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Preparation of 2- and 4-(2-alkylcarbamoyl-1-methylvinyl)-7-alkyloxybenzo[*b*]furans having potent antagonistic activity against human leukotriene B_4 BLT_1 and/or BLT_2 receptors†

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(*E*)-2-Acetyl-4-(2-diethylcarbamoyl-1-methylvinyl)-7-(1-phenylethoxy)benzo[*b*]furan (4b) with a characteristic conformation and (*E*)-2-(2-morpholinocarbo-1-methyl-vinyl)-7-ethoxycarbopropoxybenzo[*b*]furan ((*E*)-3b) were prepared and evaluated for their leukotriene B_4 (LTB_4) antagonistic activity. Compound 4b showed potent antagonistic activity against human BLT_1 and BLT_2 receptors. Compound (*E*)-3b displayed selective BLT_2 receptor antagonistic activity. Both compounds were inactive to cysteinyl LT receptors.

Leukotriene B_4 (LTB_4) plays important roles in the host defence system against infection and invasion of foreign bodies, however overproduction of LTB_4 is involved in various inflammatory diseases.[1] Thus, many attempts have been made to develop antagonists of LTB_4 as clinical drugs. Of particular interest are ONO-4057,[2a] ZK-158252,[2b] BIIL315[2c] and LY 293111[2d] (Fig. 1).

These antagonists may be grouped into two classes, aliphatic carbon chain types (ONO-4057 and ZK-158252) and ether types (BIIL315 and LY293111). No LTB_4 antagonist has yet been marketed in spite of the clinical application of an LTD_4 receptor antagonist (cysteinyl LT_1 receptor antagonist). Recently, a second LTB_4 receptor (BLT_2) was discovered and its molecular cloning was reported.[3] Current work on LTB_4 and its receptors suggest that LTB_4 selective antagonists may be applicable for treatment of arteriosclerosis,[4] rheumatoid arthritis[5] and pancreatic cancer,[6] as well as for immunosuppression[2c,7] of allograft rejection in organ transplantation. This encouraged us to find novel BLT_2 selective and dual BLT_1/BLT_2 selective receptor antagonists. BLT_2 selective antagonists may be also useful for clarifying the bioactive roles of the BLT_2 receptor in the body.

Simple heteroaromatic skeleton compounds of stable conformation may well be favored over the aliphatic carbon chain type and the ether type compounds.[8] Benzopyran[8b,8c] and dibenzofuran[8d] derivatives have been reported previously as LTB_4 antagonists. Here we used the benzo[*b*]furan derivative to search for novel LTB_4 antagonists. The combination of conjugated triene and a single C=C bond near one OH group of possible LTB_4 conformers (**A**, **B**) was based on consideration of the benzo[*b*]furan ring having an $O(CH_2)_3COOR$ group originating from the partial structure of LTB_4. The α,β-unsaturated carbamoyl group[9] modified from cinnamic acid proved to be an interesting functional group showing cysteinyl LTs antagonistic activity in our recent study.[10] Several 2-alkylcarbamoyl-1-methylvinyl groups[11] devised from the α,β-unsaturated carbamoyl group were introduced at C-2 or C-4 of the benzo[*b*]furan ring. Introduction of functional groups at C-2 or C-4 is preferable to other positions to examine the relationship between the substituent position and bioactivity because the C-2 and C-4 positions have significantly different stereochemical and electronic environments. We report here the

† Electronic supplementary information (ESI) available: Experimental details for compounds **3a**, **3b**, **3f**, **4b** and **4c**. See http://www.rsc.org/suppdata/ob/b4/b411286e/

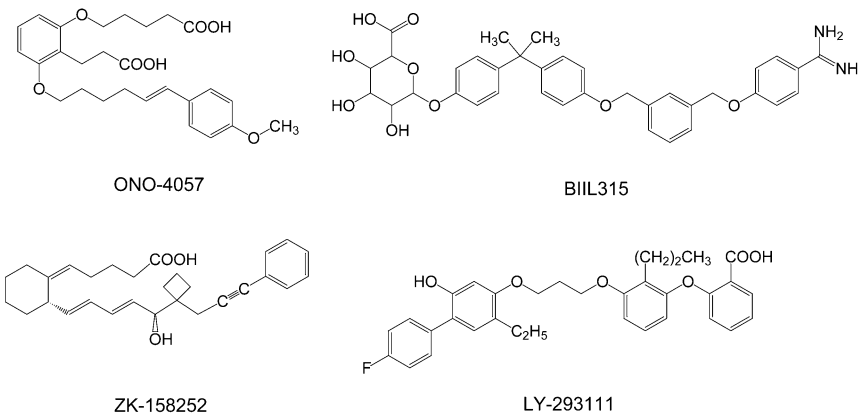

Fig. 1

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structural characteristics of **I** and **II** and their LTB_4 antagonistic activity characterized by the potency and selectivity for human BLT_1 and/or BLT_2 receptors (designed compounds **I** and **II**, Fig. 2).

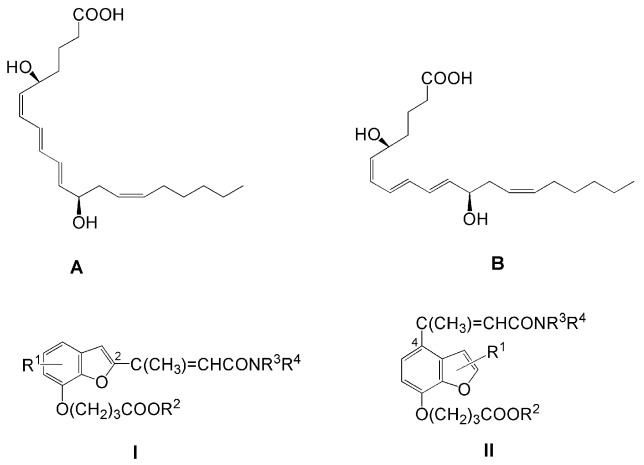


Fig. 2 Possible conformers (**A**, **B**) of LTB_4 and designed benzo[*b*]furan derivatives (**I**, **II**).

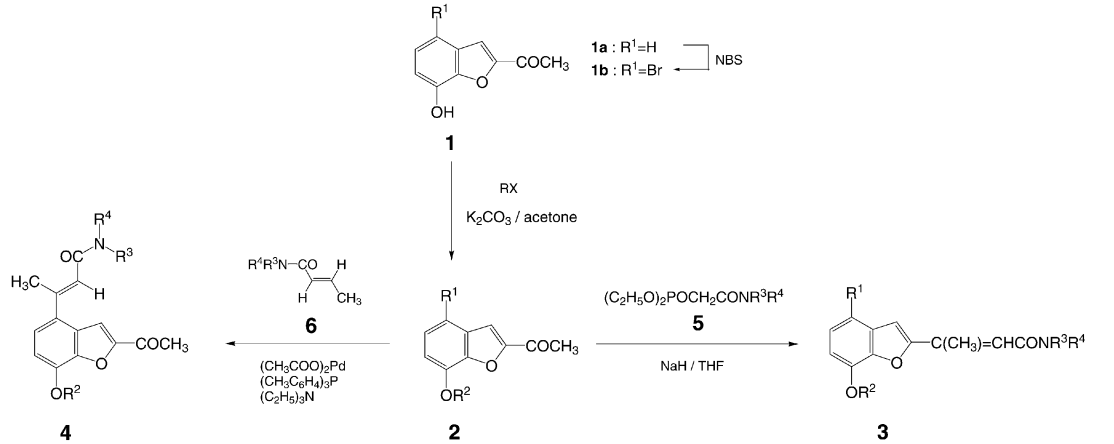
Alkylation of 2-acetyl-7-hydroxybenzo[*b*]furans (**1a**, **1b**) with alkyl halides gave the 7-alkyloxybenzo[*b*]furans (**2a**, **2c**–**2e**). Treatment of **2a** with phosphonoamides (**5**)[12] under Horner–Wadsworth–Emmons (HWE) reaction[13] conditions afforded (*E*/*Z*)-2-(2-alkylcarbamoyl-1-methylvinyl)benzo[*b*]furans ((*E*/*Z*)-**3a**, -**3b**, -**3c**). Compounds **2b**, **2c** and **2f**[14] were also subjected to HWE reaction with the phosphonoamide (**5**) to give (*E*/*Z*)-2-(2-alkylcarbamoyl-1-methylvinyl)benzo[*b*]furans ((*E*/*Z*)-**3d**, -**3e** and -**3f**), respectively. Yields and ratios of *E*/*Z* of **3** are shown in Scheme 1. (*E*)-Isomers ((*E*)-**3a**, -**3b**, -**3c**, -**3e**, -**3f**) were isolated from the corresponding *E*/*Z* mixtures ((*E*/*Z*)-**3a**, -**3b**, -**3c**, -**3e**, -**3f**). The (*E*)-isomers (*E*)-**3** showed only a nuclear Overhauser enhancement (NOE) correlation between the olefinic CH_3 and 3-H. This could be explained reasonably, since the double bond of the (*E*)-2-(2-alkylcarbamoyl-1-methylvinyl) group had an s-*trans* configuration with a double bond between C-2 and C-3, and also this group lay on approximately the same plane as the benzo[*b*]furan ring.[15] The stereostructure of (*E*)-**3f**, as a representative compound of (*E*)-**3**, was determined by X-ray analysis as shown in Fig. 3,[16] with the torsion angle being 6.9°.[15] Conformation and NOE correlation of (*E*)-**3b** are shown in Fig. 4. The main isomer of (*E*/*Z*)-**3d** was isolated and identified as s-*trans*-(*Z*)-isomer, (*Z*)-**3d**, on the basis of its ^{1}H-NMR data.

Compounds **2c**, **2d** and **2e** were treated with 2-butenamides (**6**) in the presence of $(CH_3COO)_2Pd$, $(2\text{-}CH_3C_6H_4)_3P$ and $(C_2H_5)_3N$ under the Heck coupling conditions[11b,17] to afford (*E*)-2-acetyl-4-(2-alkylcarbamoyl-1-methylvinyl)-7-alkyloxybenzo[*b*]furans (**4a**–**4d**) on the basis of their NOE analyses (Scheme 1).

In the NOE of compounds **4**, it was very interesting that both the olefinic CH_3 and olefinic H had NOE correlations with 3-H and 5-H, respectively. Consideration of the NOE correlations led to speculation concerning the molecular dissymmetry of **4** arising from restriction of the rotation about the single bond at the C-4.[18] However, this speculation was rejected by the absence of the diastereoisomer of **4b** which contained one asymmetric carbon atom in the substituent group at C-7 on the basis of ^{1}H-NMR studies. The torsion angle of representative compound **4c** between the benzo[*b*]furan plane and the 2-alkylcarbamoyl-1-methylvinyl group at C-4 estimated using MM2[15] was 44.7°. The X-ray structure of **4c** is shown in Fig. 3,[16] with the torsion angle being 45.7°. Conformation and NOE correlation of **4c** are shown in Fig. 4. The 2-alkylcarbamoyl-1-methylvinyl groups of compounds (*E*)-**3** and **4** showed distinctly different conformations from each other, because the 2-alkylcarbamoyl-1-methylvinyl group of compound **4** was subjected to significant steric hindrance from 3-H and 5-H, while there was little hindrance from 3-H on compound (*E*)-**3** (Fig. 4).

Compounds ((*E*)-**3**, **4**) were evaluated for their LTB_4 antagonistic activity by two *in vitro* methods: Method A,[19] inhibition of LTB_4-induced TXB_2 release from bronchoalveolar eosinophils of guinea pigs and Method B, inhibition of calcium mobilization in CHO-humanBLT_1 (CHO-hBLT_1) and CHO-humanBLT_2 (CHO-hBLT_2) cells by LTB_4.

Method A. Compounds (*E*)-**3a**, -**3b**, -**3c**, -**3d**, -**3e**, **4a**, **4b**, **4c** and **4d** at 100 μM completely inhibited LTB_4 (100 nM)-induced TXB_2 release from the bronchoalveolar eosinophils. Compounds (*E*)-**3a**, -**3b** and **4b** even at 1 μM produced complete inhibition, but **4c** showed 84% inhibition at the same concentration. Furthermore, the concentration-dependent antagonistic activity for (*E*)-**3a**, -**3b** and **4b** was evaluated at the concentrations of 0.1 nM, 1 nM, 10 nM, 100 nM and 1 μM. The most potent compound (*E*)-**3a**



	yield (%)
4a : R^2=$(CH_2)_3COOC_2H_5$, R^3=R^4=C_2H_5	97.0
4b : R^2=$CH(CH_3)C_6H_5$, R^3=R^4=C_2H_5	48.7
4c : R^2=$CH(C_6H_5)_2$, R^3=R^4=C_2H_5	31.7
4d : R^2=$CH(C_6H_5)_2$, R^3=$CH_2CH_2OCH_2CH_2$=R^4	15.8

1) $ClSO_3H$, 2) $C_2H_5NH_2$

		yield (%)	*E* / *Z*
2a : R^1=H, R^2=$(CH_2)_3COOC_2H_5$	(***E*/*Z*)-3a** : R^1=R^3=H, R^2=$(CH_2)_3COOC_2H_5$, R^4=C_6H_4(4-OCH_3)	96.9	72 / 28
2b : R^1=$SO_2NHC_2H_5$, R^2=$(CH_2)_3COOC_2H_5$	(***E*/*Z*)-3b** : R^1=H, R^2=$(CH_2)_3COOC_2H_5$, R^3=$CH_2CH_2OCH_2CH_2$=R^4	37.6	68 / 32
2c : R^1=Br, R^2=$(CH_2)_3COOC_2H_5$	(***E*/*Z*)-3c** : R^1=R^3=H, R^2=$(CH_2)_3COOC_2H_5$, R^4=$CH_2CH_2C_6H_3$(3-, 4-OCH_3)	87.2	81 / 19
2d : R^1=Br, R^2=$CH(CH_3)C_6H_5$			
2e : R^1=Br, R^2=$CH(C_6H_5)_2$	(***E*/*Z*)-3d** : R^1=$SO_2NHC_2H_5$, R^2=$(CH_2)_3COOC_2H_5$, R^3=R^4=C_2H_5	11.0	22 / 78
2f : R^1=H, R^2=CH_3	(***E*/*Z*)-3e** : R^1=Br, R^2=$(CH_2)_3COOC_2H_5$, R^3=R^4=C_2H_5	90.0	72 / 28
	(***E*/*Z*)-3f** : R^1=H, R^2=CH_3, R^3=$CH_2CH_2OCH_2CH_2$=R^4	78.0	57 / 43

Scheme 1

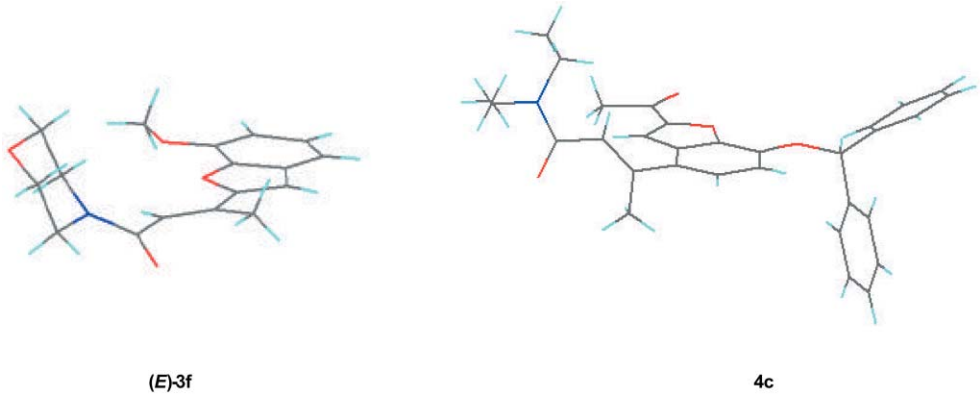


Fig. 3 X-Ray structures of (*E*)-**3f** and **4c**.

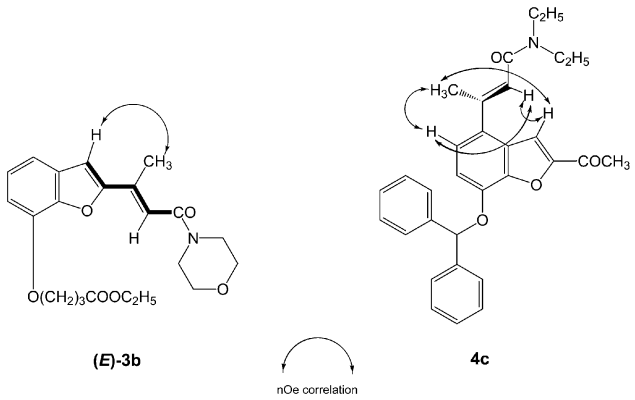


Fig. 4 Conformation and NOE correlations of (*E*)-**3b** and **4c**.

showed almost complete inhibition at 100 nM and 1 μM, and 26.8% inhibition at 10 nM. Compounds (*E*)-**3b** and **4b** were less active than (*E*)-**3a**: 18.7 and 17.1% inhibition at 10 nM, and 37.7 and 31.7% inhibition at 100 nM, respectively (Fig. 5).[20]

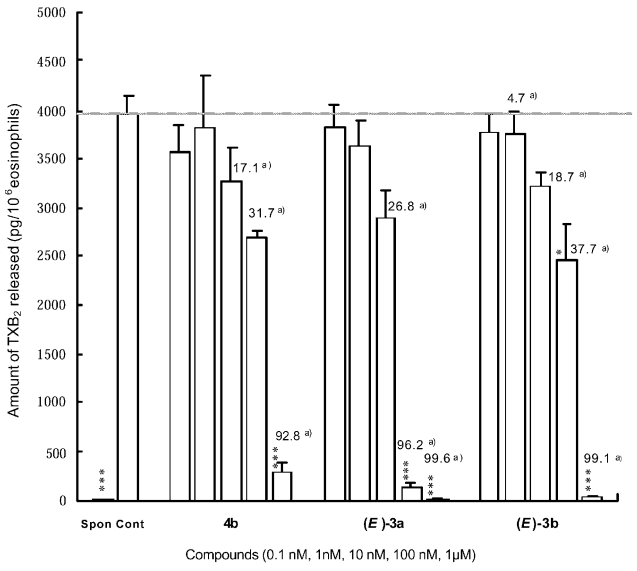


Fig. 5 Effect of (*E*)-**3a**, (*E*)-**3b**, **4b** on LTB_4-induced TXB_2 release from bronchoalveolar eosinophils harvested from Sephadex G-200-treated with guinea pigs (mean ± S.E., $n = 3$). (*E*)-**3a**, (*E*)-**3b** and **4b** was added 5 min before eosinophil stimulation by LTB_4 (100 nM). Statistically significant differences from the control are indicated ($*P < 0.05$, $***P < 0.001$, Bonferroni's multiple test). Spon: Spontaneous, Cont: Control, a) Inhibition (%).

Method B. Three compounds ((*E*)-**3a**, **-3b**, **4b**) selected from the findings with Method A were evaluated by Method B at concentrations of 10 nM, 100 nM, 1 μM and 10 μM, in comparison with standard LTB_4 antagonists (ZK-158252 and ONO-4057). Compound **4b** showed the most potent and concentration-dependent inhibition of calcium mobilization in both CHO-$hBLT_1$ and CHO-hBLT2 cells and its potency lay between ZK-158252 and ONO-4057. Characteristically, (*E*)-**3b** showed selective and concentration-dependent inhibition in CHO-$hBLT_2$ cells (Fig. 6).[21] In contrast, (*E*)-**3a** was found to be inactive by Method B.

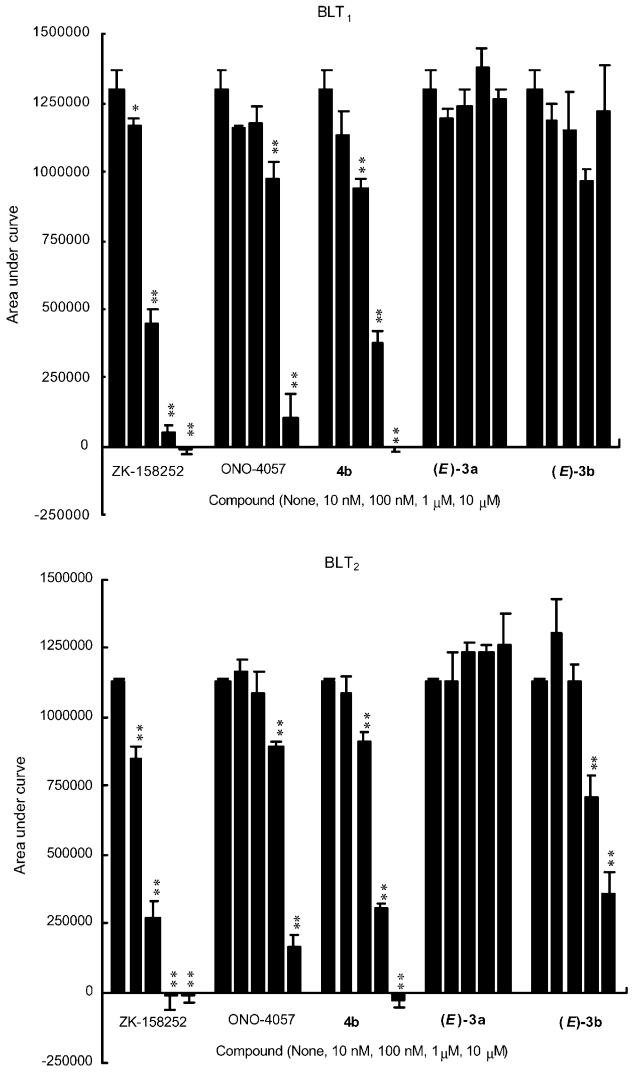


Fig. 6 Effect of (*E*)-**3a**, (*E*)-**3b**, **4b** on calcium mobilization by LTB_4 (100 nM) in CHO-hBLT1 and CHO-hBLT2 cells (mean ± S.D., $n = 3$). Statistically significant differences from the control are indicated ($*P < 0.05$, $**P < 0.01$, unpaired *t*-test).

To prove that inhibition of calcium mobilization was not due to a simple cytotoxic effect, cytotoxicity studies using ATP were performed.[22] Cysteinyl LT antagonistic activity assay[23] for the active compounds ((*E*)-**3a**, -**3b**, **4b**) was also carried out to examine their selectivity for LTB_4 antagonistic activity, and they were found to be inactive. Compound **4b**, shown to be moderately active by Method A was revealed to be the most potent compound with greater potency than the standard compound ONO-4057 using both human BLT_1 and BLT_2 in Method B. Unfortunately, the most active compound (*E*)-**3a** according to Method A was completely inactive when examined by Method B. Thus, the substituent position (at C-4) and the conformation of the 2-alkylcarbamoyl-1-methylvinyl group described above for the structure of **4** may contribute to its antagonistic potency against human BLT receptors. On the other hand, (*E*)-**3b** with the 2-morpholinocarbo-1-methylvinyl group at the C-2, which lies on nearly the same plane as the benzo[*b*]furan ring showed selective activity for human BLT_2. Compound (*E*)-**3b** is, to the best of our knowledge, the first antagonist showing selective BLT_2 antagonistic activity.

In this study, we found a potent human BLT_1 and BLT_2 receptor antagonist, (*E*)-2-acetyl-4-(2-diethylcarbamoyl-1-methylvinyl)-7-(1-phenylethoxy)benzo[*b*]furan (**4b**), and a BLT_2 selective antagonist, (*E*)-2-(2-morpholinocarbo-1-methylvinyl)-7-ethoxycarbopropoxybenzo[*b*]furan ((*E*)-**3b**). The next step would be to synthesize new derivatives and evaluate them to find more potent and selective (2-alkylcarbamoyl-1-methylvinyl)benzo[*b*]furan derivatives. Such work should clarify the relationships between the selective antagonist activities and the stereochemistry of the functional groups in a series of these derivatives.

Experimental

(*E*)-2-(2-Morpholinocarbo-1-methylvinyl)-7-ethoxycarbopropoxybenzo[*b*]furan ((*E*)-3b)

To a suspension of NaH (60% in oil, 0.24 g, 6.1 mmol) in anhydrous THF (10 ml) was added dropwise a solution of [2-(4-morpholinyl)-2-oxoethyl]phosphonic acid diethyl ester (1.6 g, 6.2 mmol) in anhydrous THF (10 ml) under N_2 atmosphere at −5 °C with stirring. The solution was then stirred at 25 °C until it became clear. A solution of **2a** (1.0 g, 3.4 mmol) in anhydrous THF (15 ml) was added dropwise at 25 °C, and the mixture was stirred at 25 °C for 3 h. The reaction mixture was worked up by an ordinary procedure to obtain a residue which was purified by silica gel column chromatography [$CHCl_3$–ethyl acetate (10 : 1)] to give (*E*)-**3b** (0.34 g, 24.6%) as colorless needles. Mp 90.9–94.8 °C.

(*E*)-2-Acetyl-4-(2-diethylcarbamoyl-1-methylvinyl)-7-(1-phenylethoxy)benzo[*b*]furan (4b)

A mixture of **2d** (1.0 g, 2.8 mmol), (*E*)-*N*,*N*-diethyl-2-butenamide (0.47 g, 3.3 mmol), palladium acetate (0.031 g, 0.14 mmol), tri-*o*-tolylphosphine (0.085 g, 0.28 mmol) and Et_3N (10.0 ml, 0.072 mol) was heated at 90–100 °C for 16 h. The precipitate was dissolved with ethyl acetate, and the insoluble portion was filtrated off. The filtrate was evaporated to dryness. The residue was poured into ice water, then made acid with 5% HCl solution and extracted with ethyl acetate. The organic layer was washed with brine and dried. The solvent was evaporated off, and the resulting residue was purified by silica gel column chromatography [hexane–ethyl acetate (5 : 1)] to give a yellow solid. The solid was recrystallized from hexane–ethyl acetate to give **4b** (0.57 g, 48.7%) as yellow prisms. Mp 118.4-121.5 °C.

Measurement of calcium mobilization in CHO cells

CHO cells stably expressing human BLT_1[24] and BLT_2,[3e] seeded on 96-well glass-bottom plate (Coster 3603) at 4×10^4 cells per well, were loaded with 4 μM Fluo-3 (Dojin, Kumamoto, Japan) in 1 × HBSS (Hanks balanced salt solution, Sigma) containing 0.04% pluoronic acid and 1% FCS at 37 °C for 1 h. The cells were washed twice with 1 × HBSS and pretreated with various concentrations of antagonists diluted in 100 μl of 1 × HBSS, 1% FCS for 30 min. A stock of BLT antagonists was prepared as a DMSO solution, and the final concentration of DMSO in the assay was adjusted to 0.1% in all wells. To each well was added 50 μl of 300 nM LTB_4 (Cayman Chemicals) to give a final concentration of 100 nM, and the LTB_4-dependent increase in the fluorescent intensity was measured using FlexStation (Molecular Devices). CHO cells transfected with empty vector did not respond to 100 nM LTB_4 (data not shown).

Acknowledgements

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16 **3f**: Formula: $C_{17}H_{19}NO_4$, formula weight: 301.34, crystal system: monoclinic, space group: $P\,2_1/a$ (# 14), a = 7.309(1) Å, b = 15.546(1) Å, c = 13.8909(9), β = 104.340(1)°, V = 1529.1(3) Å^3, Z = 4, D_{calc} = 1.309 g cm^{-3}, F_{000} = 640.00, μ(Cu-Kα) = 7.68 cm^{-1}, λ(Cu-Kα) = 1.54178 Å, ω–2θ scans at 23 °C, 2754 unique reflections (2θ < 135.1°), $R1$ = 0.066 [2240 reflections to calc. $R1$]. **4c**: Formula: $C_{31}H_{31}NO_4$, formula weight: 481.59, crystal system: triclinic, space group: $P\bar{1}$ (# 2), a = 10.259(1) Å, b = 16.120(2) Å, c = 8.654(2) Å, α = 104.45(1)°, β = 105.78(1)°, γ = 96.235(8)°, V = 1309.5(3) Å^3, Z = 2, D_{calc} = 1.221 g cm^{-3}, F_{000} = 512.00, μ(Cu-Kα) = 6.42 cm^{-1}, λ(Cu-Kα) = 1.54178 Å, ω–2θ scans at 23 °C, 4727 unique reflections (2θ < 135.20°), $R1$ = 0.059 [2503 reflections to calc. $R1$]. CCDC reference numbers 245784 (**3f**) and 236333 (**4c**). See http://www.rsc.org/suppdata/ob/b4/b411286e/ for crystallographic data in .cif or other electronic format.

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Compound	IC_{50} for $hBLT_1$/M	IC_{50} for $hBLT_2$/M
ZK-158252	5.4×10^{-8}	3.1×10^{-8}
ONO-4057	6.2×10^{-6}	4.7×10^{-6}
4b	4.2×10^{-7}	4.8×10^{-7}
(*E*)-**3a**	$>10^{-5}$	$>10^{-5}$
(*E*)-**3b**	$>10^{-5}$	8.3×10^{-7}

22 Inhibition of calcium mobilization in CHO-$hBLT_2$ by (*E*)-**3b** was not due to its cytotoxicity, because this compound at 10 μM did not affect calcium mobilization in CHO-$hBLT_1$ (Fig. 6). We also have confirmed that the inhibition by these compounds was not due to their cytotoxicity because they did not affect ATP-dependent calcium mobilization through intrinsic ATP receptors in CHO cells at the concentrations of BLT inhibition (data not shown).

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