Synthesis and biological activities of novel furo[2,3,4-*jk*][2]benzazepin-4(3*H*)-one derivatives†

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A novel seven-membered lactam formation method has been established by intramolecular ring closure reaction of 4-bromo-(*E*)-3-[(2-alkylvinyl)carbonylamino]benzo[*b*]furans (**17**) under Heck coupling conditions. A number of furo[2,3,4-*jk*][2]benzazepin-4(3*H*)-ones (**20**), tricyclicbenzo[*b*]furans, have been prepared by this method and evaluated for their leukotriene B_4 (LTB_4) receptor and poly(ADP-ribose)polymerase-1 (PARP-1) inhibitory activities.

Introduction

We previously reported the preparation of various 2- and 4-[(*E*)-2-alkylcarbamoyl-1-methylvinyl]benzo[*b*]furan derivatives and their selective LTB_4 receptor (BLT_1, BLT_2) inhibitory activities. This study revealed a significant relation between the conformation of the (*E*)-2-alkylcarbamoyl-1-methylvinyl group and BLT_1 and/or BLT_2 inhibitory activity.[1,2] The (*E*)-2-(2-alkylcarbamoyl-1-methylvinyl) group of (*E*)-2-(2-alkylcarbamoyl-1-methylvinyl)benzo[*b*]furans (**A**) showing selective BLT_2 inhibition lay on nearly the same plane as the benzo[*b*]furan ring. On the other hand, (*E*)-4-(2-alkylcarbamoyl-1-methylvinyl)benzo[*b*]furans (**C**) inhibited both BLT_1 and BLT_2, and the (*E*)-4-(2-alkylcarbamoyl-1-methylvinyl) group had a substantial torsion angle from the benzo[*b*]furan ring plane. These results suggested that conformational restriction of the (*E*)-2-alkylcarbamoyl-1-methylvinyl group by conversion of a ring-type functional group might affect the inhibitory potency and the selectivity for BLT_1 and/or BLT_2 (Fig. 1).

The ring formation originating from the (*E*)-2-alkylcarbamoyl-1-methylvinyl group of **A** affords 2,3-fused benzo[*b*]furan derivatives (**B**).[3–8] There have been many reports of the preparation and bioactivity of these derivatives (**B**).[9–11] On the other hand, ring formation of the (*E*)-2-alkylcarbamoyl-1-methylvinyl group of **C** affords tricyclicbenzo[*b*]furans (**D**) having a seven-membered lactam fused to the 3,4-position of the benzo[*b*]furan (Fig. 1). There have been few reports concerning the synthesis of 3,4-fused tricyclicbenzo[*b*]furan derivatives.[12] We were interested in both the synthesis and bioactivities of the tricyclicbenzo[*b*]-furans (**D**).

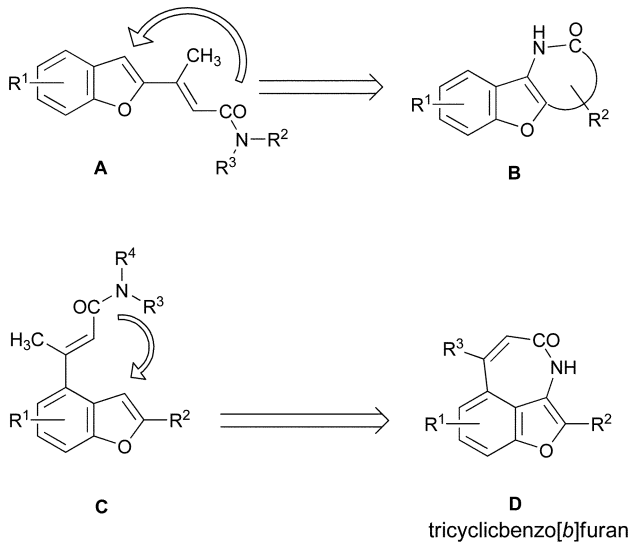

Fig. 1 Cyclization of the alkylcarbamoylvinyl group.

Recently, the PARP-1 inhibitory activity of some tricyclic lactam compounds has been reported (Fig. 2).[13–16] PARP-1 is a nuclear enzyme that is activated by DNA strand breaks. It plays an important role in the reaction that transfers the ADP-ribose moiety from nicotinamide adenine dinucleotide (NAD^+) to various proteins.[17–20] PARP-1 inhibitors are thought to be implicated in a wide spectrum of diseases, including ischemic diseases, inflammatory diseases, multiple sclerosis, arthritis and Parkinson's disease. Therefore, much work has been done toward preparing PARP-1 inhibitors.[21–30] These reports suggested that the tricyclicbenzo[*b*]furans (**D**) might possess PARP-1 inhibitory activity.

We report here the synthesis of novel tricyclicbenzo[*b*]furans (**D**) and their evaluation for LTB_4 receptor and PARP-1 inhibitory activities.

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† Electronic supplementary information (ESI) available: Physical data and yields of compounds **7**, **10–14**, **17–21**, **23** and **25**. See DOI: 10.1039/b614510h

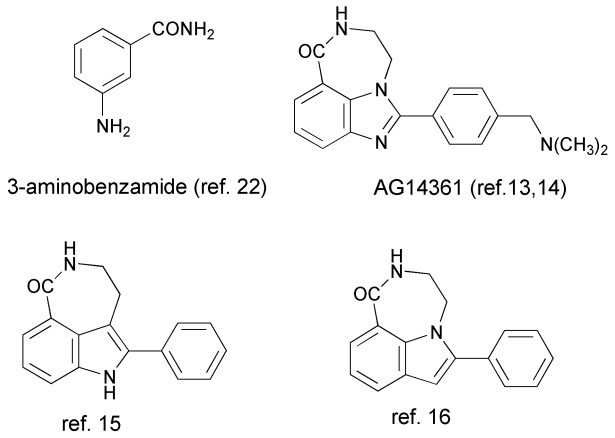


Fig. 2 Tricyclic lactams with PARP-1 inhibition activity.

Results and discussion

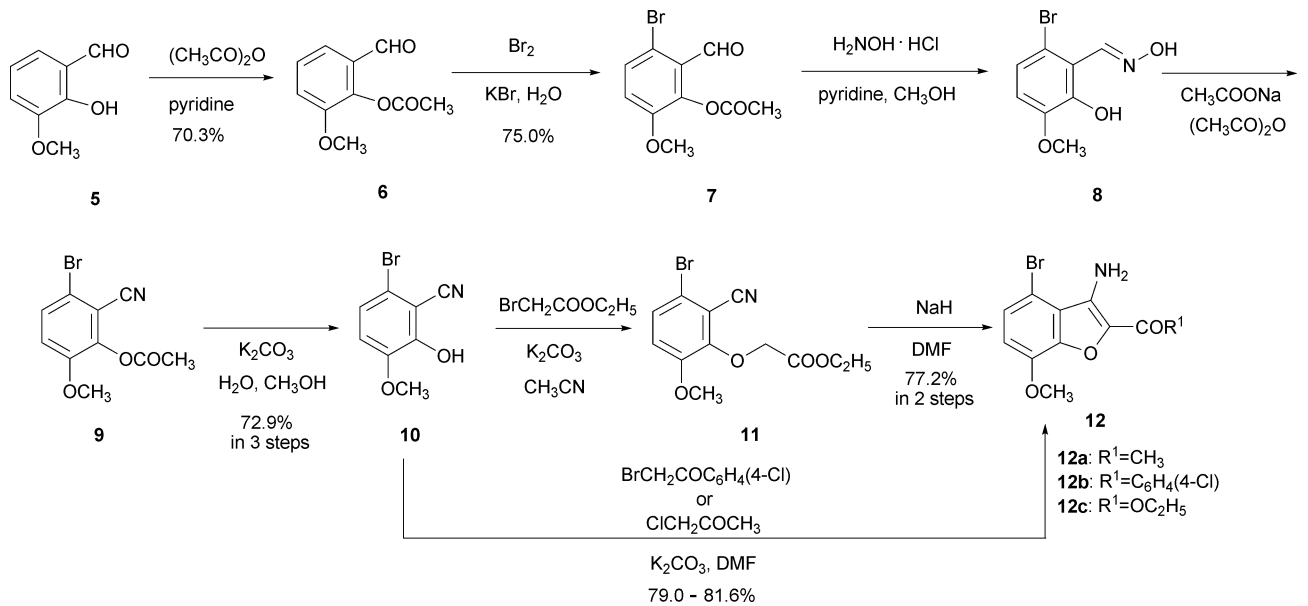
Chemistry

Tricyclic lactam compounds have been prepared using intramolecular cyclization, for example Friedel–Crafts reaction,[31] Bischler–Napieralski reaction[32,33] and lactam cyclization reaction.[34] We hypothesized that 4-bromobenzo[*b*]furans having the vinylcarbonylamino group (–NHCOCH=CH–R) at the 3-position would be subject to intramolecular cyclization between the two groups to form seven-membered lactam fused benzo[*b*]furan rings under Heck coupling conditions.[35]

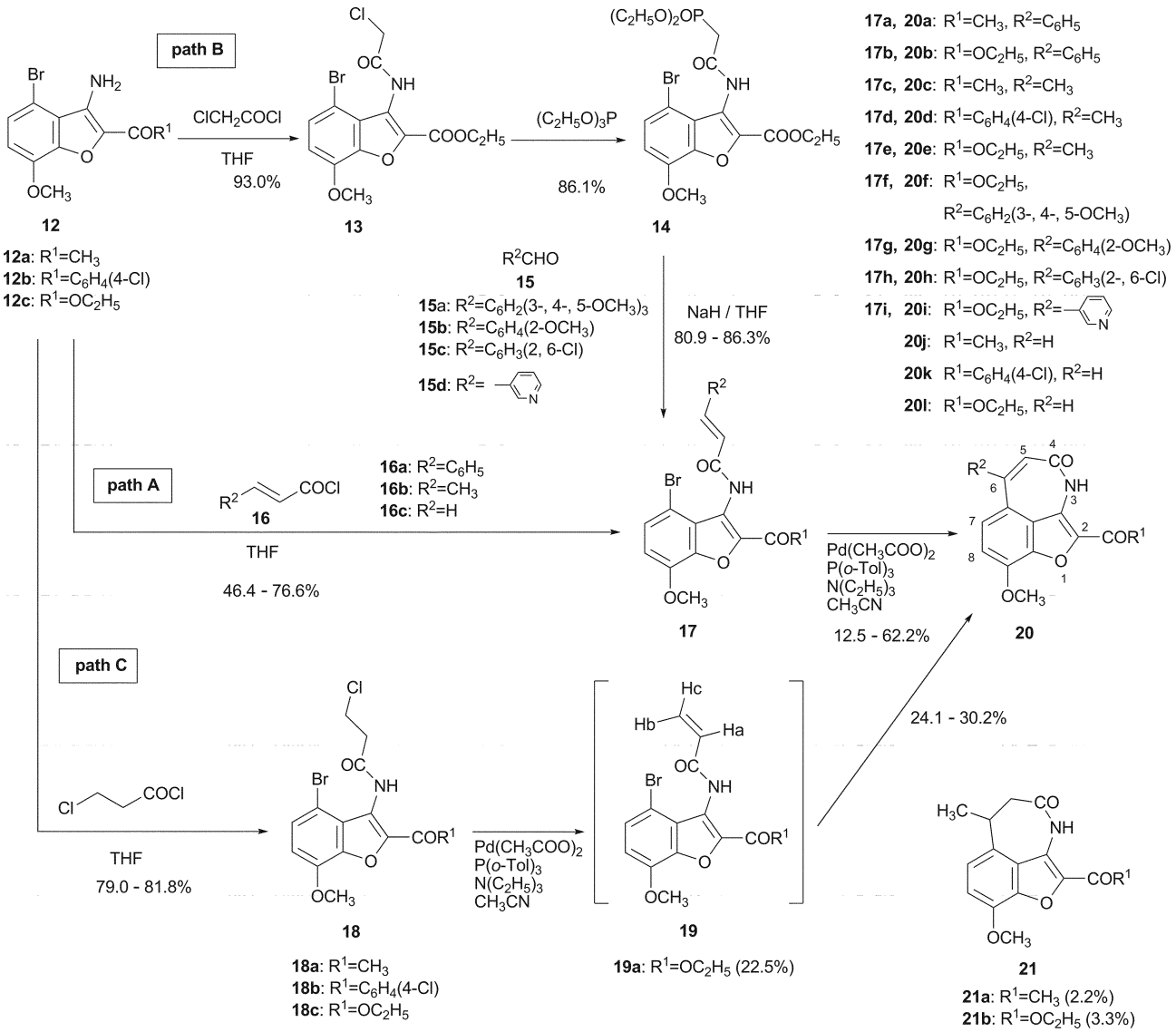
3-Amino-4-bromobenzo[*b*]furans (**12**), key intermediates to synthesize the tricyclicbenzo[*b*]furan (**D**), were prepared. Regioselective bromination of 2-acetoxy-3-methoxybenzaldehyde (**6**) with bromine in the presence of KBr gave 2-acetoxy-6-bromo-3-methoxybenzaldehyde (**7**) according to the procedure reported by Smil *et al.*[36] Compound (**7**) was converted to 6-bromo-2-hydroxy-3-methoxybenzonitrile (**10**) in three steps.[37,38] Reaction of **10** with chloroacetone and 2-bromo-4′-chloroacetophenone afforded 2-acetyl-3-amino-4-bromo-7-methoxybenzo[*b*]furan (**12a**) and 3-amino-4-bromo-2-(4-chlorobenzoyl)-7-methoxybenzo[*b*]furan (**12b**), respectively. On the other hand, treatment of **10** with ethyl bromoacetate under similar conditions afforded only the alkyloxy compound (**11**), which was converted to 3-amino-4-bromo-2-ethoxycarbonyl-7-methoxybenzo[*b*]furan (**12c**) by treatment with NaH (Scheme 1).

The key intermediates (**12a**, **12c**) were treated with cinnamoyl chloride (**16a**) to give 4-bromo-3-(*E*)-cinnamoylaminobenzo-[*b*]furans (**17a**, **17b**, respectively). A mixture of **17a** and **17b**, palladium acetate, tri-*o*-tolylphosphine and triethylamine in acetonitrile was heated at 82 °C for 4–12 h under Heck coupling conditions to afford yellow needles of **20a** (34.4%) and **20b** (62.2%) as the main product, respectively. MS, ^{1}H-NMR and elemental analysis data of **20a** and **20b** suggested them to be tricyclicbenzo[*b*]furans, 2-acetyl-9-methoxy-6-phenylfuro[2,3,4-*jk*][2]benzazepin-4(3*H*)-one (**20a**) [MS; *m*/*z* (EI) 333 (M^+, 100.00). ^{1}H-NMR; δ 5.99 (1H, d, *J* 2.3, 5-H), 9.71 (1H, br s, N*H*)] and 2-ethoxycarbonyl-9-methoxy-6-phenylfuro[2,3,4-*jk*][2]benzazepin-4(3*H*)-one (**20b**) [MS; *m*/*z* (EI) 363 (M^+, 100.00). ^{1}H-NMR; δ 5.94 (1H, d, *J* 2.2, 5-H), 9.00 (1H, br s, N*H*)]. The intramolecular cyclization forming seven-membered lactams under Heck coupling conditions proceeded successfully (Scheme 2, path A).

Syntheses of furo[2,3,4-*jk*][2]benzazepin-4(3*H*)-one derivatives having an aromatic substituent group at the 6-position were carried out. 3-Amino-4-bromo-2-ethoxycarbonyl-7-methoxybenzo[*b*]-furan (**12c**) was treated with chloroacetyl chloride to give 4-bromo-3-chloroacetylamino-2-ethoxycarbonyl-7-methoxybenzo[*b*]furan (**13**). Diethyl[2-(4-bromo-2-ethoxycarbonyl-7-methoxybenzo[*b*]-furan-3-ylamino)-2-oxoethyl]phosphonate (**14**) was obtained from **13** by treatment with triethyl phosphite. Treatment of **14** with several benzaldehydes (**15a–15d**) in the presence of NaH under the Horner–Wadsworth–Emmons (HWE) reaction conditions[39] afforded the corresponding 4-bromo-(*E*)-3-cinnamoylamino-benzo[*b*]furans (**17f–17i**). Ring closure reaction of **17f–17i** under



Scheme 1



Scheme 2

Heck coupling conditions as mentioned above afforded 6-(3,4,5-trimethoxyphenyl)-(**20f**), 6-(2-methoxyphenyl)-(**20g**), 6-(2-,6-dichlorophenyl)-(**20h**) and 6-(3-pyridyl)-2-ethoxycarbonyl-9-methoxyfuro[2,3,4-*jk*][2]benzazepin-4(3*H*)-one (**20i**), respectively (Scheme 2, path B).

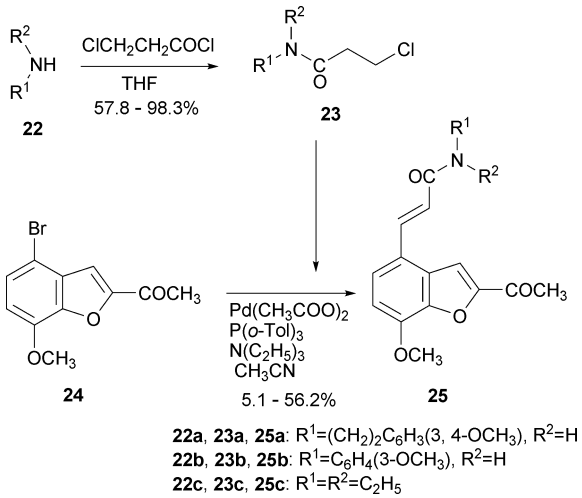
Reaction of 3-amino-4-bromobenzo[*b*]furans (**12a–12c**) with crotonyl chloride (**16b**) afforded 4-bromo-3-crotonoylamino-benzo[*b*]furans (**17c–17e**) containing a very small portion of 4-bromo-3-(3-chlorobutyrylamino)benzo[*b*]furans. The compounds (**17c–17e**) were subject to ring closure reaction under Heck coupling conditions to afford 6-methylfuro[2,3,4-*jk*][2]benzazepin-4(3*H*)-ones (**20c–20e**) accompanying formation of a small portion of 5,6-dihydro-6-methylfuro[2,3,4-*jk*][2]benzazepin-4(3*H*)-ones (**21a**, **21b**) (Scheme 2, path A).

Unfortunately, the reaction of **12a** with acryloyl chloride (**16c**) did not afford 2-acetyl-3-acryloylamino-4-bromo-7-methoxybenzo[*b*]furan. Therefore, it was difficult to synthesize furo[2,3,4-*jk*][2]benzazepin-4(3*H*)-ones (**20j–20l**) without any substituent at the 6-position *via* paths A and B. We thus devised path C to synthesize **20j–20l**. 3-Amino-4-bromobenzo[*b*]furans (**12a–12c**) were treated with chloropropionyl chloride to give 4-bromo-3-(3-chloropropionylamino)benzo[*b*]furans (**18a–18c**). The alkylchlorides (**18a–18c**) were treated with palladium acetate, tri-*o*-tolylphosphine and triethylamine under Heck coupling conditions to also afford the corresponding furo[2,3,4-*jk*][2]benzazepin-4(3*H*)-ones (**20j–20l**) (Scheme 2, path C). In these reactions, 4-bromo-3-(3-chloropropionylamino)benzo[*b*]furans (**18**) were converted to afford 3-acryloylamino-4-bromobenzo[*b*]furans (**19**) with accompanying loss of HCl. Subsequently, **19** was subjected to ring closure reaction to afford furo[2,3,4-*jk*][2]benzazepin-4(3*H*)-ones (**20j–20l**). Indeed, 3-acryloylamino-4-bromo-2-ethoxycarbonyl-7-methoxybenzo[*b*]furan (**19a**) was isolated from the reaction mixture in an early stage of the reaction process. These results suggest that not only acryloylamino (–NHCOCH=CH_2) compounds but also 3-chloropropionylamino (–$NHCOCH_2CH_2Cl$) compounds can be subjected to the Heck coupling reaction.

The representative tricyclicbenzo[*b*]furan (**20j**) showed characteristic 5-H signals (δ 5.92, dd, J 12.5 and 2.2) with coupling with the 3-N*H* in its ^{1}H-NMR spectrum. On the seven-membered lactam ring, the 5-H signals showed coupling with N*H* like meta-coupling. NOE correlations among 5-H, 6-H and 7-H were also observed (Fig. 3). These ^{1}H-NMR, ^{13}C NMR, MS and elemental analysis observations suggested **20j** to be 2-acetyl-9-methoxyfuro[2,3,4-*jk*][2]benzazepin-4(3*H*)-one.

Next, the Heck coupling reaction using alkylchlorides was examined. 3-Chloro-*N*-[2-(3,4-dimethoxyphenyl)ethyl]propionamide (**23a**), 3-chloro-*N*-(3-methoxyphenyl)propionamide (**23b**) and 3-chloro-*N*,*N*-diethylpropionamide (**23c**) were prepared from 2-(3,4-dimethoxyphenyl)ethylamine (**22a**), *m*-anisidine (**22b**) and diethylamine (**22c**), respectively, by treatment with chloropropionyl chloride. 2-Acetyl-4-bromo-7-methoxybenzo[*b*]furan (**24**)[2] treated with *N*-alkyl-3-chloropropionamides (**23a–23c**) under Heck coupling conditions afforded 4-[2-[2-(3,4-dimethoxyphenyl)ethylcarbamoyl]vinyl]-(**25a**), 4-[2-(3-methoxyphenylcarbamoyl)vinyl]-(**25b**) and 4-[2-(diethylcarbamoyl)vinyl]-2-acetyl-7-methoxybenzo[*b*]furan (**25c**), respectively, as expected (Scheme 3). These results revealed that alkylchlorides could be used for the Heck coupling reaction instead of the usually used olefinic reagents.

Finally, X-ray analysis of **20h**, as a representative compound, was examined to confirm the structure of the tricyclicbenzo-[*b*]furans. The seven-membered lactam formed between the 3- and 4-positions of the original benzo[*b*]furan skeleton was confirmed by this work. The lactam ring lay on the same plane as the benzo[*b*]furan ring (Fig. 4).[40]



Scheme 3

Biological activity

The representative tricyclicbenzo[*b*]furans (**20a–20c**, **20j–20l**) prepared were evaluated for LTB_4 receptor inhibitory activity by measurement of the inhibition of calcium mobilization in both CHO cells overexpressing human BLT_1 (CHO–hBLT_1) and human BLT_2 (CHO–hBLT_2) at the concentration of 10 μM.[41,42]

The tricyclicbenzo[*b*]furan series (**20**) was less active than the original compounds (**26** and **27**) having a diethyl group on the nitrogen atom of the carbamoylvinyl group (**26** and **27**, as representative compounds of **A** and **B** in Fig. 1). The substituent on the nitrogen of the carbamoyl group might play an important role in the appearance of inhibitory activities of hBLT_1 and/or hBLT_2.[1,2] Tricyclicbenzo[*b*]furans (**20**) don't have an appropriate substituent on the nitrogen atom of the carbamoylvinyl group for inhibitory activities of hBLT_1 and/or hBLT_2.

Tricyclicbenzo[*b*]furan (**20c**), having a methyl group at the 6-position, showed moderate inhibitory activity, but was less potent than **26** and **27**. These compounds (**26**, **27**) having a methyl group substituted on the vinyl group were most potent in our

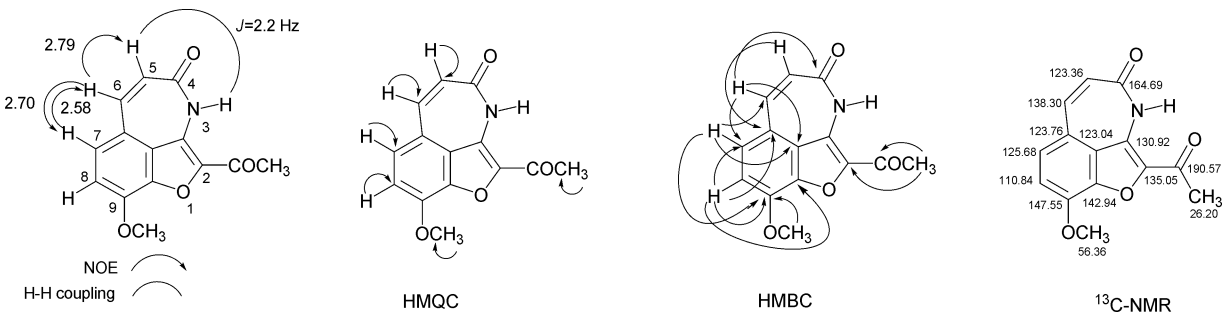


Fig. 3 Structure, ^{1}H-NMR and ^{13}C-NMR of **20j**.

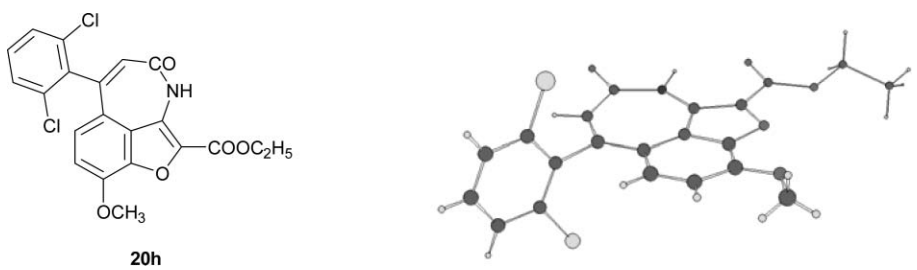


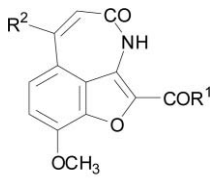
Fig. 4 Structure of 6-(2,6-dichlorophenyl)-2-ethoxycarbonyl-9-methoxyfuro[2,3,4-*jk*][2]benzazepin-4(3*H*)-one (**20h**) and its X-ray analysis

current LTB_4 study.[1,2] On the other hand, **20a** and **20b**, having a phenyl group at the 6-position, were inactive. These results suggested that the substituent on the olefinic carbon of both tricyclicbenzo[*b*]furans (**20**) and the original 2-alkylcarbamoyl-1-methylvinyl compounds (**26**, **27**) might also be an important factor in the appearance of inhibitory activities for $hBLT_1$ and/or $hBLT_2$.

The moderately active compound (**20c**) showed an approximately two-fold potency for $hBLT_1$ than for $hBLT_2$. In our previous study, the (*E*)-2-diethylcarbamoyl-1-methylvinyl group ($-C(CH_3)=CHCON(C_2H_5)_2$) of **27**, showing selective inhibition of $hBLT_2$, lay on nearly the same plane as the benzo[*b*]furan ring.[1,2] Similarly, the carbamoylvinyl moiety (–C(R)=CH–CONH–) in the seven-membered lactam of **20** lay on the same plane as the benzo[*b*]furan ring on the basis of the X-ray analysis of **20h**. These data showed good correlations between selectivity for $hBLT_2$ and stereostructure of the carbamoylvinyl group for both tricyclicbenzo[*b*]furans (**20**) and original compound (**27**) (Table 1).

Most of the tricyclicbenzo[*b*]furans (**20a–20h**, **20j–20l**) were evaluated for their PARP-1 inhibitory activity at a concentration of 10 μM (Table 1).[29,43] Compound (**20g**) having a 2-methoxyphenyl group at the 6-position among the evaluated compounds showed 17.2% inhibitory activity. It has been reported that the benzamide structure is effective for leading to inhibitory activity against PARP-1.[44] Compounds (**20**) were introduced a C=C bond as a spacer between the amide functional group (–CONH–) and the phenyl group of the benzamide structure. The

Table 1 Evaluations of **20** for LTB_4 receptor (BLT_1, BLT_2) and PARP-1 inhibitory activities[a]



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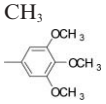
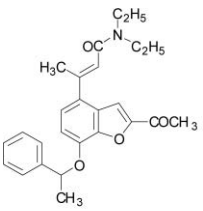
Compound	Path	R^1	R^2	Yield (%)	LTB_4 receptor inhibition (%) Inhibition (10 μM) CHO–$hBLT_1$	CHO–$hBLT_2$	PARP-1 inhibition (%) Inhibition (10 μM)
20a	A	CH_3	C_6H_5	34.4	N. I.	9.1	N. I.[b]
20b	A	OC_2H_5	C_6H_5	62.2	N. I.	2.7	N. I.[b]
20c	A	CH_3	CH_3	34.3	26.1	60.1	N. I.[b]
20d	A	C_6H_4(4-Cl)	CH_3	42.7	—	—	N. I.[b]
20e	A	OC_2H_5	CH_3	60.9	—	—	N. I.
20f	B	OC_2H_5		44.9	—	—	N. I.[b]
20g	B	OC_2H_5		51.8	—	—	17.2
20h	B	OC_2H_5		41.0	—	—	4.2
20i	B	OC_2H_5		12.5	—	—	—
20j	C	CH_3	H	30.2	N. I.	N. I.	6.1
20k	C	C_6H_4(4-Cl)	H	24.1	N. I.	3.6	N. I.[b]
20l	C	OC_2H_5	H	28.0	N. I.	N. I.	N. I.
26	—	—	—	—	92.6	92.8	N. I.
27	—	—	—	—	69.9	> 100	N. I.
3-AB	—	—	—	—	—	—	58.9



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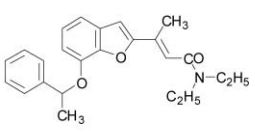

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[a] 3-AB: 3-aminobenzamide, N. I.: not inhibited, —: not tested. [b] At 1 μ M concentration.

cinnamamide lactam of **20** might not be appropriate as a PARP-1 inhibitor.

Conclusion

In this study, we established a synthetic method for 6-substituted furo[2,3,4-*jk*][2]benzazepin-4(3*H*)-ones (tricyclicbenzo[*b*]furan, **20a–20i**) from 3-[(*E*)-(2-alkylvinyl)carbonylamino]-4-bromobenzo[*b*]furans (**17**) by intramolecular cyclization under Heck coupling conditions. 4-Bromo-3-chloropropionylaminobenzo[*b*]furans (**18**) could also be used to obtain furo[2,3,4-*jk*][2]benzazepin-4(3*H*)-ones (**20j–20l**) *via* 3-acryloylamino-4-bromobenzo[*b*]furans under the same reaction conditions. The furo[2,3,4-*jk*][2]benzazepin-4(3*H*)-ones (**20**) having a characteristic seven-membered lactam showed moderate $hBLT_2$ receptor and weak PARP-1 inhibitory activities. Further work is in progress to synthesize a new series of tricyclicbenzo[*b*]furans (**20**) to find more active PARP-1 inhibitors.

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 with a JEOL JNM-ECP400, JEOL JNM-ECP500 and a JEOL PMX60FT spectrometer with tetramethylsilane as an internal standard. MS spectra (MS, HRMS) were obtained using a JEOL JMS-700 EIMS spectrometer. Elemental analyses (EA) were performed using a CHN CORDER MT-3 (Yanako). All organic extracts were dried over anhydrous $MgSO_4$. Column chromatography was carried out on Wakogel C-200. Thin layer chromatography was performed on a Merck silica gel plate (0.5 mm, 60F-254).

3-Amino-4-bromo-2-(4-chlorobenzoyl)-7-methoxybenzo[*b*]furan (12b)

A mixture of **10** (2.0 g, 8.8 mmol), K_2CO_3 (3.0 g, 21.7 mmol) and 2-bromo-4′-chloroacetophenone (2.2 ml, 9.7 mmol) in DMF (80 ml) was stirred at 92 °C for 2 h. The reaction mixture was poured into ice water, and the resulting precipitate was collected by filtration, then recrystallized from ethyl acetate to give **12b** (2.7 g, 81.6%) as yellow needles.

Compound (**12a**) was prepared according to the procedure described for **12b**.

3-Amino-4-bromo-2-ethoxycarbonyl-7-methoxybenzo[*b*]furan (12c)

A mixture of **10** (3.0 g, 13.2 mmol), K_2CO_3 (5.4 g, 39.1 mmol) and bromoethylacetate (1.7 ml, 15.5 mmol) in acetonitrile (50 ml) was stirred at 82 °C for 4.5 h. After the insoluble portion was filtered off, the filtrate was evaporated under reduced pressure to give **11** (3.8 g) as a pale yellow solid. This solid was used for the next step without further purification.

To a suspension of NaH (60% in oil, 0.57 g, 14.3 mmol) in DMF (20 ml) was added dropwise a solution of crude **11** in DMF (10 ml) under a N_2 atmosphere at −5 °C with stirring. The mixture was stirred at 0 °C for 15 min. The mixture was then quenched with H_2O, and added to saturated NH_4Cl solution. The resulting precipitate was collected by filtration and washed with water, then recrystallized from ethyl acetate to give **12c** (3.2 g, 77.2%) as pale yellow needles.

4-Bromo-3-(2-chloroacetylamino)-2-ethoxycarbonyl-7-methoxybenzo[*b*]furan (13)

To a solution of **12c** (1.1 g, 3.4 mmol) in THF (40 ml) was added dropwise chloroacetyl chloride (0.42 ml, 5.3 mmol) with vigorous stirring at 25 °C. The reaction mixture was stirred at the same temperature for 17 h and poured into ice water. The resulting precipitate was collected by filtration and washed with water, then recrystallized from acetonitrile to give **13** (1.6 g, 93.0%) as colorless needles.

Diethyl [2-(4-bromo-2-ethoxycarbonyl-7-methoxybenzo[*b*]furan-3-ylamino)-2-oxoethyl]phosphonate (14)

A mixture of **13** (12.3 g, 31.5 mmol) and triethyl phosphite (100 ml, 0.58 mol) was stirred at 140 °C for 21 h. The reaction mixture was cooled to room temperature, and the resulting precipitate was collected by filtration. The precipitate was recrystallized from ethyl acetate to give **14** (13.1 g, 86.1%) as colorless needles.

4-Bromo-3-(*E*)-cinnamoylamino-2-ethoxycarbonyl-7-methoxybenzo[*b*]furan (17b)

A mixture of **12c** (1.1 g, 3.4 mmol) and cinnamoyl chloride (**16a**) (0.57 g, 3.7 mmol) in THF (50 ml) was heated at 60 °C for 9 h. The reaction mixture was poured into ice water, and the resulting precipitate was collected by filtration, then recrystallized from acetonitrile to give **17c** (1.1 g, 74.3%) as colorless needles.

Compound (**17a**) was prepared according to the procedure described for **17b**.

4-Bromo-3-(*E*)-crotonoylamino-2-ethoxycarbonyl-7-methoxybenzo[*b*]furan (17e)

General procedure for 17c and 17d from 12. A mixture of **12c** (1.3 g, 4.1 mmol) and crotonyl chloride (**16b**) (0.51 ml, 5.4 mmol) in THF (25 ml) was heated at 66 °C for 18 h. The reaction mixture was poured into ice water, and the resulting precipitate was collected by filtration, then recrystallized from acetonitrile to give **17e** (1.21 g, 76.6%) containing a small portion of 4-bromo-2-ethoxycarbonyl-3-(3-chlorobutyrylamino)-7-methoxybenzo[*b*]furan.

4-Bromo-2-ethoxycarbonyl-7-methoxy-3-[[(*E*)-3-(2-methoxyphenyl)-1-oxo-2-propenyl]amino]benzo[*b*]furan (17g)

General procedure for 17f, 17h, and 17i from 14. To a suspension of NaH (60% in oil, 0.33 g, 8.2 mmol) in THF (20 ml) was added dropwise a solution of **14** (2.0 g, 4.1 mmol) in THF (40 ml) under a N_2 atmosphere at 0 °C with stirring. The solution was stirred at the same temperature until it became clear. A solution of *o*-anisaldehyde (**15b**) (0.59 ml, 4.9 mmol) in THF (20 ml) was added dropwise to the clear solution at 0 °C, and the mixture was stirred at the same temperature for 1 h. The mixture was then quenched with H_2O and added to saturated NH_4Cl solution. The resulting precipitate was collected by filtration and washed with water, then recrystallized from ethyl acetate to give **17h** (1.6 g, 82.5%) as colorless needles.

4-Bromo-3-(3-chloropropionylamino)-2-ethoxycarbonyl-7-methoxybenzo[*b*]furan (18c)

General procedure for 18a and 18b from 12. To a solution of **12c** (1.5 g, 3.7 mmol) in THF (40 ml) was added dropwise 3-chloropropionyl chloride (0.46 ml, 4.8 mmol) with vigorous stirring at 25 °C. The solution was heated at 60 °C for 11 h, and poured into ice water. The resulting precipitate was collected by filtration, then recrystallized from acetonitrile to give **18c** (1.5 g, 79.0%) as colorless needles.

2-Acetyl-7-methoxy-6-phenylfuro[2,3,4-*jk*][2]benzazepin-4(3*H*)-one (20a)

General procedure for 20b, 20f–20i from 17. A mixture of **17a** (0.2 g, 0.48 mmol), palladium acetate (6.0 mg, 0.024 mmol), tri-*o*-tolylphosphine (17.0 mg, 0.048 mmol) and Et_3N (2.0 ml, 14.0 mmol) in acetonitrile (50 ml) was heated at 82 °C for 12 h. The mixture was treated with chloroform, and the insoluble portion was removed by filtration. The filtrate was evaporated to dryness. The residue was poured into water, made acidic with 10% HCl solution and extracted with chloroform. The organic layer was washed with brine and dried. The solvent was evaporated off. The residue was recrystallized from ethyl acetate to give **20a** (0.06 g, 34.4%) as yellow needles (Found: C, 71.78; H, 4.44; N, 4.15. $C_{20}H_{15}NO_4$ requires C, 72.06; H, 4.54; N, 4.20); mp 246.5–248.3 °C; δ_H (500 MHz; $CDCl_3$; Me_4Si) 2.58 (3H, s, COCH_3), 4.01 (3H, s, OCH_3), 5.99 (1H, d, *J* 2.3, 5-H), 6.76 (1H, d, *J* 8.2, 7- or 8-H), 6.78 (1H, d, *J* 8.7, 7- or 8-H), 7.32–7.34 (2H, m, phenyl H), 7.42–7.45 (3H, m, phenyl H), 9.71 (1H, br s, N*H*); *m*/*z* (EI) 333 (M^+, 100.00%).

2-Acetyl-7-methoxy-6-methylfuro[2,3,4-*jk*][2]benzazepin-4(3*H*)-one (20c)

General procedure for 20d and 20e from 17. A mixture of **17c** (0.6 g, 1.7 mmol), palladium acetate (21.0 mg, 0.085 mmol), tri-*o*-tolylphosphine (62.0 g, 0.17 mmol) and Et_3N (2.0 ml, 14.0 mmol) in acetonitrile (30 ml) was heated at 82 °C for 16 h. The mixture was treated with chloroform, and the insoluble portion was removed by filtration. The filtrate was evaporated to dryness. The residue was poured into water, made acidic with 10% HCl solution and extracted with chloroform. The organic layer was washed with brine and dried. The solvent was evaporated off. The residue was recrystallized from ethyl acetate to give **20c** (0.16 g, 34.3%) as yellow needles (Found: C, 66.19; H, 4.77; N, 5.16. $C_{15}H_{13}NO_4$ requires C, 66.41; H, 4.83; N, 5.16); mp 278.8–280.2 °C; δ_H (500 MHz; $CDCl_3$; Me_4Si) 2.27 (3H, d, *J* 0.9, 6-CH_3), 2.56 (3H, s, COCH_3), 4.05 (3H, s, OCH_3), 6.04 (1H, q, *J* 0.9, 5-H), 6.92 (1H, d, *J* 8.3, 8-H), 7.20 (1H, d, *J* 8.3, 7-H), 9.63 (1H, br s, N*H*); *m*/*z* (EI) 271 (M^+, 100.00%).

2-Acetyl-5,6-dihydro-7-methoxy-6-methylfuro[2,3,4-*jk*][2]benzazepin-4(3*H*)-one (21a)

Isolation of **20c** gave a residue which was purified by silica gel column chromatography ($CHCl_3$) to give a yellow solid. The solid was recrystallized from ethyl acetate to give **21a** (10 mg, 2.2%) as yellow needles (Found: C, 65.71; H, 5.49; N, 5.10. $C_{15}H_{15}NO_4$ requires C, 65.92; H, 5.53; N, 5.13); mp 203.2–204.5 °C; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.38 (3H, d, *J* 6.9, CH(CH_3)), 2.60 (3H, s, COCH_3), 2.97–3.02 (2H, m, CH_2), 3.32–3.38 (1H, m, CH(CH$_3$)), 4.02 (3H, s, OCH_3), 6.93 (1H, d, *J* 7.8, 8-H), 7.00 (1H, d, *J* 8.3, 7-H), 9.55 (1H, br s, N*H*); *m*/*z* (EI) 273 (M^+, 85.79%), 258 (100.00).

Compound (**21b**) was prepared according to the procedure described for **21a**.

2-Acetyl-7-methoxyfuro[2,3,4-*jk*][2]benzazepin-4(3*H*)-one (20j)

General procedure for 20k, 20l from 18. A mixture of **18a** (1.5 g, 4.0 mmol), palladium acetate (49.0 mg, 0.20 mmol), tri-*o*-tolylphosphine (0.15 g, 0.40 mmol) and Et_3N (6.0 ml, 42.0 mmol) in acetonitrile (40 ml) was heated at 82 °C for 3 h. The mixture was treated with chloroform, and the insoluble portion was removed by filtration. The filtrate was evaporated to dryness. The residue was poured into water, made acidic with 10% HCl solution and extracted with chloroform. The organic layer was washed with brine and dried. The solvent was evaporated off. The residue was recrystallized from ethyl acetate to give **20j** (0.31 g, 30.2%) as yellow needles (Found: C, 65.36; H, 4.22; N, 5.42. $C_{14}H_{11}NO_4$ requires C, 65.37; H, 4.31; N, 5.44); mp 247.1–249.7 °C; δ_H (400 MHz; $CDCl_3$; Me_4Si) 2.55 (3H, s, COCH_3), 4.04 (3H, s, OCH_3), 5.92 (1H, dd, *J* 12.5 and 2.2, 5-H), 6.79 (1H, d, *J* 12.8, 6-H), 6.89 (1H, d, *J* 8.0, 8-H), 7.02 (1H, d, *J* 8.1, 7-H), 9.55 (1H, br s, N*H*); δ_C (500 MHz; $CDCl_3$; Me_4Si) 26.20, 56.36, 110.84, 123.04, 123.36, 123.76, 125.68, 130.92, 135.05, 138.30, 142.94, 147.55, 164.69, 190.57; *m*/*z* (EI) 257 (M^+, 100.00%).

2-Acetyl-4-[(*E*)-2-[2-(3,4-dimethoxyphenyl)ethylcarbamoyl]vinyl]-7-methoxybenzo[*b*]furan (25a)

General procedure for 25b, 25c from 24. A mixture of **24** (0.5 g, 1.9 mmol), **23a** (0.61 g, 2.2 mmol), palladium acetate (23.0 mg, 0.09 mmol), tri-*o*-tolylphosphine (68.0 mg, 0.19 mmol) and Et_3N (4.0 ml, 28.0 mmol) in acetonitrile (80 ml) was heated at 82 °C for 7 h. The mixture was treated with chloroform, and the insoluble portion was removed by filtration. The filtrate was evaporated to dryness. The residue was poured into water, made acidic with 10% HCl solution and extracted with chloroform. The organic layer was washed with brine and dried. The solvent was evaporated off. The residue was recrystallized from ethyl acetate to give **25a** (0.36 g, 45.6%) as pale yellow needles (Found: C, 67.47; H, 6.02; N, 3.31. $C_{24}H_{25}NO_6 \cdot 1/3H_2O$ requires C, 67.12; H, 6.02; N, 3.26); δ_H (400 MHz; $CDCl_3$; Me_4Si) 2.64 (3H, s, COCH_3), 2.86 (2H, t, *J* 6.9, NHCH$_2$CH_2), 3.67 (2H, m, NHCH_2CH$_2$), 3.87 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 5.68 (1H, br t, *J* 5.5, N*H*), 6.37 (1H, d, *J* 15.7, C*H*=CH), 6.76–6.79 (2H, m, 2′-, 6′-H), 6.83 (1H, d, *J* 7.7, 5′-H), 6.92 (1H, d, *J* 8.1, 6-H), 7.39 (1H, d, *J* 8.4, 5-H), 7.72 (1H, s, 3-H), 7.82 (1H, d, *J* 15.8, C*H*=CH); *m*/*z* (EI) 423 (M^+, 8.64%), 164 (100.00).

PARP inhibition assay

The catalytic activity of PARP-1 was measured as described previously[43] with some modifications. Human recombinant PARP-1 (800 ng ml^{-1}, Trevigen, Gaithersburg, MD) in buffer-A containing 50 mM Tris-HCl (pH 8.0), 20 μM $ZnCl_2$, 4 mM $MgCl_2$, 1 mM dithiothreitol was immobilized on an ELISA microplate for 16–18 h at 4 °C. After three washings with PBS plus 0.05%

Tween20 (PBST), 1.25 mg ml^{-1} activated DNA[29] diluted in the buffer-A plus 50 μM NAD^+ was added to each well and the reaction mixture was incubated for 15 min at 4 °C. After three washings with PBST, the synthesis of poly(ADP-ribose) by PARP-1 was detected by adding the anti-poly(ADP-ribose) pAb (1 : 2500, LP98-10, Alexis Biochemicals, Lausanne, Switzerland) in 1% BSA-PBST. After 1 h incubation at 37 °C and three washings with PBST, peroxidase-conjugated goat anti-rabbit IgG (1 : 5000, Zymed, South San Francisco, CA) in 1% BSA–PBST was added and incubated for 30 min at 37 °C. After a final series of washings (twice with PBST and once with H_2O), a positive signal was visualized by 3,3′,5,5′-tetramethyl-benzidine in the presence of H_2O_2 for 15 min at 37 °C. The reaction was terminated by adding 1 N H_2SO_4 and the absorbance was measured at 450 nm. The compounds or 3-aminobenzamide (as a positive control) were dissolved in 1% dimethyl sulfoxide, and then 10 μl (final conc. 0.1%) was added to each assay.

Measurement of calcium mobilization in CHO cells

Evaluation of tricyclicbenzo[*b*]furan (**20**) for BLT_1/BLT_2 receptor inhibitory activity was carried out according to a procedure reported previously.[1,2]

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40 Compound **20h** formula: $C_{21}H_{15}O_5NCl_2$, formula weight: 432.26, crystal color, habit: yellow, chunk, crystal dimensions: 0.20 × 0.15 × 0.05 mm, crystal system: monoclinic, lattice type: primitive, indexing images: 3 oscillations @ 180.0 seconds, detector position: 127.40 mm, pixel size: 0.100 mm, lattice parameters: $a = 8.6316(2)$ Å, $b = 14.8772(3)$ Å, $c = 14.4589(3)$ Å, $\beta = 95.3308(11)^0$, $V = 1848.70(7)$ Å^3, space group: $P2_1/n$ (#14), Z value: 4, D_{calc}: 1.553 g cm^{-3}, F_{000}: 888.00, μ(CuKα): 34.790 cm^{-1}‡.

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