Preparation of leukotriene B_4 inhibitory active 2- and 3-(2-aminothiazol-4-yl)benzo[*b*]furan derivatives and their growth inhibitory activity on human pancreatic cancer cells†

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A series of 2-(2-aminothiazol-4-yl)benzo[*b*]furan and 3-(2-aminothiazol-4-yl)benzo[*b*]furan derivatives were prepared, and their leukotriene B_4 inhibitory activity and growth inhibitory activity in cancer cell lines were evaluated. Several compounds showed strong inhibition of calcium mobilization in CHO cells overexpressing human BLT_1 and BLT_2 receptors and growth inhibition to human pancreatic cancer cells MIA PaCa-2. 3-(4-Chlorophenyl)-2-[5-formyl-2-[(dimethylamino)methyleneamino]thiazol-4-yl]-5-methoxybenzo[*b*]furan **8b** showed the most potent and selective inhibition for the human BLT_2 receptor, and its IC_{50} value was smaller than that of the selected positive control compound, ZK-158252. 3-(4-Chlorophenyl)-2-[2-[(dimethylamino)methyleneamino]-5-(2-hydroxyethyliminomethyl)thiazol-4-yl]-5-methoxybenzo[*b*]furan **9a** displayed growth inhibitory activity towards MIA PaCa-2.

Introduction

Leukotriene B_4 (LTB_4) is known as a potent mediator of the inflammatory process, playing important physiological roles on leukocytes trafficking to the site of infection and clearance of invading microorganisms.[2] On the other hand, elevated levels of LTB_4 have been observed in patients with various inflammatory diseases.[3]

Although many works have been done to develop LTB_4 receptor antagonists for clinical use as an anti-inflammatory drugs,[4] no antagonist has yet been developed for clinical applications. The structures of representative reported LTB_4 receptor antagonists are shown in Fig. 1.[5] Recently, another LTB_4 receptor (BLT_2) was found and its molecular cloning was established.[6] This encouraged new studies to find novel BLT_1 and/or BLT_2 inhibitors, which may lead to the development of new clinical drugs for immunosuppression of allograft rejection in organ transplantation,[7] arteriosclerosis,[8] psoriasis,[9] cancer,[10] and rheumatoid arthritis.[11]

We have been interested in the preparation of various types of benzo[*b*]furan derivatives in order to evaluate their biological activities, and have reported that several of their derivatives such as **1**, **2** and **3** (Fig. 2) showed selective LTB_4 receptor (BLT_1, BLT_2) inhibitory activities.[12] In an earlier paper, we described the

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† Electronic supplementary information (ESI) available: Growth inhibitory activities of **5b**, **5c**, **5f**, **7b**, **10a**, **12b**, **13** and **16** against cancer cells. See DOI: 10.1039/b803313g

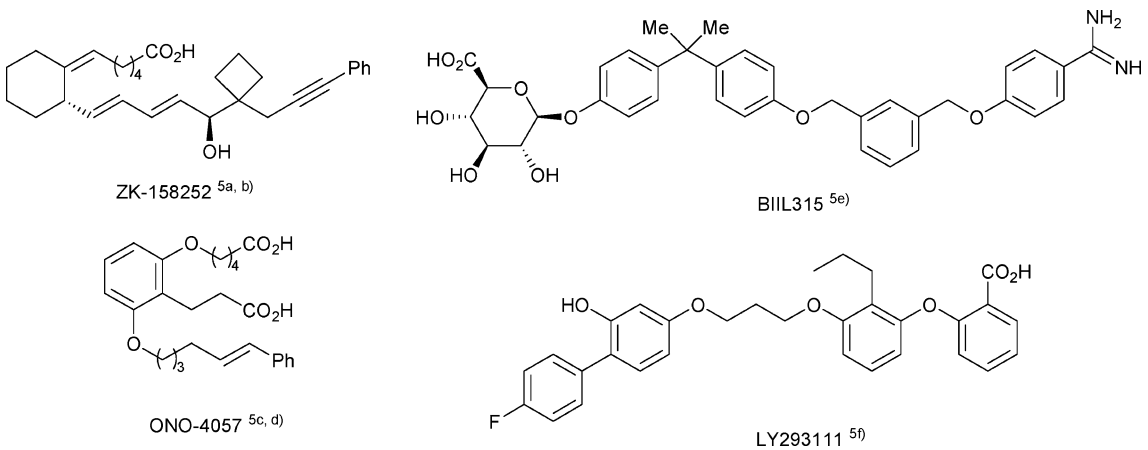


Fig. 1 Structures of representative LTB_4 receptor antagonists.

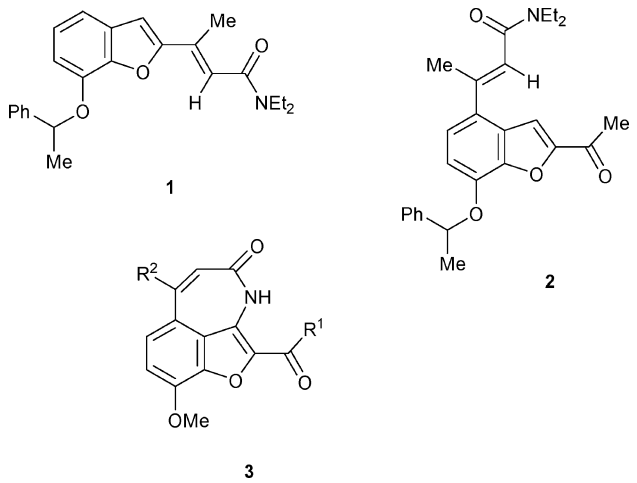


Fig. 2 Structures of the reported LTB_4 receptor–inhibitory active benzo[*b*]furan derivatives.

preparation of a new class of benzo[*b*]furan derivatives,[13] 2-(2-aminothiazol-4-yl)benzo[*b*]furans, that selectively showed highly potent and significant inhibitory activity towards BLT_2.[1]

In 2002, Adrian and co-workers reported that the LTB_4 receptor antagonist, LY293111, inhibited proliferation and induced apoptosis in human pancreatic cancer cells.[14] In the present study, we prepared 2- and 3-(2-aminothiazol-4-yl)benzo[*b*]furan derivatives showing inhibitory activities for the LTB_4 receptor and evaluated their analogues for growth inhibitory activity by using cancer cell lines including the human pancreatic cancer cells MIA PaCa-2.

Chemistry

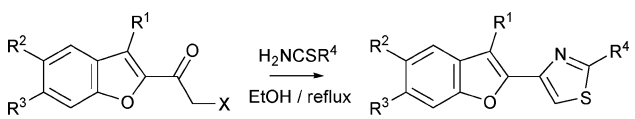
The literature has only a few examples of the preparation of 2-(2-aminothiazol-4-yl)benzo[*b*]furan derivatives.[15] We planned the preparation of a series of 2-(thiazol-4-yl)benzo[*b*]furan derivatives by applying Hantzsch thiazole synthesis as a key step in constructing the thiazole moiety at the 2-position of the benzo[*b*]furan ring.[16] The results are shown in Table 1. 2-(Haloacetyl)benzo[*b*]furans **4a-4d** were prepared according to a modification of the reported procedure[17] and were allowed to react with thioureas or thiobenzamide to obtain 2-(2-aminothiazol-4-yl)benzo[*b*]furans **5a**, **5b**, **5d**, **5e** and 2-(2-phenylthiazol-4-yl)benzo[*b*]furan **5c** in 55–86% yields as shown in Table 1.

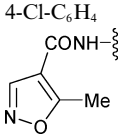
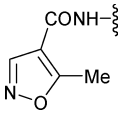
Conversion from the 2-(2-aminothiazol-4-yl)benzo[*b*]furan **5a** to several types of 2-(*N*-and/or *C*-substituted-2-aminothiazol-4-yl)benzo[*b*]furan derivatives are depicted in Scheme 1.

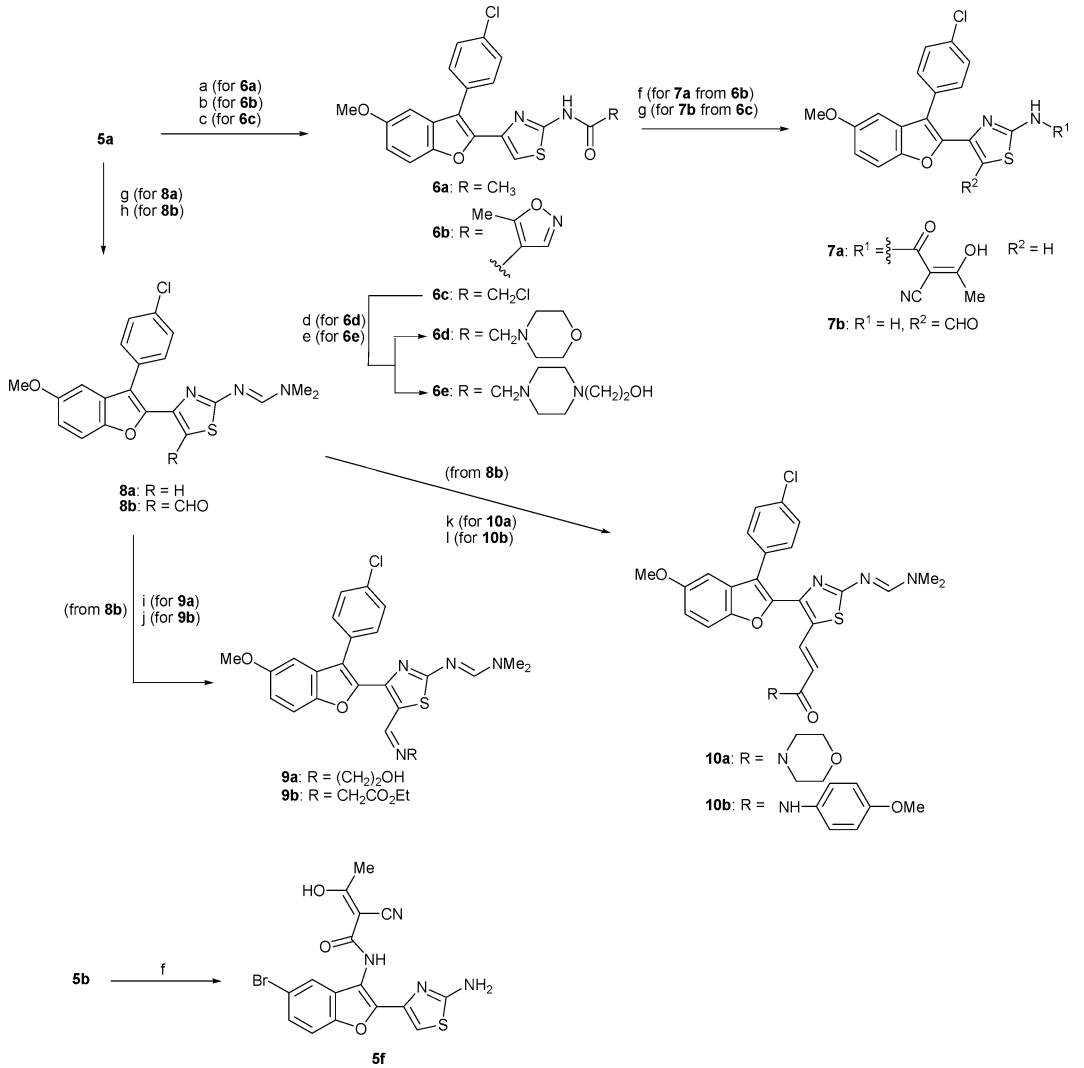
Acylation of the aminothiazole **5a** with acid chloride gave the corresponding carboxamides **6a–6c** in 31–77% yields, and the morphorinoacetamide **6d** and piperazinoacetamide **6e** were derived from **6c** in 72 and 38% yields, respectively. Treatment of the 5-methylisoxazol-4-yl derivative **5b** or **6b** with Et_3N in refluxing THF afforded the characteristic (*Z*)-2-cyano-3-hydroxybut-2-enoyl compound **5f** (60% yield) and **7a** (50% yield) by a ring opening reaction of the isoxazole ring followed by enolization of the α-cyano-β-ketoamide. The structures of **5f** and **7a** were supported by the observation of the corresponding enol OH proton at 15.75 ppm and 14.49 ppm in the ^1H NMR spectrum.[20]

In order to obtain a formylated compound at the 5-position of the thiazole ring, the reaction of aminothiazole **5a** was carried out with 4 equiv. of $POCl_3$ in DMF at −10 to −5 °C, under Vilsmeier reaction conditions. The reaction proceeded smoothly and selectively only at the primary amino group of the 2-position on the thiazole ring to give the *N*,*N*-dimethylformimidine product **8a** in 74% yield. On the other hand, the same reaction using a large excess (8 equiv.) of $POCl_3$ at room temperature provided *N*′-(5-formylthiazol-2-yl)-*N*,*N*-dimethylformimidine **8b** in 60% yield as the sole product. However, treatment of **6c** with 4 equiv. of $POCl_3$ in DMF gave *N*-formyl derivative **7b** in 34% yield, probably because the amide bond in **6c** was relatively sensitive to acidic reaction conditions. The condensation reaction of the aldehyde **8b** with 2-aminoethanol or glycine ethyl ester provided the imine derivatives **9a** (73%) and **9b** (17%). The

Table 1 Preparation of 2-(thiazol-4-yl)benzo[*b*]furan derivatives **5a–e**



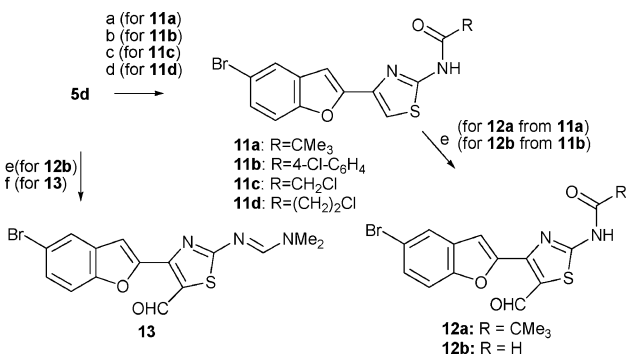
Entry	4	X	R^1	R^2	R^3	R^4	Yield of **5** (%)
1	**4a**	Cl	4-Cl-C_6H_4	OMe	H	NH_2	**5a**: 55
2	**4b**	Br		Br	H	NH_2	**5b**: 84
3	**4b**	Br		Br	H	Ph	**5c**: 86
4	**4c**[18]	Br	H	Br	H	NH_2	**5d**[19]: 85
5	**4d**	Br	H	H	OMe	NH_2	**5e**: 58



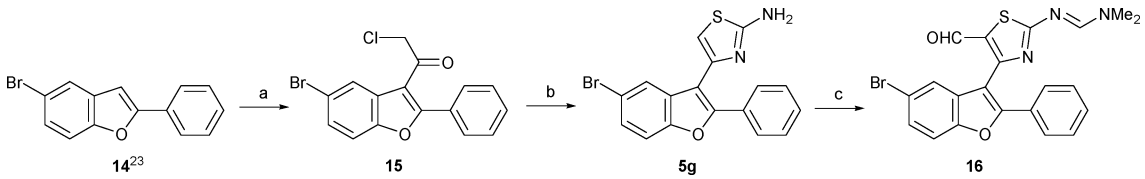
Scheme 1 Preparation of 3-substituted 2-(2-aminothiazol-4-yl)benzo[*b*]furan derivatives. *Reagents and conditions*: (a) CH_3COCl, THF, reflux, 77% (**6a**); (b) 5-methylisoxazole-4-carbonyl chloride, THF, reflux, 31% (**6b**); (c) $ClCH_2COCl$, THF, rt, 74% (**6c**); (d) morphorine, CH_3CN, reflux, 72% (**6d**); (e) 2-(piperazin-1-yl)ethanol, CH_3CN, rt, 38% (**6e**); (f) Et_3N, THF, reflux, 50% (**7a** from **6b**); 60% (**5f**); (g) 4 eqiuv. $POCl_3$, DMF, 34% (rt, **7b** from **6c**); 74% (−10 to −5 °C, **8a**); (h) 8 eqiuv. $POCl_3$, DMF, rt, 60% (**8b**); (i) $NH_2CH_2CH_2OH$, EtOH, reflux, 73% (**9a**); (j) $NH_2CH_2CO_2Et$. HCl, Et_3N, EtOH, 3Å molecular sieves, reflux, 17% (**9b**); (k) $(EtO)_2P(O)CH_2CONC_4H_8O$, NaH, THF, rt, 63% (**10a**); (l) $(EtO)_2P(O)CH_2CONHC_6H_4OMe$, NaH, THF, rt, 43% (**10b**).

Horner–Wadsworth–Emmons reaction was applied to the aldehyde **8b** with phosphonoacetamides[12b,21] to afford the amides **10a** and **10b** in moderate yields (63% and 43%).

Next, we planned the preparation of 3-unsbstituted 2-(2-amino-5-formylthiazol-4-yl)benzo[*b*]furan derivatives in order to compare their activities with 3-(4-chlorophenyl)benzo[*b*]furan derivatives (Scheme 2). *N*-Acylation of the 2-(2-aminothiazol-4-yl)benzo[*b*]furan **5d** was performed using several acid chlorides to obtain 2-(acylaminothiazol-4-yl)benzo[*b*]furan **11a–11d** in 49–68% yields. Formylation reaction of **11a** or **5d** with $POCl_3$ and DMF gave the 5-formylated thiazole derivartive **12a** and *N*′-(5-formylthiazol-2-yl)-*N*,*N*-dimethylformimidine **13** in 41% and 50% yields, respectively. However, applying the same reaction conditions to the benzamide **11b** gave a complex reaction mixture, and the product obtained was the *N*-formylthiazolylbenzo[*b*]furan **12b** in 9% isolated yield.[22]



Scheme 2 Preparation of 3-unsubstituted 2-(2-aminothiazol-4-yl)benzo-[*b*]furan derivatives. *Reagents and conditions*: (a) Me_3CCOCl, Et_3N, THF, reflux, 54% (**11a**); (b) 4-Cl-C_6H_4COCl, THF, rt, 49% (**11b**); (c) $ClCH_2$-COCl, THF, reflux, 68% (**11c**); (d) $Cl(CH_2)_2COCl$, THF, rt, 55% (**11d**); (e) $POCl_3$, DMF, rt, 41% (**12a**); 9% (**12b**); (f) $POCl_3$, DMF, 60 °C, 50% (**13**).



14[23] 15 5g 16

Scheme 3 Preparation of 3-(2-aminothiazol-4-yl)benzo[*b*]furan derivatives. *Reagents and conditions*: (a) $ClCH_2COCl$, $AlCl_3$, $CHCl_3$, 0 °C, 26%; (b) $H_2NC(S)NH_2$, EtOH, reflux, 84%; (c) $POCl_3$, DMF, rt, 33%.

We planned the preparation of the compound with a 2-aminothiazol-4-yl group attached at the 3-position on the benzo[*b*]furan ring as shown in Scheme 3. Hantzsch thiazole synthesis was also conducted with the 3-chloromethylketone **15**, derived from 5-bromo-2-phenylbenzo[*b*]furan **14**,[23] to obtain the desired 3-(2-aminothiazol-4-yl)benzo[*b*]furan **5g** in 84% yield. Treatment of **5g** with $POCl_3$ and DMF afforded 3-[2-[(dimethylamino)methyleneamino]-5-formylthiazol-4-yl]-2-phenylbenzo[*b*]furan **16** in 33% yield.

Biological study

The results of LTB_4 inhibitory activity of **5a**, **6a**, **6b**, **7a**, **8a**, **8b**, **9a** and **9b** together with **1**[12b] by inhibition of calcium mobilization in both CHO cells overexpressing human BLT_1 (CHO-hBLT_1) and human BLT_2 (CHO-hBLT_1)[24] are shown in Table 2.[1] Among the tested compounds, **8b**, **9a** and **9b**, showed potent inhibitory activity of the LTB_4 receptor and inhibited BLT_2 more potently than BLT_1. In contrast, ZK-158252 nearly equally inhibited both BLT_1 and BLT_2. Compounds **8b**, **9a** and **9b** were more potent than ZK-158252 in BLT_2 inhibition, and showed less inhibition than ZK-158252 to BLT_1.

Encouraged by these results, we planned the evaluation of the growth inhibitory activity in cancer cell lines. Fourteen compounds **5b**, **5c**, **5f**, **6c**, **6d**, **6e**, **7b**, **8b**, **9a**, **10a**, **11c**, **11d**, **12b**, **13** and **16** were assayed for activity *in vitro* against human pancreatic carcinoma (MIA PaCa-2), breast cancer (MCF-7, MDA-MB-231), human prostate carcinoma (PC-3) and normal human dermal fibroblast (NHDF) cells, and the results are summarized in Table 3 and Fig. 3.[25] Compounds **5b**, **5c**, **5f** and **7b**, *N*-unsubstituted 2-aminothiazole derivatives, had no inhibitory effect in MIA PaCa-2. However, compounds **10a**, **12b**, **13** and **16** which are fully substituted at both the *N*- and *C*-positions in the 2-aminothiazol-4-yl group at the 2- or 3-position of the benzo[*b*]furan skeleton showed an inhibitory effect on cancer cell lines. Compound **10a**, in particular, inhibited MIA PaCa-2 (79.3 ± 2.2% cell growth at 10 μM) more potently than 5-FU (81.2 ± 1.8%), while its inhibitory potency in NHDF was less than that of 5-FU (84.5 ± 1.0% *vs* 48.0 ± 2.0).[25]

Among the tested compounds, we found **6c**, **6e**, **8b**, **9a**, **11c**, and **11d** showed potent inhibitory activity on cell growth in MIA PaCa-2. Their GI_{50} values against MIA PaCa-2, PC-3, MCF-7 and NHDF cells are given in Table 3. The GI_{50} values of 2-[2-[(dimethylamino)methyleneamino]-5-substituted-thiazol-4-yl]benzo[*b*]furans **8b** and **9a** against MIA PaCa-2 were 4.84 and 3.68 μM, respectively, which were less than half the concentration

Table 2 Evaluation of prepared compounds for LTB_4 receptor (BLT_1, BLT_2) inhibitory activities[a]

	% Inhibition (10 μM)		IC_{50}/μM	
Compound	CHO-hBLT_1	CHO-hBLT_2	CHO-hBLT_1	CHO-hBLT_2
5a	4.8	70.6	—	—
6a	11.2	68.7	—	—
6b	N.I.[b]	N.I.[b]	—	—
7a	13.6	82.1	—	3.29
8a	11.6	72.0	—	—
8b	24.4	97.9	3.55	0.19
9a	51.3	100	3.19	0.20
9b	49.4	88.3	6.81	0.35
1	69.9	100	2.88	0.48
ZK-158252	—	—	1.70	1.18

[a] Effect of calcium mobilization by LTB_4 (300 nM) in CHO-hBLT_1 and CHO-hBLT_2 cells. [b] Not inhibited.

Table 3 *In vitro* cell growth inhibitory activities of **6c**, **6d**, **8b**, **9a**, **11c**, **11d** and 5-FU

	GI_{50}[a]/μM			
Compound	MIA PaCa-2	PC-3	MCF-7	NHDF
6c	8.64	[b]	7.59	>10
6d	>10	[b]	>10	>10
6e	7.92	[b]	6.99	5.53
8b	4.84	>10	[b]	>10
9a	3.68	4.17	[b]	>10
11c	6.76	[b]	3.60	9.95
11d	7.74	[b]	7.70	>10
5-FU	>10	2.05	>10	>10

[a] GI_{50} is the concentration of the compound causing 50% inhibition of cell growth compared to the negative control. [b] Not tested.

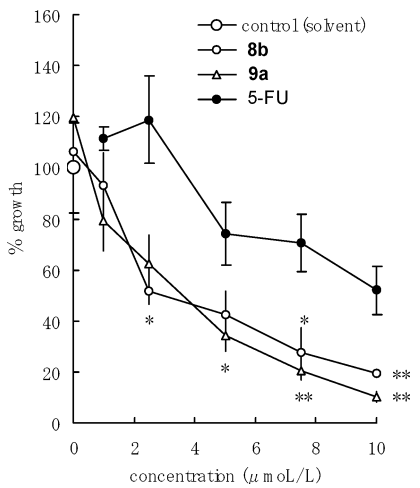


Fig. 3 Effects of **8b** and **9a** on MIA Paca-2. * P<0.05, **P<0.01 V.S. 5-FU (Tukey's test). Each data point represents the mean (% growth) ±S.E. for 6 cultures.

of 5-FU (Fig. 3, Table 3). Inhibitory activity of **8b** and **9a** against NHDF was very low ($GI_{50}>10$). From the viewpoint of reducing the risk of side effects in therapeutic treatment, such selective activity is a promising feature.

Conclusions

We have developed a method for preparing *N*-substituted 3- and 2-(2-aminothiazol-4-yl)benzo[*b*]furans. Compounds **8b** and **9a** showed potent and selective inhibitory activities for the BLT_2 receptor and inhibited cell growth of human pancreatic cancer cells. A common structural feature of the active compounds is the 2-[(dimethylamino)methyleneamino]thiazole having substituent groups at the 5-position. Their inhibitory potencies toward BLT_2 were 6.2–3.4 times more active than ZK-158252, and their activities against MIA PaCa-2 were more potent than 5-FU. Further studies with *in vivo* experiments and on their mechanism to confirm these preliminary results are in progress with the ultimate aim of developing them as agents for clinical purposes.

Experimental

Chemistry

Melting point was measured with a Yanaco MP micro-melting-point apparatus and are uncorrected. ^{1}H NMR spectra were measured with JEOL JNM-ECP500 (500 MHz) or JEOL JNM-ECP400 (400 MHz) spectrometers, with chemical shifts expressed in parts per million (ppm) downfield from tetramethylsilane as the internal standard. Mass spectra were measured on a JEOL JMS DX-303 EIMS spectrometer. Elemental analyses were performed with a CHN CORDER MT-3 (Yanaco).

2-Chloroacetyl-3-(4-chlorophenyl)-5-methoxybenzo[*b*]furan 4a. A solution of 1-(4-chlorophenyl)-2-(4-methoxyphenoxy)ethanone[26] (1.00 g, 3.62 mmol) in polyphosphoric acid (10 mL) was stirred for 30 min at 140 °C. The reaction mixture was poured into ice water, and the products were extracted with AcOEt. The organic layer was washed with saturated NaCl aqueous solution, dried over anhydrous $MgSO_4$ and evaporated to give a solid, which was recrystallized from AcOEt to give 3-(4-chlorophenyl)-5-methoxybenzo[*b*]furan (0.93 g, 99%) as colorless crystals; mp, 77–78 °C; δ_H ($CDCl_3$, 500 MHz) 3.86 (3H, s, OCH_3), 6.97 (1H, dd, $J = 9.2$, 2.7 Hz, 6-H), 7.20 (1H, d, $J = 2.8$ Hz, 4-H), 7.42–7.46 (3H, m, $J = 8.7$ Hz, 7- and Ar-H), 7.54 (2H, d, $J = 8.3$ Hz, Ar-H), 7.74 (1H, s, 2-H); Anal. Calcd for $C_{15}H_{11}ClO_2$: C, 69.64; H, 4.29 found: C, 69.67; H, 4.19%.

A solution of 3-(4-chlorophenyl)-5-methoxybenzo[*b*]furan (300 mg, 1.16 mmol) in $CHCl_3$ (10 mL) was added to a mixture of the $AlCl_3$(0.16 g, 1.20 mmol) and chloroacetyl chloride (0.11 m l, 1.40 mmol) in $CHCl_3$ (5 mL) at 0 °C. After stirring for 2.5 h at 0 °C, the reaction mixture was poured into ice water, and the products were extracted with $CHCl_3$. The organic layer was washed with saturated NaCl aqueous solution, dried over anhydrous $MgSO_4$ and evaporated to give a solid, which was recrystallized from AcOEt–*n*-hexane to give **4a** (310 mg, 80%) as yellow crystals; mp, 138–139 °C; δ_H ($CDCl_3$, 500 MHz) 3.81 (3H, s, OCH_3), 4.72 (2H, s, CH_2), 6.95 (1H, d, $J = 2.7$ Hz, 4-H), 7.18 (1H, dd, $J = 9.1$, 2.3 Hz, 6-H), 7.50–7.57 (3H, m, 7- and Ar-H), 7.55–7.57 (2H, m, Ar-H); Anal. Calcd for $C_{17}H_{12}Cl_2O_3$: C, 60.92; H, 3.61 found: C, 60.97; H, 3.45%.

5-Bromo-2-bromoacetyl-3-(5-methylisoxazole-4-carboxamido)-benzo[*b*]furan 4b. A solution of Br_2 (0.72 g, 4.51 mmol) in $CHCl_3$ (15 mL) was added to a solution of 2-acetyl-5-bromo-3-(5-methylisoxazole-4-carboxamido)benzo[*b*]furan[27] (1.50 g, 4.14 mmol) in $CHCl_3$ (30 mL). After stirring for 1.5 h at room temperature, saturated $NaHCO_3$ aqueous solution (10 mL) was added to the mixture, and the products were extracted with $CHCl_3$. The organic layer was washed with saturated NaCl aqueous solution, dried over anhydrous $MgSO_4$ and evaporated to give a solid, which was purified by column chromatography ($CHCl_3$) and recrystallized from AcOEt to give **4b** (1.28 g, 70%) as colorless crystals; mp, 195–196 °C; δ_H ($CDCl_3$, 60 MHz) 2.87 (3H, s, CH_3), 4.48 (2H, s, CH_2Br), 7.19–7.46 (1H, m, 6-H), 7.71 (1H, d, $J = 10.2$ Hz, 7-H), 8.64 and 8.85 (1H each, each s, 4-H and isoxazole-H), 10.57 (1H, br s, NH); Anal. Calcd for $C_{15}H_{10}Br_2N_2O_4$: C, 40.75; H, 2.28; N, 6.34 found: C, 40.66; H, 2.13; N, 6.35%.

2-Bromoacetyl-6-methoxybenzo[*b*]furan 4d. A solution of chloroacetone (2.01 mL, 25 mmol) in CH_3CN (8 mL) and K_2CO_3 (8.58 g, 62 mmol) were added to a solution of 4-methoxysalicylaldehyde (3.20 g, 21 mmol) in CH_3CN (45 mL). After stirring for 2 h under reflux, the reaction mixture was filtered and the solvent was evaporated. H_2O and $CHCl_3$ were added to the residue. The products were extracted with $CHCl_3$. The organic layer was washed with saturated NaCl aqueous solution, dried over anhydrous $MgSO_4$ and evaporated to give a brown solid, which was recrystallized from AcOEt–*n*-hexane to give 2-acetyl-6-methoxybenzo[*b*]furan (2.76 g, 69%) as slightly brown crystals; mp, 77–79 °C; δ_H ($CDCl_3$, 60 MHz) 2.56 (3H, s, $COCH_3$), 3.87 (3H, s, OCH_3), 6.85–7.63 (4H, m, 3-, 4-, 5-, 7-H); Anal. Calcd for $C_{11}H_{10}O_3 \cdot 0.05H_2O$: C, 69.14; H, 5.33 found: C, 69.13; H, 5.35%.

Compound **4d** was prepared under similar reaction conditions to the synthesis of **4b** starting from 2-acetyl-6-methoxybenzo[*b*]furan instead of 2-acetyl-5-bromo-3-(5-methylisoxazole-4-carboxamido)benzo[*b*]furan, and obtained as yellow crystals after recrystallization from AcOEt–*n*-hexane; yield, 62%; mp, 72–74 °C; δ_H ($CDCl_3$, 60 MHz) 3.90 (3H, s, OCH_3), 4.39 (2H, s, CH_2Br), 6.91–7.60 (4H, m, 3-, 4-, 5-, 7-H); Anal. Calcd for $C_{11}H_9BrO_3$ C, 49.10; H, 3.37 found: C, 48.98; H, 3.24%.

General procedure for the synthesis of the 2-(2-aminothiazol-4-yl)benzo[*b*]furans 5—synthesis of 2-(2-aminothiazol-4-yl)-3-(4-chlorophenyl)-5-methoxybenzo[*b*]furan 5a as an example. A solution of **4a** (0.31 g, 0.92 mmol) and thiourea (0.085 g, 1.12 mmol) in EtOH (20 mL) was refluxed for 2 h. The reaction mixture was poured into ice water, and powdered products were extracted with $CHCl_3$. The organic layer was washed with saturated NaCl aqueous solution, dried over anhydrous $MgSO_4$ and evaporated to give a solid, which was recrystallized from AcOEt to give **5a** (0.18 g, 55%) as yellow crystals; mp, 196–198 °C; δ_H ($CDCl_3$, 400 MHz) 3.80 (3H, s, OCH_3), 4.98 (2H, s, NH_2), 6.63 (1H, s, Th-H), 6.86 (1H, d, $J = 2.6$ Hz, 4-H), 6.93 (1H, dd, $J = 8.8$, 2.5 Hz, 6-H), 7.45 (1H, d, $J = 8.8$ Hz, 7-H), 7.45–7.51 (4H, m, Ar-H); Anal. Calcd for $C_{18}H_{13}ClN_2O_2S$: C, 60.59; H, 3.67; N, 7.85 found: C, 60.43; H, 3.57; N, 7.80%.

2-(2-Aminothiazol-4-yl)-5-bromo-3-(5-methylisoxazole-4-carboxamido)benzo[*b*]furan 5b. The title compound was synthesized according to the general procedure for **5a** by using **4b** instead of **4a**; yield, 84%; mp, 203–205 °C (recrystallized from MeOH); δ_H ($CDCl_3$, 400 MHz) 2.75 (3H, s, CH_3), 7.09 (1H, s, Th-H), 7.35 (2H, s, NH_2), 7.48 (1H, dd, $J = 8.8$, 2.2 Hz, 6-H), 7.56 (1H, d, $J = 8.7$ Hz, 7-H), 7.97 (1H, d, $J = 1.8$ Hz, 4-H), 9.10 (1H, s, isoxazole-H), 10.27 (1H, s, NHCO); Anal. Calcd for $C_{16}H_{11}BrN_4O_3S$: C, 45.84; H, 2.64; N, 13.36 found: C, 45.89; H, 2.51; N, 13.10%.

5-Bromo-3-(5-methylisoxazole-4-carboxamido)-2-(2-phenylthiazol-4-yl)benzo[*b*]furan 5c. The title compound was synthesized according to the general procedure for **5a** by using **4b** and thiobenzamide instead of **4a** and thiourea; yield, 86%; mp, 231–235 °C; δ_H ($CDCl_3$, 400 MHz) 2.82 (3H, s, CH_3), 7.34 (1H, d, $J = 8.7$ Hz, 7-H), 7.45 (1H, dd, $J = 8.7$, 2.0 Hz, 6-H), 7.51–7.54 (3H, m, Ar-H), 7.68 (1H, s, thiazole-H), 7.85–7.87 (2H, m, Ar-H), 8.46 (1H, d, $J = 2.0$ Hz, 4-H), 8.63 (1H, s, isoxazole-H), 10.42 (1H, s, NH); Anal. Calcd for $C_{22}H_{14}BrN_3O_3S$: C, 55.01; H, 2.94; N, 8.75 found: C, 54.81; H, 2.91; N, 8.63%.

2-(2-Aminothiazol-4-yl)-5-bromobenzo[*b*]furan 5d[19]. The title compound was synthesized according to the general procedure for **5a** by using **4c**[18] instead of **4a**; yield, 85%; mp, 234–236 °C; δ_H ($CDCl_3$, 400 MHz) 6.95 and 7.10 (1H each, each s, Th-H and 3-H), 7.21 (2H, s, NH_2), 7.42 (1H, dd, $J = 8.8$, 2.2 Hz, 6-H), 7.54 (1H, d, $J = 8.4$ Hz, 7-H), 7.84 (1H, d, $J = 1.9$ Hz, 4-H); Anal. Calcd for $C_{11}H_7BrN_2OS$: C, 44.76; H, 2.39; N, 9.49 found: C, 44.85; H, 2.36; N, 9.54%.

2-(2-Aminothiazol-4-yl)-6-methoxybenzo[*b*]furan 5e. The title compound was synthesized according to the general procedure for **5a** by using **4d** instead of **4a**; yield, 58%; mp, 190–191 °C (recrystallized from $CHCl_3$); δ_H ($CDCl_3$, 400 MHz) 3.81 (3H, s, OCH_3), 6.868 (1H, s, 3-H or Th-H), 6.871 (1H, dd, $J = 8.4$, 2.2 Hz, 5-H), 6.91 (1H, s, 3-H or Th-H), 7.15 (2H, s, NH_2), 7.18 (1H, d, $J = 1.8$ Hz, 7-H), 7.49 (1H, d, $J = 8.8$ Hz, 4-H); Anal. Calcd for $C_{12}H_{10}N_2O_2S$: C, 58.52; H, 4.09; N, 11.37 found: C, 58.32; H, 4.06; N, 11.15%.

2-(2-Acetamidothiazol-4-yl)-3-(4-chlorophenyl)-5-methoxybenzo-[*b*]furan 6a. Acetyl chloride (0.24 mL, 3.36 mmol) was added to a solution of **5a** (0.50 g, 1.40 mmol) in THF (15 mL). After stirring for 2 h under refluxing, the reaction mixture was poured into ice water, and the mixture was acidified by adding of 5% HCl aqueous solution. The products were extracted with AcOEt, and the organic layer was washed with saturated NaCl aqueous solution, dried over anhydrous $MgSO_4$ and evaporated to give a brown solid, which was recrystallized from MeOH–CCl_4 to give **6a** (0.43 g, 77%) as yellow crystals; mp, 217–218 °C; δ_H ($CDCl_3$, 400 MHz) 2.09 (3H, s, $COCH_3$), 3.81 (3H, s, OCH_3), 6.89 (1H, d, $J = 2.6$ Hz, 4-H), 6.96 (1H, dd, $J = 8.8$, 2.6 Hz, 6-H), 7.17 (1H, s, Th-H), 7.42 (2H, d, $J = 8.6$ Hz, Ar-H), 7.45 (1H, d, $J = 9.1$ Hz, 7-H), 7.49 (2H, d, $J = 8.6$ Hz, Ar-H), 9.87 (1H, s, NH); *Anal.* Calcd for $C_{20}H_{15}ClN_2O_3S$: C, 60.22; H, 3.79; N, 7.02 found: C, 60.24; H, 3.69; N, 6.92%.

3-(4-Chlorophenyl)-5-methoxy-2-[2-(5-methylisoxazole-4-carboxamido)thiazol-4-yl]benzo[*b*]furan 6b. A solution of 5-methyl-4-isoxazolecarboxylic acid (0.08 g, 0.63 mmol) and thionyl chloride (0.46 mL, 6.3 mmol) was refluxed for 2 h, and the solvent was evaporated to give crude isoxazolecarboyl chloride as a residue, which was dissolved in THF (20 mL). The compound **5a** (0.3 g, 0.84 mmol) was added to the solution, and the mixture was refluxed for 2 h. THF was evaporated and the product was extracted using AcOEt by adding a 5% HCl aqueous solution. The organic layer was washed with saturated NaCl aq. solution, dried over anhydrous $MgSO_4$ and evaporated to give a yellow solid, which was recrystallized from AcOEt to give **6b** (0.12 g, 31%) as pale yellow crystals; mp, 217–219 °C; δ_H ($CDCl_3$, 400 MHz) 2.66 (3H, s, CCH_3), 3.79 (3H, s, OCH_3), 6.81 (1H, d, $J = 2.5$ Hz, 4-H), 6.92 (1H, dd, $J = 8.8$, 2.6 Hz, 6-H), 7.13 (1H, s, Th-H), 7.33 (1H, d, $J = 9.1$ Hz, 7-H), 7.42 (4H, br s, Ar-H), 8.19 (1H, s, isoxazole-H), 11.06 (1H, s, NH); Anal. Calcd for $C_{23}H_{16}ClN_3O_4S{\cdot}0.5H_2O$: C, 58.17; H, 3.61; N, 8.85 found: C, 58.14; H, 3.44; N, 8.87%.

2-[2-(2-Chloroacetamido)thiazol-4-yl)]-3-(4-chlorophenyl)-5-methoxybenzo[*b*]furan 6c. Chloroacetyl chloride (0.10 mL, 1.26 mmol) was added to a solution of **5a** (0.30 g, 0.84 mmol) in THF (25 mL). After stirring for 8 h at room temperature, the reaction mixture was poured into ice water, and the mixture was neutralized by addition of $NaHCO_3$. The products were extracted with AcOEt, and the organic layer was washed with saturated NaCl aqueous solution, dried over anhydrous $MgSO_4$ and evaporated to give an orange solid, which was recrystallized from AcOEt to give **6c** (0.27 g, 74%) as yellow crystals; mp, 181–182 °C; δ_H ($CDCl_3$, 400 MHz) 3.81 (3H, s, CH_3), 4.25 (2H, s, CH_2), 6.88 (1H, d, $J = 2.5$, 4-H), 6.97 (1H, dd, $J = 8.8$, 2.5 Hz, 6-H), 7.08 (1H, s, Th-H), 7.47–7.50 (5H, m, 7-H and, Ar-H), 9.83 (1H, br s, NH); Anal. Calcd for $C_{20}H_{14}Cl_2N_2O_3S$: C, 55.44; H, 3.26; N, 6.47 found: C, 55.62; H, 3.24; N, 6.25%.

3-(4-Chlorophenyl)-5-methoxy-2-[2-[2-(morpholin-4-yl)acetamido]thiazol-4-yl]benzo[*b*]furan 6d. Morphorine (0.05 mL, 0.56 mmol) was added to a solution of **6c** (0.20 g, 0.46 mmol) in CH_3CN (30 mL). After stirring for 3 h under reflux, the solvent was evaporated to give a white solid, which was recrystallized from AcOEt to give **6d** (0.16 g, 72%) as yellow crystals; mp, 111–116 °C; δ_H ($CDCl_3$, 400 MHz) 2.62 (4H, t, $J = 4.8$ Hz, 2 × NCH_2CH_2), 3.26 (2H, s, $COCH_2N$), 3.79 (4H, t, $J = 4.8$ Hz, 2 × OCH_2CH_2), 3.81 (3H, s, OCH_3), 6.88 (1H, d, $J = 2.5$ Hz, 4-H), 6.96 (1H, dd, $J = 9.0$, 2.8 Hz, 6-H), 6.99 (1H, s, Th-H), 7.45–7.52 (4H, m, Ar-H), 7.50 (1H, d, $J = 7.0$, 7-H), 10.31 (1H, br s, NH); Anal. Calcd for $C_{24}H_{22}ClN_3O_4S{\cdot}0.5H_2O$: C, 58.47; H, 4.70; N, 8.52 found C, 58.64; H, 4.65; N, 8.30%.

3-(4-Chlorophenyl)-2-[2-[2-[4-(2-hydroxyethyl)piperazin-1-yl]-acetamido]thiazol-4-yl]-5-methoxybenzo[*b*]furan 6e. Compound **6e** was prepared under similar reaction conditions to the synthesis of **6d** by using 2-(piperazin-1-yl)ethanol instead of morphorine, and the reaction was conducted at room temperature; yield, 38%; mp, 101–105 °C (recrystallized from MeOH–*n*-hexane); δ_H ($CDCl_3$, 400 MHz) 1.88 (1H, br s, OH), 2.62–2.65 (10H, m, CH_2 in piperazine and NCH_2CH_2OH), 3.26 (2H, s, $COCH_2N$), 3.66 (2H, t, $J = 5.5$ Hz, CH_2OH), 3.81 (3H, s, OCH_3), 6.88 (1H, d, $J = 2.5$ Hz, 4-H), 6.96 (1H, dd, $J = 8.8$, 2.6 Hz, 6-H), 7.03 (1H, s, Th-H), 7.46–7.52 (4H, m, Ar-H), 7.49 (1H, d, $J = 7.0$ Hz, 7-H), 10.35–10.36 (1H, br s, NH); Anal. Calcd for $C_{26}H_{27}ClN_4O_4S{\cdot}\frac{1}{3}H_2O$: C, 58.58; H, 5.23; N, 10.51 found C, 58.72; H, 5.16; N, 10.34%.

(*Z*)-3-(4-Chlorophenyl)-2-[2-(2-cyano-3-hydroxybut-2-enamido)-thiazol-4-yl]-5-methoxybenzo[*b*]furan 7a. Et_3N (0.30 mL, 2.15 mmol) was added to a solution of **6b** (0.10 g, 0.22 mmol) in THF (6 mL), and the mixture was refluxed for 4.5 h. After the reaction, THF was evaporated. The product was extracted using AcOEt by addition of 5% HCl aqueous solution, and the organic layer was washed with saturated NaCl aq. solution, dried over anhydrous $MgSO_4$ and evaporated to give a brown solid, which was recrystallized from EtOH to give **7a** (0.05 g, 50%) as pale yellow crystals; mp, 227–229 °C; δ_H ($CDCl_3$, 400 MHz) 3.21 (3H, s, CCH_3), 3.75 (3H, s, OCH_3), 6.87 (1H, d, $J = 2.5$ Hz, 4-H), 6.96 (1H, dd, $J = 8.7$, 2.5 Hz, 6-H), 7.08 (1H, s, Th-H), 7.46 (1H, d, $J = 8.7$ Hz, 7-H), 7.47 (4H, br s, Ar-H), 9.66 (1H, br s, NH), 14.49 (1H, br s, OH); Anal. Calcd for $C_{23}H_{16}ClN_3O_4S$: C, 59.29; H, 3.46; N, 9.02 found: C, 59.07; H, 3.40; N, 8.93%.

General procedure for Vilsmeier reaction—synthesis of 2-(2-amino-5-formylthiazol-4-yl)-3-(4-chlorophenyl)-5-methoxybenzo-[*b*]furan 7b as an example. A solution of **6c** (0.30 g, 0.69 mmol) in DMF (10 mL) was added to a solution of $POCl_3$ (0.26 mL, 2.78 mmol) in DMF (10 mL) at 0 °C. After stirring for 30 h at room temperature, the reaction mixture was poured into 5% NaOH aqueous solution, and the resulting precipitate was collected by filtration and washed with H_2O to give a red solid, which was recrystallized from AcOEt–*n*-hexane to give **7b** (0.09 g, 34%) as red crystals; mp, 217–220 °C; δ_H (DMSO-d_6, 400 MHz) 3.80 (3H, s, OCH_3), 7.05 (1H, d, $J = 2.2$ Hz, 4-H), 7.09 (1H, dd, $J = 8.8$, 2.2 Hz, 6-H), 7.52 (2H, d, $J = 8.8$ Hz, Ar-H), 7.56 (2H, d, $J = 8.8$ Hz, Ar-H), 7.66 (1H, d, $J = 9.1$, 7-H), 8.21 (2H, s, NH_2), 9.85 (1H, s, CHO); Anal. Calcd for $C_{19}H_{13}ClN_2O_3S$: C, 59.30; H, 3.40; N, 7.28 found: C, 59.44; H, 3.34; N, 7.24%.

3-(4-Chlorophenyl)-5-methoxy-2-[2-[(dimethylamino)methylene-amino]thiazol-4-yl]benzo[*b*]furan 8a. This compound was synthesized according to the general procedure for **7b** by using **5a** instead of **6c**. The reaction was conducted at −10 to −5 °C; yield, 74%; mp, 145–147 °C (recrystallized from EtOH); δ_H ($CDCl_3$, 400 MHz) 3.05 and 3.07 (3H each, each s, $NCH_3 \times 2$), 3.30 (3H, s, OCH_3), 6.90 (1H, d, $J = 2.5$ Hz, 4-H), 6.92 (1H, dd, $J = 8.8$, 2.6 Hz, 6-H), 7.00 (1H, s, Th-H), 7.43 (1H, d, $J = 8.8$ Hz, 7-H), 7.42–7.55 (4H, m, Ar-H), 8.16 (1H, s, N=CH); Anal. Calcd for $C_{21}H_{18}ClN_3O_2S$: C, 61.23; H, 4.40; N, 10.20 found: C, 61.12; H, 4.27; N, 10.14%.

3-(4-Chlorophenyl)-2-[5-formyl-2-[(dimethylamino)methylene-amino]thiazol-4-yl]-5-methoxybenzo[*b*]furan 8b. This compound was synthesized according to the general procedure for **7b** by using **5a** instead of **6c**, and 8 equiv. of $POCl_3$ was used; yield, 60%; mp, 196–198 °C (recrystallized from MeOH–AcOE); δ_H ($CDCl_3$, 400 MHz) 3.07 (6H, s, $N(CH_3)_2$), 3.82 (3H, s, OCH_3), 6.94 (1H, d, $J = 2.8$ Hz, 4-H), 7.03 (1H, dd, $J = 9.0$, 2.6 Hz, 6-H), 7.42–7.55 (5H, m, 7- and Ar-H), 7.96 (1H, s, CH=N), 10.43 (1H, s, CHO); *m/z* 441 (40), 439 (M^+, 100), 410 (17), 395 (18), 380 (18), 283 (14), 235 (16), 208 (16), 115 (20), 98 (15); Anal. Calcd for $C_{22}H_{18}ClN_3O_3S$: C, 60.07; H, 4.12; N, 9.55 found: C, 59.99; H, 3.99; N, 9.57%.

3-(4-Chlorophenyl)-2-[5-(2-hydroxyethylimino)methyl]-2-[(dimethylamino)methyleneamino]thiazol-4-yl]-5-methoxybenzo[*b*]furan 9a. A solution of **8b** (0.30 g, 0.68 mmol) and ethanolamine (0.06 mL, 1.03 mmol) in EtOH (5 mL) was refluxed for 0.5 h. The solvent was evaporated to give a yellow residue, which was dissolved in AcOEt, washed with H_2O and saturated NaCl aqueous solution. The organic layer was dried over anhydrous $MgSO_4$ and evaporated to give an orange solid, which was recrystallized from AcOEt to give **9a** (0.24 g, 73%) as red crystals; mp, 168–169 °C; δ_H ($CDCl_3$, 400 MHz) 2.04 (1H, br s, OH), 3.06 (6H, s, N $(CH_3)_2$), 3.60–3.62 (2H, m, OCH_2CH_2N), 3.80–3.83 (2H, m, OCH_2CH_2N), 3.83 (3H, s, OCH_3), 6.98 (1H, d, $J = 1.8$ Hz, 4-H), 6.99 (1H, dd, $J = 8.7$, 2.3 Hz, 6-H), 7.41 (2H, d, $J = 8.7$ Hz, Ar-H), 7.45 (2H, d, $J = 8.7$ Hz, Ar-H), 7.46 (1H, d, $J = 8.7$ Hz, 7-H), 8.03 (1H, s, N=CH), 8.64 (1H, s, N=CH); *m/z* 484 (6), 482 (M^+, 14), 385 (17), 371 (100); Anal. Calcd for $C_{24}H_{23}ClN_4O_3S$: C, 59.68; H, 4.80; N, 11.60 found: C, 59.49; H, 4.77; N, 11.35%.

3-(4-Chlorophenyl)-2-[5-[(2-ethoxy-2-oxoethylimino)methyl]-2-[(dimethylamino)methyleneamino]thiazol-4-yl]-5-methoxybenzo-[*b*]furan 9b. A mixture of **8b** (0.50 g, 1.13 mmol), glycine ethyl ester hydrochloride (0.19 g, 1.36 mmol), Et_3N (0.31 mL, 2.22 mmol) and molecular sieves (3Å, 0.1 g) in EtOH (10 mL) was refluxed for 1 h. The solvent was evaporated to give a yellow residue, which was dissolved in AcOEt, washed with H_2O and saturated NaCl aqueous solution. The organic layer was dried over anhydrous $MgSO_4$ and evaporated to give an orange solid, which was recrystallized from AcOEt to give **9b** (0.10 g, 17%) as red crystals; mp, 162–165 °C; δ_H ($CDCl_3$, 500 MHz) 1.29 (3H, t, $J = 7.4$, CH_2CH_3), 3.05 (3H, s, NCH_3), 3.06 (3H, s, NCH_3), 3.82 (3H, s, OCH_3), 4.21 (2H, q, $J = 7.4$ Hz, CH_2CH_3), 4.23 (2H, s, NCH_2CO), 6.97–7.00 (2H, m, 4- and 6-H), 7.41 (2H, d, $J = 8.7$ Hz, Ar-H), 7.44–7.48 (3H, m, 7- and Ar-H), 8.01 (H, s, N=CH), 8.62 (H, s, N=CH); *m/z* 526 (10), 524 (M^+, 25), 427 (23), 413 (100), 356 (12), 354 (31); Anal. Calcd for $C_{26}H_{25}ClN_4O_4S{\cdot}0.2H_2O$: C, 59.07; H, 4.84; N, 10.60 found: C, 59.25; H, 4.80; N, 10.34%.

(*E*)-3-(4-Chlorophenyl)-5-methoxy-2-[-2-[(dimethylamino)methyleneamino]-5-[3-(morpholin-4-yl)-3-oxoprop-1-enyl]thiazol-4-yl]benzo[*b*]furan 10a. A solution of **8b** (0.47 g, 1.07 mmol) in THF (30 mL) was added to a mixture of [2-(4-morpholinyl)-2-oxoethyl]phosphonic acid ethyl ester[12b] (0.34 g, 1.28 mmol) and NaH (60% in oil, 0.051 g, 1.28 mmol) in THF (10 mL) at 0 °C. After stirring for 42 h at room temperature, the reaction mixture was poured into saturated NH_4Cl aqueous solution. The products were extracted with AcOEt, and the organic layer was washed with sat. NaCl aqueous solution, dried over anhydrous $MgSO_4$ and evaporated to give a yellow solid, which was recrystallized from AcOEt to give **10a** (0.37 g, 63%) as yellow crystals; mp, 142–144 °C; δ_H ($CDCl_3$, 500 MHz) 3.06 (6H, s, $N(CH_3)_2$), 3.70–3.72 (8H, m, 2 × CH_2CH_2), 3.82 (3H, s, OCH_3), 6.27 (1H, d, $J = 15.1$ Hz, CH=C*H*CO), 6.95 (1H, dd, $J = 8.5$, 2.6 Hz, 6-H), 6.98 (1H, d, $J = 2.3$ Hz, 4-H), 7.37–7.39 (2H, m, Ar-H), 7.42–7.45 (2H, m, Ar-H), 7.50 (1H, d, $J = 9.1$ Hz, 7-H), 8.01 (1H, s, N=CH), 8.19 (1H, d, $J = 15.1$ Hz, C*H*=CHCO); *m/z* 552 (M + 2, 42), 551 (M + 1, 33), 550 (M^+, 100), 464 (39), 332 (48).

(*E*)-3-(4-Chlorophenyl)-5-methoxy-2-[5-[3-(4-methoxyphenyl-amino)-3-oxoprop-1-enyl]-2-[(dimethylamino)methyleneamino]thiazol-4-yl]benzo[*b*]furan 10b. Compound **10b** was prepared under similar reaction conditions to the synthesis of **10a** by using [2-[(4-methoxyphenyl)amino]-2-oxoethyl]phosphonic acid ethyl ester[21] instead of [2-(4-morpholinyl)-2-oxoethyl]phosphonic acid ethyl

ester; yield, 43%; mp, 224–227 °C; δ_H ($CDCl_3$, 400 MHz) 3.06 (6H, s, 2 × NCH_3), 3.80 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 6.00 (1H, d, J = 15.0 Hz, CH=C*H*CO), 6.85–6.89 (2H, m, Ar-H), 6.94 (1H, d, J = 2.2 Hz, 4-H), 6.97 (1H, dd, J = 8.8, 2.5 Hz, 6-H), 7.08 (1H, br s, NH), 7.37–7.47 (6H, m, Ar-H), 7.51 (1H, d, J = 8.8 Hz, 7-H), 8.01 (1H, s, N=CH), 8.22 (1H, d, J = 15.0 Hz, C*H*=CHCO); Anal. Calcd for $C_{31}H_{27}ClN_4O_4S$: C, 63.42; H, 4.64; N, 9.54 found: C, 63.45; H, 4.57; N, 9.45%.

(*Z*)-2-(2-Aminothiazol-4-yl)-5-bromo-3-(2-cyano-3-hydroxybut-2-enamido)benzo[*b*]furan 5f. Compound **5f** was prepared under similar reaction conditions to the synthesis of **7a** by using **5b** instead of **6b**, and obtained as yellow crystals after recrystallization from AcOEt–*n*-hexane; yield, 60%; mp, 228–230 °C; δ_H ($CDCl_3$, 400 MHz) 2.38 (3H, s, CCH_3), 5.59 (2H, s, NH_2), 6.85 (1H, s, Th-H), 7.28 (1H, d, J = 8.7 Hz, 7-H), 7.91 (1H, dd, J = 8.7, 2.0 Hz, 6-H), 8.54 (1H, d, J = 2.0 Hz, 4-H), 11.76 (1H, s, NHCO), 15.75 (1H, s, OH); Anal. Calcd for $C_{16}H_{11}BrN_4O_3S$: C, 45.84; H, 2.64; N, 13.36 found: C, 45.92; H, 2.52; N, 13.25%.

5-Bromo-2-[2-(2,2-dimethylpropanamido)thiazol-4-yl]benzo[*b*]-furan 11a. Trimethylacetyl chloride (0.19 mL, 1.53 mmol) was added to a solution of **5d** (0.30 g, 1.02 mmol) and Et_3N (0.40 mL, 2.87 mmol) in THF (10 mL). After stirring for 23 h under reflux, the reaction mixture was poured into ice water. The products were extracted with $CHCl_3$, and the organic layer was washed with saturated NaCl aqueous solution, dried over anhydrous $MgSO_4$ and evaporated to give a white solid, which was recrystallized from AcOEt to give **11a** (0.21 g, 54%) as colorless crystals; mp, 184–185 °C; δ_H (DMSO-d_6, 400 MHz) 1.27 (9H, s, $C(CH_3)_2$), 7.13 (1H, s, 3-H), 7.46 (1H, dd, J = 8.7, 2.1 Hz, 6-H), 7.60 (1H, d, J = 8.7 Hz, 7-H), 7.69 (1H, s, Th-H), 7.90 (1H, d, J = 2.0, 4-H), 12.05 (1H, s, NH); m/z 380 (M + 2, 42), 378 (M^+, 41), 296 (33), 294 (32), 57 (100); HRMS Calcd for $C_{16}H_{15}BrN_2O_2S$: 378.0038; found: 378.0033.

5-Bromo-2-[2-(4-chlorobenzamido)thiazol-4-yl]benzo[*b*]furan 11b. A solution of *p*-chlorobenzoyl chloride (1.97 mL, 15.4 mmol) in THF (50 mL) was added to a solution of **5d** (0.30 g, 1.02 mmol) in THF (100 mL). After stirring for 47 h at room temperature, the reaction mixture was poured into water, and the mixture was acidified by addition of 10% HCl aqueous solution. The products were extracted with AcOEt, and the organic layer was washed with saturated NaCl aqueous solution, dried over anhydrous $MgSO_4$ and evaporated to give a yellow solid, which was recrystallized from MeOH to give **11b** (2.15 g, 49%) as pale yellow crystals; mp, 234–249 °C; δ_H (DMSO-d_6, 400 MHz) 7.18 (1H, s, 3-H), 7.48 (1H, dd, J = 8.7, 2.0 Hz, 6-H), 7.54–7.58 (2H, m, Ar-H), 7.62 (1H, d, J = 9.7 Hz, 7-H), 7.63–7.66 (2H, m, Ar-H), 7.79 (1H, s, Th-H), 7.91 (1H, d, J = 2.1 Hz, 4-H), 13.0 (1H, s, NH); m/z 436 (M + 4, 9), 434 (M + 2, 31), 432 (M^+, 22), 141 (32), 111 (23); HRMS Calcd for $C_{18}H_{10}BrClN_2O_2S$: 431.9336; found: 431.9335.

5-Bromo-2-[2-(2-chloroacetamido)thiazol-4-yl]benzo[*b*]furan 11c. Compound **11c** was prepared under similar reaction conditions to the synthesis of **11b** by using chloroacetyl chloride instead of *p*-chlorobenzoyl chloride. The reaction mixture was refluxed for 46 h and obtained as yellow crystals after recrystallization from AcOEt–*n*-hexane; yield, 68%; mp, 211 °C; δ_H ($(CD_3)_2CO$, 400 MHz) 4.31 (2H, s, CH_2), 7.01 (1H, s, 3-H), 7.39–7.42 (3H, m, 4-, 6-, 7-H), 7.73 (1H, s, Th-H), 9.72 (1H, brs, NH); Anal. Calcd for $C_{13}H_8BrClN_2O_2S$: C, 42.01; H, 2.17; N, 7.54 found: C, 42.29; H, 2.13; N, 7.51%.

5-Bromo-2-[2-(3-chloropropanamido)thiazol-4-yl]benzo[*b*]furan 11d. Compound **11d** was prepared under similar reaction conditions to the synthesis of **11b** by using 3-chloropropionyl chloride instead of *p*-chlorobenzoyl chloride, and obtained as pale yellow crystals after recrystallization from MeOH; yield, 55%; mp, 210–212 °C; δ_H (DMSO-d_6, 400 MHz) 2.99 (2H, t, J = 6.2 Hz, $COCH_2$), 3.92 (2H, t, J = 6.3 Hz, CH_2Cl), 7.12 (1H, s, 3-H), 7.47 (1H, dd, J = 8.7, 2.1 Hz, 6-H), 7.60 (1H, d, J = 8.4 Hz, 7-H), 7.71 (1H, s, Th-H), 7.89 (1H, d, J = 1.8 Hz, 4-H), 12.65 (1H, s, NH); Anal. Calcd for $C_{14}H_{10}BrClN_2O_2S$: C, 43.60; H, 2.61; N, 7.26 found: C, 43.67; H, 2.66; N, 7.12%.

5-Bromo-2-[5-formyl-2-(2,2-dimethylpropanamido)thiazol-4-yl-]benzo[*b*]furan 12a. This compound was synthesized according to the general procedure for **7b** by using **11a** instead of **6c**; yield, 41%; mp, 244–245 °C (recrystallized from MeOH–AcOEt); δ_H ($CDCl_3$, 400 MHz) 1.38 (9H, s, $C(CH_3)_3$), 7.30 (1H, s, 3-H), 7.42 (1H, d, J = 8.8 Hz, 7-H), 7.48 (1H, dd, J = 8.8, 1.9 Hz, 6-H), 7.80 (1H, d, J = 1.8 Hz, 4-H), 9.03 (1H, br s, NH), 10.70 (1H, s, CHO); Anal. Calcd for $C_{17}H_{15}BrN_2O_3S{\cdot}0.2H_2O$: C, 49.69; H, 3.78; N, 6.82 found: C, 49.82; H, 3.71; N, 6.72%.

5-Bromo-2-(2-formamido-5-formylthiazol-4-yl)benzo[*b*]furan 12b. This compound was synthesized according to the general procedure for **7b** by using **11b** instead of **6c**; yield, 9%; mp, 250–254 °C; δ_H ($(CD_3)_2CO$, 400 MHz) 7.46 (1H, s, 3-H), 7.59 (1H, dd, J = 8.8, 2.2 Hz, 6-H), 7.70 (1H, d, J = 8.8 Hz, 7-H), 7.95 (1H, d, J = 2.2 Hz, 4-H), 8.79 (1H, br s, NCHO), 10.74 (1H, s, CCHO), 11.66 (1H, br s, NH); m/z 352 (M + 2, 100), 350 (M^+, 99), 324 (38), 322 (37), 280 (28), 278 (27), 254 (62), 252 (62), 145 (33), 144 (30).

5-Bromo-2-[5-formyl-2-[(dimethylamino)methyleneamino]thiazol-4-yl]benzo[*b*]furan 13. This compound was synthesized according to the general procedure for **7b** by using **5d** instead of **6c**, and the reaction was conducted at 60 °C; yield, 50%; mp, 187 °C (recrystallized from AcOEt); δ_H ($CDCl_3$, 500 MHz) 3.17 and 3.21 (3H each, each s, $N(CH_3)_2$), 7.37 (1H, s, 3-H), 7.40 (1H, d, J = 8.7 Hz, 7-H), 7.46 (1H, dd, J = 8.9, 2.1 Hz, 6-H), 7.78 (1H, d, J = 1.8, 4-H), 8.35 (1H, s, N=CH), 10.61 (1H, s, CHO); Anal. Calcd for $C_{15}H_{12}BrN_3O_2S$: C, 47.63; H, 3.20; N, 11.11 found: C, 47.47; H, 2.98; N, 10.91%.

5-Bromo-3-(2-chloroacetyl)-2-phenylbenzo[*b*]furan 15. A solution of **14**[23] (1.00 g, 3.67 mmol) in $CHCl_3$ (18 mL) was added to a solution of $AlCl_3$ (1.94 g, 15 mmol) and chloroacetyl chloride (0.35 mL, 4.42 mmol) at 0 °C. After stirring for 2 h at 0 °C, the reaction mixture was poured into ice water, and the products were extracted with AcOEt. The organic layer was washed with saturated NaCl aqueous solution, dried over anhydrous $MgSO_4$ and evaporated to give a yellow solid, which was purified by column chromatography (AcOEt–*n*-hexane = 3 : 7) and recrystallized from AcOEt–*n*-hexane to give **15** (0.33 g, 26%) as pale yellow crystals; mp, 104–106 °C; δ_H ($CDCl_3$, 400 MHz) 4.28 (2H, s, CH_2), 7.41 (1H, d, 7-H, J = 8.8 Hz), 7.52 (1H, dd, 6-H, J = 8.4, 1.9 Hz), 7.54–7.63 (3H, m, Ar-H), 7.71–7.74 (2H, m, Ar-H),

8.24 (1H, d, 4-H, J = 1.8 Hz); Anal. Calcd for $C_{16}H_{10}BrClO_2$: C, 54.97; H, 2.88 found: C, 54.85; H, 2.79%.

3-(2-Aminothiazol-4-yl)-5-bromo-2-phenylbenzo[*b*]furan 5g. This compound was synthesized according to the general procedure for **5a** by using **15** instead of **4a**; yield, 84%; mp, 203–205 °C (recrystallized from AcOEt–*n*-hexane); δ_H (DMSO-d_6, 400 MHz) 6.73 (1H, s, Th-H), 7.12 (2H, br s, NH_2), 7.42–7.49 (3H, m, Ar-H), 7.51 (1H, dd, J = 8.6, 2.1 Hz, 6-H), 7.63 (1H, d, J = 8.8 Hz, 7-H), 7.81–7.83 (2H, m, Ar-H), 7.92 (1H, d, J = 2.2 Hz, 4-H); Anal. Calcd for $C_{17}H_{11}BrN_2OS$: C, 55.00; H, 2.99; N, 7.55 found: C, 54.94; H, 2.93; N, 7.48%.

5-Bromo-3-[5-formyl-2-[(dimethylamino)methyleneamino]thiazol-4-yl]-2-phenylbenzo[*b*]furan 16. This compound was synthesized according to the general procedure for **7b** by using **5g** instead of **6c**; yield, 33%; mp, 151–154 °C (recrystallized from MeOH–*n*-hexane); δ_H ($CDCl_3$, 400 MHz) 3.19 (6H, s, 2 × NCH_3), 7.34–7.39 (2H, m, 6-, 7-H), 7.44–7.45 (3H, m, Ar-H), 7.67 (1H, d, J = 1.5 Hz, 4-H), 7.68–7.69 (2H, m, Ar-H), 8.49 (1H, s, N=CH), 9.46 (1H, s, CHO); Anal. Calcd for $C_{21}H_{16}BrN_3O_2S{\cdot}0.4H_2O$: C, 54.65; H, 3.67; N, 9.10 found: C, 54.94; H, 3.48; N, 8.81%.

Biology

Measurement of calcium mobilization in CHO cells. The prepared compounds were evaluated for BLT1/BLT2 receptor inhibitory activity according to a procedure reported previously.[12a,b]

Materials and methods for measurement of growth inhibitory activity to cancer cell lines.

Reagents. 5-Fluorouracil (5-FU) and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. Stock solutions of the synthesized compounds or 5-FU were prepared by dissolving each compound in DMSO at 10 μM. Some of the dilutions were subsequently prepared in growth medium (D-MEM or E-MEM). The final concentration of DMSO in growth medium was made to be 0.25% or less.

Cell lines. NHDF "neonatal normal human dermal fibroblasts", MIA Paca-2 "human pancreatic carcinoma" and MCF-7 "human adenocarcinoma of breast" were purchased from Japan Health Sciences Foundation. MDA-MB-231 "human adenocarcinoma of breast" was purchased from American Type Culture Collection. NHDF and MCF-7 were grown in E-MEM. MIA Paca-2 was grown in D-MEM. Each medium was supplemented with 10% of fetal calf serum (MultiSer™) and 6 mL of antibiotic-antimycotic 100× (GIBCO).

AlamarBlue™ assay for cell cytotoxicity. We used an alamarBlue™ (Biosource) assay to measure cell cytotoxicity. The human cells were seeded at 1 × 10^4 cells in 200 μL of growth medium per well in 96 well flat bottom tissue culture plates (Nunc). The cells were incubated for 24 h at 37 °C in a humidified atmosphere of 5% CO_2 in air. Next, the growth media from the plates were eliminated, and 180 μL of growth medium containing drug was added to triplicate wells. The cells were incubated continuously for 72 h. Following incubation of plates, 20 μL of alamarBlue™ was added to all wells, and the plates were set in an incubator for three additional hours. The live cells were counted on a microplate reader (Spectra Max M5, Molecular Devices), using an excitation wavelength of 530 nm and emission wavelength of 590 nm.

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