGCG (21-fold) > **8a** (25-fold) > **14** (37-fold) > **16a** (46-fold) > **17a** (48-fold). Although **16a** and **17a** exhibited potent inhibitory activity on RANKL-induced osteoclast formation by TRAP assay with an IC_{50} of 2.58 and 5.70 μM, respectively, they are inactive in the inhibitory effect of the RANKL-induced NFAT activation. Compound **8a**, which inhibited RANKL-induced osteoclast formation in RAW 264.7 with an IC_{50} of 2.0 μM, ~15-fold more potent than the positive EGCG (29.8 μM), was less active than EGCG in the inhibitory effect of the RANKL-induced NFAT activation. These results indicated that the antiosteoclastogenic effect of **16a** and **17a** is not related to the suppression of NFAT, while the effect of **8a** is only partly through the suppression of NFAT. Therefore, **8a** and **16a** have been identified as new leads for the discovery of antiosteoclastogenic agents. Further studies of their molecular mechanisms are ongoing.

■ CONCLUSION

We have synthesized certain indeno[1,2-*c*]quinoline derivatives and evaluated their inhibition on RANKL-induced osteoclast formation in RAW 264.7 cells and the inhibitory effect of RANKL-induced NFAT activation. Among them, **16a** and **17a** exhibited potent inhibitory activity on RANKL-induced osteoclast formation by TRAP assay with an IC_{50} of 2.58 and 5.70 μM, respectively. Both compounds are inactive in the inhibition of the RANKL-induced NFAT activation. Compound **8a**, which

inhibited RANKL-induced osteoclast formation in RAW 264.7 with an IC_{50} of 2.0 μM, ~15-fold more potent than the positive EGCG (29.8 μM), was less active than EGCG in the inhibitory effect of the RANKL-induced NFAT activation. These results indicated that the antiosteoclastogenic effect of **16a** and **17a** is not related to the suppression of NFAT while the effect of **8a** is only partly through the suppression of NFAT. Therefore, indeno[1,2-*c*]-quinoline, as examples of **8a** and **16a**, has been identified as a novel structural type of potential antiosteoclastogenic agent. Further mechanism studies of these potential leads are ongoing.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures, spectroscopic data, and elemental analysis data of all new compounds; procedures for biological studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

HRT, hormone replacement therapy; SERM, selective estrogen receptor modulator; TRAP, tartrate resistant acid phosphatase; RANKL, receptor activator for nuclear factor κB ligand; EGCG, epigallocatechin gallate; NFAT, nuclear factor of activated T-cells

■ REFERENCES

(1) Rodan, G. A.; Martin, T. J. Therapeutic approaches to bone diseases. *Science* **2000**, *289*, 1508–1514.

(2) Sato, M.; Grese, T. A.; Dodge, J. A.; Bryant, H. U.; Turner, C. H. Emerging therapies for the prevention or treatment of postmenopausal osteoporosis. *J. Med. Chem.* **1999**, *42*, 1–24.

(3) Kauffman, R. F.; Bryant, H. U.; Yang, N.; Harper, K. D.; Huster, W. J.; Walls, E. L.; et al. Preventing post-menopausal osteoporosis: an update on raloxifene. *Drug News Perspect.* **1999**, *12*, 223–233.

(4) Colditz, G. A.; Hankinson, S. E.; Hunter, D. J.; Willett, W. C.; Manson, J. E.; Stampfer, M. J.; Hennekens, C.; Rosner, B.; Speizer, F. E. The use of estrogens and progestin and the risk of breast cancer in post-menopausal women. *N. Engl. J. Med.* **1995**, *332*, 1589–1593.

(5) Beresford, S. A.; Weiss, N. S.; Voigt, L. F.; McKnight, B. Risk of endometrial cancer in relation to use of oestrogen combined with cyclic progestagen therapy in postmenopausal women. *Lancet* **1997**, *349*, 458–461.

(6) Sun, W.; Cama, L. D.; Birzin, E. T.; Warrier, S.; Locco, L.; Mosley, R.; Hammond, M. L.; Rohrer, S. P. 6*H*-Benzo[*c*]chromen-6-one derivatives as selective ERbeta agonists. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1468–1472.

(7) O'Neill, K.; Chen, S.; Brinton, R. D. Impact of the selective estrogen receptor modulator, raloxifene, on neuronal survival and outgrowth following toxic insults associated with aging and Alzheimer's disease. *Exp. Neurol.* **2004**, *185*, 63–80.

(8) Yamaguchi, M.; Gao, Y. H. Inhibitory effect of genistein on bone resorption in tissue culture. *Biochem. Pharmacol.* **1998**, *55*, 71–76.

(9) Delcanale, M.; Amari, G.; Armani, E.; Lipreri, M.; Civelli, M.; Galbiati, E.; Giossi, M.; Caruso, P. L.; Crivori, P.; Carrupt, P.-A.; Testa, B. Novel basic isoflavones as inhibitors of bone resorption. *Helv. Chim. Acta* **2001**, *84*, 2417–2429.

(10) Wang, S. F.; Jiang, Q.; Ye, Y. H.; Li, Y.; Tan, R. X. Genistein derivatives as selective estrogen receptor modulators: sonochemical synthesis and in vivo anti-osteoporotic action. *Bioorg. Med. Chem.* **2005**, *13*, 4880–4890.

(11) Shino, A.; Tsukuda, R.; Odaka, H.; Kitazaki, T.; Tsuda, M.; Matsuo, T. Suppressive effect of ipriflavone on bone depletion in the experimental diabetic rat: dose response of ipriflavone. *Life Sci.* **1988**, *42*, 1123–30.

(12) Agnusdei, D.; Zacchei, F.; Bigazzi, S.; Cepollaro, C.; Nardi, P.; Montagnani, M.; Gennari, C. Metabolic and clinical effects of ipriflavone in established post-menopausal osteoporosis. *Drugs Exp. Clin. Res.* **1989**, *15*, 97–104.

(13) Reginster, J. Y. Ipriflavone: pharmacological properties and usefulness in postmenopausal osteoporosis. *Bone Miner.* **1993**, *23*, 223–232.

(14) Tseng, C. H.; Chen, Y. L.; Lu, C. M.; Wang, C. K.; Tsai, Y. T.; Lin, R. W.; Chen, C. F.; Chang, Y. F.; Wang, G. J.; Ho, M. L.; Tzeng, C. C. Synthesis and anti-osteoporotic evaluation of certain 3-amino-2-hydroxypropoxyisoflavone derivatives. *Eur. J. Med. Chem.* **2009**, *44*, 3621–3626.

(15) Zhu, M.; Kim, M. H.; Lee, S.; Bae, S. J.; Kim, S. H.; Park, S. B. Discovery of novel benzopyranyl tetracycles that act as inhibitors of osteoclastogenesis induced by receptor activator of NF-κB ligand. *J. Med. Chem.* **2010**, 8760–8764.

(16) Kamijo, T.; Ujiie, A.; Harada, H.; Tsutsumi, N.; Tsubaki, A.; Yamaguchi, T.; Nagata, H. Acylated Benzofuro[3,2-*c*]quinoline Compounds with Utility as Treatments for Osteoporosis. U.S. Patent 5,023,261, 1991.

(17) Tseng, C. H.; Chen, Y. L.; Lu, P. J.; Yang, C. N.; Tzeng, C. C. Synthesis and antiproliferative evaluation of certain indeno[1,2-*c*]-quinoline derivatives. *Bioorg. Med. Chem.* **2008**, *15*, 3153–3162.

(18) Tseng, C. H.; Chen, Y. L.; Chung, K. Y.; Cheng, C. M.; Wang, C. H.; Tzeng, C. C. Synthesis and antiproliferative evaluation of 6-arylindeno[1,2-*c*]quinoline derivatives. *Bioorg. Med. Chem.* **2009**, *17*, 7465–7476.

(19) Tseng, C. H.; Tzeng, C. C.; Yang, C. L.; Lu, P. J.; Chen, H. L.; Li., H. Y.; Chuang, Y. C.; Yang, C. N.; Chen, Y. L. Synthesis and antiproliferative evaluation of certain indeno[1,2-*c*]quinoline derivatives. Part 2. *J. Med. Chem.* **2010**, *53*, 6164–6179.

(20) Boyle, W. J.; Simonet, W. S.; Lacey, D. L. Osteoclast differentiation and activation. *Nature* **2003**, *423*, 337–342.

(21) Lee, J. H.; Jin, H.; Shim, H. E.; Kim, H. N.; Na, H.; Lee, Z. H. Epigallocatechin-3-gallate inhibits osteoclastogenesis by down-regulating c-Fos expression and suppressing the nuclear factor-κB signal. *Mol. Pharmacol.* **2010**, *77*, 17–25.