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A novel oxazine ring closure reaction affording (*Z*)-((*E*)-2-styrylbenzo[*b*]furo[3,2-*d*][1,3]oxazin-4-ylideno)acetaldehydes and their anti-osteoclastic bone resorption activity

Yuko Ando,[a] Kumiko Ando,[a] Mami Yamaguchi,[a] Jun-ichi Kunitomo,[a] Masao Koida,[b] Ryo Fukuyama,[c] Hiromichi Nakamuta,[c] Masayuki Yamashita,[d] Shunsaku Ohta[d] and Yoshitaka Ohishi[a,*]

[a]*Department of Medicinal and Synthetic Chemistry, Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11-68 Koshien Kyuban-cho, Nishinomiya 663-8179, Japan*
[b]*Department of Pharmacology, Faculty of Pharmaceutical Sciences, Setsunan University, 45-1, Nagaotoge cho, Hirakata, Osaka 573-1010, Japan*
[c]*Laboratory of Pharmacology, Department of Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, Hiroshima International University, 5-1-1 Hirokoshinkai, Kure, Hiroshima 737-0112, Japan*
[d]*Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8412, Japan*

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Abstract—A novel oxazine ring formation method was established using the reaction of 2-acetyl-(*E*)-3-styrylcarbonylaminobenzo[*b*]furans (**4**) with Vilsmeier–Haack–Arnold reagent to afford (*E* and *Z*)-((*E*)-2-styrylbenzo[*b*]furo[3,2-*d*][1,3]oxazin-4-ylideno)acetaldehydes (**5**). (*Z*)-4-(8-Bromo-(*E*)-2-styrylbenzo[*b*]furo[3,2-*d*][1,3]oxazin-4-ylideno)but-(*E*)-2-enoic acid ethyl ester (**6b**), derived from (*Z*)-**5a**, showed significantly potent anti-osteoclastic bone resorption activity comparable to 17β-estradiol (E_2).

Various oxazine compounds have been found to show versatile bioactivities.[1] This prompted us to establish a novel oxazine ring formation method to find promising bioactive oxazine compounds. We chose the Vilsmeier–Haack–Arnold reagent ($(CH_3)_2N^+{=}CHCl{\cdot}Cl^- \leftrightarrow (CH_3)_2N{-}C^+HCl{\cdot}Cl^-$) (VM reagent) which is extensively used for the formylation of activated aromatic, heteroaromatic, and carbonyl compounds.[2] We focused on the oxazine ring formation using aromatic *ortho*-keto amide compounds (2-acetyl-3-alkylcarbonylaminobenzo[*b*]furans) under the Vilsmeier reaction conditions. 2-Acetyl-3-cyanomethylcarbonylaminobenzo[*b*]furans (**1a**, **1b**) and 2-acetyl-3-ethoxycarbocarbonylaminobenzo[*b*]furan (**1c**) were treated with VM reagent at 24 °C. Unfortunately, 4-chloro-3-formylbenzo[*b*]furo[3,2-*b*]pyridines (**2**) were isolated in low yield, and no oxazine derivatives were obtained. These findings led us to hypothesize that the 3-amide moiety stabilized by a conjugate system (3-styrylcarbonylamino group) would be more advantageous for oxazine ring formation than the cyanomethylcarbonylamino and ethoxycarbocarbonylamino groups. We thus prepared four 2-acetyl-(*E*)-3-styrylcarbonylaminobenzo[*b*]furans (**4a**–**4d**) from 2-acetyl-3-aminobenzo[*b*]furans (**3a**, **3b**)[3] by reactions with *trans*-cinnamoyl chlorides. To a VM reagent prepared from $POCl_3$ with dry *N*,*N*-dimethylformamide (DMF) was added **4a** at 6 °C. The reaction mixture was stirred at 25 °C for 30 h and an orange precipitate was formed but was difficult to purify because of its chemical instability. A suspension of this precipitate in water was treated with 10% NaOH aqueous solution or triethylamine with vigorous stirring to give an orange powder. This orange powder was recrystallized from ethyl acetate–chloroform (5:1) to yield orange needles (**5a**, mp 213–216 °C, 46% (by treatment with triethylamine)). ^{1}H NMR (HMBC, HMQC), MS, and elemental

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* Corresponding author. Tel.: +798 45 9953; fax: +798 41 2792; e-mail: yohishi@mukogawa-u.ac.jp

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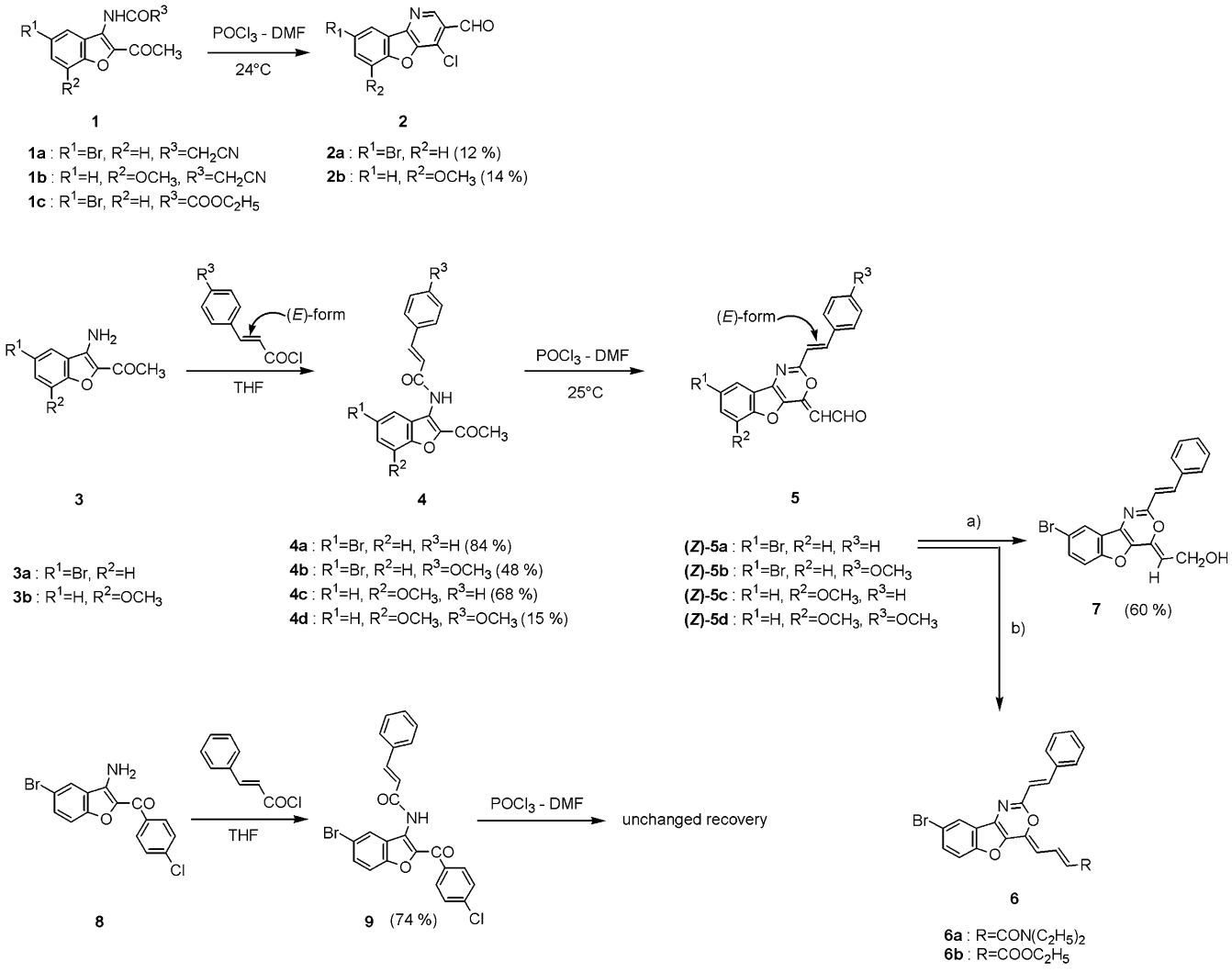
analysis data suggested **5a** to be a novel (*E* or *Z*)-(8-bromo-(*E*)-2-styrylbenzo[*b*]furo[3,2-*d*][1,3]oxazin-4-ylideno)acetaldehyde with a characteristic *exo*-formylmethylene group on the oxazine ring (Scheme 1).

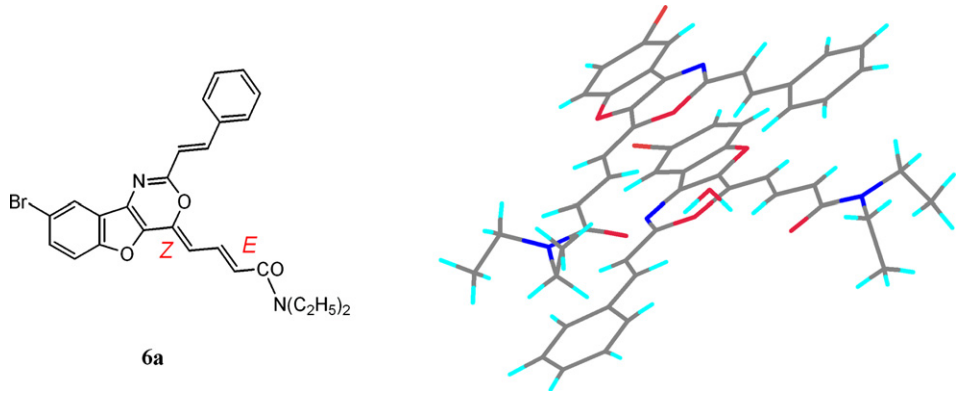
These findings were not sufficient to confirm the presence of an oxazine ring in **5a** and also did not define the isomeric form (*E* or *Z*) of the *exo*-formylmethylene group. Two butadiene derivatives (**6a**,**6b**) were prepared from **5a**, because of the difficulty of single crystal preparation of **5a** for X-ray analysis. The formyl group of **5a** was allowed to react with *N*,*N*-diethylphosphonoacetamide and ethyl diethylphosphonoacetate under the Horner–Wadsworth–Emmons (HWE) reaction conditions to give the corresponding butadiene derivatives (**6a**, **6b**) (Scheme 1). The structure of compound (**6a**) was confirmed to be (*Z*)-4-(8-bromo-(*E*)-2-styrylbenzo[*b*]furo[3,2-*d*][1,3]oxazin-4-ylideno)-*N*,*N*-diethylbut-(*E*)-2-enamide on the basis of its X-ray analysis as shown in Figure 1.[4] This result and the physical data of **5a** demonstrated it to be (*Z*)-(8-bromo-(*E*)-2-styrylbenzo[*b*]furo[3,2-*d*][1,3]oxazin-4-ylideno)acetaldehyde ((***Z***)-**5a**).

Compounds (**4b**–**4d**) were also treated with VM reagent to afford (***Z***)-**5b**, (***Z***)-**5c** and (***Z***)-**5d**, respectively. These oxazine ring closure reactions to **5** from **4** afforded mixtures consisting of the predominant *Z*-isomer and the *E*-isomer (*Z*-isomer:*E*-isomer = 98:2–95:5 by ^{1}H NMR). Each predominant (*Z*)-isomer was isolated from the respective mixture (Scheme 1) (Table 1). We were thus able to establish a novel oxazine preparation method based on the reaction of **4** with VM reagent. This oxazine cyclization reaction was markedly dependent on the chemical property of the 3-carbonylamino functional group. 3-Styrylcarbonylamino group was favorable for the oxazine ring formation.

(***Z***)-**5a** showed isomerization to its *E*-isomer ((***E***)-**5a**) in dimethylformamide or $CHCl_3$ solution. The more stable (***Z***)-**5a** reached an equilibrium with the less stable (***E***)-**5a** at the ratio of (***Z***)-**5a**:(***E***)-**5a** = 5:2 in these solutions after 15–48 h.[5] The isomerization of (***Z***)-**5a** to (***E***)-**5a** is considered to be caused by the formyl group.[6] Evidence for this came from the absence of isomerization of (*Z*)-2-(8-bromo-(*E*)-2-styrylbenzo[*b*]furo[3,2-*d*][1,3]oxazin-4-ylideno)ethanol (**7**) to its *E*-isomer. 5-Bromo-2-



Scheme 1. Reagents: (a) $NaBH_4$/THF; (b) $(C_2H_5O)_2P(O)CH_2CON(C_2H_5)_2$ and $(C_2H_5O)_2P(O)CH_2COOC_2H_5$, NaH/THF.



6a

Figure 1. Structure of (*Z*)-4-(8-bromo-(*E*)-2-styrylbenzo[*b*]furo[3,2-*d*][1,3]oxazin-4-ylideno)-*N*,*N*-diethylbut-(*E*)-2-enamide (**6a**) and its X-ray analysis.

Table 1. Physical data and yields of compounds (*Z*)-**5** and **6**

Compd	Yield (%)	Mp (°C)	^{1}H NMR	MS *m*/*z* (int.)	Formula HR-MS *m*/*z* M^+ Calcd (Found) or Anal. Calcd (Found)
(*Z*)-**5a**	46	213–216	δ_H(400 MHz; $CDCl_3$; Me_4Si) 5.72 (1H, d, *J* = 7.7, C*H*CHO), 6.79 (1H, d, *J* = 16.2, C*H*=CHC$_6$H$_5$), 7.43–7.45 (3H, m, 3′-, 4′-, 5′-H), 7.45 (1H, d, *J* = 8.5, 6-H), 7.59–7.62 (2H, m, 2′-, 6′-H), 7.63 (1H, dd, *J* = 8.8 and 1.8, 7-H) 7.77 (1H, d, *J* = 16.1, CH=C*H*C$_6$H$_5$), 8.05 (1H, d, *J* = 2.1, 9-H), 10.3 (1H, d, *J* = 8.1, CHC*H*O)	393 (M^+, 100.00)	$C_{20}H_{12}BrNO_3$ C; 60.93 (60.74) H; 3.07 (2.88) N; 3.55 (3.54)
(*Z*)-**5b**	35	215–218	δ_H(400 MHz; $CDCl_3$; Me_4Si) 3.87 (3H, s, OC*H*$_3$), 5.71 (1H, d, *J* = 8.1, C*H*CHO), 6.65 (1H, d, *J* = 16.1, C*H*=CHC$_6$H$_4$(4′-OCH$_3$)), 6.94–6.97 (2H, m, 2′-, 6′-H or 3′-, 5′-H), 7.45 (1H, d, *J* = 8.8, 6-H), 7.54–7.57 (2H, m, 2′-, 6′-H or 3′-, 5′-H), 7.62 (1H, d, *J* = 8.8 and 2.2, 7-H), 7.72 (1H, d, *J* = 16.1, CH=C*H*C$_6$H$_4$(4′-OCH$_3$)), 8.04 (1H, d, *J* = 1.8, 9-H), 10.3 (1H, d, *J* = 7.7, CHC*H*O)	423 (M^+, 99.72) 425 (100.00)	$C_{21}H_{14}BrNO_4$ C; 59.45 (58.92) H; 3.33 (3.11) N; 3.30 (3.10)
(*Z*)-**5c**	39	239–241	δ_H(400 MHz; $CDCl_3$; Me_4Si) 4.05 (3H, s, OC*H*$_3$), 5.81 (1H, d, *J* = 7.7, C*H*CHO), 6.82 (1H, d, *J* = 16.1, C*H*=CHC$_6$H$_5$), 7.04 (1H, dd, *J* = 8.1 and 0.8, 7-H or 9-H), 7.34 (1H, dd, *J* = 8.1 and 8.1, 8-H), 7.41–7.44 (3H, m, 3′-, 4′-, 5′-H), 7.49 (1H, dd, *J* = 7.9 and 0.9, 7-H or 9-H), 7.59–7.62 (2H, m, 2′-, 6′-H), 7.77 (1H, d, *J* = 16.2, CH=C*H*C$_6$H$_5$), 10.3 (1H, d, *J* = 8.1, CHC*H*O)	345 (M^+, 100.00)	$C_{21}H_{15}NO_4 \cdot 2/3H_2O$ C; 70.58 (70.40) H; 4.61 (4.01) N; 3.92 (3.96)
(*Z*)-**5d**	17	242–246	δ_H(400 MHz; $CDCl_3$; Me_4Si) 3.87 (3H, s, OC*H*$_3$), 4.05 (3H, s, OC*H*$_3$), 5.79 (1H, d, *J* = 8.0, C*H*CHO), 6.68 (1H, d, *J* = 16.1, C*H*=CHC$_6$H$_4$(4′-OCH$_3$)), 6.94–6.97 (2H, m, 2′-, 6′-H or 3′-, 5′-H), 7.04 (1H, dd, *J* = 7.9 and 1.0, 7-H or 9-H), 7.33 (1H, t, *J* = 8.1, 8-H), 7.48 (1H, dd, *J* = 7.9 and 1.0, 7-H or 9-H), 7.53–7.57 (2H, m, 3′-, 5′-H or 2′-, 6′-H), 7.72 (1H, d, *J* = 16.1, CH=C*H*C$_6$H$_4$(4′-OCH$_3$)), 10.3 (1H, d, *J* = 7.7, CHC*H*O)	375 (M^+, 100.00)	$C_{22}H_{17}NO_5$ C; 70.39 (70.24) H; 4.56 (4.41) N; 3.73 (3.77)
6a	49	191–192	δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.21–1.25 (6H, m, N(CH$_2$C*H*$_3$)$_2$ × 2), 3.44–3.52 (4H, m, N(C*H*$_2$CH$_3$)$_2$ × 2), 5.88 (1H, dd, *J* = 12.1 and 0.7, =C*H*CH=CHCO), 6.37 (1H, dd, *J* = 14.6 and 0.7, =CHCH=C*H*CO), 6.71 (1H, d, *J* = 16.1, C*H*=CHC$_6$H$_5$), 7.34 (1H, d, *J* = 8.8, 6-H), 7.37–7.43 (3H, m, 3′-, 4′-, 5′-H), 7.48 (1H, dd, *J* = 8.8 and 2.2, 7-H), 7.60-7.63 (2H, m, 2′-, 6′-H), 7.74 (1H, d, *J* = 16.1, CH=C*H*C$_6$H$_5$), 7.93 (1H, d, *J* = 2.2, 9-H), 7.95 (1H, dd, *J* = 14.6 and 12.1, CHC*H*=CHCO)	490 (M^+, 76.46), 391 (100.00)	$C_{26}H_{23}BrN_2O_3 \cdot 1/2\ H_2O$ C; 62.41 (62.17) H; 4.83 (4.57) N; 5.60 (5.59)
6b	71	199–200	δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.35 (3H, t, *J* = 7.1, OCH$_2$C*H*$_3$), 4.26 (2H, q, *J* = 7.2, OC*H*$_2$CH$_3$), 5.84 (1H, dd, *J* = 11.8 and 0.8, =C*H*CH=CHCO), 5.96 (1H, dd, *J* = 15.4 and 0.7, =CHCH=C*H*CO), 6.73 (1H, d, *J* = 16.1, C*H*=CHC$_6$H$_5$), 7.36 (1H, d, *J* = 8.8, 6-H), 7.39–7.45 (3H, m, 3′-, 4′-, 5′-H), 7.51 (1H, dd, *J* = 8.8 and 2.2, 7-H), 7.59-7.62 (2H, m, 2′-, 6′-H), 7.72 (1H, d, *J* = 16.2, CH=C*H*C$_6$H$_5$), 7.89 (1H, dd, *J* = 15.4 and 12.1, =CHC*H*=CHCO), 7.94 (1H, d, *J* = 1.9, 9-H)	463 (M^+, 100.00)	$C_{24}H_{18}BrNO_4$ C; 62.08 (62.03) H; 3.91 (3.71) N; 3.02 (2.90)

(4-chlorobenzoyl)-(*E*)-3-styrylcarbonylaminobenzo[*b*]furan (**9**)[3] prepared from **8**[3] was treated with VM reagent under the same reaction conditions as the reaction of **4a** with VM reagent. However, only starting material **9** was recovered unchanged (Scheme 1). This showed that reaction of the acetyl group with VM reagent is the driving force for the oxazine cyclization reaction. The formation mechanism of (*Z*)-**5** and (*E*)-**5** from **4** is likely to occur as follows. Both the enol 2-acetyl group and 3-carbonylamino group of **4** reacted with VM reagent to form **10**[2] which is converted into two geometrical isomers (**11A**, **11B**). Because the heat of formation of **12A** is lower than that of **12B**,[7] **11A** is converted faster to **12A** than **11B**. Both **12A** and **12B** produced labile immonium salts (**13**) consisting mostly of (*Z*)-**13** with a little (*E*)-**13**. The immonium salts (**13**) are deposited as an orange precipitate in the reaction mixture, as described above. Treatment of **13** with base afforded a mixture of (*Z*)-**5** (predominant isomer) and (*E*)-**5** (Scheme 2). The predominant formation of (*Z*)-**5** over (*E*)-**5** can be explained by the lower heat of formation of **12A** compared to **12B**.

The oxazine ring moiety of compounds **5** and **6** was similar to the ring type design devised from the (*Z*)-2-cyano-3-hydroxybut-2-enonylamino group of 2-(4-chlorobenzoyl)-(*Z*)-3-(2-cyano-3-hydroxybut-2-enonyl)aminobenzo[*b*]furan[3] which had potent anti-osteoclastic bone resorption activity in vitro and exhibited an anti-osteoporosis effect in vivo in our recent work.[8] Therefore, representative compounds ((*Z*)-**5a**, **6a**, **6b**) were tested with an in vitro assay of anti-osteoclastic bone resorption activity. In coculture of fresh bone marrow preosteoclasts expressing the receptor activator of NF-κB (RANK) with calvarial osteoblasts that express the ligand for RANK (RANKL), bone resorbing osteoclasts developed and formed resorption pits on a dentin slice. PGE_2 stimulated pit formation, and estrogens (e.g., E_2)

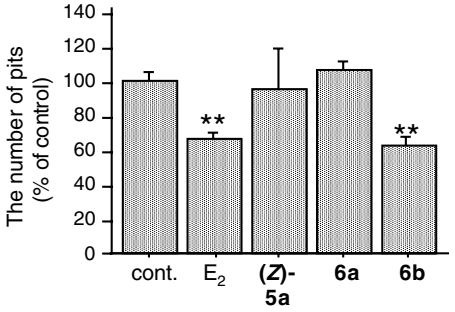
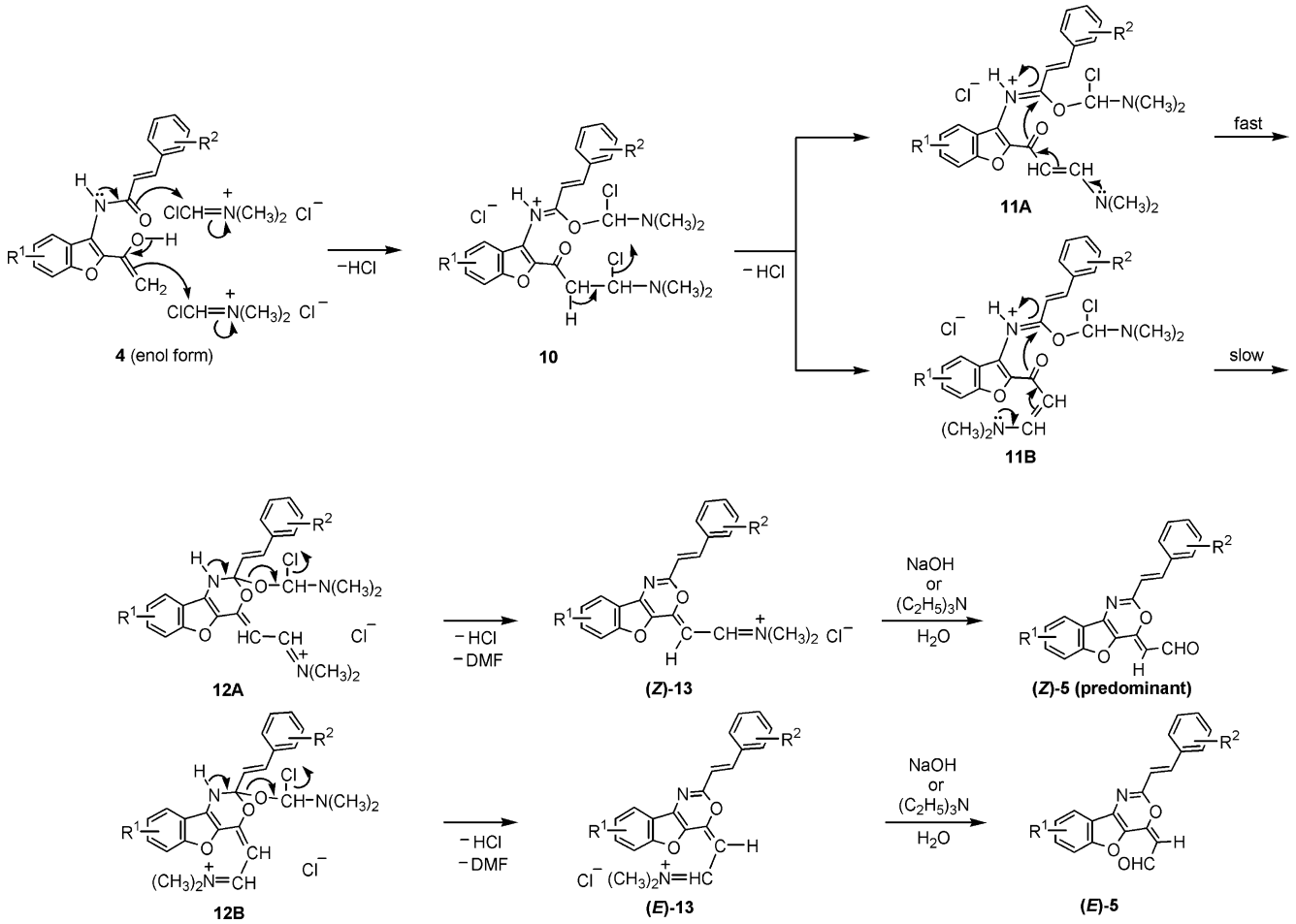


Figure 2. Anti-osteoclastic bone resorption activities of the oxazine derivatives ((*Z*)-**5a**, **6a**, **6b**) cont.: control E_2: 17β-estradiol.



Scheme 2. Proposed mechanism of formation of (*E* and *Z*)-((*E*)-2-styrylbenzo[*b*]furo[3,2-*d*][1,3]-oxazin-4-ylideno)acetoaldehydes (**5**) from **4**.

inhibited PGE_2-stimulated pit formation by suppressing the RANKL effect.[9] Among the compounds tested, the butadiene ester derivative (**6b**) showed significantly potent inhibition activity comparable to E_2, but the *exo*-formylmethylene compound ((***Z***)-**5a**) was inactive (Fig. 2).[10] This suggested that the butadiene moiety having particular polar functional groups might play an important role in inhibiting osteoclasts.

We found the novel compound (**6b**) which possesses significantly potent anti-osteoclastic bone resorption activity. It shows promise as a lead compound to develop new osteoporosis treatment agents, because of its significant potency and favorable balance of lipophilic and hydrophilic properties at the molecular level. In this work, we developed a novel oxazine ring preparation method by reaction of 2-acetyl-(*E*)-3-styrylcarbonylaminobenzo [*b*]furans (**4**) with VM reagent. This is a new application of the VM reaction. The (***Z***)-**5** prepared had the characteristic *exo*-formylmethylene group on the oxazine moiety.[11] This group enabled further synthesis of versatile oxazine compounds. The butadiene ester derivative (**6b**) prepared from (***Z***)-**5a** showed significantly potent anti-osteoclastic bone resorption activity comparable to E_2. The potent anti-osteoclastic bone resorption activity of oxazine derivative (**6b**) encouraged us to synthesize additional (*Z*)-benzo[*b*]furo[3,2-*d*][1,3]oxazin-4-ylidene derivatives having various butadiene groups and to evaluate their anti-osteoclastic bone resorption activity. Work continues on study of the mechanism of the inhibitory action of **6b**.

Acknowledgments

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4. Compound **6a** formula: $C_{26}H_{23}BrN_2O_3 \cdot 1/2\ H_2O$, formula weight: 500.39, crystal color, habit: red, platelet, crystal dimensions: $0.25 \times 0.05 \times 0.03$ mm, crystal system: monoclinic, lattice type: primitive, indexing images: 3 oscillations at 30.0 s, Detector position: 127.40 mm, Pixel size: 0.100 mm, lattice parameters: $a = 16.7921(11)$ Å, $b = 11.3491(7)$ Å, $c = 25.3011(16)$ Å, $\beta = 109.668(4)°$, $V = 4540.5(5)$ Å^3, space group: $P2_1/a$ (#14), Z value: 8, D_{calc}: 1.464 g/cm^3, F_{000}: 2056.00, μ(CuKα): 27.447 cm^{-1}. CCDC Deposit No. 600565.
5. The heat of formation of (***Z***)-5a was 0.5 kcal/mol lower than that of (***E***)-5a. The calculation was performed by using Spartan 2004, Wavefunction, Inc., Irvine, CA. Hehre J. W., 'A Guide to Molecular Mechanics and Quantum Chemical Calculations', Wavefunction, Irvine, 2003.
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7. The heat of formation of **12A** was about 2 kcal/mol lower than that of **12B**. The calculation was performed in a similar manner to that described in Ref. 5.
8. Paper in preparation. A part of this work was presented at the 'International Symposium of Maxillofacial and Oral Regenerative Biology in Okayama (Japan) 2005'. The proceeding version of the presentation was published in (Koida, M.; Nakamuta, H.; Ohishi, Y.) *J. Hard Tissue Biol.* (special issue) **2005**, *14*, 160.
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10. In vitro assay of anti-osteoclastic bone resorption activity: preconfluent primary calvarial osteoblasts from 1 to 2-day-old ddY mice (Japan SLC, Shizuoka, Japan) and fresh bone marrow cells from 5-week-old ddY male mice were cocultured in α-MEM (pH 7.0; Sigma Chemical Co., St. Louis, MO, USA) containing 10% fetal calf serum (FCS, Moregate, Australia and New Zealand), 10 nM calcitriol (Wako Pure Chemical Ind., Osaka, Japan), and 1.0 μM prostaglandin E_2 (PGE_2, Sigma Chemical Co.) on a 100 mm dish (Greiner, Tokyo, Japan) precoated with collagen (cell matrix Type I-A, Nitta Gelatin Inc., Osaka, Japan), for 7 days. The cells were then resuspended by collagenase (Wako Pure Chemical Ind.) digestion and each 0.30 ml suspension was plated over a dentin slice (10 mm in diameter and 200 μm in thickness) in 0.50 ml α-MEM (pH 7.0) containing 10% FCS and 20 mM HEPES on a well of a 48-well dish (Greiner) then subjected to 3 h of incubation. The dentin slice was transferred to a well of a 24-well dish filled with α-MEM (pH 7.0) containing 10% FCS and 20 mM HEPES for 3 days to examine pit formation. Each dentin slice was sonicated in 0.01 N NaOH, washed with water, and stained with 0.1% toluidine blue (Sigma Chemical Co.) in 1.0% sodium borate (Wako Pure Chemical Ind.) for pit counting. The average number of pits on a slice was 187 ± 8 in the control experiment ($n = 10$) and each value was expressed as the percentage to the corresponding control. All the data were expressed as means ± SE and the significance of the difference was analyzed by the Tukey–Kramer method at the level of $P < 0.05$.: Takahashi, N.; Akatsu,

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11. A few compounds somewhat similar to the oxazine derivatives prepared here have been reported. However the starting materials and reaction conditions were quite different from those of our preparation method. Costa, M.; Della Cà, N.; Gabriele, B.; Massera, C.; Salerno, G.; Soliani, M. *J. Org. Chem.* **2004**, *69*, 2469. Echavarren, M. A. *J. Org. Chem.* **1990**, *55*, 4255.