ARTICLE

OBC
www.rsc.org/obc

Synthesis of 2-, 4- and 5-(2-alkylcarbamoyl-1-methylvinyl)-7-alkyloxybenzo[*b*]furans and their leukotriene B_4 receptor antagonistic activity†‡

Kumiko Ando,[a] Eriko Tsuji,[a] Yuko Ando,[a] Jun-ichi Kunitomo,[a] Reina Kobayashi,[b] Takehiko Yokomizo,[b,c] Takao Shimizu,[b] Masayuki Yamashita,[d] Shunsaku Ohta,[d] Takeshi Nabe,[d] Shigekatsu Kohno[d] and Yoshitaka Ohishi*[a]

[a] *School of Pharmaceutical Sciences, Mukogawa Women's University, 11-68 Koshien Kyuban-cho, Nishinomiya 663-8179, Japan. E-mail: yohishi@mwu.mukogawa-u.ac.jp; Fax: +798 41 2792; Tel: +798 45 9953*

[b] *Department of Biochemistry and Molecular Biology, Faculty of Medicine, The University of Tokyo, 7-3-1 Hongo Bunkyo-ku, Tokyo, 113-0033, Japan*

[c] *PRESTO, 7-3-1 Hongo Bunkyo-ku, Tokyo 113-0033, Japan*

[d] *Kyoto Pharmaceutical University, Misasagi, Yamashinaku, Kyoto 607-8412, Japan*

Received 10th March 2005, Accepted 21st April 2005
First published as an Advance Article on the web 16th May 2005

Variable benzo[*b*]furan derivatives having (*E*)- and (*Z*)-2-alkylcarbamoyl-1-methylvinyl groups at the 2-, 4- and 5-positions and a carboxylpropoxy or (1-phenyl)ethoxy group at the 7-position were prepared to find novel and selective leukotriene B_4 (LTB_4) receptor antagonists. (*E*)-2-(2-Diethylcarbamoyl-1-methylvinyl)-7-(1-phenylethoxy)benzo[*b*]furan (**4v**) showed selective inhibition to the human BLT_2 receptor ($hBLT_2$). On the other hand, (*E*)-2-acetyl-4-(2-diethylcarbamoyl-1-methylvinyl)-7-(1-phenylethoxy)benzo[*b*]furan (**7v**) inhibited both human BLT_1 receptor ($hBLT_1$) and $hBLT_2$. The (*E*)-2-(2-diethylcarbamoyl-1-methylvinyl) group lay on approximately the same plane as the benzo[*b*]furan ring, whereas the (*E*)-4-(2-diethylcarbamoyl-1-methylvinyl) group had the torsion angle (45.7°) from the benzo[*b*]furan ring plane. However, the (*Z*)-(2-alkylcarbamoyl-1-methylvinyl)benzo[*b*]furans were inactive. The inhibitory activity depended on the conformation of the 2-diethylcarbamoyl-1-methylvinyl group.

Introduction

Leukotriene B_4 is a dihydroxy fatty acid produced mainly by macrophages and neutrophils. LTB_4 plays important physiological roles on leukocytes trafficking to the site of infection and clearance of invaded microorganisms. However, overproduction of LTB_4 is reported to cause inflammatory diseases including bronchial asthma and inflammatory bowel diseases.[2] Thus, much work has been done to prepare LTB_4 antagonists for clinical use as anti-inflammatory drugs. The structures of several antagonistic active compounds are presented in Fig. 1.[3]

The particularly interesting antagonists are grouped into two classes, aliphatic carbon chain types (LY-255283, ONO-4057, ZK-158252) and ether types (BIIL315, LY-29311). Unfortunately, no antagonist has yet been developed for clinical medicinal applications. Recently, a novel cell surface receptor (BLT_2) for LTB_4 has been isolated and its molecular cloning has also been established.[4] Current studies on LTB_4 antagonists and its receptors (BLT_1, BLT_2) suggest the possibility of the development of new clinical drugs for treating arteriosclerosis,[5] immunosuppression of allograft rejection in organ transplantation,[6] psoriasis,[7] cancer,[8] and rheumatoid arthritis.[9] Stable compounds with definite conformations should be favorable as antagonists. Thus, simpler heteroaromatic skeleton compounds should be better than aliphatic carbon chain type and ether type compounds which may have plural conformations.[10a–10g] Cyclization of three partial structures (conjugated triene, single C=C bond and OH group) originating from possible LTB_4 stable conformers (**A**, **B**) formed a benzo[*b*]furan ring with a $O(CH_2)_3COOH$ group. Cyclized aliphatic compounds as LTB_4 antagonists designed on the basis of the LTB_4 molecular structure were previously reported to be finding antagonistic active compounds.[10h] This study supported partially our design using a benzo[*b*]furan skeleton to find novel LTB_4 antagonists. We recently found the α,β-unsaturated carbamoyl group[11] to be an interesting bioactive functional group.[12] This led us to design and prepare benzo[*b*]furan derivatives with various 2-alkylcarbamoyl-1-methylvinyl groups[13] devised from the α,β-unsaturated carbamoyl group at C-2, C-4 and C-5. (Designed compounds **I**, **II**, **III** in Fig. 2.)

We describe here the synthesis of the designed compounds and their antagonistic potency and selectivity for $hBLT_1$ and/or $hBLT_2$.

Results and discussion

Chemistry

Bromination of 2-acetyl-7-hydroxybenzo[*b*]furan (**1a**)[14] with NBS in an equivalent amount and 2 equivalent amounts in the presence of $AlCl_3$ gave the 4-bromo compound (**1b**)[15] and the 4,6-dibromo compound (**1c**), respectively. Alkylation of **1** with various alkyl halides afforded the corresponding 7-alkyloxybenzo[*b*]furans (**2a–2q**) (Table S1, ESI†). Reactions of the 2-acetyl-7-alkyloxybenzo[*b*]furans (**2a**, **2c–2e**, **2g–2j**, **2n**, **2p**, **2q**) with *N*-monoalkyl or *N*,*N*-dialkyl diethylphosphonoacetamides (**3a–3g**)[16] prepared in our laboratories in the presence of NaH under Horner–Wadsworth–Emmons (HWE) reaction conditions[17] afforded the corresponding (*E*/*Z*)-2-(2-alkylcarbamoyl-1-methylvinyl)-7-alkyloxybenzo[*b*]furans (**4a–4z**, **4α**) (Scheme 1).

Most of the (*E*/*Z*)-mixtures (**4**) could be separated into *E*- and *Z*-isomers (Table S2, ESI†). The *E*-isomers (**4**) showed only a nuclear Overhauser enhancement (NOE) correlation between CH_3 on the vinyl group (olefinic CH_3) and 3-H, and the olefinic CH_3 of *Z*-isomers (**4**) showed NOE correlations

† Electronic supplementary information (ESI) available: Tables S1–S6. See http://www.rsc.org/suppdata/ob/b5/b503615a/
‡ See ref. 1

DOI:10.1039/b503615a

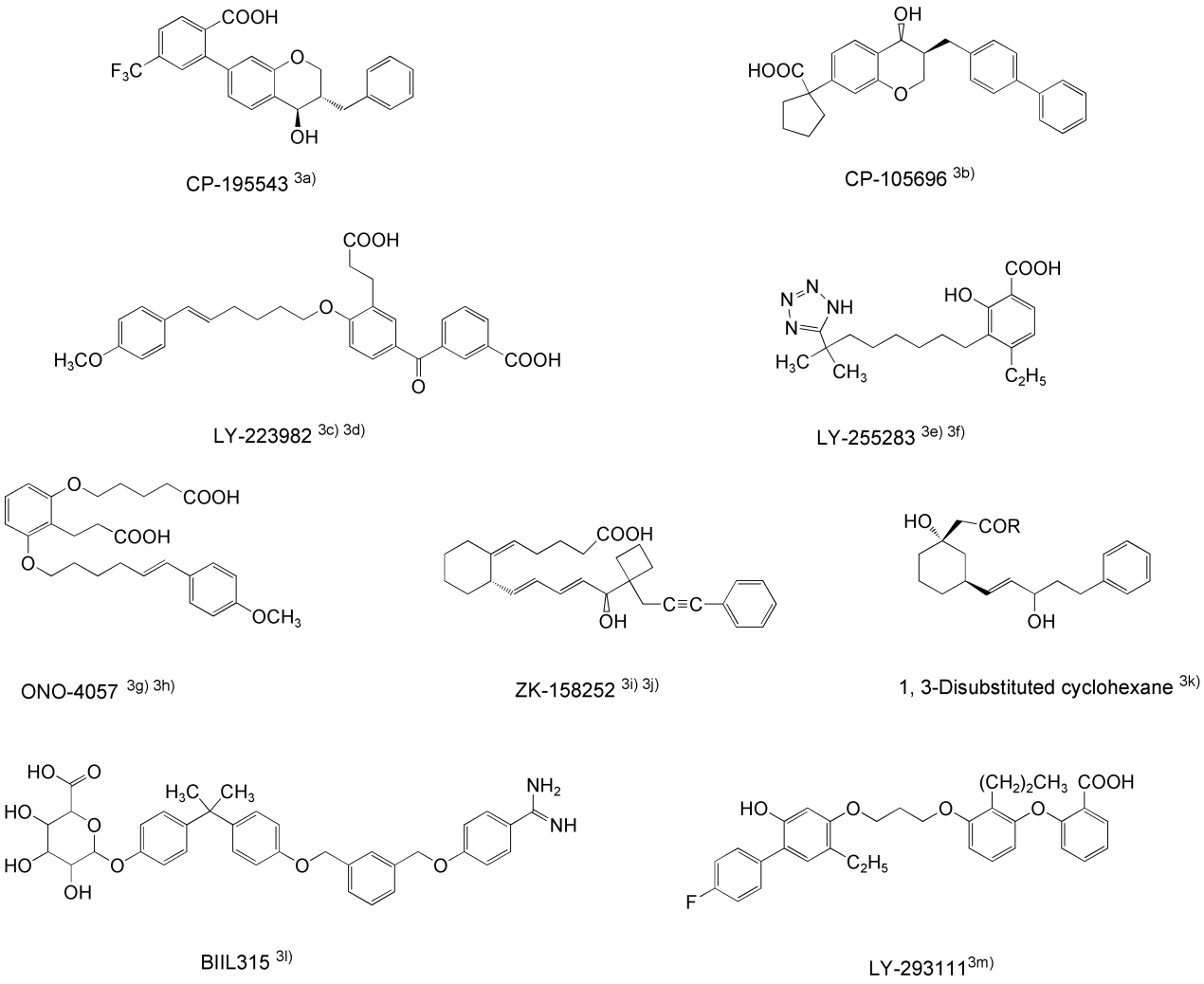


Fig. 1 Structures of reported LTB_4 receptor antagonists.

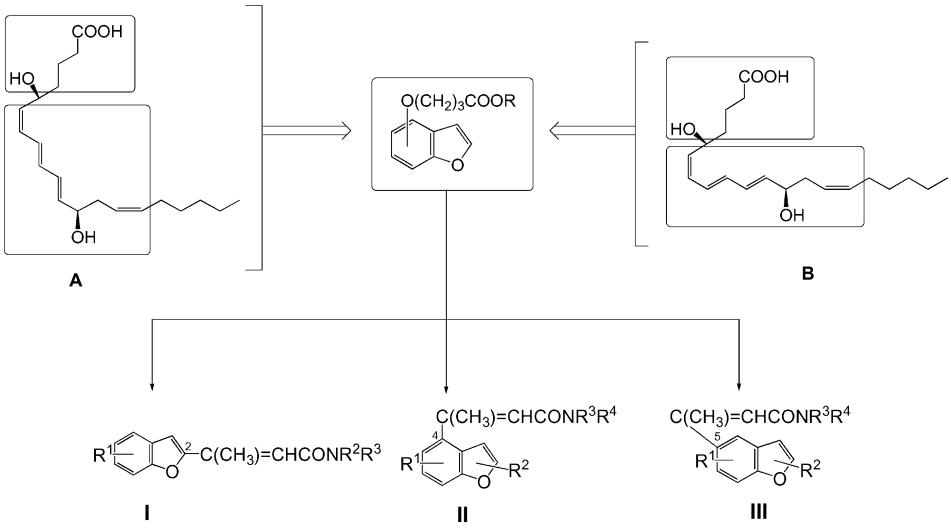
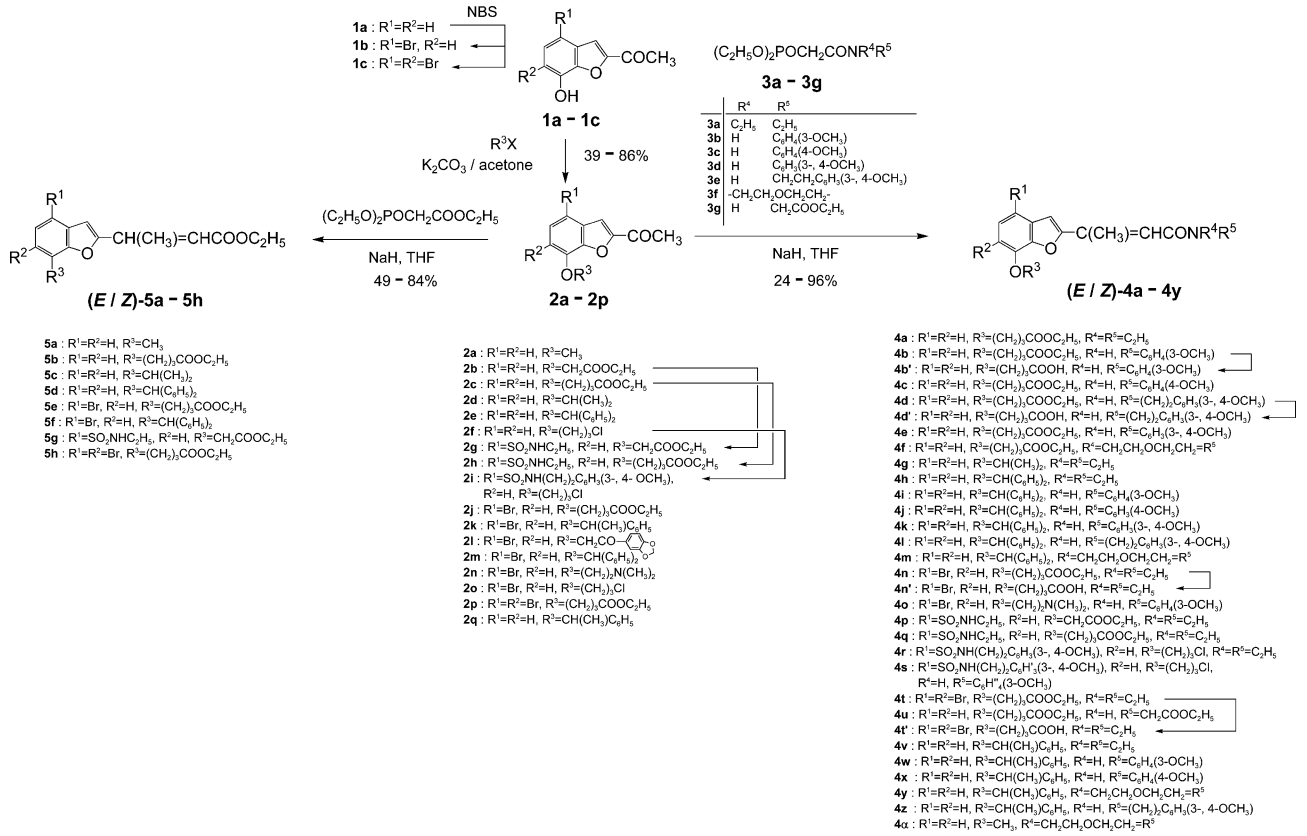


Fig. 2 Designed benzo[*b*]furan compounds (**I**, **II**, **III**) and possible conformers (**A**, **B**) of LTB_4.

with both 3-H and olefinic H in their ^{1}H-NMR. These NOE data showed that the carbon–carbon double bond of both the (*E*)- and (*Z*)-2-(2-alkylcarbamoyl-1-methylvinyl) group had an s-*trans* configuration with a double bond between C-2 and C-3. The structures of (*E*)-**4α** and (*Z*)-**4α**, as representative compounds, and their NOE correlations are shown in Fig. 3. It was interesting that (*Z*)-isomers (**4**) were easily converted into a mixture (1 : 1) of the (*E*)-isomer and the (*Z*)-isomer at room temperature when exposed to light. Estimation of the torsion angle between the (*E*)- and (*Z*)-2-(2-alkylcarbamoyl-1-methylvinyl) groups, and the benzo[*b*]furan ring plane was found to be 0–4° for the (*E*)-isomers and 25–27° for the (*Z*)-isomers.[18] The stereostructure of (*E*)-**4α** was confirmed by X-ray analysis (Fig. 4).[19] The torsion angle (6.9°) found by X-ray



Scheme 1

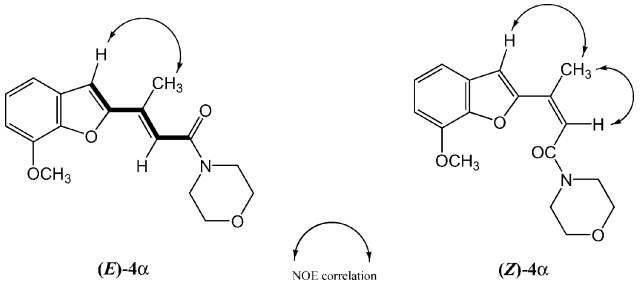


Fig. 3 Structures of (*E*)- and (*Z*)-**4α**, and NOE correlations.

analysis was close to the estimated value (4.4°). Thus, the 2-(2-alkylcarbamoyl-1-methylvinyl) group of (*E*)-isomer (**4**) lay on nearly the same plane as the benzo[*b*]furan ring.

E-isomers (**4**) were obtained preferentially under HWE reaction conditions using NaH (*E* selectivity: 51–100%) except for several sulfonylamide compounds (**4q–4s**) (*E* selectivity: 11–34%).[17a,20] LiCl and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) used in the place of NaH gave (*E*)-isomers (**4a**, **4c**, **4e**, **4i–4m**) more selectively (*E* selectivity: 67–100%) (Table S3, ESI†).[21] The reactions of several 2-acetylbenzo[*b*]furans (**2a**, **2c**, **2d**, **2e**, **2g**, **2j**, **2m**, **2p**) with ethyl diethylphosphonoacetate[22] instead of *N*-monoalkyl or *N*,*N*-dialkyl diethylphosphonoacetamides (**3**) in the presence of NaH or LiCl–DBU under HWE reaction conditions also resulted predominantly in the formation of *E*-isomers (**5a–5h**) (Scheme 1) (Tables S3 and S4, ESI†).

2-Acetyl-7-alkyloxy-4-bromobenzo[*b*]furans (**2j–2m**, **2o**) were treated with *N*-alkylcrotonamides (**6a–6e**) prepared in our laboratories in the presence of palladium acetate, tri-*o*-tolylphosphine and triethylamine under Heck coupling conditions selectively affording only (*E*)-2-acetyl-4-(2-alkylcarbamoyl-1-methylvinyl)-7-alkyloxybenzo[*b*]furans (**7a–7o**).[23] The 2-acetyl compound (**7a**) was derived to similar cinnamic acid compounds (**8b**) *via* ester (**8a**) (Scheme 2) (Table S5, ESI†).

Unlike (*E*)-**4**, the olefinic CH_3 and H of (*E*)-**7** showed particular NOE correlations with both 3-H and 5-H, respectively (Fig. 5). Some examination of the conformation of the 4-(2-alkylcarbamoyl-1-methylvinyl) group using MM2[18] and Dreiding Stereomodels (BÜCHI) revealed the great difficulty of free rotation of the vinyl carbon and C-4 because of

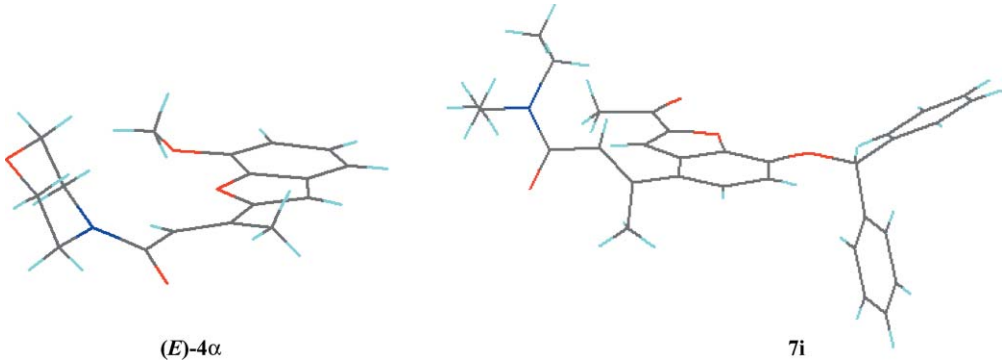


Fig. 4 X-Ray structures of (*E*)-**4α** and **7i**.

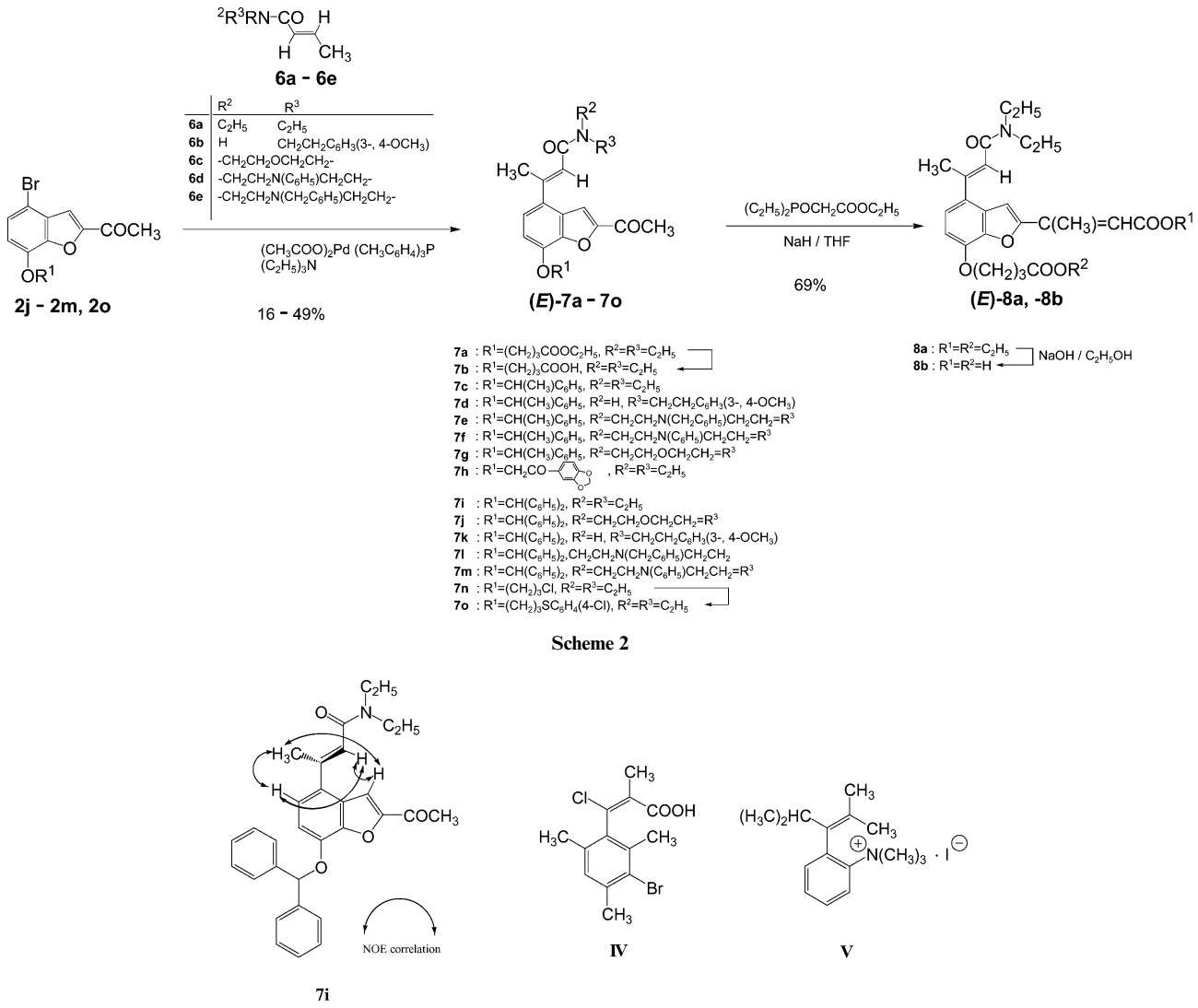


Fig. 5 NOE correlation and structure of **7i**, and structure of molecular dissymmetrical β-chloro-β-(2,4,6-trimethyl-3-bromophenyl)-α-methylacrylic acid (**IV**) and *o*-(β, β-dimethyl-α-isopropylvinyl)phenyltrimethylammonium iodide (**V**).

steric repulsion between the 4-(2-alkylcarbamoyl-1-methylvinyl) group and both 3-H and 5-H. Similar olefinic molecular dissymmetrical compounds, β-chloro-β-(2,4,6-trimethyl-3-bromophenyl)-α-methylacrylic acid (**IV**)[24] and *o*-(β,β-dimethyl-α-isopropylvinyl)phenyltrimethylammonium iodide (**V**)[25] were resolved readily through the appropriate salt because of the restriction of free rotation (Fig. 5).

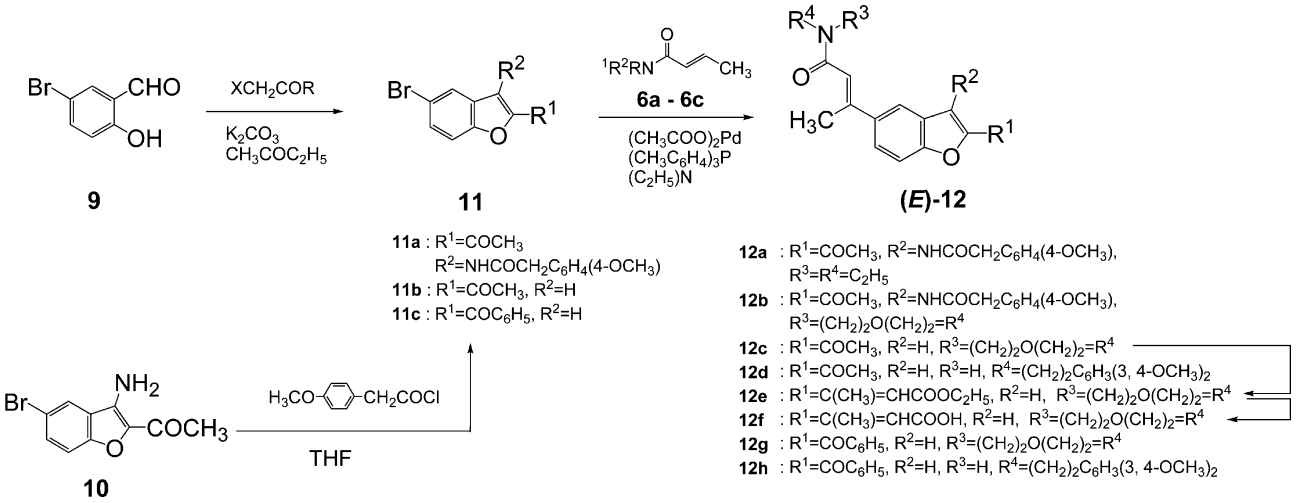
Thus, several 7-(1-phenylethoxy) derivatives (**7c–7g**) having an asymmetric carbon in the substituent group at C-7 were prepared to examine molecular dissymmetry due to restriction of free rotation between the vinyl carbon and C-4. However, no diastereoisomers of compounds (**7c–7g**) have been discovered through their ^{1}H-NMR. This suggested that restriction of the rotation was not enough to increase the molecular dissymmetry of **7**. It was presumably due to insufficient overlap of the 4-(2-alkylcarbamoyl-1-methylvinyl) group with both 3-H and 5-H.[26] The stereostructure of representative compound **7i** was determined by X-ray analysis as shown in Fig. 4.[19] The torsion angle between the 4-(2-diethylcarbamoyl-1-methylvinyl) group and the benzo[*b*]furan ring of **7i** was 45.7°. The torsion angle of several (*E*)-4-(2-alkylcarbamoyl-1-methylvinyl)-7-alkyloxybenzo[*b*]furans (**7**) was estimated as being 38.8–47.9° by MM2.[18,27] The conformation of the 4-(2-alkylcarbamoyl-1-methylvinyl) group considered from the torsion angle could account for the NOE relations of (*E*)-4-(2-alkylcarbamoyl-1-methylvinyl) compounds (**7**). These results demonstrated that the (*E*)-2-(2-alkylcarbamoyl-1-methylvinyl) group of **4** lay on approximately the same plane as the benzo[*b*]furan ring, while the (*E*)-4-(2-alkylcarbamoyl-1-methylvinyl) group of **7** was favorably located at 45° from the benzo[*b*]furan ring plane.

5-Bromobenzo[*b*]furans (**11a**, **11b**, **11c**) were prepared from **10**[12] and **9**.[28] Heck coupling of **11** using *N*-alkylcrotonamides (**6a**, **6b**, **6c**) selectively afforded (*E*)-5-(2-alkylcarbamoyl-1-methylvinyl)benzo[*b*]furans (**12a–12d**, **12g**, **12h**). The 2-acetyl compound (**12c**) was subjected to the HWE reaction to give **12e**, which was converted into **12f** by hydrolysis (Scheme 3) (Table S6, ESI†). In the ^{1}H-NMR spectrum of (*E*)-**12**, olefinic CH_3 and H showed NOE correlations with both 4-H and 6-H, respectively. The torsion angle between the (*E*)-5-(2-alkylcarbamoyl-1-methylvinyl group) and the benzo[*b*]furan ring of **12** was estimated as being 31.3–38.9° by MM2.

Our previous X-ray analysis of 2-(4-cyanobenzoyl)-3-(*Z*)-(2-cyano-3-hydroxybuto-2-enonyl)amino-5-(*E*)-(2-diethylcarbamoyl-1- methylvinyl)benzo[*b*]furan (torsion angle: 33.5°)[12b] supported these estimated values. These results suggested that the (*E*)-5-(2-alkylcarbamoyl-1-methylvinyl) group was less sterically hindered than that at C-4.

Bioactivity

Most of the compounds prepared were first evaluated for their LTB_4 antagonistic activity by Method A[29] examining the inhibition of LTB_4-induced TXB_2 release from bronchoalveolar eosinophils of guinea pigs. Fourteen compounds (**4b′**, **4c**, **4d**,



Scheme 3

Table 1 LTB_4 antagonistic activity (ratio of amount of TXB_2 released against control (%))

Compound[a]	Concentration 100 μM	1 μM[b]	10 nM[b]
4b′	0.2	0.3	47.9
4c	0.1	0.2	77.8
4d	2.7	40.9	57.1
4e	45.8	—	—
4f	0.5	0.3	48.6
4g	0.2	56.6	90.1
(*Z*)-**4g**	0.3	84.5	94.1
4h	16.0	—	—
4i	91.8	—	—
4j	118.6	—	—
4k	115.4	—	—
(*Z*)-**4k**	115.4	—	—
4l	127.3	—	—
4m	112.4	—	—
(*Z*)-**4m**	14.1	—	—
4n	1.4	64.7	87.2
4n′	0.07	44.9	96.3
4o	n. d.[c]	60.7	93.1
(*Z*)-**4q**	54.0	—	—
4r	39.0	—	—
(*Z*)-**4r**	60.7	—	—
4u	0.2	69.1	97.4
7a	45.0	—	—
7c	0.8	3.7	89.1
7d	41.9	—	—
7e	23.7	—	—
7f	81.7	—	—
7g	1.0	65.9	96.3
7h	32.1	—	—
7i	n. d.[c]	16.2	83.6
7j	10.2	—	—
7k	105.2	—	—
7l	122.0	—	—
7m	39.1	—	—
8a	101.3	—	—
8b	6.1	77.8	97.4
12a	56.7	—	—
12b	113.9	—	—

[a] Compounds without indication of (*Z*) are (*E*)-isomers. [b] Fourteen potent compounds were further evaluated at concentrations of 1 μM and 10 nM. [c] n. d.: no TXB_2 release was detected.

4f, **4g**, (*Z*)-**4g**, **4n**, **4n′**, **4o**, **4u**, **7c**, **7g**, **7i**, **8b**) at a concentration of 100 μM completely inhibited LTB_4 (100 nM)-induced TXB_2 release from the bronchoalveolar eosinophiles (Table 1). Next, these active compounds were evaluated at 1 μM and 10 nM and the (*E*)-2-(2-alkylcarbamoyl-1-methylvinyl) compounds (**4b′**, **4c**, **4f**) and (*E*)-4-(2-alkylcarbamoyl-1-methylvinyl) compound (**7c**) were found to be active at these concentrations. The concentration-dependent activity for these four compounds (**4b′**, **4c**, **4f**, **7c**) was evaluated at concentrations of 0.1 nM, 1 nM, 10 nM, 100 nM and 1 μM. The most active compound **4c** completely inhibited LTB_4-dependent TXB_2 release at 1 μM, and showed 96.2% inhibition at 100 nM and 26.8% inhibition at 10 nM. Other compounds, **4b′**, **4f** and **7c**, showed almost complete inhibition at 1 μM, but were less potent than **4c** at 100 nM (Fig. 6). These studies showed that (*E*)-2-(2-alkylcarbamoyl-1-methylvinyl) compounds (**4**) and (*E*)-4-(2-alkylcarbamoyl-1-methylvinyl) compound (**7**) were potent and (*E*)-5-(2-alkylcarbamoyl-1-methylvinyl) compounds (**12**) were inactive.[30]

Subsequently, a detailed study was achieved by evaluation of the inhibition activity for $hBLT_1$ and/or $hBLT_2$ (Method B, inhibition of calcium mobilization in both CHO cells overexpressing human BLT_1 (CHO–$hBLT_1$) and human BLT_2 (CHO–$hBLT_2$).[4e,31] At first, (*E*)-2-(2-alkylcarbamoyl-1-methylvinyl) compounds (**4a**, **4b′**, **4c**, **4d**, **4d′**, **4e**, **4f**, **4g**, **4n′**, **4u**, **4v**, **4w**, **4y**, **4z**), (*Z*)-2-(2-alkylcarbamoy-1-methylvinyl) compounds ((*Z*)-**4a**, (*Z*)-**4v**), (*E*)-4-(2-alkylcarbamoyl-1-methylvinyl) compounds (**7a**, **7c**, **7d**, **7g**, **7i**, **7j**, **7n**, **8b**) and (*E*)-5-(2-alkylcarbamoyl-1-methylvinyl) compound (**12c**) were evaluated at a concentration of 10 μM, and ten (*E*)-compounds (**4a**, **4b′**, **4d**, **4d′**, **4e**, **4f**, **4g**, **4v**, **4y**, **7c**) showed more than 70% inhibition of calcium mobilization in CHO–$hBLT_1$ and/or CHO–$hBLT_2$ cells. However, (*Z*)-2-(2-alkylcarbamoyl-1-methylvinyl) compounds ((*Z*)-**4a**, (*Z*)-**4v**) were inactive to both $hBLT_1$ and $hBLT_2$. Nine (*E*)-2-(2-alkylcarbamoyl-1-methylvinyl) compounds (**4a**, **4b′**, **4d**, **4d′**, **4e**, **4f**, **4g**, **4v**, **4y**) were more potent for $hBLT_2$ than $hBLT_1$. On the other hand, the (*E*)-4-(2-alkylcarbamoyl-1-methylvinyl) compound (**7c**) inhibited both $hBLT_1$ and $hBLT_2$. A combination of the (*E*)-2- or (*E*)-4-(2-alkylcarbamoyl-1-methylvinyl) group having a diethyl or morpholino on the nitrogen atom, and 1-phenylethoxy or 3-carboxypropoxy group at the 7-position was favorable for increasing the inhibitory activity. The potent compounds (**4a**, **4b′**, **4d**, **4f**, **4v**, **4y**, **7c**) were also evaluated for their antagonistic activity on cysteinyl leukotriene receptors and found to be inactive.[32] Unfortunately, the (*E*)-5-(2-alkylcarbamoyl-1-methylvinyl) compound (**12c**) was also found to be inactive by Method B, similar to the results of Method A (Table 2).

(*E*)-2-(2-Alkylcarbamoyl-1-methylvinyl) compounds (**4a**, **4b′**, **4d**, **4f**, **4v**, **4y**) and (*E*)-4-(2-alkylcarbamoyl-1-methylvinyl) compound (**7c**) selected based on the results of the above screening were evaluated at concentrations of 1 nM, 10 nM, 100 nM, 1 μM and 10 μM. All of the compounds evaluated exhibited dose-dependent inhibition of $hBLT_1$ and/or $hBLT_2$. The IC_{50} values of these compounds are shown in Table 3.

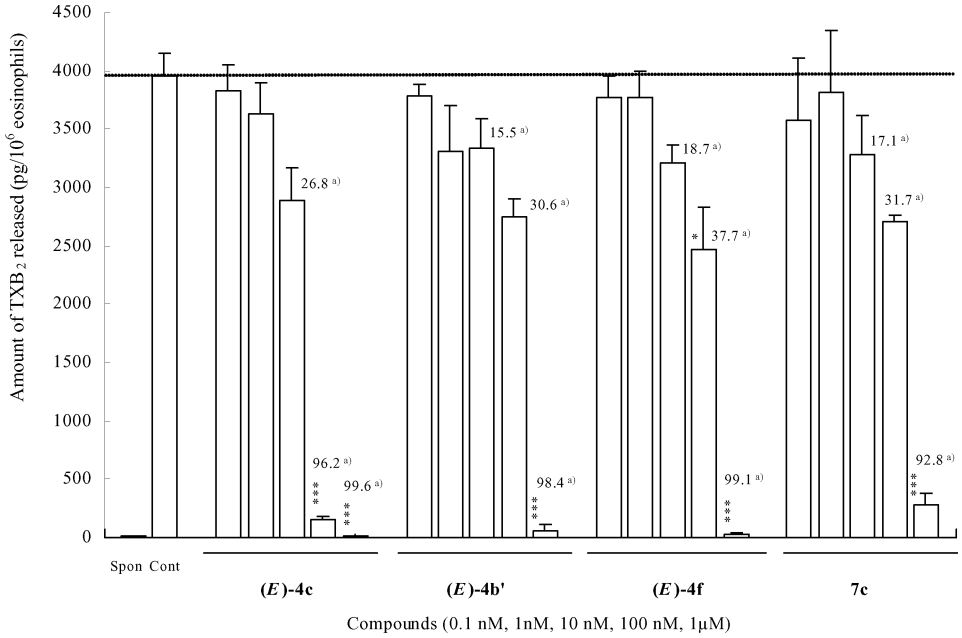


Fig. 6 Effect of (*E*)-**4c**, (*E*)-**4b**′, (*E*)-**4f**, **7c** on LTB_4-induced TXB_2 release from bronchoalveolar eosinophils harvested from Sephadex G-200-treated guinea pigs (mean ± S. E., $n = 3$). (*E*)-**4c**, (*E*)-**4b**′, (*E*)-**4f**, **7c** were added 5 min before eosinophil stimulation 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 (%).

Table 2 Inhibition of calcium mobilization in CHO–$hBLT_1$ and CHO–$hBLT_2$ cells at 300 nM LTB_4

Compound[e] (10 μM)	Inhibition (%)	
	CHO-$hBLT_1$	CHO-$hBLT_2$
4a	21.9	88.1
4b′[a]	35.8	71.7
4c[a]	1.7	6.6
4d	41.3	89.3
4d′	16.5	71.8
4e	22.1	83.9
4f[a]	24.1	72.1
4g	9.0	70.2
4n′	7.4	4.1
4u	7.0	34.6
4v	69.9	com.[b]
4w	n. i.[c]	5.0
4y	53.0	84.5
4z	8.6	53.6
(*Z*)-**4a**	9.2	34.4
(*Z*)-**4v**	8.4[d]	10.8[d]
7a	8.1	33.3
7c[a]	92.6	92.8
7d	10.8	29.7
7g	8.4	21.0
7i	12.6	9.3
7j	20.9	53.8
7n	19.2	33.3
8b	5.7	4.4
12c	8.0	4.8
ZK158252[a]	92.3	92.7
ZK158252	56.6	61.3

[a] Concentration of LTB_4: 100 nM. [b] Calcium mobilization was completely inhibited. [c] Not inhibited. [d] Concentration of (*Z*)-**4v**: 5 μM. [e] Compounds without indication of (*Z*) are (*E*)-isomers.

All (*E*)-2-(2-alkylcarbamoyl-1-methylvinyl) compounds (**4a**, **4b**′, **4d**, **4f**, **4v**, **4y**) showed BLT_2 selective inhibition. The most potent (*E*)-2-(2-diethylcarbamoyl-1-methylvinyl)-7-(1-phenylethoxy)benzo[*b*]furan (**4v**) inhibited $hBLT_2$ more than $hBLT_1$. The inhibition for $hBLT_2$ was superior to ZK-158252 but the inhibition for $hBLT_1$ was below ZK-158252. Interestingly, (*E*)-4-(2-alkylcarbamoyl-1-methylvinyl) compound (**7c**) showed high activity for both $hBLT_1$ and $hBLT_2$ (Table 3 and Fig. 7).

Table 3 IC_{50} for CHO-$hBLT_1$ and CHO-$hBLT_2$

Compound	IC_{50}/μM	
	CHO-$hBLT_1$	CHO-$hBLT_2$
(*E*)-**4a**[a]	—	4.84
(*E*)-**4d**[a]	—	1.78
(*E*)-**4y**[a]	$>10^{-5}$	7.97
(*E*)-**4v**[a]	2.88	0.68
ZK158252[a]	1.70	1.18
(*E*)-**4b**′[b]	$>10^{-5}$	6.41
(*E*)-**4f**[b]	$>10^{-5}$	0.83
7c[b]	0.42	0.48
ZK158252[b]	0.054	0.031

[a] Stimulated by LTB_4 at 300 nM. [b] Stimulated by LTB_4 at 100 nM.

To prove that inhibition of calcium mobilization was not due to a simple cytotoxic effect to the CHO cells, the effects of these compounds on ATP-dependent calcium mobilization were examined. Weak inhibition of calcium mobilization was observed only at a concentration of 10 μM, with no inhibition being detected at 1 μM.[33,34]

Conclusions

This study revealed a significant relationship between stereostructure and $hBLT_1$ and/or $hBLT_2$ inhibitory activity. (*E*)-2-(2-Alkylcarbamoyl-1-methylvinyl) compounds (**4a**, **4b**′, **4d**, **4f**, **4v**, **4y**) showed selective $hBLT_2$ inhibition, and their (*E*)-2-(2-alkylcarbamoyl-1-methylvinyl) groups lay on nearly the same plane as the benzo[*b*]furan ring. The (*E*)-4-(2-alkylcarbamoyl-1-methylvinyl) compound (**7c**) inhibited both $hBLT_1$ and $hBLT_2$, and its (*E*)-4-(2-alkylcarbamoyl-1-methylvinyl) group had the torsion angle (*ca.* 45°) from the benzo[*b*]furan ring plane. Both **4v** and **7c** had the same (*E*)-2-(2-diethylcarbamoyl-1-methylvinyl) group at C-2 or C-4, respectively, and the 1-phenylethoxy group at C-7. Thus, the difference of conformation of the (*E*)-(2-diethylcarbamoyl-1-methylvinyl) group may significantly affect the selectivity of the antagonistic activity to $hBLT_1$ and/or $hBLT_2$. To examine the relationship between the conformation of the (*E*)-(2-alkylcarbamoyl-1-methylvinyl) group and antagonistic activity, some novel benzo[*b*]furan derivatives having

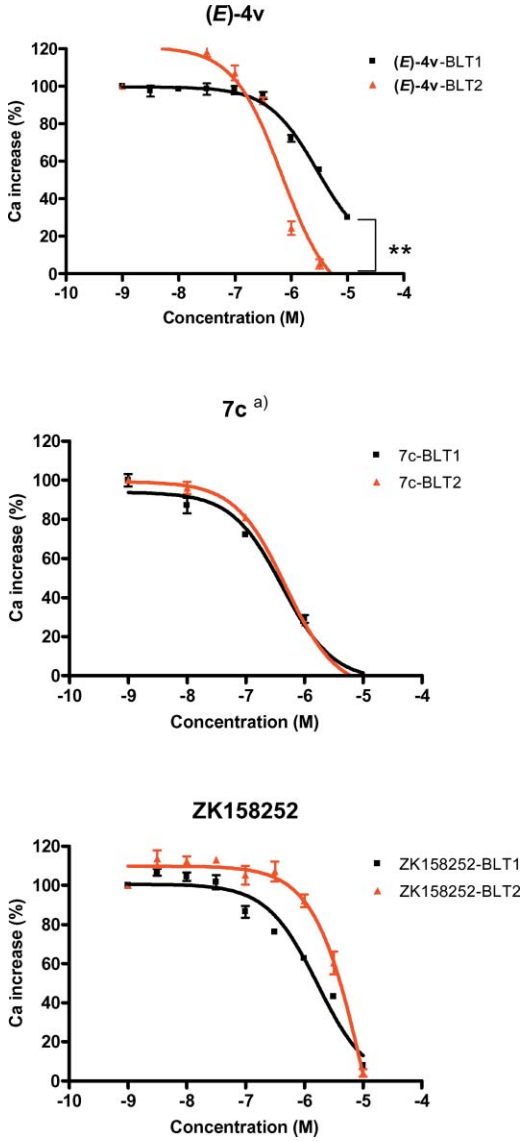


Fig. 7 Effect of (*E*)-**4v**, **7c**, **ZK158252** on calcium mobilization by LTB_4 (300 nM) in CHO–hBLT1 and CHO–hBLT2 cells (mean ± S. D., n = 3,4). a) Stimulated by LTB_4 at 100 nM. $^{**}p$ <0.001 (two-way ANOVA).

more characteristic conformations are being prepared and will be evaluated for their antagonistic activity.

Experimental

All melting points were determined using a Yanako microscopic hot-stage apparatus and are uncorrected. ^{1}H-NMR, ^{13}C-NMR, HMBC and HMQC spectra were obtained on a JEOL GSX-500 and a JNM-PMX 60 spectrometer with tetramethylsilane as an internal standard. MS spectra (MS, HRMS) were obtained using a JEOL JMS DX-303 EIMS spectrometer. Elemental analyses were performed on a CHN CORDER MT-3 (Yanako). All organic extracts were dried over anhydrous $MgSO_4$. Column chromatography was carried out on Wakogel C-200 (100–200). Thin layer chromatography was performed on an E. Merck silica gel plate (0.5 mm, 60F-254).

2-Acetyl-4-bromo-7-hydroxybenzo[*b*]furan (1b)

To a solution of **1** (7.3 g, 0.041 mol) in CH_2Cl_2 (300 ml) was added $AlCl_3$ (21.9 g, 0.16 mol) and the mixture was stirred for 1 h at 40 °C. Br_2 (8.0 g, 0.05 mol) was added dropwise to the reaction mixture over 1 h at 26 °C. The mixture was stirred for 3 h at 26 °C, poured into ice water, and treated with 6 N HCl solution (300 ml). The resulting precipitate was collected by filtration and washed with water, and recrystallized from ethyl acetate to give **1b** (9.8 g, 93.3%) as yellow needles: mp 224.3–226.6 °C; δ_H (500 MHz; DMSO; Me_3Si) 2.60 (3H, s, $COCH_3$), 6.91 (1H, d, J 8.6, 6-H), 7.35 (1H, d, J 8.6, 5-H), 7.74 (1H, s, 3-H), 10.7 (1H, br s, OH); m/z (EI) 253.9585 (M^+. $C_{10}H_7BrO_3$ requires 253.9579), 254 (M^+, 100.00%), 239 (89.88), 211 (17.03), 183 (27.65).

2-Acetyl-4,6-dibromo-7-hydroxybenzo[*b*]furan (1c)

To a solution of **1** (22.0 g, 0.125 mol) in CH_2Cl_2 (1200 ml) was added $AlCl_3$ (66.7 g, 0.5 mol) and the mixture was stirred for 1 h at 40 °C. Br_2 (40.0 g, 0.25 mol) was added dropwise over 45 min to the reaction mixture at 26 °C. The mixture was stirred for 16 h at 26 °C, and poured into ice water, and treated with 6 N HCl solution (500 ml). The resulting precipitate was collected by filtration and washed with water, then recrystallized from ethyl acetate to give **1c** (33.9 g, 81.8%) as colorless needles: mp 214.6–216.5 °C; δ_H (500 MHz; DMSO; Me_3Si) 2.60 (3H, s, $COCH_3$), 7.65 (1H, s, 5-H), 7.75 (1H, s, 3-H); m/z (EI) 331.8682 (M^+. $C_{10}H_6Br_2O_3$ requires 331.8684), 334 (100.00%), 332 (M^+, 60.78), 319 (89.98), 291 (10.79), 263 (21.07), 211 (17.03), 183 (27.65)

2-Acetyl-7-ethoxycarbonylmethoxybenzo[*b*]furan (2b)

General procedure for 2a, 2c–2f, 2q, 2j–2o, 2p. A mixture of **1a** (5.7 g, 32.4 mmol), K_2CO_3 (13.0 g, 93.9 mmol), and ethyl bromoacetate (3.8 ml, 34.1 mmol) in dry acetone (120 ml) was stirred at 54 °C for 2 h. After an insoluble portion was filtered off, the filtrate was distilled under reduced pressure. The ethyl acetate solution of the resulting residue was washed with 5% NaOH solution and brine, and dried. Concentration of the solution gave **2b** (5.1 g, 60.1%) as pale yellow needles.

In a similar manner to that described above, **1a** gave **2a**, **2c–2f**, **2q**, **1b** gave **2j–2o**, and **1c** gave **2p**.

2-Acetyl-7-(ethoxycarbonylmethoxy)-4-ethylsulfamoylbenzo[*b*]furan (2g)

General procedure for 2h, 2i. The fine powder **2b** (5.0 g, 0.019 mol) was carefully added to chlorosulfonic acid (10.1 ml, 0.152 mol) in portions at 26–30 °C over 3 h under vigorous stirring. After the mixture was stirred at the same temperature for 5 min, it was poured into ice water and extracted with ethyl acetate. The organic layer was washed with brine, then dried, and evaporated quickly to give the intermediate sulfonyl chloride as a yellow solid, R_f = 0.74, benzene–methanol (2 : 1), which was suitable for use in subsequent reactions. To a mixture of ethylamine hydrochloride (2.0 g, 0.082 mol) and triethylamine (4.5 g, 0.044 mol) in CH_2Cl_2 (30 ml) was added dropwise the sulfonyl chloride in dry $CHCl_3$ (30 ml) at 27–30 °C with stirring. After the mixture was stirred at 25 °C for 1 h, it was poured into ice water and made acidic with 5% HCl solution, then extracted with ethyl acetate. The extract was washed with brine, then dried. The solvent was evaporated off, giving a residue which was recrystallized from ethyl acetate to give **2g** (1.9 g, 27.0%) as yellow prisms.

In a similar manner to that described above, **2c** gave **2h**, and **2f** gave **2i**.

N, *N*-Diethyl diethylphosphonoacetamide (3a)

A mixture of *N*,*N*-diethylchloroacetamide (6.9 ml, 50.0 mmol) and triethyl phosphite (8.8 ml, 50.0 mmol) was stirred at 180 °C for 8 h. The reaction mixture was cooled to room temperature and distilled to give **3a** (8.8 g, 70.0%) as a colorless oil: bp_3 136–140 °C; δ_H (60 MHz; $CDCl_3$; Me_3Si) 0.90–1.40 (12H, m, CH_2CH_3 × 4), 3.00 (2H, d, J 20.2, PCH_2), 3.10–3.60 (4H, m, NCH_2CH_3 × 2), 3.92–4.50 (4H, m, OCH_2CH_3 × 2); m/z (EI) 251 (M^+, 24.24%), 179 (21.28), 79 (100.00).

Diethyl [2-(3-methoxyphenylamino)-2-oxoethyl]phosphonate (3b)

General procedure for 3c–3g. To a solution of *m*-anisidine (15.0 g, 0.12 mol) in dry THF (200 ml) was added dropwise chloroacetyl chloride (10.0 ml, 0.13 mol) in dry THF (20 ml) over 15 min at −5–0 °C. The reaction mixture was stirred at 0–10 °C for 3 h, poured into ice water, and extracted with ethyl acetate. The organic layer was washed with brine and dried. Concentration of the extract gave a brown solid. The solid was recrystallized from ethyl acetate to give a white solid (21.2 g, 92.0%, mp 83.0–85.0 °C). The solid and triethyl phosphite (18.8 ml, 0.12 mol) were stirred at 130 °C for 15 h. The reaction mixture was cooled to room temperature, and the resulting precipitate was recrystallized from ethyl acetate to give **3b** (21.9 g, 65.8%) as colorless prisms: mp 86.5–88.8 °C; δ_H (500 MHz; $CDCl_3$; Me_3Si) 1.36 (6H, t, *J* 7.3, $CH_2CH_3 \times 2$), 2.99 (2H, d, *J* 20.6, PCH_2), 3.79 (3H, s, OCH_3), 4.14–4.21 (4H, m, $CH_2CH_3 \times 2$), 6.65–7.26 (4H, m, 2-, 4-, 5-, 6-H); *m*/*z* (EI) 301 (M^+, 82.37%), 123 (100.00).

Diethyl [2-(4-methoxyphenylamino)-2-oxoethyl]phosphonate (3c)

Yield: 4.1 g (33.0%), white needles; mp 79.5–82.5 °C; δ_H (500 MHz; $CDCl_3$; Me_3Si) 1.35 (3H, t, *J* 6.9, CH_2CH_3), 1.35 (3H, t, *J* 6.9, CH_2CH_3), 2.98 (2H, d, *J* 20.6, PCH_2), 3.78 (3H, s, OCH_3), 4.18 (4H, m, $CH_2CH_3 \times 2$), 6.84 (2H, d, *J* 8.9, 2-, 6-H or 3-, 5-H), 7.43 (2H, d, *J* 8.9, 2-, 6-H or 3-, 5-H), 8.68 (1H, br s, NH); *m*/*z* (EI) 301 (M^+, 48.00%), 152 (18.67), 123 (100.00), 108 (36.59).

Diethyl [2-(3,4-dimethoxyphenylamino)-2-oxoethyl]phosphonate (3d)

Yield: 4.8 g (60.0%), pale yellow needles; mp 117.8–119.9 °C; δ_H (500 MHz; $CDCl_3$; Me_3Si) 1.30 (6H, t, *J* 7.0, $CH_2CH_3 \times 2$), 3.0 (2H, d, *J* 24.0, PCH_2), 3.80 (6H, s, $OCH_3 \times 2$), 4.10 (2H, q, CH_2CH_3), 4.25 (2H, q, CH_2CH_3), 6.75 (1H, d, *J* 8.0, 5-H), 6.98 (1H, d, *J* 2.0, 2-H), 7.25 (1H, dd, *J* 8.0 and 2.0, 6-H), 9.1 (1H, br s, NH); *m*/*z* (EI) 331 (M^+, 100.00%), 179 (4.42), 153 (56.15), 138 (39.55), 125 (26.99).

Diethyl [2-(2-(3,4-dimethoxyphenyl)ethylamino)-2-oxoethyl]phosphonate (3e)

Yield: 0.7 g (96.9%), white needles; mp 84.2–85.9 °C; δ_H (500 MHz; $CDCl_3$; Me_3Si) 0.95–1.43 (6H, m, $CH_2CH_3 \times 2$), 2.82 (2H, d, *J* 20.0, PCH_2), 2.60–3.00 (2H, m, NCH_2CH_2), 3.35–3.70 (2H, m, NCH_2CH_2), 3.80 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.90–4.35 (4H, m, $CH_2CH_3 \times 2$), 6.80–6.90 (3H, m, arom-H); *m*/*z* (EI) 359 (M^+, 12.37%), 164 (100.00), 151 (15.04).

Diethyl [2-(morpholino)-2-oxoethyl]phosphonate (3f)

Yield: 3.8 g (87.8%), yellow oil; δ_H (500 MHz; $CDCl_3$; Me_3Si) 1.32 (6H, m, $CH_2CH_3 \times 2$), 2.95 (2H, d, *J* 22.0, PCH_2), 3.50 (8H, br s, $NCH_2CH_2O \times 2$), 3.80–4.45 (4H, m, $CH_2CH_3 \times 2$); *m*/*z* (EI) 265 (M^+, 30.61%), 235 (70.43), 197 (40.07), 179 (100.00), 151 (48.13).

Diethyl [2-(3,4-dimethoxyphenylamino)-2-oxoethyl]phosphonate (3g)

Yield: 0.60 g (76.0%), yellow oil; δ_H (60 MHz; $CDCl_3$; Me_3Si) 1.00–1.60 (9H, m, $CH_2CH_3 \times 3$), 2.95 (2H, d, *J* 22.0, PCH_2), 3.90–4.40 (8H, m, $OCH_2CH_3 \times 3$, NCH_2), 7.80 (1H, br s, NH); *m*/*z* (EI) 281 (M^+, 25.71%), 236 (19.45), 208 (52.91), 179 (100.00), 152 (17.48).

(*E*/*Z*)-2-(Diethylcarbamoyl-1-methylvinyl)-7-(3-ethoxycarbopropoxy)benzo[*b*]furan (4a)

General procedure for 4b–4z, 4α. To a suspension of NaH (60% in oil, 0.23 g, 6.2 mmol) in anhydrous THF (10 ml) was added dropwise a solution of *N*,*N*-diethyl phosphonoacetamide (1.6 g, 6.2 mmol) in anhydrous THF (10 ml) under an N_2 atmosphere at −3 °C with stirring. The solution was then stirred at 25 °C until it became clear. A solution of **2c** (1.5 g, 5.2 mmol) in anhydrous THF (10 ml) was added dropwise to the clear solution at 25 °C, and the mixture stirred at 25 °C for 4 h. The mixture was then quenched with H_2O, and evaporated. The residue was added to saturated NH_4Cl solution and extracted with ethyl acetate. The organic layer was washed with brine, then dried. The solvent was evaporated off, giving a residue which was purified with silica gel column chromatography [$CHCl_3$–ethyl acetate (5 : 2)] to give *E*-**4a** (0.62 g, 30.8%) as a colorless oil, and *Z*-**4a** (0.16 g, 8.0%) as a colorless oil.

In a similar manner to that described above, **2c** gave **4b–4f**, **4u**, **2e** gave **4g–4m**, **2j** gave **4n**, **2n** gave **4o**, **2g** gave **4p**, **2h** gave **4q**, **2i** gave **4r**, **4s**, **2p** gave **4t**, **2q** gave **4v–4z**, and **4a** gave **4α**.

The acids **4b′**, **4d′**, **4n′**, **4t′** were obtained according to the usual manner from **4b**, **4d**, **4n**, **4t**, respectively.

(*E*/*Z*)-2-(2-Ethoxycarbonyl-1-methylvinyl)-7-(3-ethoxycarbopropoxy)benzo[*b*]furan (5b)

General procedure for 5a, 5c–5h. To a suspension of NaH (60% in oil, 0.08 g, 3.5 mmol) in anhydrous THF (3 ml) was added dropwise a solution of ethyl diethylphosphonoacetamide (0.77 g, 3.5 mmol) in anhydrous THF (10 ml) under an N_2 atmosphere at 5 °C with stirring. The solution was then stirred at 25 °C until it became clear. A solution of **2d** (0.2 g, 0.69 mmol) in anhydrous THF (4 ml) was added dropwise at 25 °C, and the mixture stirred at 25 °C for 30 min. The mixture was then quenched with saturated NH_4Cl solution and extracted with ethyl acetate. The organic layer was washed with brine, then dried. The solvent was evaporated off, giving a residue which was purified with silica gel column chromatography [hexane–ethyl acetate (10 : 1)] to give a mixture of *E*-**5b** and *Z*-**5b** (0.13 g, 52.0%) as a colorless oil.

In a similar manner to that described above, **2a** gave **5a**, **2d** gave **5c**, **2e** gave **5d**, **2j** gave **5e**, **2m** gave **5f**, **2g** gave **5g**, and **2p** gave **5h**.

(*E*/*Z*)-2-(Diethylcarbamoyl-1-methylvinyl)-7-diphenylmethoxybenzo[*b*]furan (4h) (procedure using LiCl–DBU instead of NaH)

General procedure for 4a, 4c, 4e, 4i-4m. To a solution of LiCl (0.19 g, 4.5 mmol) in acetonitrile (10 ml) was added dropwise *N*,*N*-diethyl phosphonoacetamide (0.47 g, 1.9 mmol) in acetonitrile (5 ml) at 25 °C. After the solution was stirred for 5 min, DBU (0.34 g, 2.3 mmol) and **2e** (0.32 g, 0.94 mmol) in acetonitrile (5 ml) were added to the solution. The mixture was heated at 83 °C for 5.5 h, and the solvent was evaporated. The residue was added to saturated NH_4Cl solution and extracted with ethyl acetate. The organic layer was washed with brine, then dried. The solvent was evaporated off, giving a residue which was purified with silica gel column chromatography [hexane–ethyl acetate (20 : 1)] to give *E*-**4h** (0.12 g, 29.3%) and *Z*-**4h** (0.09 g, 22.0%) as a colorless oil.

In a similar manner to that described above, **2c** gave **4a**, **4c**, **4e**, and **2e** gave **4i–4m**.

(*E*)-*N*-[2-(3,4-Dimethoxyphenyl)ethyl]-2-butenamide (6b)

General procedure for 6a, 6c–6e. To a mixture of 3,4-dimethoxyphenylethylamine (6.9 ml, 40.0 mmol) and Et_3N (5.5 ml) in dry benzene (180 ml) was added dropwise crotonyl chloride (4.2 ml, 44.0 mmol) in dry benzene (40 ml) over 30 min at 0 °C. The reaction mixture was stirred at 3–5 °C for 1 h. The solvent was evaporated off. The residue was recrystallized from ethyl acetate to give **6b** (9.6 g, 96.0%) as colorless needles: mp 76.8–79.1 °C; δ_H (500 MHz; $CDCl_3$; Me_3Si) 1.82 (3H, dd, *J* 8.0 and 2.0, CH_3), 2.78 (2H, t, CH_2CH_2N), 3.50 (2H, m,

CH_2CH_2NH), 3.82 (6H, s, $OCH_3 \times 2$), 5.93 (1H, dd, *J* 16.0 and 2.0, CH=$CHCH_3$), 6.75 (1H, m, CH=$CH$$CH_3$), 6.85 (3H, s, arom-H); *m*/*z* (EI) 249 (M^+, 35.46%), 164 (100.00).

(*E*)-*N*,*N*-Diethyl-2-butenamide (6a)

Yield: 50.8 g (56.8%), colorless oil; bp_8 88.0 °C; δ_H (500 MHz; $CDCl_3$; Me_3Si) 1.11–1.20 (6H, m, $CH_2CH_3 \times 2$), 1.88 (3H, dd, *J* 7.2 and 1.7, CH_3), 3.31–3.43 (4H, m, $CH_2CH_3 \times 2$), 6.22 (1H, dq, *J* 15.0 and 1.7, CH=$CHCH_3$), 6.90 (1H, dq, *J* 15.0 and 6.8, CH=$CH$$CH_3$); *m*/*z* (EI) 141 ($M^+$, 100.00%).

(*E*)-4-(1-Oxo-2-butenyl)morpholine (6c)

Yield: 18.7 g (93.7%), white needles; mp 55.8–58.0 °C; δ_H (60 MHz; $CDCl_3$; Me_3Si) 1.81 (3H, br s, CH_3), 3.55 (8H, br s, $CH_2CH_2 \times 2$), 6.20–7.09 (2H, m, CH=CH); *m*/*z* (EI) 155 (M^+, 61.77%), 140 (100.00).

(*E*)-1-(1-Oxo-2-butenyl)-4-phenylpiperazine (6d)

Yield: 1.4 g (69.0%), yellow needles; mp 124.3–125.4 °C; δ_H (60 MHz; $CDCl_3$; Me_3Si) 1.90 (3H, dd, *J* 6.6 and 1.8, CH_3), 3.17–3.19 (4H, m, $NCH_2 \times 2$), 3.70–3.86 (4H, m, $NCH_2 \times 2$), 6.10–6.50 (1H, m, CH=$CHCH_3$), 6.80–7.48 (6H, m, CH=$CH$$CH_3$ and phenyl H), *m*/*z* (EI) 230 (M^+, 91.40%), 161 (29.32), 132 (100.00).

(*E*)-1-(1-Oxo-2-butenyl)-4-(phenylmethyl)piperazine (6e)

Yield: 18.0 g (90.0%), white needles; mp 79.6–81.2 °C; δ_H (60 MHz; $CDCl_3$; Me_3Si) 1.81 (3H, d, *J* 6.0, CH_3), 2.40 (4H, t, *J* 5.0, $NCH_2 \times 2$), 3.53 (2H, s, CH_2), 3.59 (4H, t, *J* 5.0, $NCH_2 \times 2$), 6.50–6.90 (2H, m, CH=CH), 7.40 (5H, m, phenyl H); *m*/*z* (EI) 244 (M^+, 48.83%), 91 (100.00).

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

General procedure for 7a, 7c–7h, 7j–7n, 12a–12d, 12g, 12h. A mixture of **2m** (2.0 g, 4.8 mmol), **6a** (1.4 g, 9.9 mmol), palladium acetate (0.054 g, 0.24 mmol), tri-*o*-tolylphosphine (0.42 g, 1.4 mmol) and Et_3N (4.0 ml, 0.028 mol) was heated at 90–100 °C for 8 h. The mixture was treated with ethyl acetate, and the insoluble portion was removed by filtration. The filtrate was evaporated to dryness. The residue was poured into ice water, made acidic with 1 N HCl solution and extracted with ethyl acetate. The organic layer was washed with brine and dried. The solvent was evaporated off. The residue was purified with silica gel column chromatography [$CHCl_3$–ethyl acetate (5 : 2)] to give **7i** (0.73 g, 31.7%) as a yellow solid.

In a similar manner to that described above, **2j** gave **7a**, **2k** gave **7c–7g**, **2l** gave **7h**, **2m** gave **7i–7m**, **2o** gave **7n**, **11a** gave **12a**, **12b**, **11b** gave **12c**, **12d**, and **11c** gave **12g**, **12h**. **7b** was obtained according to the usual manner from **7a**.

(*E*)-2-Acetyl-7-[3-(4-chlorophenylsulfanyl)propoxy]-4-(diethylcarbamoyl-1-methylvinyl)benzo[*b*]furan (7o)

A mixture of **7n** (0.24 g, 0.61 mmol), K_2CO_3 (0.67 g, 4.8 mmol) and 4-chlorobenzenethiol (0.26 g, 1.8 mmol) in dry acetone (50 ml) was stirred at 54 °C for 48 h. After an insoluble portion was filtered off, the filtrate was distilled under reduced pressure. The residue was purified with silica gel column chromatography [$CHCl_3$–ethyl acetate (10 : 1)] to give a pale yellow solid. The solid was washed with hexane to give **7o** (0.12 g, 38.7%) as a white solid.

4-(2-Diethylcarbamoyl-1-methylvinyl)-7-ethoxycarbopropoxy-2-(2-ethoxycarbonyl-1-methylvinyl)benzo[*b*]furan (8a)

To a suspension of NaH (60% in oil, 0.56 g, 14.0 mmol) in anhydrous THF (10 ml) was added dropwise a solution of ethyl diethylphosphonoacetamide (3.1 g, 14.0 mmol) in anhydrous THF (10 ml) under an N_2 atmosphere at 5 °C with stirring. The solution was then stirred at 25 °C until it became clear. A solution of **7a** (2.0 g, 4.7 mmol) in anhydrous THF (20 ml) was added dropwise to the clear solution at 25 °C, and the mixture was stirred at 25 °C for 40 min. The mixture was then quenched with saturated NH_4Cl solution and extracted with ethyl acetate. The organic layer was washed with brine, then dried. The solvent was evaporated off, giving a residue which was purified with silica gel column chromatography [$CHCl_3$–ethyl acetate (5 : 2)] to give *E*-**8a** (1.3 g, 53.5%) as a yellow oil.

2-(2-Carboxy-1-methylvinyl)-7-(3-carboxypropoxy)-4-(2-diethylcarbamoyl-1-methylvinyl)benzo[*b*]furan (8b)

8b was obtained according to the usual manner from **8a**. Yield: 0.37 g (46.4%).

2-Acetyl-5-bromo-3-[2-(4-methoxyphenyl)-acetylamino]benzo[*b*]furan (11a)

To a solution of **10** (1.5 g, 5.9 mmol) in anhydrous THF (80 ml) was added dropwise 4-methoxyphenylacetic acid chloride [prepared by treatment of 4-methoxyphenylacetic acid (2.2 g, 11.8 mmol) with $SOCl_2$ (5 ml, 4.8 mmol)] under an N_2 atmosphere with stirring at 25 °C. The mixture was stirred at 25 °C for 20 h. The solvent was evaporated off. An ethyl acetate solution of the resulting residue was washed with 5% NaOH solution and brine, and dried. The solvent was evaporated off, giving a residue which was recrystallized from ethyl acetate to give **11a** (1.3 g, 55.6%) as pale yellow prisms: mp 192.1–194.7 °C; δ_H (60 MHz; $CDCl_3$; Me_3Si) 2.64 (3H, s, $COCH_3$), 7.28–8.10 (6H, m, 5-, 6-H and 2′-, 3′-, 5′-, 6′-H), 8.93 (1H, d, *J* 2.4, 4-H), 10.29 (1H, br s, N*H*); *m*/*z* (EI) 390.9607 (M^+. $C_{17}H_{11}BrClNO_3$ requires 390.9611), 391 (M^+, 32.45%), 139 (100.00).

2-(2-Carboxy-1-methylvinyl)-5-(2-morpholinocarbo-1-methylvinyl)benzo[*b*]furan (12f)

To a suspension of NaH (60% in oil, 0.07 g, 1.76 mmol) in anhydrous THF (8 ml) was added dropwise a solution of ethyl diethylphosphonoacetamide (0.39 g, 1.8 mmol) in anhydrous THF (10 ml) under an N_2 atmosphere at 5 °C with stirring. The solution was then stirred at 10 °C until it became clear. A solution of **12c** (0.5 g, 1.6 mmol) in anhydrous THF (10 ml) was added dropwise to the clear solution at 10 °C, and the mixture was stirred at 20 °C for 1.5 h. The mixture was then quenched with saturated NH_4Cl solution and extracted with ethyl acetate. The organic layer was washed with brine, then dried. The solvent was evaporated off, and the crude product (**12e**) was used for the next step without further purification. A mixture of **12e** (0.46 g), NaOH (1.0 g, 0.025 mol), methanol (40 ml) and H_2O (10 ml) was heated at 75 °C for 2.5 h. The solvent was evaporated off, and made acidic with 5% HCl solution. The resulting precipitate was collected by filtration and washed with water, and recrystallized from ethyl acetate to give **12f** (0.068 g, 11.9% from **12c**) as white needles.

Measurement of LTB_4-induced TXB_2 release from bronchoalveolar eosinophils

Bronchoalveolar eosinophils were harvested from guinea pigs treated with Sephadex G-200.[29] After preincubation at 37 °C for 5 min, the test compound was added to the purified eosinophils (1.1×10^6 cells per ml), and the mixture was incubated for 5 min. Next, LTB_4 (100 nM) was added to the mixture, and the reaction was allowed to proceed at 37 °C for 15 min. The reaction was stopped by cooling in ice–water, followed by centrifugation at 1700 × g for 10 min at 4 °C. The resultant supernatant was stored at −80 °C until assay of TXB_2. The TXB_2 in the supernatant was measured by a TXB_2 enzyme immunoassay (EIA) kit (Cayman Chem.) and expressed as pg/10^6 eosinophils.

Measurement of calcium mobilization in CHO cells

CHO cells stably expressing human BLT_1[31] or BLT_2[2f], seeded on a 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 in 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 (or 150 μl) of 300 nM LTB_4 (Cayman Chem.) to give a final concentration of 100 nM (or 300 nM), and the LTB_4-dependent increase in fluorescent intensity was measured using FlexStation (Molecular Devices). CHO cells transfected with an empty vector did not respond to 100 nM LTB_4 (data not shown).

Acknowledgements

This work was supported in part by a grant from the Ministry of Education, Culture, Sports, Science and Technology for the "University–Industry Joint Research" Project (2004–2008). We thank the staff of the instrumental analysis center of Mukogawa Women's University for the ^{1}H-NMR and MS measurements and elemental analyses.

References

1 Part of this work has been published as a preliminary communication: K. Ando, E. Tsuji, Y. Ando, J. Kunitomo, M. Yamashita, S. Ohta, T. Nabe, S. Kohno, T. Yokomizo, T. Shimizu and Y. Ohishi, *Org. Biomol. Chem.*, 2004, **2**, 3427–3431.

2 (*a*) C. R. Turner, R. Breslow, M. J. Conklyn, C. J. Andresen, D. K. Patterson and A. Lopez-Anaya, *J. Clin. Invest.*, 1996, **97**, 381–387; (*b*) P. Sharon and W. F. Stenson, *Gastroenterology*, 1984, **86**, 453–460; (*c*) A. Nakao, K. Nasaka, N. Ohishi, E. Noiri, T. Suzuki and S. Taniguchi, *Kidney Int., Suppl.*, 1997, **63**, S236–238; (*d*) R. J. Griffiths, E. R. Pettipher, K. Kock, C. A. Farrell, R. Breslow and M. Conklyn, *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 517–521; (*e*) R. P. Gladue, L. A. Carroll, A. J. Milici, D. N. Scampoli, H. A. Stukenbrok and E. R. Pettipher, *J. Exp. Med.*, 1996, **183**, 1893–1898; (*f*) T. Yokomizo, K. Kato, K. Terawaki, T. Izumi and T. Shimizu, *J. Exp. Med.*, 2000, **192**, 421–431.

3 **CP-195543:** (*a*) L. A. Reiter, K. Koch, A. D. Piscopio, H. J. Showell, R. Alpert, M. S. Biggers, R. J. Chambers, M. J. Conklyn, K. Cooper, S. R. Cortina, J. N. Dibrino, B. W. Dominy, C. A. Farrell, G. P. Hingorani, G. J. Martinelli, M. Ramchandani and K. F. Wright, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 1781–1786. **CP-105696:**; (*b*) K. Koch, L. S. Melvin, Jr., L. A. Reiter, M. S. Biggers, H. J. Showell, R. J. Griffiths, E. R. Pettipher, J. B. Cheng, A. J. Milici and R. Breslow, *J. Med. Chem.*, 1994, **37**, 3197–3199. **LY-223982:**; (*c*) D. M. Gapinski, B. E. Mallett, L. L. Froelich and W. T. Jackson, *J. Med. Chem.*, 1999, **33**, 2807–2813; (*d*) W. T. Jackson, R. J. Boyd, L. L. Froelich, D. M. Gapinski, B. E. Mallett and J. S. Sawyer, *J. Med. Chem.*, 1993, **36**, 1726–1734. **LY-255283:**; (*e*) D. K. Herron, T. Goodson, N. G. Bollinger, D. Swanson-Bean, I. G. Wright, G. S. Staten, A. R. Thompson, L. L. Froelich and W. T. Jackson, *J. Med. Chem.*, 1992, **35**, 1818–1828; (*f*) J. S. Sawyer, N. J. Bach, S. R. Baker, R. F. Baldwin, P. S. Borromeo, S. L. Cockerham, J. H. Fleisch, P. Floreancig, L. L. Froelich and W. T. Jackson, *J. Med. Chem.*, 1995, **38**, 4411–4432. **ONO-4057:**; (*g*) K. Kishikawa, S. Nakao, S. Matsumoto, K. Kondo and N. Hamanaka, *Adv. Prostaglandin, Thromboxane, Leukotriene Res.*, 1995, **23**, 279–281; (*h*) K. Kishikawa, N. Tateishi, T. Maruyama, R. Seo, M. Toda and T. Miyamoto, *Prostaglandins*, 1992, **44**, 261–275. **ZK-158252:**; (*i*) R. D. Pallavi, K. H. Abdelmadjid, P. Mai, D. S. Wolf-Dieter, M. S. Bruce and W. Walter, *J. Biol. Chem.*, 1999, **274**, 23341–23348; (*j*) M. Matousek, K. Mitsube, M. Mikuni and M. Brannstrom, *Mol. Hum. Reprod.*, 2001, **7**, 35–42. **1,3-Disubstituted cyclohexane:**; (*k*) J.-M. Poudrel, P. Hullot, J.-P. Vidal, J.-P. Girard, J.-C. Rossi, A. Muller, C. Bonne, V. Bezuglov, I. Serkov, P. Renard and B. Pfeiffer, *J. Med. Chem.*, 1999, **42**, 5289–5310. **BIIL315:**; (*l*) F. W. Birke, C. J. Meade, R. Anderskewitz, G. A. Speck and H.-M. Jennewein, *J. Pharmacol. Exp. Ther.*, 2002, **297**, 458–466. **LY293111:**; (*m*) M. Philip, S. J. Scott, F. L. Larry, M. L. Larry and S. M. Stephen, *Biochem. Pharmacol.*, 1995, **49**, 1683–1690.

4 (*a*) C. Brink, S.-E. Dahlen, J. F. Evans, D. W. P. Hay, S. Nicosia, C. N. Serhan, T. Shimizu and T. Yokomizo, *Pharmacol. Rev.*, 2003, **55**, 195–227; (*b*) A. Toba, T. Yokomizo and T. Shimizu, *Prostaglandins & Other Lipid Mediators*, 2002, **68–69**, 575–585; (*c*) T. Yokomizo, K. Masuda, K. Toda, T. Izumi and T. Shimizu, *Am. J. Respir. Crit. Care Med.*, 2000, **161**, S51–55; (*d*) T. Yokomizo, T. Izumi and T. Shimizu, *Arch. Biochem. Biophys.*, 2001, **385**, 231–241; (*e*) T. Yokomizo, K. Kato, K. Terawaki, T. Izumi and T. Shimizu, *J. Exp. Med.*, 2000, **192**, 421–431.

5 R. J. Aiello, P. A. Bourassa, S. Lindsey, W. Weng, A. Freeman and H. J. Showell, *Arterioscler., Thromb., Vasc. Biol.*, 2002, **22**, 443–449; A. Mennander, S. Tiisala, J. Ustinov, A. Räisänen, T. Paavonen and P. Häyry, *Arterioscler. Thromb.*, 1992, **12**, 1380–1386.

6 H. Takatsuka, Y. Takemoto, S. Yamada, T. Wakae, A. Mori, M. Okada, N. Iwata and T. Okamoto, *Drugs Exp. Clin. Res.*, 2002, **28**, 121–125; M. Tanaka, T. Tamaki, Y. Konoeda, Y. Uchida, T. Kaizu and A. Kawamura, *Transplant. Proc.*, 2000, **32**, 2340.

7 L. Iversen, K. Kragballe and V. A. Ziboh, *Skin Pharmacol.*, 1997, **10**, 169–177.

8 W.-G. Tong, X.-Z. Ding, R. Hennig, R. C. Witt, J. Standop, P. M. Pour and T. E. Adrian, *Clin. Cancer Res.*, 2002, **8**, 3232–3242; M.-H. Yoo, H. Song, C.-H. Woo, H. Kim and J.-H. Kim, *Oncogene*, 2004, **23**, 9259–9268.

9 Y. Kurihara, H. Endo, T. Akahoshi and H. Kondo, *Clin. Exp. Immunol.*, 2001, **123**, 323–330; A. Hashimoto, H. Endo, I. Hayashi, Y. Murakami, H. Kitasato, S. Kono, T. Matsui, S. Tanaka, A. Nishimura, K. Urabe, M. Itoman and H. Kondo, *J. Rheumatol.*, 2003, **30**, 1712–1718; Y. Murakami, T. Akahoshi, I. Hayashi, H. Endo, A. Hashimoto, S. Kono, H. Kondo, S. Kawai, M. Inoue and H. Kitasato, *Arthritis Rheum.*, 2003, **48**, 2931–2941; R. Alten, E. Gromnica-Ihle, C. Pohl, J. Emmerich, J. Steffgen, R. Roscher, R. Sigmund, B. Schmolke and G Steinmann, *Ann. Rheum. Dis.*, 2004, **63**, 170–176.

10 Several heteroaromatic and cyclized aliphatic compounds as an LTB_4 antagonist were reported previously: (*a*) D. Delorme, Y. Ducharme, C. Brideau, C.-C. Chan, N. Chauret, S. Desmarais, D. Dube, J.-P. Falgueyret and R. Relean, *J. Med. Chem.*, 1996, **39**, 3951–3970. (L 708780, naphthalene derivative with furan ring); (*b*) S. W. Djuric, S. H. Docter, S. S. Yu, D. Spangler, B. S. Tsai, C. P. Anglin, T. S. Gaginella, J. F. Kachur and R. H. Keith, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 811–816. (SC-53228, benzopyran derivative); (*c*) S. W. Djuric, P. W. Collins, P. T. Jones, R. L. Shone, B. S. Tsai, D. J. Fretland, G. M. Butchko, D. Villani-Prince and R. H. Keith, *J. Med. Chem.*, 1989, **32**, 1145–1147. (SC 41939, benzopyran derivative); (*d*) T. S. Shoupe, S. M. Coutts, D. C. Baker and E. S. Hand, *Can. Pat. Appl.*, 1991, 63 pp. CPXXEB CA 2013960, AA 19910319, CAN 115:158949, AN 1991: 558949. (PF 10042, dibenzofuran derivative); (*e*) C. D. Wright, P. J. Kuipers, M. D. Hoffman, D. O. Thueson and M. C. Conroy, *Biochem. Biophys. Res. Commun.*, 1990, **167**, 828–834. (CI 949, indole derivative); (*f*) J. M. Musser, U. R. Chakraborty, S. Sciortino, R. J. Gordon, A. Khandwala, E. S. Neiss, T. P. Pruss, R. V. Inwegen, I. Weinryb and S. M. Coutts, *J. Med. Chem.*, 1997, **30**, 96–104. (PF 5901, quinoline derivative); (*g*) C. Can, M. G. Cinar, S. Ulker, A. Evinc and S. Kosay, *Eur. J. Pharmacol.*, 1998, **350**, 223–228. (MK 886, indole derivative); (*h*) J. M. Poudrel, P. Hullot, J. P. Vidal, J. P. Girard, J. C. Rossi, A. Muller and C. Bonne, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 2349–2354.

11 H. Nakai, M. Konno, S. Kosuge, S. Sakuyama, M. Toda, Y. Arai, T. Obata, N. Katsube, T. Miyamoto, T. Okegawa and A. Kawasaki, *J. Med. Chem.*, 1988, **31**, 84–91.

12 (*a*) E. Tsuji, K. Ando, J. Kunitomo, M. Yamashita, S. Ohta, S. Kohno and Y. Ohishi, *Org. Biomol. Chem.*, 2003, **1**, 3139–3141; (*b*) K. Ando, E. Tsuji, Y. Ando, N. Kuwata, J. Kunitomo, M. Yamashita, S. Ohta, S. Kohno and Y. Ohishi, *Org. Biomol. Chem.*, 2004, **2**, 625–635.

13 P. D. Greenspan, A. J. Main, S. S. Bhagwat, L. I. Barsky, R. A. Doti, A. R. Eagle, L. M. Frey, H. Zhou, K. E. Lipson, M. H. Chin, R. H. Jackson and S. Uziel-Fusi, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 949–954; P. D. Greenspan, R. A. Fujimoto, P. J. Marshall, A. Raychaudhuri, K. E. Lipson, H. Zhou, R. A. Doti, D. V. Coppa, L. Zhu, R. Pelletier, S. Uziel-Fusi, R. H. Jackson, M. H. Chin, B. L. Kotyuk and J. J. Fitt, *J. Med. Chem.*, 1999, **42**, 164–172.

14 Y. Ohishi, T. Mukai, M. Nagahara, M. Yajima and N. Kajikawa, *Chem. Pharm. Bull.*, 1989, **37**, 2393–2405.

15 The 3-H signal (7.73 ppm) showed no NOE correlation between other aromatic signals (6.9 ppm, d and 7.4 ppm, d) of **1b**. This supported the structure of **1b**.

16 M. Watanabe, S. Hisamatsu, H. Hotokezaka and S. Furukawa, *Chem. Pharm. Bull.*, 1986, **34**, 2810–2820.

17 (*a*) J. Boutagy and R. Thomas, *Chem. Rev.*, 1974, **74**, 87–99; (*b*) N. Matsuura, Y. Yashiki, S. Nakashima, M. Maeda and S. Sasaki, *Heterocycles*, 1999, **51**, 975–978; (*c*) J. K. F. Geirsson, B. Ö. Gudmundsson and R. Sigurdardottir, *Acta Chem. Scand.*, 1993, **47**, 1112–1116.

18 The torsion angle between the 2-(2-alkylcarbamoyl-1-methylvinyl) group and the benzo[*b*]furan ring of (*E*)-isomers ((*E*)-**4c**, (*E*)-**4f**, (*E*)-**4***α*) and of (*Z*)-isomers ((*Z*)-**4c**, (*Z*)-**4f**, (*Z*)-**4***α*) was estimated using Cambridge Soft, Chem3D 5.0, MM2. (*E*)-**4c**: 0.3°, (*E*)-**4f**: 3.5°, (*E*)-**4***α*: 4.4°; (*Z*)-**4c**: 28.4°, (*Z*)-**4f**: 26.9°, (*Z*)-**4***α*: 24.9°.

19 X-Ray essential data of (*E*)-**4***α* and **7i** were reported in our previous communication (ref. 1).

20 S. Sano, *Yakugaku Zasshi*, 2000, **120**, 432–444.

21 M. A. Blanchette, W. Choy, J. T. Davis, A. P. Essenfeld, S. Masamune, W. R. Roush and T. Sakai, *Tetrahedron Lett.*, 1984, **25**, 2183–2186; D. F. Nets and J. L. Seidel, *Tetrahedron Lett.*, 1992, **33**, 1957–1958.

22 K. Ando, *J. Org. Chem.*, 1997, **62**, 1934–1939; K. Ando, *Tetrahedron Lett.*, 1995, **36**, 4105–4108; W. C. Still and C. Gennari, *Tetrahedron Lett.*, 1983, **24**, 4405–4408.

23 F. R. Heck, *Organic Reactions*, John Wiley & Sons Publishers, New York, 1982, vol. 27, pp. 345-390; P. D. Greenspan, R. A. Fujimoto, P. J. Marshall, A. Raychaudhuri, K. F. Lipson, H. Zhou, R. A. Doti, D. E. Coppa, L. Zhu, R. Pelletier, S. Uziel-Fusi, R. H. Jackson, M. H. Chin, B. L. Kotyuk and J. J. Fitt, *J. Med. Chem.*, 1999, **42**, 164–172.

24 R. Adams and M. W. Miller, *J. Am. Chem. Soc.*, 1940, **62**, 53–57.

25 W. H. Mills and G. H. Dazeley, *J. Chem. Soc.*, 1936, 460–463.

26 *o*-(β-Methyl-α-ethylvinyl)phenyltrimethylammonium iodide having a less bulky substituent groupthan *o*-(β,β-dimethyl-α-isopropylvinyl)-phenyltrimethylammonium iodide (**V**) was unresolvable.[25]

27 The torsion angle between the (*E*)-4-(2-alkylcarbamoyl-1-methylvinyl) group and the benzo[*b*]furan ring plane of several (*E*)-4-(2-alkylcarbamoyl-1-methylvinyl) compounds (**7a**, **7c**, **7g**, **7i**, **7j**, **7n**) was estimated using MM2. **7a**: 47.9°, **7c**: 39.7°, **7g**: 40.2°, **7i**: 40.2°, **7j**: 38.8° and **7n**: 43.4°. The estimated torsion angle (40.2°) of **7i** was approximately that (45.7°) found by X ray analysis.

28 C. Paizs, M. Tosa, C. Majdik, P. Moldovan, L. Novak, P. Kolonits, A. Marcovici, F.-D. Irimie and L. Poppe, *Tetrahedron: Asymmetry*, 2000, **14**, 1495–1501; J. P. Bachelet, P. Demerseman, R. Royer, R. Cavier and J. Lemoine, *Eur. J. Med. Chem.*, 1982, **17**, 323–325.

29 T. Nabe, H. Yamamura and S. Kohno, *Jpn. J. Pharmacol.*, 1996, **70**, 337–345.

30 Several (*E*)-5-(2-alkylcarbamoyl-1-methylvinyl) compounds previously prepared in our laboratories (ref. 12*b*) were also evaluated and found to be inactive.

31 T. Yokomizo, T. Izumi, K. Chang, Y. Takuwa and T. Shimizu, *Nature*, 1997, **387**, 620–624.

32 A calcium assay and binding assay for evaluation of cysteinyl leukotriene antagonistic activity were performed according to Nothacker's procedure: H.-P. Nothacker, Z. Wang, Y. Zhu, R. K. Reinscheid, S. H. S. Lin and O. Civelli, *Mol. Pharmacol.*, 2000, **58**, 1601–1608.

33 ATP (10 μM)-dependent calcium mobilization through an intrinsic ATP receptor in CHO cells was evaluated at concentrations of 10 nM, 100 nM, 1 μM and 10 μM of each compound.

34 Inhibition of calcium mobilization in the CHO–hBLT$_2$ cell by compounds (**4a**, **4b′**, **4d**, **4f**, **4y**) was not due to its cytotoxicity, because these compounds did not affect calcium mobilization in the CHO–hBLT$_1$ cell at 10 μM.