Synthesis and Structure Activity Relationship Studies of Benzothieno[3,2-*b*]furan Derivatives as a Novel Class of IKKβ Inhibitors

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As a novel class of IKKβ inhibitors, a series of tricyclic furan derivatives was designed and synthesized based on the structure of known thiophene IKKβ inhibitors. Among the various fused furan derivatives synthesized, a benzothieno[3,2-*b*]furan derivative 13a displayed potent inhibitory activity towards IKKβ in enzymatic and cellular assays. The potent inhibitory activity originates from an intramolecular non-bonded S···O interaction which was confirmed by the X-ray structure of JNK3 with 16k. The introduction of further substituents on the core structure led to the discovery of the 6-alkoxy derivatives, which possessed a comparable IKKβ inhibitory activity to 13a and an improved metabolic stability. Among these, appropriately lipophilic compounds 16a, h, i, and 13g (log D>2) were found to possess good oral bioavailability.

Key words IKK; kinase; inhibitor; benzothieno[3,2-*b*]furan; non-bonded interaction

IκB kinase (IKK) has been identified as a serine–threonine kinase complex, which phosphorylates the inhibitor of NF-κB (IκB).[1—3] This complex is composed of three subunits, *i.e.* the catalytic subunits IKKα and IKKβ, and the regulatory subunit IKKγ, also called IKK-1, IKK-2, and NEMO, respectively. The complex plays a critical role in a signaling pathway leading to NF-κB (nuclear factor-κB) activation.[4] The activation of IKK by cytokine signals (tumor necrosis factor (TNF)-α, interleukin (IL)-1 *etc.*), lipopolysaccharide (LPS), and stress leads to phosphorylation of IκB, which triggers poly-ubiquitinylation and proteasome-dependent degradation of IκB, resulting in transcriptional activation of NF-κB. It has been recognized that the kinase IKKβ plays a major role in activation of NF-κB *via* classical (canonical) pathway, and activation of the classical pathway promotes the production of TNF-α, IL-1, intercellular adhesion molecule (ICAM)-1, and cyclooxygenase (COX)-2, which indicates that this pathway is important for inflammation and the innate immune system. Therefore, IKKβ inhibitors are expected to be useful for the treatment of acute and chronic inflammatory diseases and autoimmune disorders, such as rheumatoid arthritis (RA).[5] Alternatively, IKKβ inhibitors are also thought to be useful for the treatment of type 2 diabetes because activation or overexpression of the IKKβ attenuated insulin signaling.[6] In addition, IKKβ inhibitors may also have application for the treatment of asthma, chronic obstructive pulmonary disease (COPD),[7] and cancer.[8] Because of the key role played by the IKK complex in the NF-κB mediated transcription, and of the druggability of kinases as a target class, numerous companies have been pursuing discovery programs aimed at identifying small molecule inhibitors of this enzyme.[9,10]

As part of our program to discover new IKK inhibitors, we identified **1** (MAYBRIDGE) as a potent hit compound. However, AstraZeneca has previously reported that the structurally-analogous urea derivative **2**,[11] which could be derived from the compound **1**, exhibited excellent inhibitory activity. The introduction of a urea group resulted in the improvement of activity by as much as 100-fold compared with the amino derivative **1** (Fig. 1). Because the benzene ring of **2** was considered to be coplanar to the thiophene ring in it's active conformation,[11] it seemed that the relative orientation of the urea, carbamoyl, and benzene was important for IKKβ inhibition. Thus, it was also thought that constraining the terminal benzene to be in the active conformation could improve activity. From this point of view and taking into consideration of patentability issues, we designed tricyclic fused furan derivatives for the development of novel IKK inhibitors. In this manuscript, we describe the synthesis and the SAR of the series of tricyclic furan derivatives.

Chemistry

The synthesis of benzothieno[3,2-*b*]furans **13a**—**i** and **16a**—**o** having a 2-carbamoyl and 3-urea group, is outlined in Chart 1. The substituted thiophenols **8b**—**g** were synthesized from the substituted salicylic acids **3** or anthranilic acids **6** according to the known procedures.[12—14] These thiophenol derivatives were alkylated with 2-bromoacetonitrile and cyclized using sodium ethoxide to afford benzothiophene derivatives **10a**—**g**. The bromo substituted derivatives **10f**, **g** were coupled with phenylboronic acid to afford **10h**, **i**. These phenol derivatives **10a**—**i** were alkylated with 2-bromo- (or 2-iodo-) acetamide followed by cyclization to afford the benzothieno[3,2-*b*]furan **12a**—**i**. Finally, the amino group of **12a**—**i** was converted into an urea group using potassium cyanate or trichloroacetyl isocyante to afford **13a**—**i**. To allow further modification of 6-alkoxy derivatives, **12c** was demethylated, and selective alkylation using various alkylating reagents with sodium hydride was carried out to give **15a**—**j**. These 3-amino derivatives were converted to the urea derivatives **16a**—**j** in a similar mannar. The *tert*-butoxycarbonyl (Boc) group of **16a**, **b** was cleaved to give amines **16k**, **m**, respectively, and the acetylation afforded acetamide **16l**. The ester derivatives **16d**, **e** were converted to the corresponding carboxylic acid **16n** and alcohol **16o**, respectively.

A 5-phenyl furan derivative **21** was synthesized as shown in Chart 2. The amino group of the known furan derivative **17**[15] was protected with Boc group, and the resulting ester **18** was converted to amide **19** by alkaline hydrolysis and subsequent amidation using 1-ethyl-3-(3-dimethylamino-

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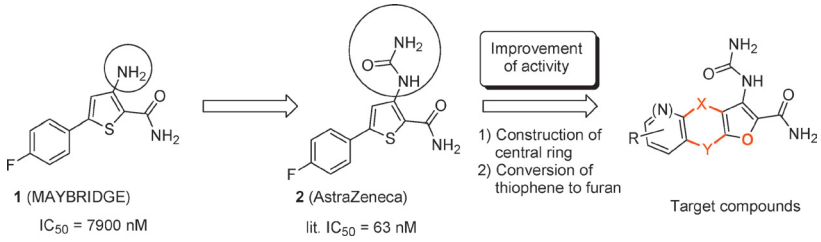


Fig. 1. Drug Design of the Tricyclic Furan Derivative

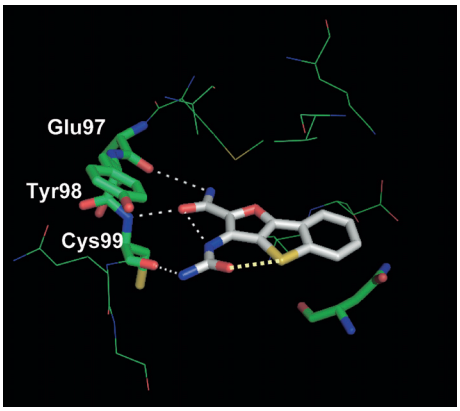


Fig. 2. Predicted Binding Mode of **13a** with IKKβ

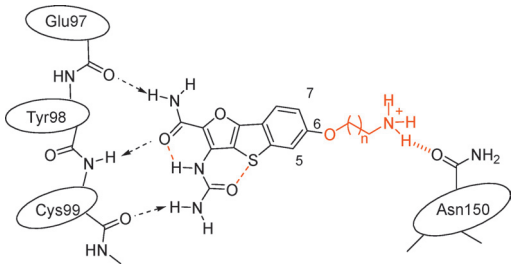


Fig. 3. Predicted Binding Model Orientated to Interact with Asn150

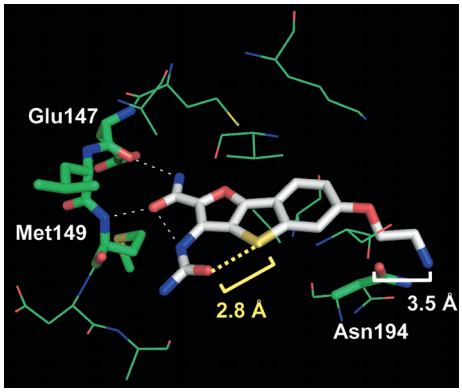


Fig. 4. The X-Ray Structure of JNK3 with **16k**

propyl)carbodiimide hydrochloride (WSC) and ammonium 1*H*-1,2,3-benzotriazol-1-olate (HOBt·NH_3). *N*-Deprotection of **19** by treatment with trifluoroacetic acid and subsequent acylation of the resulting amine **20** provided urea **21**.

Synthesis of the 4,5-dihydronaphtho[1,2-*b*]furan **26a** and 5,6-dihydro-4*H*-benz[6,7]cyclohepta[1,2-*b*]furan **26b** is shown in Chart 3. The Mitsunobu reaction of the known cyanoketones **22a**,[16] **b**[17] and ethyl glycolate afforded esters **23a**, **b**, which were converted to amides **24a**, **b**, respectively, by the aminolysis using aqueous ammonia. The resulting amides were converted to **26a**, **b** in a manner similar to that described for the synthesis of benzothieno[3,2-*b*]furans in Chart 1.

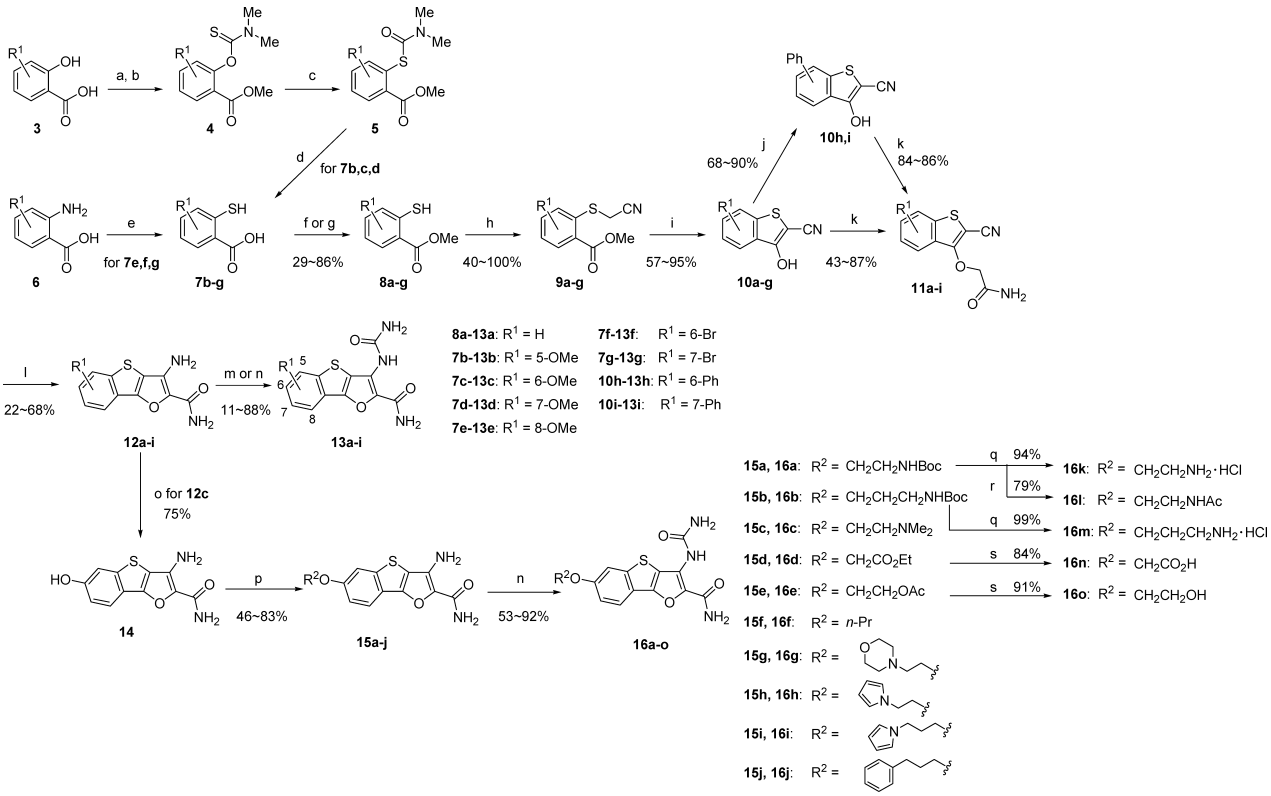
The naphthofuran derivative **32** was synthesized as shown in Chart 4. Naphthoic acid **27** was converted to the amide **28**, which after alkylation of the phenolic hydroxy group followed by the treatment with thionyl chloride gave the naphthonitrile **30**. Cyclization using sodium ethoxide afforded the naphthofuran **31**, in which the cyano group was simultaneously hydrolyzed to the amide. Finally, the amino group of **31** was converted to an urea group to afford **32**.

The furo[2′,3′:4,5]thieno[2,3-*b*]pyridine **39**, which possesses a pyridine ring instead of a benzene ring in **13a**, was synthesized as shown in Chart 5. Introduction of a sulfur atom to 2-chloropyridine **33** was carried out using sodium hydrogen sulfide, and the resulting thiol **34** was instantly subjected to the alkylation to give **35** because **34** was easily oxidized when isolated. The obtained **35** was converted to **39** using a method similar to that described in Chart 1.

Results and Discussion

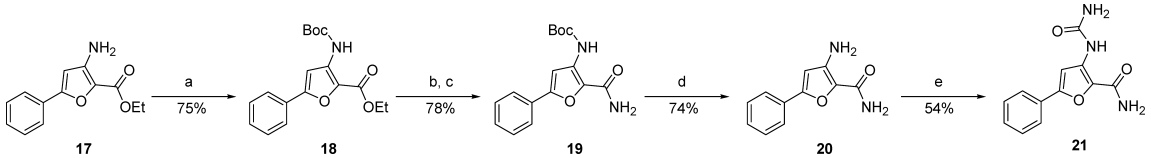
The compounds were evaluated *in vitro* for inhibitory potency against the recombinant IKKβ activity in the non-RI kinase assay using a Kinase-Glo reagent (Promega, U.S.A.) with the addition of 0.1 mg/ml bovine serum albumin (BSA) to reduce false positive activity.[18] To examine the secondary cellular efficacy assays of IKKβ inhibition, the inhibitory activity of TNF-α production in LPS-stimulated THP-1 cells was evaluated.

The effect of altering the central core of the furan derivatives on inhibitory activity was evaluated using 3-aminofuran-2-carboxamide derivatives, which were either substituted or fused with other rings (Table 1). The benzothieno[3,2-*b*]furan **12a** exhibited moderate inhibitory activity (IC_{50}=5500 nM). The 5-phenyl furan **20** was as potent as **12a**, furthermore, **25a**, **b**, and **31** which contain a saturated or unsaturated ring in their central core had the comparable potency to **12a**. The thienopyridine derivative **38** was also as potent as **12a**. Next, we examined the 3-ureido derivatives of the 3-aminofurans. All of the 3-ureido compounds exhibited an improvement in activity compared to the corresponding 3-amino compounds. The effect was significant on the thiophene-fused furans **13a** and **39**. Notably, benzothieno-[3,2-*b*]furan **13a** exhibited excellent potency (IC_{50}=45 nM), which was 100-fold greater than the corresponding 3-amino derivative **12a** (IC_{50}=5500 nM), while the increase on furo[2′,3′:4,5]thieno[2,3-*b*]pyridine **39** was 72-fold. The ob-



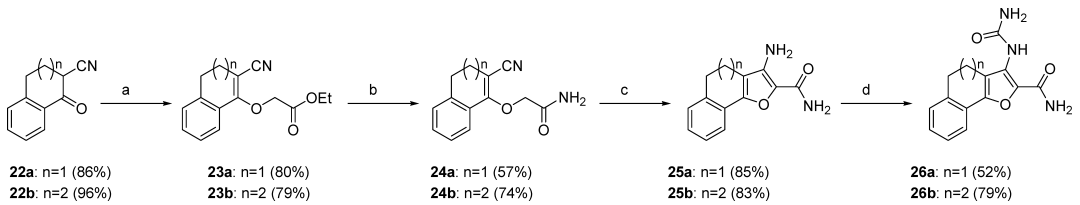
Reagents and conditions: (a) $SOCl_2$, MeOH, reflux; (b) Me_2NCSCl, DBU, DMF; (c) 210 °C; (d) aq. NaOH, reflux; (e) i) $NaNO_2$, aq. HCl, 0 °C; ii) KSCSOEt, 85 °C; iii) aq. NaOH, 80 °C; (f) conc. H_2SO_4, MeOH, reflux; (g) HCl/MeOH, reflux; (h) bromoacetonitrile, K_2CO_3, THF; (i) NaOEt, EtOH, reflux; (j) phenylboronic acid, $Pd(Ph_3P)_4$, K_2CO_3, aq. THF, reflux; (k) 2-iodoacetamide, K_2CO_3, THF, reflux; (l) NaOEt, EtOH, reflux; (m) KNCO, AcOH, reflux; (n) $Cl_3CCONCO$, THF, −78 to −30 °C then 2 N NH_3 in MeOH; (o) BBr_3, CH_2Cl_2, reflux; (p) NaH, R^2X (X=I, Br, Cl), (NaI), DMF, rt to 60 °C; (q) aq. HCl, MeOH, 60 °C; (r) i) TFA, $CHCl_3$, rt, ii) Ac_2O, Et_3N, DMF, rt; (s) 2 N aq. NaOH, MeOH, rt; the positional numbers of R^1 are based on the [1]benzothieno[3,2-*b*]furan structure.

Chart 1. Synthesis of Benzothieno[3,2-*b*]furans



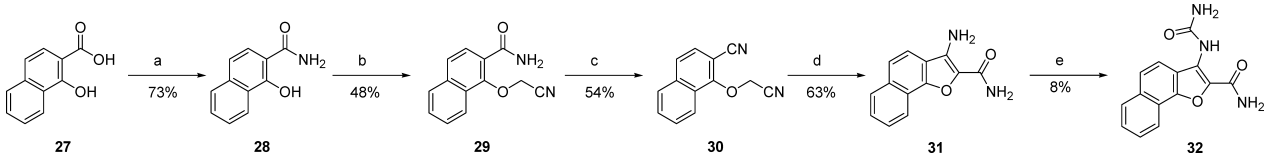
Reagents and conditions: (a) Boc_2O, THF; (b) 8 N aq. NaOH, EtOH; (c) WSC, HOBt·NH_3, CH_3CN–DMF; (d) TFA, $CHCl_3$; (e) $Cl_3CCONCO$, THF, −78 to 0 °C, then 2 N NH_3 in MeOH.

Chart 2. Synthesis of 5-Phenyl Furan Derivative



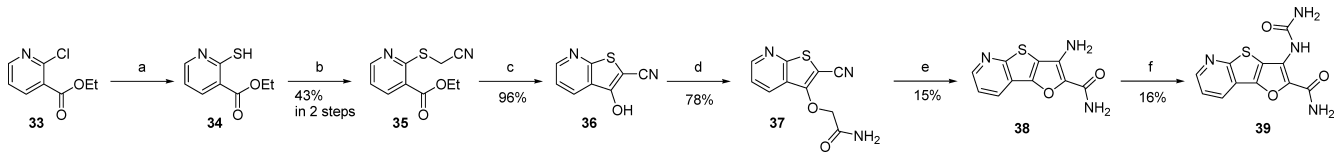
Reagents and conditions: (a) ethyl glycolate, Ph_3P, DEAD, THF, 0 °C; (b) 28% aq. NH_3, MeOH; (c) NaOEt, EtOH, reflux; (d) $Cl_3CCONCO$, THF, −78 to −30 °C then 2 N NH_3 in MeOH.

Chart 3. Synthesis of Tricyclic Furans Having Saturated Hydrocarbon Ring



Reagents and conditions: (a) WSC, HOBt·NH_3, DMF; (b) $BrCH_2CN$, K_2CO_3, THF; (c) $SOCl_2$, DMF; (d) NaOEt, EtOH, reflux; (e) KCNO, AcOH, reflux.

Chart 4. Synthesis of Naphthofuran Derivative



Reagents and conditions: (a) NaSH, EtOH, reflux; (b) chloroacetonitrile, NaI, K_2CO_3, THF; (c) NaOEt, EtOH, reflux; (d) 2-iodoacetamide, K_2CO_3, THF, reflux; (e) NaOEt, EtOH, reflux; (f) $Cl_3CCONCO$, THF, -78 to -30 °C then 2 N NH_3 in MeOH.

Chart 5. Synthesis of Furo[2′,3′:4,5]thieno[2,3-*b*]pyridine Derivative

Table 1. Evaluation of Furan-2-carboxamide Derivatives with Various Skeletons

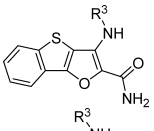
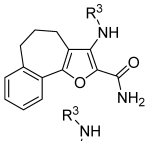
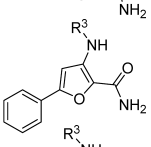
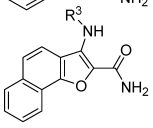
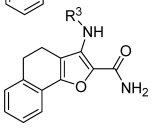
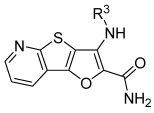
No.	Structure	IKKβ IC_{50} (nM)	No.	Structure	IKKβ IC_{50} (nM)
12a: R^3=H		5500	**25b**: R^3=H		13000
13a: R^3=$CONH_2$		45	**26b**: R^3=$CONH_2$		3400
20: R^3=H		2100	**31**: R^3=H		3500
21: R^3=$CONH_2$		120	**32**: R^3=$CONH_2$		160
25a: R^3=H		7100	**38**: R^3=H		7200
26a: R^3=$CONH_2$		150	**39**: R^3=$CONH_2$		100

Table 2. Profile of **13a**

In vitro		PK Rat cassette (1 mg/kg, *p.o.*)[a]			Metabolic stability (μl/min/mg)[b]		Solubility (μg/ml)		
IKKβ IC_{50} (nM)	THP-1 (TNF-α) IC_{50} (nM)	*AUC* (ng·h/ml)	*MRT* (h)	BA (%)	Rat	Human	JP1	JP2	JP2+GCDC
19—45	310	ND	ND	0	71	55	3.4	3.5	>63

a) Administration substrate: i.v., dimethyl sulfoxide; *p.o.*, 0.5% methyl cellulose. *b*) Liver microsomes clearance.

served increase of the other 3-ureido compounds **21**, **26a**, **b**, and **32** was smaller than that of **13a**. This effect could be explained by the following hypothesis of the intramolecular non-bonded S···O interaction.

The docking model of **13a** with IKKβ (using SCWRL2.9,[19,20] based on the crystal structure of PKA, PDB ID, 1ATP[21]) is shown in Fig. 2. In this model, **13a** can form hydrogen bonds with the kinase back bone *via* the urea and carbamoyl moieties. Furthermore, it is likely that the urea group of the tricyclic furan is constrained to lie in-plane with the benzothienofuran ring by two intramolecular interactions: i) a hydrogen bond between the urea proton and the amide carbonyl and ii) a non-bonded S···O interaction[22] between the oxygen atom of urea carbonyl and the sulfur atom on the thiophene ring. The later is particularly important to increase IKKβ inhibition, which is confirmed by the reduced potency observed when the intramolecular non-bonded S···O interaction is lost by conversion of the thiophene to other rings (**13a** *vs.* **21**, **26a**, **b**, and **32**). This hypothetical S···O interaction was confirmed by X-ray crystallography of a complex between one of the benzothienofuran derivatives **16k** and the related kinase (JNK3, see below).

The result of further profiling of the potent benzothieno[3,2-*b*]furan **13a** is shown in Tables 2 and 3. The compound **13a** exhibited good cellular activity (inhibitory activity of TNF-α production in THP-1 cell line, IC_{50}=310 nM), and high selectivity more than 44-fold for IKKβ against several other serine/threonine kinases. However, **13a** was not detected in rat plasma after oral administration, may be due to it's metabolic instability, although it showed acceptable solubility at pH 6.8 with glycochenodeoxycholic acid (GCDC).

Table 3. Selectivity Profile of **13a** for Representative Kinases

Kinases	IC_{50} (μM)[a]	Kinases	IC_{50} (μM)[a]
IKKβ	0.022	ERK1	15
ASK1	1.2	p38α	>100
TAK1	1.6	PKCθ	18
MEKK1	>100	GSK3β	1.6
JNK1	0.97		

a) RI-method.

The result of introduction of substituents on the terminal benzene ring to improve the bioavailability of **13a** is shown in Table 4. In these derivatives, substitution at the 5- or 6-position (**13b**, **c**) was well tolerated without reduction of the activity. In contrast, introduction of substituents at the 8-position caused decrease of activity (**13e**). Although introduction of a bromine at the 6- or 7-position was tolerated (**13f**, **g**), direct attachment of a phenyl group to the benzene ring caused

a decrease in activity (**13h**, **i**). In the cellular assay, the 6-MeO (**13c**) and 7-MeO (**13d**) derivatives had comparable potency to **13a**, and 7-bromo derivative **13g** (IC_{50}=120 nM) was more potent than **13a**. These results suggested that only small substituents are tolerated at 7-position, and the 6-position is most favorable for further modification.

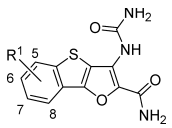
With the aim of improving activity, we investigated the potential of interaction with Asn150 of IKKβ. This residue is almost completely conserved throughout the kinase family, and coordinated with Mg^{2+} in the phosphate binding site.[21] Therefore, it was considered that the basic moieties would interact with Asn, and the modeling study suggested that introduction of an aminoethoxy group at the 6-position of the tricyclic furan would provide a suitable distance to interact with Asn150 (Fig. 3, n=1).

According to this hypothesis, we introduced various kinds of basic substituents on the 6-position, including the aminoethoxy group, and SAR are shown in Table 5. Aminoethoxy derivative **16k** exhibited slightly increased activity (IC_{50}=11 nM), however, a large decrease of cellular potency was observed. The other compounds having a basic moiety (**16c**, **g**, **m**) showed only moderate inhibition of the enzyme (>10-fold less potent than **16k**), and introduction of hydrophobic substituents caused a decrease in enzymatic activities (**16f**, **j**). Interestingly, enzymatic potency was retained by carboxylic acid **16n** and alcohol **16o**. The carbamate **16a** and amide **16l** exhibited good enzyme inhibition, and also showed moderate inhibition of TNF-α production (10^{-7} M of IC_{50}'s). Enzymatic and cellular activities were also retained after introduction of nitrogen-containing aromatic substituents (**16h**, **i**). These results suggested that the presence of certain hetero atoms is essential for enzymatic potency because these substituents are located in a hydrophilic phosphate binding site.

A crystal structure of IKKβ is not available yet, however, it was possible to obtain structural data from the related kinase, *i.e.* JNK3. From the X-ray structure of a complex of JNK3 and **16k** (Fig. 4; 2.0 Å resolution), the binding mode of **16k** to the ATP binding site was confirmed as predicted by the binding model (Fig. 3) with the two hydrogen bonds. However, no obvious interaction between the amine moiety on the substituent and the Asn194 of JNK3 (Asn150 of IKKβ) was observed. On the other hand, an intramolecular nonbonded S···O interaction between the urea carbonyl and the sulfur atom on the thiophene ring predicted in the model was clearly confirmed in this crystal structure, and the S···O distance was 2.8 Å shorter than the corresponding van der Waals radii (3.3 Å).

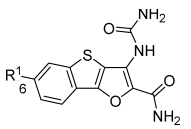
The ADMETox parameters of the selected compounds exhibiting potent cellular activity (IC_{50}<1 μM) are summarized in Table 6. Introduction of substituents generally resulted in the improvement of metabolic stability, thus the main site of metabolism is presumed to be on the benzene ring. A clear tendency was observed that the lipophilic compounds ($\log D$>2) **16a**, **h**, **i**, and **13g** possessed good oral bioavailability (BA 7—32%) in rat. In contrast, the compounds **13a**, **16k**, and **16l** with $\log D$'s less than 2 did not show oral bioavailability regardless of potency in cellular assay. It is assumed that the membrane permeability of compounds **13a**

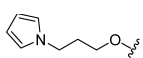
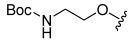
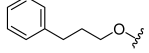
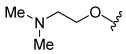
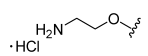
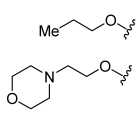
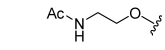
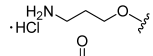
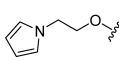
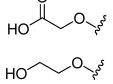
Table 4. SAR of the Substituents on the Benzene Ring of Benzo[3,2-*b*]furans



No.	R^1	IKKβ IC_{50} (nM)	THP-1 (TNF-α) IC_{50} (nM)
13a	H	19—45	180—310
13b	5-OMe	57	600
13c	6-OMe	26	450
13d	7-OMe	110	330
13e	8-OMe	670	23000
13f	6-Br	57	1200
13g	7-Br	30	120
13h	6-Ph	120	1400
13i	7-Ph	970	2500

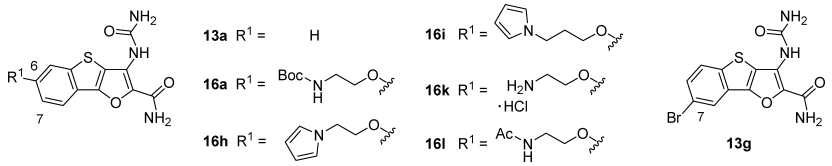
Table 5. SAR for Various 6-Alkoxy Tricyclic Furans



No.	R^1	IKKβ IC_{50} (nM)	TNF-α IC_{50} (nM)	No.	R^1	IKKβ IC_{50} (nM)	TNF-α IC_{50} (nM)
13a	H	19—45	180—310	**16i**		25	1000
16a		44	660	**16j**		990	NT[a]
16c		220	NT[a]	**16k**		11	>50000
16f		480	1900	**16l**		37	390
16g		240	1300	**16m**		110	NT[a]
				16n		29	25000
16h		34	590	**16o**		35	1700

a) NT means not tested.

Table 6. Pharmacokinetic Profiles of Tricyclic Furans



No.	*In vitro* IKKβ IC_{50} (nM)	*In vitro* THP-1 (TNF-α) IC_{50} (nM)	PK Rat cassette (0.1 mg/kg, i.v., 1 mg/kg, *p.o.*)[a] Vd_{ss} (ml/kg)	CL_{total} (ml/h/kg)	C_{max} (ng/ml)	$AUC_{p.o.}$ (ng·h/ml)	$MRT_{p.o.}$ (h)	BA (%)	Metabolic stability (μl/min/mg)[c] Rat/Human	log *D* at pH 7.4
13a	19—45	180—310	2884	5120	ND[b]	ND[b]	ND[b]	0	71/55	1.44
16k	11	>50000	17274	102148	ND[b]	ND[b]	ND[b]	0	9/9	−1.11
16l	37	390	394	1463	ND[b]	ND[b]	ND[b]	0	−14/2	0.23
16a	44	660	240	119	114	736	3.9	8	9/21	2.01
16h	34	590	856	1182	12	59	3.3	7	36/21	2.12
16i	25	1000	677	643	95	368	2.8	24	29/27	2.01
13g	30	120	1358	2373	59	119	1.7	32	10/14	2.46

a) Administration substrate; i.v., dimethyl sulfoxide; *p.o.*, 0.5% methyl cellulose. *b*) ND means not detected. *c*) Liver microsomes clearance.

and **16l** is enough for cellular potency, however, they don't have sufficient lipophilicity for oral bioavailability.

Conclusion

A novel class of IKK inhibitors, tricyclic furan derivatives were designed based on the known thiophenecarboxamide IKK inhibitors, synthesized and evaluated for their inhibitory activity against IKKβ. Among the various fused furan derivatives, the benzothieno[3,2-*b*]furan derivative **13a**, possessing an intramolecular non-bonded S···O interaction, displayed high inhibitory activity for IKKβ in enzyme and cell-based assays. Introduction of substituents onto the benzothieno[3,2-*b*]furan to overcome the low metabolic stability led to the discovery of a series of 6-alkoxy derivatives. Furthermore, it was found that the lipophilic compounds ($\log D > 2$), **16a**, **h**, **i**, and **13g** showed an improved oral bioavailability with potent enzymatic inhibitory activity. Future efforts will focus on further improvements in potency in order to evaluate the therapeutic application of these IKK inhibitors.

Experimental

Reagents were purchased from Wako Pure Chemical Industries, Ltd., Tokyo Chemical Industry, Aldrich Chemical Company, Inc. and Lancaster and used without further purification. All reagents and solvents were of commercial quality. ^{1}H-NMR spectra were recorded on a Varian Gemini-200 (200 MHz), a Varian Mercury-300 (300 MHz) or a Brucker 300 (300 MHz) with tetramethylsilane (TMS) as the internal standard. Chemical shifts are reported in parts per million (ppm) units. Coupling constants (*J*) are reported in units of hertz (Hz). Splitting patterns are designated as: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Elemental analyses were determined by Takeda Analytical Research Laboratories, Ltd., Osaka, and within ±0.4% of the theoretical values for the elements indicated unless otherwise noted. When air or moisture-sensitive reagents were used, reactions were run under argon. Column chromatography was performed on columns of Kieselgel 60 (230—400 mesh) and Chromatorex (NH_2-coated silica gel, 100—200 mesh). The yields reported are not optimized.

Abbreviations: rt=room temperature, aq.=aqueous, Et_3N=triethylamine, HOBt=1-hydroxy-1*H*-benzotriazole monohydrate, DMAP=4-dimethylaminopyridine, DMA=*N,N*-dimethylacetamide, WSC=1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, IPE=diisopropyl ether, DMSO=dimethyl sulfoxide, AcOH=acetic acid, EtOAc=ethyl acetate, THF=tetrahydrofuran, TFA=trifluoroacetic acid, DMF=dimethylformamide, MeOH=methanol, EtOH=ethanol, Et_2O=diethyl ether, CH_3CN=acetonitrile, Ph=phenyl.

Typical Procedure A.[12,13] **Methyl 2-{[(Dimethylamino)carbonothioyl]oxy}-4-methoxybenzoate (4c)** To a stirred solution of **3c** (100 g) and *N*,*N*-dimethylthiocarbamoyl chloride (88.2 g) in DMF (200 ml) was added dropwise 1,8-diazabicyclo[5.4.0]undec-7-ene (88.2 g) at 0 °C, and the mixture was stirred at room temperature for 3 d. The mixture was poured into ice-water, and extracted with EtOAc. The extract was washed with 1 N aq. HCl and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was crystallized EtOAc/hexane to afford the crude title compound (128 g). This was purified by the column chromatography on silica gel with EtOAc, and recrystallization from EtOAc/hexane afforded the title compound (123 g, 83%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 3.40 (3H, s), 3.47 (3H, s), 3.81 (3H, s), 3.85 (3H, s), 6.64 (1H, d, *J*=3 Hz), 6.82 (1H, dd, *J*=9, 3 Hz), 7.97 (1H, d, *J*=9 Hz).

Methyl 2-{[(Dimethylamino)carbonyl]thio}-4-methoxybenzoate (5c) A compound **4c** (123 g) was heated to 210 °C for 14 h, and then cooled to room temperature. This was purified by column chromatography on silica gel with EtOAc/hexane (1/4 to 2/3) to afford the title compound (85.4 g, 70%) as a reddish oil. ^{1}H-NMR ($CDCl_3$) δ: 3.00—3.20 (6H, br), 3.84 (6H, s), 6.91 (1H, dd, *J*=9, 2 Hz), 7.17 (1H, d, *J*=3 Hz), 7.93 (1H, d, *J*=9 Hz).

2-Mercapto-4-methoxybenzoic Acid (7c) A mixture of **5c** (85.3 g) and 4 N aq. NaOH (250 ml) was heated to reflux for 1 d, and then acidified with conc. HCl (100 ml) at 0 °C. The precipitate was collected by filtration and washed with H_2O. The moist cake was dissolved in THF/EtOAc, and the solution was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was recrystallized from EtOAc/hexane to afford the title compound (51.9 g, 89%) as a pale brown solid. ^{1}H-NMR ($CDCl_3$) δ: 3.79 (3H, s), 6.73 (1H, dd, *J*=9, 2 Hz), 7.10 (1H, d, *J*=2 Hz), 7.87 (1H, d, *J*=9 Hz).

Methyl 2-Mercapto-4-methoxybenzoate (8c) A mixture of **7c** (10.6 g) and 10% HCl–MeOH (200 ml) was stirred at 60 °C for 24 h, and the solvent was evaporated off. The residue was chromatographed on silica gel with EtOAc/hexane (1/4) to give the title compound as an oil (6.4 g, 56%). ^{1}H-NMR ($CDCl_3$) δ: 3.83 (3H, s), 3.89 (3H, s), 5.10 (1H, s), 6.67 (1H, dd, *J*=9, 2 Hz), 6.79 (1H, d, *J*=2 Hz), 7.98 (1H, d, *J*=9 Hz).

Similarly, compounds **8b**, **d** were prepared.

Methyl 2-Mercapto-3-methoxybenzoate (8b) Crystals (37%). ^{1}H-NMR ($CDCl_3$) δ: 3.92 (3H, s), 3.94 (3H, s), 5.31 (1H, s), 7.00—7.02 (1H, m), 7.11 (1H, d, *J*=8 Hz), 7.66 (1H, dd, J=8, 2 Hz).

Methyl 2-Mercapto-5-methoxybenzoate (8d) A pale green oil (34%). ^{1}H-NMR ($CDCl_3$) δ: 3.81 (3H, s), 3.93 (3H, s), 4.48 (1H, s), 6.94 (1H, dd, *J*=9, 3 Hz), 7.21 (1H, d, *J*=9 Hz), 7.52 (1H, d, *J*=3 Hz).

Typical Procedure B.[12,14] **5-Bromo-2-mercaptobenzoic Acid (7g)** A solution of **6g** (47.5 g), NaOH (8.98 g), and $NaNO_2$ (15.2 g) in H_2O (220 ml) was added dropwise to a mixture of conc. HCl (66 ml) and ice (*ca.* 30 g). The internal temperature was kept at 3—6 °C by the addition of ice. The mixture was stirred at 0 °C for 30 min, and then neutralized with potassium acetate (70 g). This solution was added to a solution of potassium *O*-ethylxanthate (106 g) in H_2O (200 ml) which had preheated to 90 °C. The mixture

was stirred at the same temperature for 30 min, cooled to 0 °C, and acidified with conc. HCl. The aqueous phase was decanted from the resulting semi-solid sludge. This was dissolved in 10% aq. NaOH (200 ml), and heated to 85 °C for 2 h. To this mixture was added portionwise $NaHSO_3$ (20 g), and the mixture was heated to 85 °C for 10 min. The mixture was filtered, cooled to 0 °C, and acidified with conc. HCl. The precipitate was collected by filtrarion and washed with H_2O. The moist cake was dissolved in THF/EtOAc, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to afford crude **7g** (50.3 g, 98%) as a pale brown solid. This material was used for the next reaction without further purification.

Methyl 5-Bromo-2-mercaptobenzoate (8g) A mixture of crude **7g** (50.3 g) and conc. H_2SO_4 (20 ml) in MeOH (500 ml) was heated to reflux overnight. The mixture concentrated *in vacuo*, diluted with ice-H_2O, and the whole mixture was extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with EtOAc/hexane (1/10) to afford the title compound (46.2 g, 88%) as a yellow solid. ^{1}H-NMR ($CDCl_3$) δ: 3.93 (3H, s), 4.78 (1H, s), 7.18 (1H, d, J=9 Hz), 7.42 (1H, dd, J=9, 3 Hz), 8.14 (1H, d, J=2 Hz).

Similarly, compounds **8e**, **f** were prepared.

Methyl 2-Mercapto-6-methoxybenzoate (8e) A yellow oil (42%). ^{1}H-NMR ($CDCl_3$) δ: 3.69 (3H, s), 3.82 (3H, s), 3.95 (3H, s), 6.74—6.77 (1H, m), 6.94 (1H, dd, J=8, 1 Hz), 7.21 (1H, t, J=8 Hz).

Methyl 4-Bromo-2-mercaptobenzoate (8f) A yellow oil (38%). ^{1}H-NMR ($CDCl_3$) δ: 3.92 (3H, s), 4.83 (1H, s), 7.28 (1H, dd, J=14, 3 Hz), 7.49 (1H, d, J=3 Hz), 7.87 (1H, d, J=13 Hz).

Typical Procedure C. Methyl 2-[(Cyanomethyl)thio]benzoate (9a) A mixture of **8a** (18.0 g), bromoacetonitrile (9.70 g), and K_2CO_3 (29.6 g) in THF (200 ml) was heated to reflux for 8 h. The mixture was diluted with H_2O, and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The obtained solid was washed with EtOH/hexane to afford the title compound (17.4 g, 78%). mp 105—106 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.72 (2H, s), 3.94 (3H, s), 7.34 (1H, td, J=8, 1 Hz), 7.43 (1H, d, J=8 Hz), 7.58 (1H, td, J=8, 1 Hz), 8.04 (1H, dd, J=8, 1 Hz).

Similarly, compounds **9b**—**g** were prepared.

Methyl 2-[(Cyanomethyl)thio]-3-methoxybenzoate (9b) Crystals (91%). ^{1}H-NMR ($CDCl_3$) δ: 3.70 (3H, s), 3.90 (5H, s), 6.79 (1H, dd, J=8, 2 Hz), 6.87 (1H, d, J=2 Hz), 8.04 (1H, d, J=8 Hz).

Methyl 2-[(Cyanomethyl)thio]-4-methoxybenzoate (9c) Crystals (81%). ^{1}H-NMR ($CDCl_3$) δ: 3.70 (3H, s), 3.90 (5H, s), 6.79 (1H, dd, J=9, 2 Hz), 6.87 (1H, d, J=2 Hz), 8.04 (1H, d, J=9 Hz).

Methyl 2-[(Cyanomethyl)thio]-5-methoxybenzoate (9d) A white solid (98%). ^{1}H-NMR ($CDCl_3$) δ: 3.69 (2H, s), 3.86 (3H, s), 3.94 (3H, s), 7.09 (1H, dd, J=9, 3 Hz), 7.44 (1H, d, J=3 Hz), 7.54 (1H, d, J=9 Hz).

Methyl 2-[(Cyanomethyl)thio]-6-methoxybenzoate (9e) A yellow oil (100%). ^{1}H-NMR ($CDCl_3$) δ: 3.59 (2H, s), 3.86 (3H, s), 3.95 (3H, s), 7.02 (1H, dd, J=8, 1 Hz), 7.32 (1H, dd, J=7, 1 Hz), 7.42 (1H, t, J=8 Hz).

Methyl 4-Bromo-2-[(cyanomethyl)thio]benzoate (9f) A white solid (91%). mp 129—130 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.72 (2H, s), 3.93 (3H, s), 7.44—7.48 (1H, m), 7.49 (1H, d, J=2 Hz), 7.89—7.92 (1H, m).

Methyl 5-Bromo-2-[(cyanomethyl)thio]benzoate (9g) A white solid (40%). mp 161—162 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.70 (2H, s), 3.94 (3H, s), 7.29 (1H, d, J=9 Hz), 7.66—7.70 (1H, m), 8.15 (1H, d, J=2 Hz). *Anal.* Calcd for $C_{10}H_8BrNO_2S$: C, 41.97; H, 2.82; N, 4.89. Found: C, 41.89; H, 2.78; N, 4.88.

Typical Procedure D. 3-Hydroxy-1-benzothiophene-2-carbonitrile (10a) A mixture of **9a** (17.4 g) and sodium ethoxide (8.6 g) in EtOH (200 ml) was refluxed 2 h, and the solvent was evaporated off. The residue was diluted with EtOAc/THF, and acidified with 1 N aq. HCl. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* to afford the title compound (8.36 g, 57%) as crystals. mp 173—174 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.48 (1H, t, J=8 Hz), 7.58 (1H, t, J=7 Hz), 7.96 (1H, d, J=8 Hz), 8.01 (1H, d, J=8 Hz), 12.25 (1H, s).

Similarly, compounds **10b**—**g** were prepared.

3-Hydroxy-7-methoxy-1-benzothiophene-2-carbonitrile (10b) Crystals (75%). ^{1}H-NMR (DMSO-d_6) δ: 3.97 (3H, s), 7.17 (1H, d, J=8 Hz), 7.44—7.51 (1H, m), 7.63 (1H, d, J=8 Hz), 12.28 (1H, s).

3-Hydroxy-6-methoxy-1-benzothiophene-2-carbonitrile (10c) Crystals (75%). ^{1}H-NMR ($CDCl_3$) δ: 3.89 (3H, s), 7.05 (1H, d, J=9 Hz), 7.13 (1H, s), 7.76 (1H, d, J=9 Hz).

3-Hydroxy-5-methoxy-1-benzothiophene-2-carbonitrile (10d) A pale brown solid (95%). mp 195—196 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.83 (3H, s), 7.22—7.25 (1H, m), 7.53 (1H, d, J=2 Hz), 7.86 (1H, d, J=9 Hz), 12.16 (1H, br). *Anal.* Calcd for $C_{10}H_7NO_2S$: C, 58.52; H, 3.44; N, 6.82. Found: C, 58.59; H, 3.39; N, 6.73.

3-Hydroxy-4-methoxy-1-benzothiophene-2-carbonitrile (10e) A pale purple solid (81%). mp 132—133 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.91 (3H, s), 6.95 (1H, d, J=7 Hz), 7.46—7.53 (2H, m), 10.88 (1H, br). *Anal.* Calcd for $C_{10}H_7NO_2S$: C, 58.52; H, 3.44; N, 6.82. Found: C, 58.61; H, 3.44; N, 6.86.

6-Bromo-3-hydroxy-1-benzothiophene-2-carbonitrile (10f) A white solid (85%). mp 232—236 °C (decomposed). ^{1}H-NMR (DMSO-d_6) δ: 7.64—7.69 (1H, m), 7.95 (1H, dd, J=8, 1 Hz), 8.33—8.34 (1H, m), 12.45 (1H, br s). *Anal.* Calcd for C_9H_4BrNOS: C, 42.54; H, 1.59; N, 5.51. Found: C, 42.71; H, 1.67; N, 5.74.

5-Bromo-3-hydroxy-1-benzothiophene-2-carbonitrile (10g) A white solid (90%). mp 262 °C (decomposed). ^{1}H-NMR ($CDCl_3$) δ: 3.72 (2H, s), 3.93 (3H, s), 7.29 (1H, dd, J=8, 2 Hz), 7.34 (1H, d, J=2 Hz), 8.00 (1H, d, J=8 Hz). *Anal.* Calcd for C_9H_4BrNOS: C, 42.54; H, 1.59; N, 5.51. Found: C, 42.79; H, 1.67; N, 5.58.

3-Hydroxy-6-phenyl-1-benzothiophene-2-carbonitrile (10h) A mixture of **10f** (1.78 g, 7.0 mmol), phenylboronic acid (1.11 g), and K_2CO_3 (2.90 g) in THF (36.0 ml) and H_2O (12.0 ml) was degassed, and N_2 was introduced. To this mixture was added $Pd(Ph_3P)_4$ (243 mg, 3 mol%), and the mixture was heated to reflux for 18 h. The mixture was partitioned between 1 N aq. HCl and EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered through the short pad of silica gel eluting with EtOAc, and concentrated *in vacuo*. The residue was recrystallized from EtOAc/hexane to afford the title compound (1.59 g, 90%) as a brown solid (90%). mp 213—218 °C (decomposed). ^{1}H-NMR (DMSO-d_6) δ: 7.40—7.54 (3H, m), 7.76—7.84 (3H, m), 8.11 (1H, t, J=8 Hz), 8.31 (1H, d, J=2 Hz), 12.33 (1H, s). *Anal.* Calcd for $C_{15}H_9NOS$: C, 71.18; H, 3.66; N, 5.53. Found: C, 71.06; H, 3.69; N, 5.53.

3-Hydroxy-5-phenyl-1-benzothiophene-2-carbonitrile (10i) A mixture of **10g** (152 mg, 0.60 mmol), phenylboronic acid (110 mg), and K_2CO_3 (250 mg) in THF (4.0 ml) and H_2O (1.8 ml) was degassed, and N_2 was introduced. To this mixture was added $Pd(Ph_3P)_4$ (35.0 mg), and the mixture was heated to reflux for 18 h. The mixture was partitioned between 1 N aq. $KHSO_4$ and EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with EtOAc/hexane (20—30%), and the obtained solid was recrystallized from EtOAc/hexane to afford the title compound (103 mg, 68%) as a white solid (68%). mp 181—181 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.36—7.50 (3H, m), 7.62—7.66 (2H, m), 7.74—7.81 (2H, m), 8.09 (1H, t, J=1 Hz). *Anal.* Calcd for $C_{15}H_9NOS$: C, 71.69; H, 3.61; N, 5.57. Found: C, 71.53; H, 3.66; N, 5.50.

Typical Procedure E. 2-[(2-Cyano-1-benzothien-3-yl)oxy]acetamide (11a) A mixture of **10a** (2.21 g), bromoacetamide (2.09 g), and K_2CO_3 (3.48 g) in THF (50.0 ml) was heated to reflux for 14 h. The mixture was diluted with H_2O, and extracted with EtOAc. The extract was washed with saturated brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was recrystallized from THF/hexane to afford the title compound (1.82 g, 62%) as crystals. mp 165—166 °C. ^{1}H-NMR (DMSO-d_6) δ: 5.07 (2H, s), 7.50—7.55 (2H, m), 7.64 (1H, t, J=8 Hz), 7.74 (1H, s), 8.01 (1H, d, J=8 Hz), 8.09 (1H, d, J=8 Hz).

Similarly, compounds **11b—i** were prepared.

2-[(2-Cyano-7-methoxy-1-benzothien-3-yl)oxy]acetamide (11b) Crystals (75%). mp 255—257 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.99 (3H, s), 5.07 (2H, s), 7.23 (1H, d, J=8 Hz), 7.51 (1H, t, J=8 Hz), 7.53 (1H, s), 7.69 (1H, d, J=8 Hz), 7.74 (1H, s).

2-[(2-Cyano-6-methoxy-1-benzothien-3-yl)oxy]acetamide (11c) Crystals (43%). ^{1}H-NMR (DMSO-d_6) δ: 3.85 (3H, s), 5.03 (2H, s), 7.11 (1H, dd, J=9, 2 Hz), 7.54 (1H, s), 7.59 (1H, d, J=2 Hz), 7.73 (1H, s), 7.98 (1H, d, J=9 Hz).

2-[(2-Cyano-5-methoxy-1-benzothien-3-yl)oxy]acetamide (11d) A white solid (87%). mp 181—182 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.86 (3H, s), 5.07 (2H, s), 7.28 (1H, dd, J=9, 3 Hz), 7.52 (1H, d, J=3 Hz), 7.58 (1H, br), 7.79 (1H, br), 7.91 (1H, d, J=9 Hz). *Anal.* Calcd for $C_{12}H_{10}N_2O_3S$: C, 54.95; H, 3.84; N, 10.68. Found: C, 54.85; H, 3.81; N, 10.67.

2-[(2-Cyano-4-methoxy-1-benzothien-3-yl)oxy]acetamide (11e) A white solid (68%). mp 200—202 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.92 (3H, s), 4.85 (2H, s), 7.02—7.06 (1H, m), 7.37 (1H, br), 7.56—7.58 (2H, m), 7.64 (1H, br). *Anal.* Calcd for $C_{12}H_{10}N_2O_3S$: C, 54.95; H, 3.84; N, 10.68. Found: C, 54.67; H, 3.64; N, 10.60.

2-[(6-Bromo-2-cyano-1-benzothien-3-yl)oxy]acetamide (11f) A pale brown solid (57%). mp 228—232 °C. ^{1}H-NMR (DMSO-d_6) δ: 5.07 (2H, s), 7.57 (1H, br), 7.70 (1H, dd, J=9, 2 Hz), 8.06 (1H, d, J=9 Hz), 8.38 (1H, d, J=2 Hz). *Anal.* Calcd for $C_{11}H_7BrN_2O_2S$: C, 42.46; H, 2.27; N, 9.00. Found:

C, 42.54; H, 2.22; N, 9.10.

2-[(5-Bromo-2-cyano-1-benzothien-3-yl)oxy]acetamide (11g) A white solid (81%). mp 191—192 °C. ^{1}H-NMR (DMSO-d_6) δ: 5.07 (2H, s), 7.60 (1H, br s), 7.79 (1H, dd, J=9, 2 Hz), 7.87 (1H, br s), 8.01 (1H, d, J=9 Hz), 8.41 (1H, d, J=2 Hz). *Anal.* Calcd for $C_{11}H_7BrN_2O_2S \cdot 0.1H_2O$: C, 42.36; H, 2.48; N, 8.82. Found: C, 42.45; H, 2.52; N, 8.63.

2-[(2-Cyano-6-phenyl-1-benzothien-3-yl)oxy]acetamide (11h) A pale brown solid (84%). mp 201—203 °C. ^{1}H-NMR (DMSO-d_6) δ: 5.09 (2H, s), 7.41—7.57 (4H, m), 7.77—7.81 (3H, m), 7.85 (1H, d, J=8 Hz), 8.18 (1H, d, J=8 Hz), 8.36 (1H, s). *Anal.* Calcd for $C_{17}H_{12}N_2O_2S$: C, 66.22; H, 3.92; N, 9.08. Found: C, 65.94; H, 3.94; N, 9.17.

2-[(2-Cyano-5-phenyl-1-benzothien-3-yl)oxy]acetamide (11i) A pale yellow solid (86%). mp 186—187 °C. ^{1}H-NMR (DMSO-d_6) δ: 5.11 (2H, s), 7.39—7.44 (1H, m), 7.49—7.54 (2H, m), 7.61 (1H, br), 7.78 (2H, d, J=7 Hz), 7.87 (1H, br), 7.94—7.98 (1H, m), 8.12 (1H, d, J=9 Hz), 8.35 (1H, d, J=9 Hz). *Anal.* Calcd for $C_{17}H_{12}N_2O_2S$: C, 66.22; H, 3.92; N, 9.08. Found: C, 66.01; H, 3.93; N, 9.02.

Typical Procedure F. 3-Amino[1]benzothieno[3,2-*b*]furan-2-carboxamide (12a) A mixture of **11a** (1.0 g) and sodium ethoxide (440 mg) in EtOH (50.0 ml) was heated to reflux for 3 h, and then concentrated *in vacuo*. The residue was washed with H_2O, and purified by column chromatography on silica gel with EtOAc to afford the title compound (420 mg, 42%) as crystals. mp 222—224 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.12 (2H, s), 7.19 (2H, s), 7.41—7.53 (2H, m), 7.87 (1H, d, J=8 Hz), 8.05 (1H, d, J=8 Hz).

Similarly, compounds **12b**—**i** were prepared.

3-Amino-5-methoxy[1]benzothieno[3,2-*b*]furan-2-carboxamide (12b) Crystals (22%). mp 239—241 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.00 (3H, s), 6.11 (2H, s), 7.05—7.08 (1H, m), 7.19 (2H, s), 7.46—7.48 (2H, m).

3-Amino-6-methoxy[1]benzothieno[3,2-*b*]furan-2-carboxamide (12c) Crystals (43%). mp 231—233 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.84 (3H, s), 6.06 (2H, s), 7.09—7.12 (3H, m), 7.66 (1H, d, J=2 Hz), 7.73 (1H, d, J=9 Hz).

3-Amino-7-methoxy[1]benzothieno[3,2-*b*]furan-2-carboxamide (12d) A pale brown solid (56%). mp 246—248 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.85 (3H, s), 6.09 (2H, br s), 7.04—7.08 (1H, m), 7.16 (2H, br), 7.33 (1H, d, J=2 Hz), 7.91 (1H, d, J=9 Hz). *Anal.* Calcd for $C_{12}H_{10}N_2O_3S$: C, 54.95; H, 3.84; N, 10.68. Found: C, 54.66; H, 3.82; N, 10.57.

3-Amino-8-methoxy[1]benzothieno[3,2-*b*]furan-2-carboxamide (12e) A gray solid (68%). mp 219—221 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.97 (3H, s), 6.08 (2H, br s), 6.99—7.02 (3H, m), 7.36 (1H, d, J=8 Hz), 7.57 (1H, d, J=8 Hz). *Anal.* Calcd for $C_{12}H_{10}N_2O_3S$: C, 54.95; H, 3.84; N, 10.68. Found: C, 55.04; H, 4.04; N, 10.42.

3-Amino-6-bromo[1]benzothieno[3,2-*b*]furan-2-carboxamide (12f) A mixture of **11f** (16.8 g) and sodium ethoxide (428 mg) in EtOH (20 ml) was heated to reflux for 1 h. The mixture was diluted with H_2O, and the precipitate was collected by filtration, washed with H_2O and EtOH. The solid was dissolved in MeOH/THF/EtOAc, filtered through the short pad of NH_2-coated silica gel eluting with THF/EtOAc, and the filtrate was concentrated *in vacuo*. The residue was recrystallized from THF/EtOH and DMF/H_2O to afford a crude **12f** (0.345 g) as a white solid. This material was used for the next reaction without further purification.

3-Amino-7-bromo[1]benzothieno[3,2-*b*]furan-2-carboxamide (12g) A pale brown solid (40%). mp 249 °C (decomposed). ^{1}H-NMR (DMSO-d_6) δ: 6.12 (2H, s), 7.19 (2H, br s), 7.57 (1H, dd, J=9, 2 Hz), 7.96 (1H, d, J=2 Hz), 8.03 (1H, d, J=9 Hz). *Anal.* Calcd for $C_{11}H_7BrN_2O_2S$: C, 42.46; H, 2.27; N, 9.00. Found: C, 42.52; H, 2.22; N, 8.99.

3-Amino-6-phenyl[1]benzothieno[3,2-*b*]furan-2-carboxamide (12h) This compound was prepared from **11h** by a similar procedure described in the synthesis of **12a** as a pale brown solid (35%). mp 266—268 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.13 (2H, br s), 7.20 (2H, br), 7.40 (1H, t, J=7 Hz), 7.51 (2H, t, J=7 Hz), 7.77—7.83 (3H, m), 7.92 (1H, d, J=8 Hz), 8.38—8.39 (1H, m). *Anal.* Calcd for $C_{17}H_{12}N_2O_2S$: C, 66.22; H, 3.92; N, 9.08. Found: C, 66.20; H, 4.03; N, 9.16.

3-Amino-7-phenyl[1]benzothieno[3,2-*b*]furan-2-carboxamide (12i) A pale brown solid (28%). mp 269—271 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.13 (2H, br s), 7.18 (2H, br), 7.41—7.56 (3H, m), 7.74—7.70 (3H, m), 8.08 (1H, s), 8.14 (1H, d, J=9 Hz). *Anal.* Calcd for $C_{17}H_{12}N_2O_2S \cdot 0.2$EtOH: C, 65.81; H, 4.19; N, 8.82. Found: C, 65.60; H, 3.97; N, 8.79.

Typical Procedure G. 3-[(Aminocarbonyl)amino][1]benzothieno-[3,2-*b*]furan-2-carboxamide (13a) Method A: A mixture of **12a** (220 mg) and potassium cyanate (153 mg) in AcOH (3.0 ml) was heated to 110 °C for 3 d, and then concentrated *in vacuo*. The residue was dissolved in THF, washed with aq. $NaHCO_3$ and saturated brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with THF/toluene to afford the title compound (40 mg, 15%).

Method B: To a solution of **12a** (300 mg) in THF (8.0 ml) was added $Cl_3CCONCO$ (365 mg) at −60 °C. After stirring at −30 °C for 1 h, 2 N NH_3–EtOH (5.0 ml) was added to the reaction mixture. After stirring at rt overnight, the reaction mixture was poured into H_2O. The precipitate was collected by filtration and washed with H_2O and EtOH to give the title compound (200 mg, 56%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.87 (2H, s), 7.38—7.52 (2H, m), 7.46 (2H, s), 7.60—7.80 (2H, br s), 7.86 (1H, d, J=8 Hz), 7.98 (1H, d, J=8 Hz), 9.24 (1H, s). *Anal.* Calcd for $C_{12}H_9N_3O_3S$: C, 52.36; H, 3.30; N, 15.26. Found: C, 52.01; H, 3.42; N, 15.01.

Compound **13c** was prepared according to the typical procedure G (method A), and compounds **13b**, **13d**—**i** were prepared according to the typical procedure G (method B).

3-[(Aminocarbonyl)amino]-5-methoxy[1]benzothieno[3,2-*b*]furan-2-carboxamide (13b) Crystals (39%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.98 (3H, s), 6.90 (2H, br s), 7.05 (1H, s), 7.46 (2H, s), 7.56 (1H, br s), 7.80 (1H, br s), 9.25 (1H, s). *Anal.* Calcd for $C_{13}H_{11}N_3O_4S \cdot 0.25H_2O$: C, 50.40; H, 3.74; N, 13.56. Found: C, 50.56; H, 3.71; N, 13.42.

3-[(Aminocarbonyl)amino]-6-methoxy[1]benzothieno[3,2-*b*]furan-2-carboxamide (13c) Crystals (11%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.85 (3H, s), 6.85 (2H, s), 7.08 (1H, dd, J=9, 2 Hz), 7.40—7.70 (3H, m), 7.73 (1H, d, J=9 Hz), 9.22 (1H, s). *Anal.* Calcd for $C_{13}H_{11}N_3O_4S$: C, 51.14; H, 3.63; N, 13.76. Found: C, 51.05; H, 3.93; N, 13.88.

3-[(Aminocarbonyl)amino]-7-methoxy[1]benzothieno[3,2-*b*]furan-2-carboxamide (13d) A pale pink solid (88%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.85 (3H, s), 6.86 (2H, br), 7.05 (1H, dd, J=9, 2 Hz), 7.32 (1H, d, J=2 Hz), 7.55 (1H, br), 7.76 (1H, br), 7.85 (1H, d, J=9 Hz), 9.22 (1H, s). *Anal.* Calcd for $C_{13}H_{11}N_3O_4S \cdot 0.6H_2O$: C, 49.39; H, 3.89; N, 13.29. Found: C, 49.30; H, 4.12; N, 13.47.

3-[(Aminocarbonyl)amino]-8-methoxy[1]benzothieno[3,2-*b*]furan-2-carboxamide (13e) A gray solid (55%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.98 (3H, s), 6.88 (2H, br s), 6.97 (1H, d, J=8 Hz), 7.35 (1H, t, J=8 Hz), 7.48—7.51 (3H, m), 9.25 (1H, s). *Anal.* Calcd for $C_{13}H_{11}N_3O_4S \cdot 0.2$EtOH $\cdot 0.4H_2O$: C, 50.02; H, 4.07; N, 13.06. Found: C, 50.05; H, 4.01; N, 12.90.

3-[(Aminocarbonyl)amino]-6-bromo[1]benzothieno[3,2-*b*]furan-2-carboxamide (13f) This compound was prepared from crude **12f** by a similar procedure described in the synthesis of **13a** as a pale brown solid (15% from **11f**). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.90 (2H, br), 7.50—7.90 (2H, br), 7.63 (1H, d, J=9 Hz), 7.76 (1H, d, J=8 Hz), 8.30 (1H, s), 9.23 (1H, s). *Anal.* Calcd for $C_{12}H_8BrN_3O_3S$: C, 40.69; H, 2.28; N, 11.86. Found: C, 40.44; H, 2.29; N, 11.69.

3-[(Aminocarbonyl)amino]-7-bromo[1]benzothieno[3,2-*b*]furan-2-carboxamide (13g) A pink solid (40%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.89 (2H, br s), 7.54—7.80 (3H, m), 7.94—7.98 (2H, m), 9.20 (1H, s). *Anal.* Calcd for $C_{12}H_8BrN_3O_3S \cdot 1.0H_2O$: C, 38.72; H, 2.71; N, 11.29. Found: C, 38.87; H, 2.84; N, 10.99.

3-[(Aminocarbonyl)amino]-6-phenyl[1]benzothieno[3,2-*b*]furan-2-carboxamide (13h) A solid (87%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.87 (2H, br), 7.38—7.82 (8H, m), 7.93 (1H, d, J=8 Hz), 8.31—8.32 (1H, m), 9.27 (1H, s). *Anal.* Calcd for $C_{18}H_{13}N_3O_3S$: C, 61.53; H, 3.73; N, 11.96. Found: C, 61.23; H, 3.66; N, 11.87.

3-[(Aminocarbonyl)amino]-7-phenyl[1]benzothieno[3,2-*b*]furan-2-carboxamide (13i) A pale brown solid (85%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.90 (2H, br), 7.18 (2H, br), 7.41 (1H, t, J=7 Hz), 7.50—7.76 (7H, m), 8.06—8.08 (2H, m), 9.25 (1H, s). *Anal.* Calcd for $C_{18}H_{13}N_3O_3S \cdot 0.3H_2O$: C, 60.60; H, 3.84; N, 11.78. Found: C, 60.62; H, 3.78; N, 11.70.

3-Amino-6-hydroxy[1]benzothieno[3,2-*b*]furan-2-carboxamide (14) To a stirred solution of **12c** (10.2 g) in CH_2Cl_2 (200 ml) was added dropwise BBr_3 (25.0 g) at 0 °C, and the mixture was heated to reflux for 1 d. After cooling, the reaction was quenched with ice, and the precipitate was collected by filtration. The solid was dissolved in DMF, and filtered through the short pad of NH_2-coated silica gel with MeOH/EtOAc (20%). The filtrate was concentrated *in vacuo*, and the residue was recrystallized from DMF/MeOH/EtOH to afford the title compound (7.28 g, 75%) as crystals. mp 285—290 °C (decomposed). ^{1}H-NMR (DMSO-d_6) δ: 6.04 (2H, br s), 6.96 (1H, d, J=8 Hz), 7.06 (2H, br), 7.33 (1H, s), 7.65 (1H, d, J=9 Hz), 10.00 (1H, br). *Anal.* Calcd for $C_{11}H_8N_2O_3S$: C, 53.22; H, 3.25; N, 11.28. Found: C, 53.22; H, 3.19; N, 11.20.

Typical Procedure H. *tert*-Butyl (2-{[3-Amino-2-(aminocarbonyl)-[1]benzothieno[3,2-*b*]furan-6-yl]oxy}ethyl)carbamate (15a) To a stirred solution of **14** (300 mg) in DMF (5.0 ml) was added NaH (72.5 mg, 60% in oil), and the mixture was heated to 60 °C for 20 min. To this mixture was

added a solution of *tert*-butyl (2-bromoethyl)carbamate (325 mg) in DMF (1.0 ml), and the mixture was heated to 60 °C for 6 h. The mixture was partitioned between H_2O and EtOAc. The organic phase was separated, washed with 1 N aq. $KHSO_4$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on NH_2-coated silica gel with EtOAc/hexane (90—100%), and recrystallization from EtOAc/hexane to afford the title compound (391 mg, 83%) as a white solid. mp 212—214 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.38 (9H, s), 3.31—3.34 (2H, m, overlapped with DMSO), 4.01—4.07 (2H, m), 6.07 (2H, br s), 7.05—7.11 (3H, m), 7.65 (1H, d, J=2 Hz), 7.72 (1H, d, J=9 Hz). *Anal.* Calcd for $C_{18}H_{21}N_3O_5S$: C, 55.23; H, 5.41; N, 10.73. Found: C, 55.16; H, 5.56; N, 10.49.

***tert*-Butyl (3-{[3-Amino-2-(aminocarbonyl)[1]benzothieno[3,2-*b*]furan-6-yl]oxy}propyl)-carbamate (15b)** This compound was prepared from **14** and *tert*-butyl (3-bromopropyl)carbamate by a similar procedure described in the synthesis of **15a** as a white solid (79%). mp 159—161 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (9H, s), 1.84—1.99 (2H, m), 3.10 (2H, q, J=6 Hz), 4.06 (2H, t, J=6 Hz), 6.06 (2H, br s), 6.91—6.93 (3H, m), 7.07—7.10 (3H, m), 7.63 (1H, d, J=2 Hz), 7.72 (1H, d, J=9 Hz). *Anal.* Calcd for $C_{19}H_{23}N_3O_5S \cdot 0.5$EtOH: C, 56.06; H, 6.12; N, 9.81. Found: C, 55.81; H, 5.85; N, 9.74.

3-Amino-6-[2-(dimethylamino)ethoxy][1]benzothieno[3,2-*b*]furan-2-carboxamide (15c) This compound was prepared from **14**, 2-dimethylaminoethyl chloride hydrochloride and sodium iodide by a similar procedure described in the synthesis of **15a** as a brown solid (71%). mp 184—186 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.23 (6H, s), 2.66 (2H, t, J=6 Hz), 4.14 (2H, t, J=6 Hz), 6.06 (2H, br s), 7.07—7.12 (3H, m), 7.66 (1H, d, J=2 Hz), 7.72 (1H, d, J=8 Hz). *Anal.* Calcd for $C_{15}H_{17}N_3O_3S \cdot 0.1H_2O$: C, 56.09; H, 5.20; N, 13.08. Found: C, 56.11; H, 5.22; N, 13.15.

Ethyl {[3-Amino-2-(aminocarbonyl)[1]benzothieno[3,2-*b*]furan-6-yl]-oxy}acetate (15d) This compound was prepared from **14** and ethyl bromoacetate by a similar procedure described in the synthesis of **15a** as a pale brown solid (68%). mp 174—175 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.23 (3H, t, J=7 Hz), 4.19 (2H, q, J=7 Hz), 4.89 (2H, s), 6.07 (2H, br s), 7.11—7.15 (3H, m), 7.66 (1H, d, J=2 Hz), 7.74 (1H, d, J=8 Hz), 7.95 (1H, s). *Anal.* Calcd for $C_{15}H_{14}N_2O_5S \cdot 0.6$DMF: C, 53.35; H, 4.85; N, 9.63. Found: C, 53.08; H, 5.01; N, 9.76.

2-{[3-Amino-2-(aminocarbonyl)[1]benzothieno[3,2-*b*]furan-6-yl]-oxy}ethyl Acetate (15e) This compound was prepared from **14** and 2-bromoethyl acetate by a similar procedure described in the synthesis of **15a** as a white solid (76%). mp 173—176 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.05 (3H, s), 4.27—4.29 (2H, m), 4.36—4.38 (2H, m), 6.07 (1H, br s), 7.10—7.14 (3H, m), 7.69 (1H, d, J=2 Hz), 7.73 (1H, d, J=9 Hz). *Anal.* Calcd for $C_{15}H_{14}N_2O_5S$: C, 53.88; H, 4.22; N, 8.38. Found: C, 53.91; H, 4.25; N, 8.10.

3-Amino-6-propoxy[1]benzothieno[3,2-*b*]furan-2-carboxamide (15f) This compound was prepared from **14** and 1-iodopropane by a similar procedure described in the synthesis of **15a** as a pale brown solid (71%). mp 171—173 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.00 (3H, t, J=7 Hz), 1.73—1.81 (2H, m), 4.02 (2H, t, J=7 Hz), 6.06 (2H, br s), 7.08—7.11 (3H, m), 7.64 (1H, s), 7.72 (1H, d, J=8 Hz). *Anal.* Calcd for $C_{14}H_{14}N_2O_3S \cdot 0.6$EtOH: C, 57.83; H, 4.99; N, 9.50. Found: C, 57.60; H, 4.83; N, 9.48.

3-Amino-6-(2-morpholin-4-ylethoxy)[1]benzothieno[3,2-*b*]furan-2-carboxamide (15g) This compound was prepared from **14**, *N*-(2-chloroethyl)morpholine hydrochloride and sodium iodide by a similar procedure described in the synthesis of **15a** as a pale green solid (55%). mp 170—172 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.49—2.50 (2H, m), 2.73 (2H, t, J=6 Hz), 3.58 (4H, t, J=4 Hz), 4.18 (2H, t, J=6 Hz), 6.06 (2H, br s), 7.08—7.12 (3H, m), 7.67 (1H, s), 7.70—7.73 (1H, m). *Anal.* Calcd for $C_{17}H_{19}N_3O_4S$: C, 56.50; H, 5.30; N, 11.60. Found: C, 56.36; H, 5.44; N, 11.35.

3-Amino-6-[2-(1*H*-pyrrol-1-yl)ethoxy][1]benzothieno[3,2-*b*]furan-2-carboxamide (15h) This compound was prepared from **14** and 1-(2-bromoethyl)-1*H*-pyrrole by a similar procedure described in the synthesis of **15a** as crystals (46%). mp 158—160 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.31 (4H, s), 6.00 (2H, t, J=2 Hz), 6.05 (2H, s), 6.11 (2H, s), 6.85 (2H, t, J=2 Hz), 7.07—7.10 (3H, m), 7.63 (1H, s), 7.72 (1H, d, J=8 Hz).

3-Amino-6-[3-(1*H*-pyrrol-1-yl)propoxy][1]benzothieno[3,2-*b*]furan-2-carboxamide (15i) This compound was prepared from **14** and 1-(3-bromopropyl)-1*H*-pyrrole by a similar procedure described in the synthesis of **15a** as crystals (54%). mp 197—198 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.13—2.20 (2H, m), 3.98 (2H, t, J=6 Hz), 4.07 (2H, t, J=7 Hz), 5.99 (2H, s), 6.06 (2H, s), 6.77 (2H, s), 7.08 (2H, s), 7.13 (1H, s), 7.61 (1H, s), 7.73 (1H, d, J=9 Hz). *Anal.* Calcd for $C_{18}H_{17}N_3O_3S$: C, 60.83; H, 4.82; N, 11.82. Found: C, 60.53; H, 5.05; N, 11.57.

3-Amino-6-(3-phenylpropoxy)[1]benzothieno[3,2-*b*]furan-2-carboxamide (15j) This compound was prepared from **14** and 1-bromo-3-phenylpropane by a similar procedure described in the synthesis of **15a** as a pale brown solid (50%). mp 185—187 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.03—2.09 (2H, m), 2.77 (2H, t, J=8 Hz), 4.06 (2H, t, J=6 Hz), 6.06 (2H, br s), 7.04—7.34 (8H, m), 7.63 (1H, d, J=2 Hz), 7.72 (1H, d, J=9 Hz). *Anal.* Calcd for $C_{20}H_{18}N_2O_3S \cdot 0.1H_2O$: C, 65.23; H, 4.98; N, 7.61. Found: C, 65.00; H, 5.02; N, 7.62.

Compounds **16a—j** were prepared according to the typical procedure G (method B).

***tert*-Butyl [2-({2-(Aminocarbonyl)-3-[(aminocarbonyl)amino][1]benzothieno[3,2-*b*]furan-6-yl}oxy)ethyl]carbamate (16a)** A white solid (81%). mp 209—211 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.39 (9H, s), 3.31—3.33 (2H, m, overlapped with DMSO), 4.05 (2H, t, J=6 Hz), 6.65—6.95 (2H, br), 7.04—7.09 (2H, m), 7.40—7.70 (2H, br), 7.57 (1H, d, J=2 Hz), 7.72 (1H, d, J=9 Hz), 9.22 (1H, s). *Anal.* Calcd for $C_{19}H_{22}N_4O_6S \cdot 1.0H_2O$: C, 50.43; H, 5.35; N, 12.38. Found: C, 50.38; H, 5.36; N, 12.34.

***tert*-Butyl [3-({2-(Aminocarbonyl)-3-[(aminocarbonyl)amino][1]benzothieno[3,2-*b*]furan-6-yl}oxy)propyl]carbamate (16b)** A white solid (79%). mp 221—223 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (9H, s), 1.86—1.91 (2H, m), 3.11 (2H, q, J=6 Hz), 4.05 (2H, t, J=6 Hz), 6.70—7.00 (3H, br), 7.04 (1H, dd, J=9, 3 Hz), 7.31 (1H, d, J=3 Hz), 7.56 (1H, br), 7.76 (1H, br), 7.84 (1H, d, J=9 Hz), 9.22 (1H, s). *Anal.* Calcd for $C_{20}H_{24}N_4O_6S \cdot 0.2H_2O$: C, 53.14; H, 5.44; N, 12.39. Found: C, 52.90; H, 5.53; N, 12.32.

3-[(Aminocarbonyl)amino]-6-[2-(dimethylamino)ethoxy][1]benzothieno[3,2-*b*]furan-2-carboxamide (16c) A pale green solid (77%). mp 290 °C (decomposed). ^{1}H-NMR (DMSO-d_6) δ: 2.33 (6H, s), 2.65 (2H, t, J=6 Hz), 4.13 (2H, t, J=6 Hz), 6.85 (2H, br), 7.08 (1H, dd, J=9, 2 Hz), 7.40—7.75 (2H, br), 7.58 (1H, d, J=2 Hz), 7.72 (1H, d, J=9 Hz), 9.22 (1H, s). *Anal.* Calcd for $C_{16}H_{18}N_4O_4S \cdot 0.6H_2O$: C, 51.49; H, 5.19; N, 15.01. Found: C, 51.35; H, 5.17; N, 15.04.

Ethyl ({2-(Aminocarbonyl)-3-[(aminocarbonyl)amino][1]benzothieno-[3,2-*b*]furan-6-yl}oxy)acetate (16d) A pale brown solid (80%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.23 (3H, t, J=7 Hz), 4.19 (2H, q, J=7 Hz), 4.88 (2H, s), 6.87 (2H, br), 7.09—7.13 (1H, m), 7.35—7.80 (2H, br), 7.57 (1H, d, J=2 Hz), 7.74 (1H, d, J=8 Hz), 9.22 (1H, s). *Anal.* Calcd for $C_{16}H_{15}N_3O_6S \cdot 0.5H_2O$: C, 49.74; H, 4.17; N, 10.88. Found: C, 49.85; H, 4.03; N, 10.88.

2-({2-(Aminocarbonyl)-3-[(aminocarbonyl)amino][1]benzothieno[3,2-b]furan-6-yl}oxy)ethyl Acetate (16e) A white solid (92%). mp 225—227 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.05 (3H, s), 4.29 (2H, br), 4.37 (2H, br), 6.86 (2H, br), 7.08—7.12 (1H, m), 7.40—7.80 (2H, br), 7.61 (1H, s), 7.73 (1H, d, J=9 Hz), 9.22 (1H, s). *Anal.* Calcd for $C_{16}H_{15}N_3O_6S \cdot 0.3H_2O$: C, 50.21; H, 4.11; N, 10.98. Found: C, 50.10; H, 4.05; N, 10.95.

3-[(Aminocarbonyl)amino]-6-propoxy[1]benzothieno[3,2-*b*]furan-2-carboxamide (16f) A white solid (78%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.00 (3H, t, J=8 Hz), 1.71—1.85 (2H, m), 4.02 (2H, t, J=7 Hz), 6.86 (2H, br), 7.08 (1H, dd, J=9, 2 Hz), 7.40—7.80 (2H, br), 7.56 (1H, d, J=2 Hz), 7.72 (1H, d, J=9 Hz), 9.22 (1H, s). *Anal.* Calcd for $C_{15}H_{15}N_3O_4S \cdot 0.2H_2O$: C, 53.47; H, 4.61; N, 12.47. Found: C, 53.40; H, 4.50; N, 12.51.

3-[(Aminocarbonyl)amino]-6-(2-morpholin-4-ylethoxy)[1]benzothieno[3,2-*b*]furan-2-carboxamide (16g) A pale green solid (53%). mp 299—300 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.47—2.51 (4H, m), 2.73 (2H, t, J=6 Hz), 3.58 (4H, t, J=5 Hz), 4.18 (2H, t, J=6 Hz), 6.86 (2H, br), 7.09 (1H, dd, J=9, 2 Hz), 7.40—7.75 (2H, br), 7.59 (1H, d, J=2 Hz), 7.72 (1H, d, J=9 Hz), 9.22 (1H, s). *Anal.* Calcd for $C_{18}H_{20}N_4O_5S \cdot 0.8H_2O$: C, 51.64; H, 5.53; N, 12.81. Found: C, 51.45; H, 5.26; N, 12.51.

3-[(Aminocarbonyl)amino]-6-[2-(1*H*-pyrrol-1-yl)ethoxy][1]benzothieno[3,2-*b*]furan-2-carboxamide (16h) Crystals (88%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.31 (4H, s), 6.00 (2H, t, J=2 Hz), 6.85 (2H, t, J=2 Hz), 6.86 (2H, s), 7.05—7.09 (1H, m), 7.55 (1H, d, J=2 Hz), 7.40—7.70 (2H, br s), 9.21 (1H, s). *Anal.* Calcd for $C_{18}H_{16}N_4O_4S \cdot 0.75H_2O$: C, 54.33; H, 4.43; N, 14.08. Found: C, 54.23; H, 4.29; N, 14.27.

3-[(Aminocarbonyl)amino]-6-[3-(1*H*-pyrrol-1-yl)propoxy][1]benzothieno[3,2-*b*]furan-2-carboxamide (16i) Crystals (67%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.15—2.19 (2H, m), 3.98 (2H, t, J=6 Hz), 4.07 (2H, t, J=7 Hz), 5.98 (2H, t, J=2 Hz), 6.83 (2H, br s), 7.07—7.11 (1H, m), 7.40—7.74 (4H, m), 9.22 (1H, s). *Anal.* Calcd for $C_{19}H_{18}N_4O_4S \cdot 0.75H_2O$: C, 55.40; H, 4.77; N, 13.60. Found: C, 55.45; H, 4.58; N, 13.79.

3-[(Aminocarbonyl)amino]-6-(3-phenylpropoxy)[1]benzothieno[3,2-*b*]furan-2-carboxamide (16j) A pale brown solid (90%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.01—2.10 (2H, m), 2.77 (2H, t, J=6 Hz), 4.06 (2H, t, J=6 Hz), 6.84 (2H, br), 7.09 (1H, dd, J=9, 3 Hz), 7.16—7.32 (5H, br m), 7.40—7.80 (2H, br), 7.55 (1H, d, J=2 Hz), 7.73 (1H, d, J=9 Hz), 9.22

(1H, s). *Anal.* Calcd for $C_{21}H_{19}N_3O_4S \cdot 0.1H_2O$: C, 61.33; H, 4.71; N, 10.22. Found: C, 61.28; H, 4.62; N, 10.12.

3-[(Aminocarbonyl)amino]-6-(2-aminoethoxy)[1]benzothieno[3,2-*b*]furan-2-carboxamide Hydrochloride (16k) To a stirred solution of **16a** (100 mg) in MeOH (2.0 ml) was added 6 N aq. HCl (1.5 ml), and the mixture was heated to 50 °C for 3 h. The mixture was concentrated *in vacuo*, and the residue was recrystallized from MeOH to afford the title compound (80 mg, 94%) as a white crystal. mp 293—294 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.25—3.27 (2H, m), 4.25 (2H, t, J=5 Hz), 6.86 (2H, br), 7.15 (1H, d, J=9 Hz), 7.50—7.80 (2H, br), 7.64 (1H, s), 7.77 (1H, d, J=9 Hz), 8.20 (3H, br), 9.23 (1H, s). *Anal.* Calcd for $C_{14}H_{15}ClN_4O_4S \cdot 0.5H_2O$: C, 44.27; H, 4.25; N, 14.75. Found: C, 44.02; H, 4.19; N, 14.61.

6-[2-(Acetylamino)ethoxy]-3-[(aminocarbonyl)amino][1]benzothieno[3,2-*b*]furan-2-carboxamide (16l) To a stirred suspension of **16a** (113 mg) in $CHCl_3$ (1.0 ml) was added trifluoroacetic acid (1.0 ml) at rt, and the mixture was stirred for 14 h. The mixture was concentrated *in vacuo*, and the residue was recrystallized from EtOH to afford 3-[(aminocarbonyl)-amino]-6-(2-aminoethoxy)[1]benzothieno[3,2-*b*]furan-2-carboxamide trifluoroacetate (106 mg, 91%) as a pale yellow crystal. mp 275—280 °C (decomposed). ^{1}H-NMR (DMSO-d_6) δ: 3.20—3.30 (2H, m), 4.26 (2H, t, J=5 Hz), 6.88 (2H, br), 7.14 (1H, dd, J=9, 2 Hz), 7.50—7.70 (2H, br), 7.64 (1H, d, J=2 Hz), 7.77 (1H, d, J=9 Hz), 8.02 (3H, br), 9.23 (1H, s). *Anal.* Calcd for $C_{16}H_{15}F_3N_4O_6S \cdot 1.0H_2O$: C, 41.20; H, 3.67; N, 12.01. Found: C, 41.20; H, 3.65; N, 11.95. To a stirred solution of this TFA salt (52 mg, 0.12 mmol) in DMF (1.5 ml) were added acetic anhydride (14.3 μl, 0.151 mmol) and Et_3N (40.4 μl, 0.29 mmol) at rt, and the mixture was stirred at rt for 14 h. The mixture was diluted with H_2O, and the precipitate was collected by filtration, washed with H_2O and EtOH to give the title compound (38 mg, 87%) as a white solid. mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.83 (3H, s), 3.40—3.46 (2H, m), 4.07 (2H, t, J=6 Hz), 6.87 (2H, br), 7.09 (1H, dd, J=9, 1 Hz), 7.45—7.75 (2H, br), 7.59 (1H, s), 7.72 (1H, d, J=9 Hz), 8.10—8.18 (1H, m), 9.22 (1H, s). *Anal.* Calcd for $C_{16}H_{16}N_4O_5S \cdot 0.1H_2O$: C, 50.81; H, 4.32; N, 14.81. Found: C, 50.55; H, 4.36; N, 14.79.

3-[(Aminocarbonyl)amino]-6-(3-aminopropoxy)[1]benzothieno[3,2-*b*]furan-2-carboxamide Hydrochloride (16m) This compound was prepared from **16b** by a similar procedure described in the synthesis of **16k** as a pale brown solid (99%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.04—2.11 (2H, m), 2.94—3.01 (2H, m), 4.17 (2H, t, J=6 Hz), 6.86 (2H, br), 7.11 (1H, dd, J=9, 2 Hz), 7.40—7.80 (2H, br), 7.58 (1H, d, J=2 Hz), 7.74 (1H, d, J=9 Hz), 7.99 (3H, br), 9.22 (1H, s). *Anal.* Calcd for $C_{15}H_{17}ClN_4O_4S \cdot 0.5H_2O$: C, 45.74; H, 4.61; N, 14.23. Found: C, 45.67; H, 4.60; N, 14.18.

({2-(Aminocarbonyl)-3-[(aminocarbonyl)amino][1]benzothieno[3,2-*b*]furan-6-yl}oxy)acetic Acid (16n) To a stirred solution of **16d** (53 mg, 0.14 mmol) in MeOH was added 2 N aq. NaOH (0.5 ml) at rt, and the mixture was stirred at rt for 3 d. The mixture was acidified with 1 N aq. HCl, and the precipitate was collected by filtration, washed with H_2O and EtOH to give the title compound (41 mg, 84%) as a pale brown solid. mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.78 (2H, s), 6.86 (2H, br), 7.08—7.12 (1H, m), 7.40—7.80 (2H, br), 7.55 (1H, s), 7.74 (1H, d, J=9 Hz), 9.22 (1H, s), 13.00 (1H, br). *Anal.* Calcd for $C_{14}H_{11}N_3O_6S \cdot 0.2EtOH \cdot 1.5H_2O$: C, 44.86; H, 3.97; N, 10.90. Found: C, 44.69; H, 3.88; N, 10.74.

3-[(Aminocarbonyl)amino]-6-(2-hydroxyethoxy)[1]benzothieno[3,2-*b*]furan-2-carboxamide (16o) This compound was prepared from **16e** by a similar procedure described in the synthesis of **16n** as a white solid (91%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.75 (2H, q, J=5 Hz), 4.08 (2H, t, J=5 Hz), 4.91 (1H, t, J=5 Hz), 6.85 (2H, br), 7.07—7.10 (1H, m), 7.35—7.80 (2H, br), 7.57 (1H, d, J=2 Hz), 7.72 (1H, d, J=8 Hz), 9.22 (1H, s). *Anal.* Calcd for $C_{14}H_{13}N_3O_5S \cdot 0.2H_2O$: C, 49.61; H, 3.98; N, 12.40. Found: C, 49.52; H, 3.91; N, 12.30.

Ethyl 3-[(*tert*-Butoxycarbonyl)amino]-5-phenyl-2-furoate (18) To a stirred solution of **17**[11] (647 mg) and DMAP (68.0 mg) in THF (12.0 ml) was added a solution of di-*tert*-butyl dicarbonate (1.83 g) in THF (2.0 ml) at 0 °C, and the mixture was stirred at rt for 16 h. The mixture was concentrated *in vacuo*, and the residue was purified by column chromatography on silica gel with EtOAc/hexane (5—25%). The obtained solid was recrystallized from EtOAc/hexane to afford the title compound (704 mg, 76%) as a white crystal. mp 109—110 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, t, J=7 Hz), 1.45 (9H, s), 4.35 (2H, q, J=7 Hz), 6.64 (1H, s), 7.33—7.45 (5H, m), 7.76—7.79 (2H, m). *Anal.* Calcd for $C_{18}H_{21}NO_5 \cdot 0.5EtOAc$: C, 63.99; H, 6.71; N, 3.73. Found: C, 63.92; H, 6.69; N, 3.19.

***tert*-Butyl [2-(Aminocarbonyl)-5-phenyl-3-furyl]carbamate (19)** To a stirred solution of **18** (746 mg) in EtOH (6.0 ml) was added 8 N aq. NaOH (1.0 ml) at rt, and the mixture was stirred for 3 h. The mixture was concentrated *in vacuo*, and the residue was acidified with 1 N aq. $KHSO_4$ (10 ml). The mixture was extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to give 3-[(*tert*-butoxycarbonyl)amino]-5-phenyl-2-furoic acid (590 mg). This crude acid (590 mg) was dissolved in CH_3CN/DMF (8.0/2.0 ml), and then cooled to 0 °C. To this mixture were added WSC (431 mg) and HOBt·NH_3 (342 mg), and the mixture was stirred at rt for 1 d. The mixture was diluted with H_2O and extracted with EtOAc. The organic phase was washed with 1 N aq. $KHSO_4$, saturated aq. $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated *in vacuo.* The residue was purified by column chromatography on silica gel with EtOAc/hexane (5—55%) and recrystallized from EtOAc/hexane to afford the title compound (530 mg, 78% from **18**) as a white crystal. mp 179—181 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.53 (9H, s), 7.33—7.44 (3H, m), 7.51 (1H, br), 7.69—7.73 (2H, m), 8.66 (1H, br). *Anal.* Calcd for $C_{16}H_{18}N_2O_4$: C, 63.56; H, 6.00; N, 9.27. Found: C, 63.70; H, 6.11; N, 9.07.

3-Amino-5-phenyl-2-furamide (20) To a stirred solution of **19** (111 mg) in $CHCl_3$ (1.5 ml) was added TFA (1.0 ml) at room temperature, and the mixture was stirred for 4 h. The mixture was concentrated *in vacuo*, and the residue was purified by column chromatography on NH_2-coated silica gel with EtOAc/hexane (10—100%), and recrystallized from EtOAc/hexane to afford the title compound (55 mg, 74%) as a pale yellow crystal. mp 159—160 °C. ^{1}H-NMR ($CDCl_3$) δ: 4.71 (2H, br s), 5.52 (2H, br), 6.34 (1H, s), 7.31—7.44 (3H, m), 7.63—7.68 (2H, m). *Anal.* Calcd for $C_{11}H_{10}N_2O_2$: C, 65.34; H, 4.98; N, 13.85. Found: C, 65.24; H, 5.04; N, 13.76.

3-[(Aminocarbonyl)amino]-5-phenyl-2-furamide (21) To a stirred solution of **20** (49 mg) in THF (1.5 ml) was added trichloroacetyl isocyanate (27.4 μl) at −70 °C, and the mixture was allowed to warm up to 0 °C over 3 h. To this mixture was added 2 N NH_3 in MeOH (1.0 ml), and the mixture was stirred at rt for 16 h. The mixture was concentrated *in vacuo*, and the residue was diluted with H_2O. The precipitate was collected by filtration, and washed with H_2O. The solid was recrystallized from EtOAc/hexane to afford the title compound (32 mg, 54%) as a pale brown crystal. mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.63 (2H, br), 7.34—7.48 (4H, m), 7.60 (1H, s), 7.75 (1H, br), 7.91—7.93 (2H, m), 8.78 (1H, s). *Anal.* Calcd for $C_{12}H_{11}N_3O_3 \cdot 0.2EtOAc \cdot 0.2H_2O$: C, 58.09; H, 4.87; N, 15.88. Found: C, 58.11; H, 4.67; N, 16.11.

Ethyl [(2-Cyano-3,4-dihydronaphthalen-1-yl)oxy]acetate (23a) A solution of Ph_3P (3.93 g) in THF (30.0 ml) was cooled to 0 °C, and then diethyl azodicarboxylate (6.53 ml, 40% solution in toluene) and ethyl glycolate (1.42 ml) were added. To this mixture was added dropwise a solution of **22a**[16] (1.71 g) in THF (1.5 ml) at 0 °C, and the mixture was stirred at rt for 8 h. The mixture was concentrated *in vacuo*, and the residue was purified by column chromatography on silica gel with EtOAc/hexane (10—25%) and recrystallized from EtOAc/hexane to afford the title compound (2.06 g, 80%) as a white crystal. mp 50—51 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, t, J=7 Hz), 2.54—2.60 (2H, m), 2.84 (2H, t, J=8 Hz), 4.29 (2H, q, J=7 Hz), 5.00 (2H, s), 7.16 (1H, d, J=7 Hz), 7.25—7.34 (2H, m), 7.72—7.75 (1H, m). *Anal.* Calcd for $C_{15}H_{15}NO_3$: C, 70.02; H, 5.88; N, 5.44. Found: C, 70.11; H, 5.88; N, 5.42.

Ethyl [(8-Cyano-6,7-dihydro-5*H*-benzo[7]annulen-9-yl)oxy]acetate (23b) This compound was prepared from **22b**[17] as described in the synthesis of **23a** as a yellow oil (79%). ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7 Hz), 2.04 (2H, t, J=7 Hz), 2.17—2.26 (2H, m), 2.70 (2H, t, J=8 Hz), 4.21 (2H, q, J=7 Hz), 4.57 (2H, s), 7.26—7.42 (4H, m).

2-[(2-Cyano-3,4-dihydronaphthalen-1-yl)oxy]acetamide (24a) To a stirred solution of **23a** (2.90 g) in EtOH (38.0 ml) was added 28% aq. NH_3 (14.0 ml) at 0 °C, and the mixture was stirred at rt for 16 h. The mixture was concentrated *in vacuo*, and the residue was purified by column chromatography on silica gel with EtOAc/hexane (25—50%) and on NH_2-coated silica gel with EtOAc/hexane (10—90%). The obtained solid was recrystallized from EtOAc/hexane to afford the title compound (1.48 g, 57%) as a white crystal. mp 139—141 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.54—2.60 (2H, m), 2.87 (2H, t, J=9 Hz), 4.63 (2H, s), 5.74 (1H, br), 6.83 (2H, br), 7.21—7.43 (4H, m). *Anal.* Calcd for $C_{13}H_{12}N_2O_2$: C, 68.41; H, 5.30; N, 12.27. Found: C, 68.36; H, 5.30; N, 12.23.

2-[(8-Cyano-6,7-dihydro-5*H*-benzo[7]annulen-9-yl)oxy]acetamide (24b) This compound was prepared from **23b** by a similar procedure described in the synthesis of **24a** as a white solid (74%). mp 108—109 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.06 (2H, t, J=7 Hz), 2.18—2.28 (2H, m), 2.68 (2H, t, J=8 Hz), 4.30 (2H, s), 5.69 (1H, br), 6.70 (1H, br), 7.29—7.44 (4H, m). *Anal.* Calcd for $C_{14}H_{14}N_2O_2$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.17; H, 5.76; N, 11.55.

3-Amino-4,5-dihydronaphtho[1,2-*b*]furan-2-carboxamide (25a) This

compound was prepared from **24a** according to the typical procedure F. A pale yellow solid (85%). mp 133—134 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.64 (2H, t, J=8 Hz), 3.01 (2H, t, J=8 Hz), 4.65 (2H, br s), 5.53 (2H, br), 7.20—7.28 (3H, m), 7.51 (1H, d, J=7 Hz). *Anal.* Calcd for $C_{13}H_{12}N_2O_2$: C, 68.41; H, 5.30; N, 12.27. Found: C, 68.62; H, 5.29; N, 12.38.

3-Amino-5,6-dihydro-4H-benzo[6,7]cyclohepta[1,2-*b*]furan-2-carboxamide (25b) This compound was prepared from **24b** according to the typical procedure F. A white solid (83%). mp 157—159 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.01—2.09 (2H, m), 2.59 (2H, t, J=7 Hz), 2.88—2.91 (2H, m), 4.56 (2H, br s), 5.54 (2H, br), 7.13—7.29 (3H, m), 7.83 (1H, dd, J=8, 2 Hz). *Anal.* Calcd for $C_{14}H_{14}N_2O_2\cdot 0.3H_2O$: C, 67.89; H, 5.94; N, 11.31. Found: C, 68.06; H, 5.81; N, 11.07.

3-[(Aminocarbonyl)amino]-4,5-dihydronaphtho[1,2-*b*]furan-2-carboxamide (26a) This compound was prepared from **25a** according to the typical procedure G (method B). A white solid (52%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.79—2.89 (4H, m), 6.46 (2H, br s), 7.20—7.31 (3H, m), 7.39 (1H, br), 7.62—7.66 (2H, m), 8.52 (1H, s). *Anal.* Calcd for $C_{14}H_{13}N_3O_3\cdot 0.3H_2O$: C, 60.78; H, 4.95; N, 15.19. Found: C, 60.97; H, 4.85; N, 14.94.

3-[(Aminocarbonyl)amino]-5,6-dihydro-4*H*-benzo[6,7]cyclohepta[1,2-*b*]furan-2-carboxamide (26b) This compound was prepared from **25b** according to the typical procedure G (method B). A white solid (79%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.84—1.88 (2H, m), 2.71 (2H, t, J=6 Hz), 2.86—2.90 (2H, m), 6.31 (2H, br s), 7.20—7.32 (3H, m), 7.44 (1H, br), 7.84 (1H, br), 8.03 (1H, s), 8.16 (1H, d, J=7 Hz). *Anal.* Calcd for $C_{15}H_{15}N_3O_3\cdot 0.2H_2O$: C, 62.36; H, 5.37; N, 14.54. Found: C, 62.35; H, 5.35; N, 14.41.

1-Hydroxy-2-naphthamide (28) A mixture of **27** (1.0 g) and WSC (1.22 g), and HOBt·NH_3 (0.97 g) in DMF (30.0 ml) was stirred at 80 °C for 1 h. The reaction mixture was poured into 1 N aq. HCl and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with EtOAc/hexane (2/1) to afford the title compound (720 mg, 73%) as crystals. mp 181—182 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.00 (2H, br s), 7.28—7.32 (2H, m), 7.50—7.62 (2H, m), 7.65 (1H, d, J=7 Hz), 8.41—8.44 (1H, m).

1-(Cyanomethoxy)-2-naphthamide (29) A mixture of **28** (3.0 g), bromoacetonitrile (3.71 g) and K_2CO_3 (4.43 g) in THF (50 ml) was refluxed for 8 h. The reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with EtOAc to afford the title compound (1.74 g, 48%) as crystals. mp 151—152 °C. ^{1}H-NMR ($CDCl_3$) δ: 4.90 (2H, s), 5.99 (2H, s), 7.00 (1H, m), 7.62—7.69 (2H, m), 7.79 (1H, d, J=8 Hz), 7.90—7.98 (2H, m), 8.18—8.21 (1H, m).

1-(Cyanomethoxy)-2-naphthonitrile (30) To a stirred solution of **29** (1.65 g) in DMF (10 ml) was added $SOCl_2$ (1.5 ml) at 0 °C. After stirring at 60 °C for 30 min, the reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with EtOAc/hexane (33%) to afford the title compound (820 mg, 54%) as crystals. mp 120—121 °C. ^{1}H-NMR ($CDCl_3$) δ: 5.19 (2H, s), 7.55 (1H, d, J=8 Hz), 7.69—7.78 (3H, m), 7.90—7.93 (1H, m), 8.26—8.30 (1H, m).

3-Aminonaphtho[1,2-*b*]furan-2-carboxamide (31) A mixture of **30** (0.79 g) and sodium ethoxide (0.39 g) in EtOH (20 ml) was refluxed 3 h, and the solvent was evaporated off. The residue was diluted with EtOAc/THF, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* to give crystals. This crude crystals were purified by column chromatography on silica gel with EtOAc/hexane (20%) to afford the title compound (0.54 g, 63%) as crystals. mp 205—207 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.06 (2H, s), 7.33 (2H, s), 7.59—7.72 (3H, m), 7.89 (1H, d, J=9 Hz), 8.03 (1H, d, J=8 Hz), 8.36 (1H, d, J=8 Hz).

3-[(Aminocarbonyl)amino]naphtho[1,2-*b*]furan-2-carboxamide (32) A mixture of **31** (0.20 g) and potassium cyanate (0.22 g) in AcOH (10 ml) was stirred at 100 °C for 15 min, and the solvent was evaporated off. The residue was diluted with THF, washed with aq. $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with EtOAc to afford the title compound (18 mg, 8%) as crystals. mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.70 (2H, s), 7.62—7.71 (4H, m), 8.01—8.21 (3H, m), 8.45 (1H, d, J=7 Hz), 9.01 (1H, s). *Anal.* Calcd for $C_{14}H_{11}N_3O_3\cdot 0.3H_2O$: C, 61.22; H, 4.26; N, 15.30. Found: C, 61.15; H, 4.33; N, 14.92.

Ethyl 2-[(Cyanomethyl)thio]nicotinate (35) To a stirred solution of **33** (3.72 g) in EtOH (40.0 ml) was added NaSH (3.36 g) at rt, and the mixture was heated to reflux for 16 h. NaSH (2.24 g) was added to this mixture, because TLC showed the **33** was remained. After further refluxing for 1 d, the mixture was acidified with 1 N aq. $KHSO_4$ (100 ml) at 0 °C. The mixture was extracted with EtOAc. The extract was washed with brine (50.0 ml), dried over Na_2SO_4, and concentrated *in vacuo* to give **34** (3.86 g) as a yellow oil. This was dissolved in DMF/THF (20.0/20.0 ml), and chloroacetonitrile (1.52 ml) and NaI (3.60 g) were added to this solution. The mixture was stirred at 50 °C for 14 h, and then partitioned between H_2O and EtOAc. The organic phase was washed with saturated brine (30.0 ml), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with EtOAc/hexane (5—50%) and recrystallized from EtOAc/hexane to afford the title compound (2.35 g, 53% from **33**) as a white crystal. mp 72—73 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (3H, t, J=7 Hz), 3.93 (2H, s), 4.42 (2H, q, J=7 Hz), 7.19 (1H, dd, J=8, 5 Hz), 8.30 (1H, dd, J=8, 2 Hz), 8.66 (1H, dd, J=5, 2 Hz). *Anal.* Calcd for $C_{10}H_{10}N_2O_2$: C, 54.04; H, 4.53; N, 12.60. Found: C, 54.18; H, 4.46; N, 12.76.

3-Hydroxythieno[2,3-*b*]pyridine-2-carbonitrile (36) This compound was prepared from **35** accordingyo to the typical procedure D. A white solid (96%). mp 196—198 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.54—7.59 (1H, m), 8.43 (1H, dd, J=8, 1 Hz), 8.75—7.78 (1H, m), 12.68 (1H, br s). *Anal.* Calcd for $C_8H_4N_2OS$: C, 54.53; H, 2.29; N, 15.90. Found: C, 54.71; H, 2.47; N, 15.83.

2-[(2-Cyanothieno[2,3-b]pyridin-3-yl)oxy]acetamide (37) This compound was prepared from **36** according to the typical procedure E. A white solid (78%). mp 249—252 °C. ^{1}H-NMR (DMSO-d_6) δ: 5.06 (2H, s), 7.59—7.63 (2H, m), 7.78 (1H, br s), 8.53—8.56 (1H, m), 8.81—8.82(1H, m). *Anal.* Calcd for $C_{10}H_7N_3O_2S$: C, 51.49; H, 3.02; N, 18.02. Found: C, 51.29; H, 3.03; N, 17.90.

3-Aminofuro[2′,3′:4,5]thieno[2,3-*b*]pyridine-2-carboxamide (38) This compound was prepared from **37** according to the typical procedure F. A brown solid (15%). mp 265 °C (decomposed). ^{1}H-NMR (DMSO-d_6) δ: 6.16 (2H, br s), 7.25 (2H, br s), 7.55 (1H, dd, J=8, 5 Hz), 8.24 (1H, dd, J=8, 2 Hz), 8.59 (1H, dd, J=5, 2 Hz). *Anal.* Calcd for $C_{10}H_7N_3O_2S$: C, 51.49; H, 3.02; N, 18.02. Found: C, 51.53; H, 3.18; N, 17.90.

3-[(Aminocarbonyl)amino]furo[2′,3′:4,5]thieno[2,3-*b*]pyridine-2-carboxamide (39) To a stirred solution of **38** (26.0 mg) in THF/DMF (3.0/1.0 ml) was added dropwise trichloroacetyl isocyanate (19.0 μl) at 0 °C, and the mixture was allowed to warm up to rt over 3 h. After the mixture was cooled to 0 °C, trichloroacetyl isocyanate (10.0 μl) was added, and then the mixture was stirred at rt for 2 h. To this mixture was added 2 N NH_3–MeOH (2 ml), and the mixture was stirred at rt for 2 h. The mixture was diluted with H_2O, and extracted with THF/EtOAc. The extract was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was recrystallized from THF/EtOH to afford the title compound (5.0 mg, 16%) as a pale brown solid. mp 262—264 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.91 (2H, br s), 7.52 (1H, dd, J=8, 5 Hz), 7.62 (1H, br s), 7.87 (1H, br s), 8.21 (1H, d, J=8 Hz), 8.59 (1H, d, J=5 Hz), 9.26 (1H, s). *Anal.* Calcd for $C_{11}H_8N_4O_3S\cdot 0.25H_2O$: C, 47.06; H, 3.05; N, 19.95. Found: C, 47.24; H, 3.30; N, 19.70.

Preparation of Recombinant IKKβ cDNA encoding human IKKβ was isolated by PCR with primers containing sequences encoding a FLAG-tag in the carboxy-terminal region and subcloned into an insect cell expression vector, pFASTBAC1 (Invitrogen, U.S.A.). Sf21 cells were infected with IKKβ recombinant baculovirus and cultured at 28 °C for 72 h. Cells were lysed and FLAG-tagged IKKβ protein was purified by affinity chromatography using anti-FLAG M2 affinity gel (Sigma, U.S.A.).

***In Vitro* Phosphorylation Assay** Kinase reaction of purified human IKKβ was performed at room temperature for 1 h in kinase reaction buffer (25 mmol/l HEPES, pH 7.5, 10 mmol/l magnesium acetate, 1 mM dithiothreitol, 0.01% bovine serum albumin, 0.01% Tween20) containing 500 nM ATP, and bacterially expressed GST-IκBα (1—54), and then terminated by adding the same volume of Kinase-Glo™ reagent (Promega, U.S.A.). After incubating at rt for 10 min the luminescent signal correlated with the amount of ATP remaining in solution following the reaction was measured by Wallac Arvo HTS multilabel counter (Perkin Elmer, U.S.A.).

Quantitation of TNF-α Human monocytic THP-1 cells were suspended in RPMI1640 medium (GIBCO, U.S.A.) containing 1% fetal bovine serum and 50 μg/ml gentamicin. Aliquots of 1×10^5 cells were seeded in 96 well plates (Corning Coster, U.S.A.), and then incubated with test compounds for 1 h, subsequently stimulated with 5 μg/ml Lipopolysaccharide (Wako, Japan) for 4 h. After centrifugation, the quantity of TNF-α in the medium was measured by a human TNF-α ELISA kit (Diaclone, France).

X-Ray Crystallography JNK3 was purified following the published protocol.[23] Crystals of the JNK3:AMPPNP complex were obtained by vapor diffusion in hanging drops with micro-seeding method. The complex was

equilibrated at 20 °C against a reservoir containing 20% Pentaerythritol ethoxylate (15/4 EO/OH), 0.1 M ammonium sulfate in 0.1 M Bis-Tris, pH 6.5. Crystals of the **16k** compolex were obtained by soaking the AMPPNP-containing crystals overnight in 1 mM compound in mother liquor. They belong to the orthorhombic space group $P2_12_12_1$, with unit cell parameters a=49.2 Å, b=71.5 Å, c=107.4 Å and one molecule per asymmetric unit. Diffraction data set was collected on single crystals, vitrified at 100 K, using synchrotron radiation at beamline BL32B2 in the facilities of Pharmaceutical Consortium at SPring-8. The structure was initially solved by molecular replacement with CNX (Accelrys, U.S.A.) using the coordinates of the JNK3:AMPPNP complex (PDB code: 1JNK) as a starting model. The structure was further refined and rebuilt to the crystallographic R-factor/free-R of 0.278/0.328, with rmsd bond length/angle of 0.008 Å/1.3°, at 2.0 Å resolution using CNX and QUANTA (Accelrys, U.S.A.).

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References

1) Mercurio F., Zhu H., Murray B. W., Shevchenko A., Bennett B. L., Jian W. L., Young D. B., Barbosa M., Mann M., Manning A., Rao A., *Science*, **278**, 860—866 (1997).
2) Woronicz J. D., Gao X., Cao Z., Rothe M., Goeddel D. V., *Science*, **278**, 866—869 (1997).
3) Zandi E., Rothwarf D. M., Delhase M., Hayakawa M., Karin M., *Cell*, **91**, 243—252 (1997).
4) Hayden M. S., Ghosh S., *Genes Dev.*, **18**, 2195—2224 (2004).
5) Blake S. M., Swift B. A., *Curr. Opin. Pharmacol.*, **4**, 276—280 (2004).
6) Yuan M., Konstantopoulos N., Lee J., Hansen L., Li Z. W., Karin M., Shoelson S. E., *Science*, **293**, 1673—1677 (2001).
7) Caramori G., Adcock I. M., Ito K., *Curr. Opin. Investig. Drugs*, **5**, 1141—1147 (2004).
8) Viatour P., Merville M. P., Bours V., Chariot A., *Trends Biochem. Sci.*, **30**, 43—52 (2005).
9) Karin M., Yamamoto Y., Wang Q. M., *Nat. Rev. Drug Discov.*, **3**, 17—26 (2004).
10) Coish P. D. G., Wickens P. L., Lowinger T. B., *Expert Opin. Ther. Patens*, **16**, 1—12 (2006).
11) Baxter A., Brough S., Cooper A., Floettmann E., Foster S., Harding C., Kettle J., McInally T., Martin C., Mobbs M., Needham M., Newham P., Paine S., St-Gallay S., Salter S., Unitt J., Xue Y., *Bioorg. Med. Chem. Lett.*, **14**, 2817—2822 (2004).
12) Kajino M., Kawada A., Nakayama Y., Kimura H., PCT Int. Appl. WO03020719 (2003).
13) Newman M. S., Karnes H. A., *J. Org. Chem.*, **31**, 3980—3984 (1966).
14) Katz L., Karger L. S., Schroeder W., Cohen M. S., *J. Org. Chem.*, **18**, 1380—1401 (1953).
15) Redman A. M., Dumas J., Scott W. J., *Org. Lett.*, **2**, 2061—2063 (2000).
16) Johnson W. S., Shelberg W. E., *J. Am. Chem. Soc.*, **67**, 1745—1754 (1945).
17) Hirota T., Ieno K., Sasaki K., *J. Heterocyclic Chem.*, **23**, 1685—1687 (1986).
18) McGovern S. L., Caselli E., Grigorieff N., Shoichet B. K., *J. Med. Chem.*, **45**, 1712—1722 (2002).
19) Bower M. J., Cohen F. E., Dunbrack R. L., Jr., *J. Mol. Bio.*, **267**, 1268—1282 (1997).
20) Dunbrack R. L., Jr., *Proteins*, **3**, 81—87 (1999).
21) Zheng J., Trafny E. A., Knighton D. R., Xuong N. H., Taylor S. S., Eyck L. F. T., Sowadski J. M., *Acta Cryst.*, D**49**, 362—365 (1993).
22) Nagao Y., Hirata T., Goto S., Sano S., Kakehi A., Iizuka K., Shir M., *J. Am. Chem. Soc.*, **120**, 3104—3110 (1998).
23) Xie X., Gu Y., Fox T., Coll J. T., Fleming M. A., Markland W., Caron P. R., Wilson K. P., Su M. S., *Structure*, **6**, 983—991 (1998).