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Discovery and structure–activity relationship of thienopyridine derivatives as bone anabolic agents

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ABSTRACT

A cell-based assay was performed for the discovery of novel bone anabolic agents. Alkaline phosphatase (ALPase) activity of ST2 cells was utilized as an indicator of osteoblastic differentiation, and thienopyridine derivative **1** was identified as a hit compound. 3-Aminothieno[2,3-*b*]pyridine-2-carboxamide was confirmed to be a necessary core structure for the enhancement of ALPase activity, and then optimization of the C4-substituent on the thienopyridine ring was carried out. Introduction of cyclic amino groups to the C4-position of the thienopyridine ring improved the activity. Especially, *N*-phenyl-homopiperazine derivatives were found to be strong enhancers of ALPase among this new series. Furthermore, 3-amino-4-(4-phenyl-1,4-diazepan-1-yl)thieno[2,3-*b*]pyridine-2-carboxamide (**15k**) was orally administered to ovariectomized (OVX) rats over 6 weeks for evaluating the effects on areal bone mineral density (aBMD), and statistically significant improvements in aBMD were observed from the dosage of 10 mg/kg/day.

1. Introduction

Osteoporosis is a skeletal disorder characterized by low bone mass and deterioration of bone microarchitecture.[1] Under these pathological conditions, bones are more fragile and susceptible to fractures compared to healthy conditions.[2] It is estimated that over 200 million people worldwide have osteoporosis, and the number is growing with the increasing life expectancy.[3]

Bone is a dynamically remodeling tissue balanced by the coordinated actions of bone resorbing cells (osteoclasts) and bone forming cells (osteoblasts). In osteoporosis bone resorption exceeds bone formation resulting in bone loss. Agents for the treatment of osteoporosis are classified as either antiresorptive or anabolic.[4] Current therapeutic agents are mainly antiresorptive agents[5] such as bisphosphonates, calcitonins, estrogens, selective estrogen receptor modulators and the RANKL monoclonal antibody. On the other hand, recombinant human PTH is the only approved anabolic agent. PTH therapy has demonstrated that anabolic agents could increase bone mass to a greater degree than antiresorptive agents and also reduce fracture incidence.[6] However, the administration period of PTH is limited due to safety concerns.[6] Under these circumstances, the calcium-sensing receptor, the Wnt signaling pathway and β-adrenergic receptor have gained attention as molecular targets for developing bone anabolic agents.[7] However, mechanisms contributing to bone formation are complex and not yet completely understood. Therefore, we utilized a cell-based screening strategy instead of a target-based strategy. We believe that a cell-based screening, which detects differentiation of stromal cells to osteoblast cells, may provide compounds with a novel mechanism of action, and those compounds would lead to drug candidates.

For the screening, the stromal cell line ST2, derived from mouse bone marrow, was utilized, and alkaline phosphatase (ALPase) activity was detected as an indicator of differentiation to osteoblasts.[8] By a high-throughput screening of our corporate library, we identified 3-amino-4-(dimethylamino)thieno[2,3-*b*]pyridine-2-carboxamide (**1**)[9] as a hit compound with an EC_{200} = 0.17 μg/mL, a concentration to enhance ALPase activity to 200% of control (Fig. 1). Furthermore, hit compound **1** was evaluated with regard to calcification in rat bone marrow cells,[10] and found to induce the formation of mineralized nodules. These results indicated that

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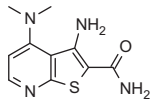


1

Figure 1. Hit compound **1**.

compound **1** was a promising bone anabolic candidate and encouraged us to initiate chemical modification for potentiating the activity.

In this paper, we report the synthesis of thienopyridine derivatives and structure–activity relationships (SARs) with regard to enhancement of ALPase activity. In addition, the results of selected compounds **1** and **15k** evaluated in an in vivo animal osteoporosis model are described.

2. Chemistry

The synthetic routes for the compounds in Tables 1 and 2 are outlined in Schemes 1–3.

Scheme 1 describes the preparation of *N*-aryl-homopiperazine derivatives, which are employed as the C4-substituent of the thienopyridine ring. Appropriate halobenzenes **2** were coupled with *N*-protected homopiperazines **3** using transition metal catalysts,[11] or by nucleophilic aromatic substitution, and followed by cleavage of the protecting groups to give *N*-aryl-homopiperazines **5**. Benzamides **10a** and **10b** were prepared from 2,2,2,4′-tetrafluoroacetophenone (**6**).[12] Compound **6** was reacted with benzyl 1-homopiperazinecarboxylate to afford **7**. Compound **7** was treated with aqueous NaOH to give carboxylic acid **8**, and subsequent condensation with suitable amines, followed by deprotecting the benzyloxycarbonyl group furnished homopiperazines **10a** and **10b** in good yields.

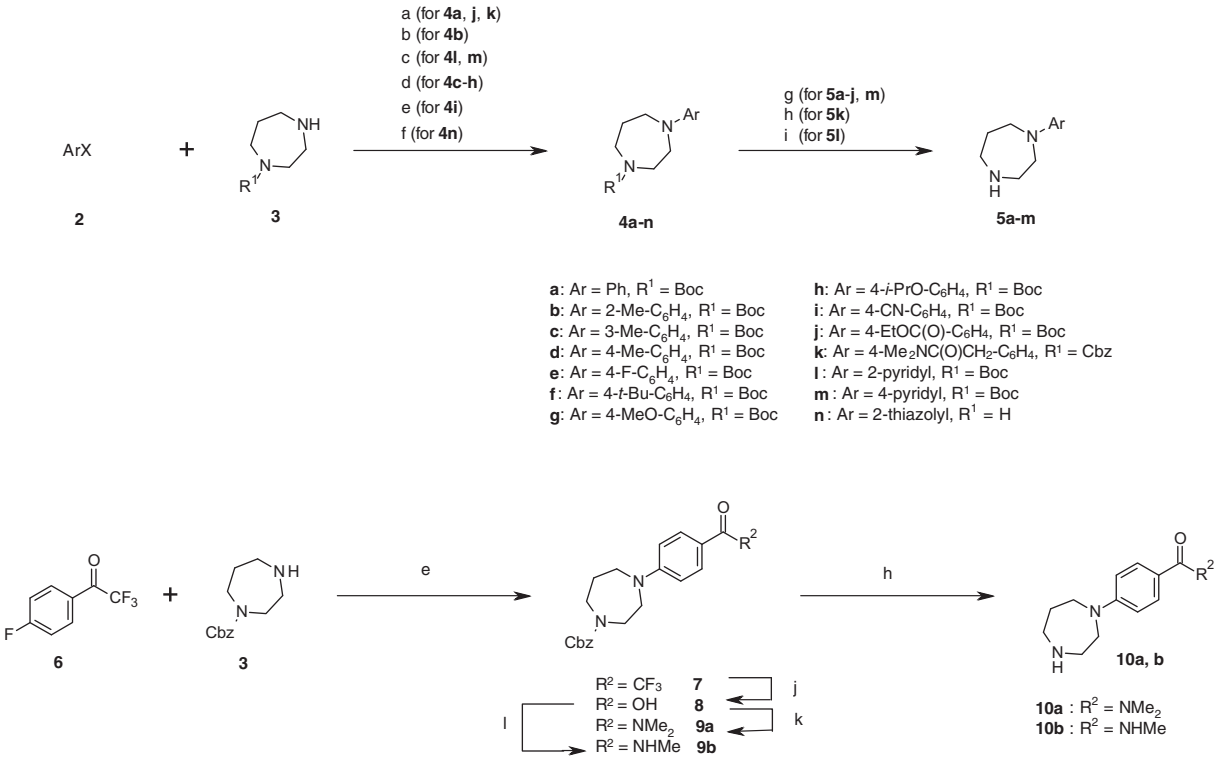
The synthesis of thienopyridine derivatives is shown in Scheme 2. Compound **11**[13] was treated with suitable amines to give enaminothioamides **12**. Condensation of **12** with *N*,*N*-dimethylformamide dimethyl acetal (DMF–DMA), and subsequent refluxing in aqueous NaOH afforded thiopyridones **14** via the intermediates **13**.[9] Cyclization to the thiopyridone ring was also performed in DMF or DMA under heating conditions without a base. Eventually, the resulting thiopyridones **14** were reacted with 2-chloroacetamide in the presence of bases to furnish thienopyridines **1** and **15**. Since the major product was 4-(dimethylamino)thiopyridone **14a** when 3-morpholinobutenethioamide **12g** was reacted with DMF–DMA, 4-(dimethoxymethyl)morpholine was used instead of DMF–DMA in the preparation of morpholine derivative **15g**.

The preparation of compounds **15aa**, **15ab** and **15ac** is shown in Scheme 3. Deprotection of the *tert*-butoxycarbonyl group of **15h** provided **15aa**. Saponification of ester **15t** furnished carboxylic acid **15ab**. Carboxamide **15ac** was prepared by the hydration of nitrile **15s**.

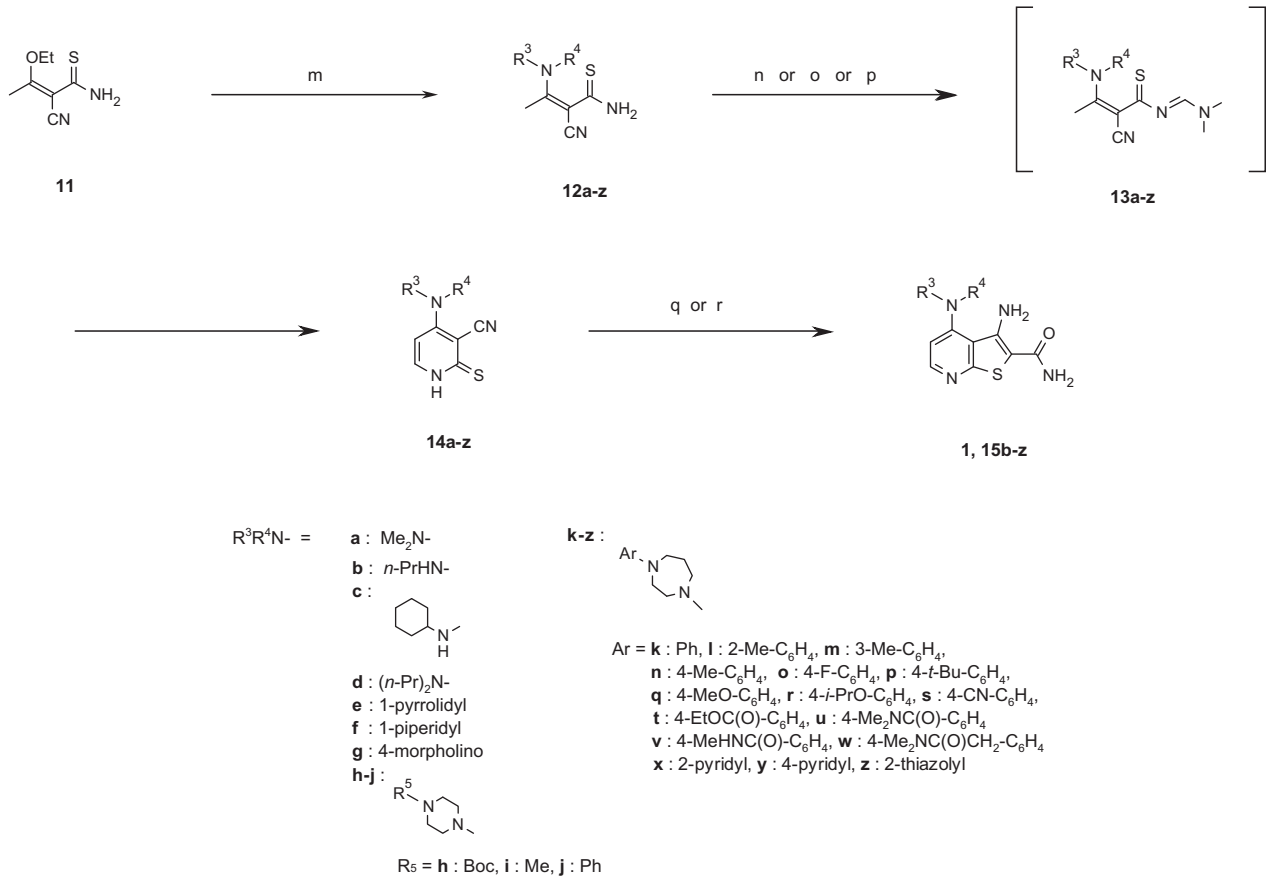
3. Results and discussion

The effects of the synthesized compounds on cellular ALPase activity, an indicator of differentiation to osteoblasts, were evaluated using the stromal cell line ST2, derived from mouse bone marrow. The results are shown in Tables 1 and 2, and the potency is expressed as EC_{200}, concentrations to enhance ALPase activity to 200% of control.

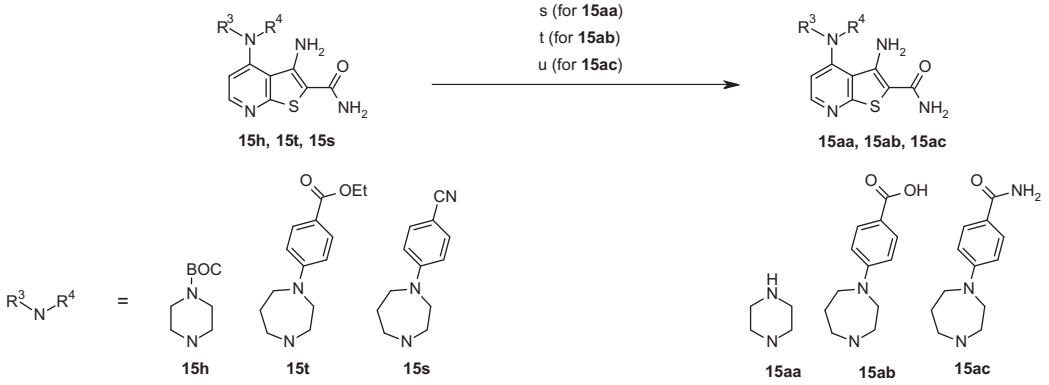
Initial chemical efforts were focused on the modification of the thienopyridine core of hit compound **1**. Replacement of the ring



Scheme 1. Reagents and conditions: (a) $Pd_2(dba)_3$, 2-dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl, NaO*t*-Bu, *t*-BuOH, 1,4-dioxane, 100 °C; (b) $Pd(OAc)_2$, 2,8,9-triisobutyl-2,5,8,9-tetraaza-1-phosphabicyclo[3.3.3]undecane, NaO*t*-Bu, toluene, 80 °C; (c) $Pd_2(dba)_3$, 1,3-bis-(2,6-diisopropylphenyl)-imidazolium chloride, KO*t*-Bu, 1,4-dioxane, 90 °C; (d) CuI, K_2CO_3, ethylene glycol, *i*-PrOH, 80 °C; (e) Et_3N, DMSO, 100 °C; (f) *n*-BuOH, reflux; (g) HCl, MeOH, 1,4-dioxane, rt; (h) H_2, Pd/C, EtOH, rt; (i) TFA, CH_2Cl_2, rt; (j) aq NaOH, DMF, 80 °C, 93%; (k) CDI, Me_2NH, THF, rt, 100%; (l) CDI, $MeNH_2$, THF, rt, 100%.



Scheme 2. Reagents and conditions: (m) R^3R^4NH, EtOH, rt; (n) *N*,*N*-dimethylformamide dimethyl acetal, toluene, reflux, then aq NaOH, reflux; (o) *N*,*N*-dimethylformamide dimethylacetal, DMF or EtOH, rt, then DMF or DMA, Δ; (p) 4-(dimethoxymethyl)morpholine, toluene, reflux, then aq NaOH, reflux; (q) 2-chloroacetamide, aq NaOH, DMF, rt; (r) 2-chloroacetamide, NaOEt, DMF, rt.



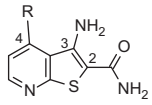
Scheme 3. Reagents and conditions: (s) HCl, MeOH, 1,4-dioxane, rt, 100%; (t) aq NaOH, EtOH, reflux, 79%; (u) 30% H_2O_2, K_2CO_3, DMSO, rt, 71%.

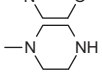
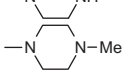
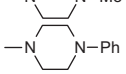
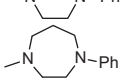
nitrogen by a carbon atom leading to benzothiophene reduced potency substantially. The same reduction in potency was found for thieno[3,2-*c*]pyridine, a structural isomer of thieno[2,3-*b*]pyridine, with a different location of the ring nitrogen atom. Conversion of the thienopyridine ring to a thienopyrimidine ring also resulted in a complete loss of activity (data not shown). These results suggest that the ring nitrogen atom plays a pivotal role for its activity.

We then investigated the effects of substituents on the thienopyridine ring. Replacement of the carboxamide group at the C2-position and the amino group at the C3-position with the other substituents, such as hydrogen, methyl, cyano, ester and carboxylic acid, resulted in significantly less active or even inactive compounds (data not shown). On the other hand, manipulation of the dimethyl amino group at the C4-position was found to be flexible. Hence, this region was examined extensively.

The results are summarized in Table 1. We first introduced primary amines to the C4-position. Although *n*-propyl-amino analog **15b** was less active, balkier cyclohexyl-amino analog **15c** showed moderate activity with an EC_{200} value of 0.24 μg/mL. However, compound **15c** was still less potent than **1**. We then incorporated secondary amino groups. The activity of *N*,*N*-dipropyl-amino analog **15d** was dramatically reduced from the *N*,*N*-dimethyl-amino analog **1**. However, cyclic amino groups were found to be effective. Especially, piperidine analog **15f** was threefold more potent than **1**.

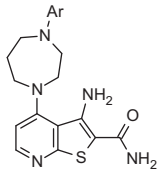
Table 1
Effects of 4-aminothienopyridine derivatives on ALP activity in ST2 cells[a]



Compound	–R	EC_{200}[b] (μg/mL)	Metabolic stability[c] (%)
1	$-NMe_2$	0.17	48
15b		134%[d]	–
15c		0.24	–
15d		110%[d]	–
15e		0.29	–
15f		0.067	2
15g		0.44	82
15aa		105%[d]	–
15i		117%[d]	–
15j		0.10	91
15k		0.051	71

[a] Values are the arithmetic means of at least two experiments. Standard deviation of assay is less than ±10%.
[b] Concentration to enhance ALP activity in ST2 cell to 200% of control.
[c] Stability is described as % remaining after 30 min incubation with the rat liver microsome.
[d] % Enhancement at 0.30 μg/mL.

Table 2
Effects of *N*-aryl-homopiperazine derivatives on ALP activity in ST2 cells[a]



Compound	Ar	EC_{200}[b] (μg/mL)
15k	Ph	0.051
15l	2-Me-C_6H_4	>1.0
15m	3-Me-C_6H_4	0.074
15n	4-Me-C_6H_4	0.044
15o	4-F-C_6H_4	0.082
15p	4-*t*-Bu-C_6H_4	0.27
15q	4-MeO-C_6H_4	0.035
15r	4-*i*-PrO-C_6H_4	>1.0
15s	4-CN-C_6H_4	0.099
15t	4-EtOC(O)-C_6H_4	0.193
15ab	4-HOC(O)-C_6H_4	>1.0
15u	4-$Me_2NC(O)$-C_6H_4	0.014
15v	4-MeHNC(O)-C_6H_4	0.023
15ac	4-$H_2NC(O)$-C_6H_4	0.079
15w	4-$Me_2NC(O)CH_2$-C_6H_4	0.003
15x		0.059
15y		0.091
15z		0.036

[a] Values are the arithmetic means of at least two experiments. Standard deviation of assay is less than ±10%.
[b] Concentration to enhance ALP activity in ST2 cell to 200% of control.

However, this compound was metabolically unstable. Whereas, morpholine analog **15g** was less potent than **1** but metabolically more stable. These results led us to investigate piperazine derivatives to both enhance the activity and improve the metabolic stability. Although the unsubstituted and *N*-methyl-piperazine derivatives (**15aa** and **15i**) showed very weak activities, *N*-phenyl-piperazine analog **15j** demonstrated higher activity than **1** with an EC_{200} value of 0.10 μg/mL. We next attempted to expand the six-membered piperazine ring to a seven-membered homopiperazine ring. *N*-Phenyl-homopiperazine analog **15k** showed good activity, which was more than threefold as potent as that of **1** (EC_{200} = 0.051 μg/mL). Furthermore, compound **15k** was metabolically moderately stable. Therefore, *N*-phenyl-homopiperazine derivatives were examined in detail.

We first examined the effect of a methyl group placed on the benzene ring of **15k**. C2-Substiution (**15l**) resulted in a significant loss of activity. Although C3-substituted compound **15m** showed slightly lower activity, C4-substituted compound **15n** had slightly higher activity than unsubstituted compound **15k**. We then focused on the steric and electronic effects of the substituents at the C4-position of the benzene ring. Fluorine substitution (**15o**) was detrimental. Introduction of a methoxy group (**15q**) resulted in a slight increase in activity. The activity of *i*-propoxy analog **15r** was dramatically reduced from the methoxy analog **15q**. A similar tendency was observed with alkyl derivatives (**15n** vs **15p**). These results suggest that sterically hindered and lipophilic groups are disfavored around this position. We then examined electron withdrawing groups. Nitrile analog **15s** and ethyl ester analog **15t** showed lower activity than **15k**, and hydrolysis of **15t** into the carboxylic acid **15ab** resulted in a substantial loss of activity. Although carboxamide analog **15ac** showed moderate activity, *N*-methylamide **15v** and *N*,*N*-dimethylamide **15u** provided a major boost in activity, and **15u** was fourfold more potent than **15k**. Furthermore, insertion of a methylene group between the *N*,*N*-dimethylcarboxamide group and the benzene ring of **15u** produced compound **15w**, which gave 15-fold higher activity (EC_{200} = 0.003 μg/mL) than **15k**. These results suggest that the incorporation of hydrogen bond acceptors into its proper location

Table 3
PK parameters of **1** and **15k** in F344 rat[a]

Compound	*c*log*P*[c]	Cl (mL/min/kg)		V_d (L/kg)		$t_{1/2}$ (h)		C_{max} (μg/mL)		AUC (μg h/mL)		*F* (%)
		iv	po	iv	po	iv	po	iv	po	iv	po	
1[b]	1.7	39	45	1.1	3.1	0.33	0.78	–	5.59	0.87	9.4	87
15k[b]	3.0	29	34	4.2	8.1	2.77	3.22	–	2.54	1.1	9.5	71

[a] Mean (n = 3).
[b] po 25 mg/kg, 0.5% carboxymethyl cellulose; iv 2 mg/kg, *N*,*N*-dimethylacetamide/distilled water (5/95).
[c] The *c*log*P* values were calculated by CLOGP software (CLOGP Daylight Version 4.83, Daylight Chemical Information Systems, Inc.).

is beneficial to enhance the activity. Replacement of the benzene ring of **15k** with heteroaryl rings (**15x–z**) resulted in comparable activities.

With the promising in vitro activities, compounds **1**, **15k** and **15w** were selected for pharmacokinetics studies in the rat (Table 3). Hit compound **1** had good oral exposure with a bioavailability of 86%, but also had a short plasma half-life (0.78 h). The bioavailability and oral exposure for compound **15k** were also favorable, furthermore, **15k** had a longer plasma half-life (3.22 h). These PK profiles indicated that compound **15k** could be a candidate for in vivo pharmacological evaluation with once daily dosing. Unfortunately, compound **15w**, which showed the most potent activity, was inferior in PK with low C_{max} (0.08 μg/mL, 3 mg/kg, po).

We next sought to test compounds **1** and **15k** in vivo. Compounds **1** and **15k** were administered to ovariectomized (OVX) rats, a model of postmenopausal osteoporosis, at the doses of 60 mg/kg/day (po, b.i.d) and 10, 30 mg/kg/day (po, q.d.), respectively, over 6 weeks. Their areal bone mineral density (aBMD) of distal femurs, femoral diaphyses and proximal tibiae, and serum osteocalcin (a marker for the bone formation)[14] concentrations after six weeks were compared to those of vehicle control and sham-operated rats. As shown in Figure 2, vehicle control OVX rats showed significant decreases in aBMD compared to sham-operated rats, and treatment with these two compounds significantly increased all parts of aBMD. Especially, aBMD recovered almost equal to that of sham-operated rats upon treatment with 30 mg/kg/day of **15k**. Both of the thienopyridine derivatives increased the aBMD of femoral diaphyses remarkably. Furthermore, these compounds also increased the serum osteocalcin concentration, consistent with an elevation in bone formation activity (Fig. 3). The observed in vivo pharmacodynamic effects with 10 mg/kg/day (q.d.) of **15k** were comparable to those with 60 mg/kg/day (b.i.d.) of **1**, consistent with their in vitro activities and pharmacokinetics parameters. It is fair to say that these results provided proof-of-concept for this lead discovery and optimization strategy of novel bone anabolic agents.

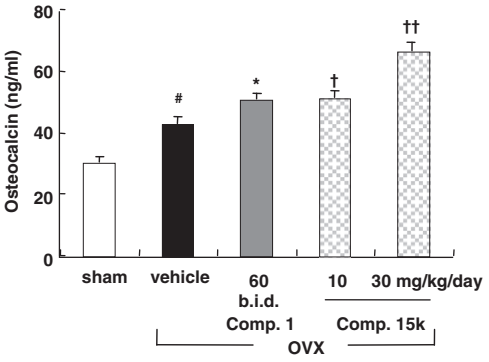


Figure 3. Effects of compound **1** and **15k** on serum osteocalcin concentration in OVX rat model. Data are presented as the mean ± SE (n = 8–9). $^{\#}p$ <0.05 versus sham group, $^{*}p$ <0.05 versus vehicle group, as determined using Student's t-test. $^{\dagger}p$ <0.05, $^{\dagger\dagger}p$ <0.01 versus vehicle group, as determined using Dunnett's test.

4. Conclusion

Thienopyridine derivative **1** was identified as a compound stimulating osteoblastic differentiation by an assay evaluating alkaline phosphatase (ALPase) activity in ST2 cells. Extensive SAR studies led to the discovery of 3-amino-4-(4-phenyl-1,4-diazepan-1-yl)thieno[2,3-*b*]pyridine-2-carboxamide (**15k**), which was found to be a stronger enhancer of ALPase and had better pharmacokinetic properties compared to **1**. Furthermore, **15k** showed statistically significant improvements of aBMD from the dosage of 10 mg/kg/day in an in vivo study using a rat model of postmenopausal osteoporosis. The mechanism of action has not yet been made clear, and mechanism investigations are underway. Also, further structural optimization and biological evaluations of the novel thienopyridine derivatives are ongoing. These results will be reported in due course.

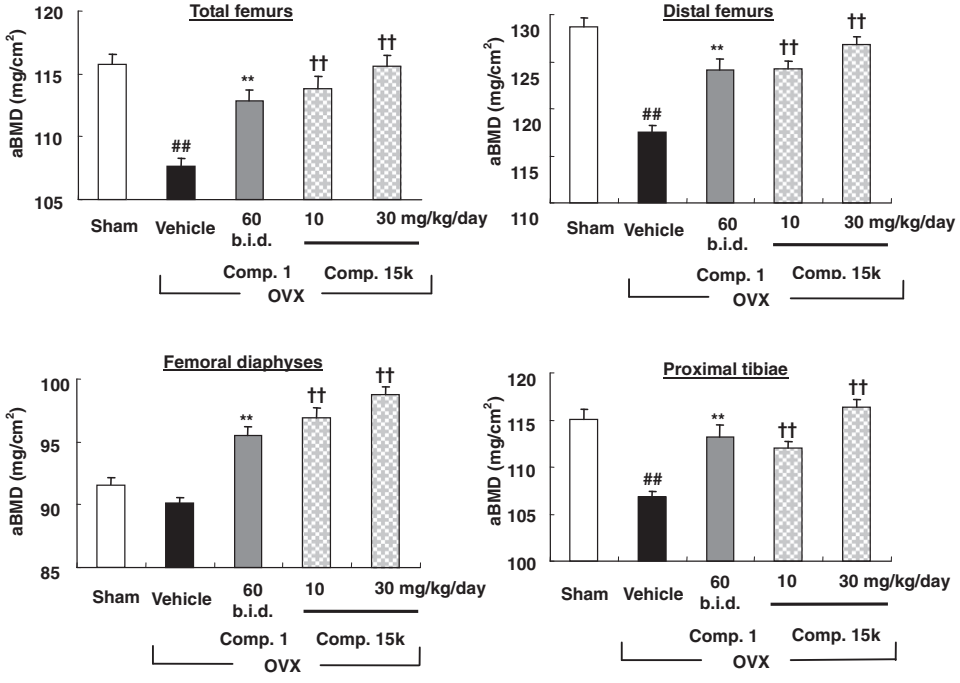


Figure 2. Effects of compound **1** and **15k** on aBMD in the OVX rat model. Data are presented as the mean ± SE (n = 8–9). $^{\#\#}p$ <0.01 versus sham group, $^{**}p$ <0.01 versus vehicle group, as determined using Student's t-test. $^{\dagger\dagger}p$ <0.01 versus vehicle group, as determined using Dunnett's test.

5. Experimental

5.1. Chemistry

5.1.1. General

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Melting points were determined in a Yanaco micro melting point apparatus and are uncorrected. IR absorption spectra were recorded on a Jasco FT/IR-610 spectrophotometer. NMR spectra were recorded on a Varian Mercury 400 or 500 spectrometer with tetramethylsilane as an internal reference. The mass spectra (Low- or High-resolution mass) spectroscopy was carried out with a JEOL GCmate or JEOL JMS-AX505H. Elemental analyses were performed by the analytical department of Daiichi Sankyo RD Novare Co., Ltd. TLC analysis was performed on 60F254 plates (Merck 5715). Separation of the compounds by column chromatography was carried out with silica gel 60 (Merck, 230–400 mesh ASTM).

5.1.2. *tert*-Butyl 4-phenyl-1,4-diazepane-1-carboxylate (4a)

Tris(dibenzylideneacetone)dipalladium(0) (458 mg, 0.500 mmol), 2-dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl (787 mg, 2.00 mmol) and sodium *tert*-butoxide (1.35 g, 14.0 mmol) were put into an oven-dried flask. *tert*-Butanol (10 mL), 1,4-dioxane (20 mL), iodobenzene (1.12 mL, 10.0 mmol) and 1-(*tert*-butoxycarbonyl)homopiperazine (2.40 g, 12.0 mmol) were successively added to the flask at room temperature, and the mixture was stirred under reflux for 2 h under N_2 atmosphere. After cooling to room temperature, diethyl ether was added to the reaction mixture, the insolubles were removed by filtration, and the filtrate was concentrated in vacuo. The obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate = 10:1) to give the title compound (2.76 g, 100%) as pale yellow oil. IR (film) 2974, 1694, 1599, 1506, 1415, 1237, 1169, 930, 748, 692 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.37 (4.5H, s), 1.44 (4.5H, s), 1.93–2.00 (2H, m), 3.19 (1H, t, J = 5.9 Hz), 3.80 (1H, t, J = 5.9 Hz), 3.51–3.57 (6H, m), 6.63–6.69 (3H, m), 7.19 (2H, t, J = 7.1 Hz); HRMS m/z calcd for $C_{16}H_{24}O_2N_2$ 276.1835, found 276.1833; Anal. Calcd for $C_{16}H_{24}N_2O_2{\cdot}0.14H_2O$: C, 68.90; H, 8.77; N, 10.04. Found: C, 68.96; H, 8.75; N, 9.85.

5.1.3. *tert*-Butyl 4-(*o*-tolyl)-1,4-diazepane-1-carboxylate (4b)

To a toluene (20 mL) solution of 2-iodotoluene (2.18 g, 10.0 mmol) was added 1-(*tert*-butoxycarbonyl)homopiperazine (2.40 g, 12.0 mmol), palladium(II) acetate (89.8 mg, 0.400 mmol), 2,8,9-triisobutyl-2,5,8,9-tetraaza-1-phosphabicyclo[3.3.3]undecane (137 mg, 0.400 mmol) and sodium *tert*-butoxide (1.35 g, 14.0 mmol). The mixture was stirred at 80 °C for 18 h under N_2 atmosphere. After cooling to room temperature, diethyl ether was added to the reaction mixture, the insolubles were removed by filtration, and the filtrate was concentrated in vacuo. The obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate, 5:1) to give the title compound (754 mg, 26%) as pale yellow oil. IR (film) 2975, 1695, 1492, 1413, 1159, 762, 725 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.47–1.48 (9H, m), 1.87–1.97 (2H, m), 2.29 (3H, s), 3.00–3.10 (4H, m), 3.52–3.60 (4H, m), 6.92 (1H, t, J = 7.8 Hz), 7.01 (1H, d, J = 7.8 Hz), 7.08–7.14 (2H, m); HRMS m/z calcd for $C_{17}H_{26}O_2N_2$ 290.1994, found 290.1977.

5.1.4. *tert*-Butyl 4-(*m*-tolyl)-1,4-diazepane-1-carboxylate (4c)

CuI (80.0 mg, 0.420 mmol) and K_3PO_4 (3.40 g, 16.0 mmol) were put into an oven-dried flask. 2-Propanol (8.0 mL), ethylene glycol (0.90 mL, 16.0 mmol), 3-iodotoluene (1.03 mL, 8.00 mmol) and 1-(*tert*-butoxycarbonyl)-homopiperazine (1.89 mL, 9.6 mmol) were successively added to the flask at room temperature. The reaction mixture was stirred at 80 °C for 10 h under N_2 atmosphere. After cooling to room temperature, water was added to the reaction mixture, and the mixture was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate = 3:1) to give the title compound (812 mg, 35%) as pale yellow oil. IR (film) 2974, 1695, 1602, 1498, 1415, 1175, 930, 692 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.36 (4.5H, s), 1.44 (4.5H, s), 1.92–1.98 (2H, m), 2.28 (3H, s), 3.19 (1H, t, J = 5.9 Hz), 3.29 (1H, t, J = 5.9 Hz), 3.49–3.54 (6H, m), 6.46–6.48 (3H, m), 7.06 (1H, t, J = 7.1 Hz); HRMS m/z calcd for $C_{17}H_{26}O_2N_2$ 290.1994, found 290.1981; Anal. Calcd for $C_{17}H_{26}N_2O_2{\cdot}0.16H_2O$: C, 69.62; H, 9.05; N, 9.55. Found: C, 69.71; H, 9.36; N, 9.28.

5.1.5. *tert*-Butyl 4-(*p*-tolyl)-1,4-diazepane-1-carboxylate (4d)

The title compound was prepared in a similar manner described for **4c**. Yield: 36%; colorless oil; IR (film) 2974, 2929, 1695, 1619, 1521, 1415, 1365, 1237, 1169 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.38 (4.5H, s), 1.44 (4.5H, s), 1.91–2.01 (2H, m), 2.23 (3H, s), 3.18 (1H, t, J = 5.9 Hz), 3.20 (1H, t, J = 5.9 Hz), 3.46–3.59 (6H, m), 6.60 (2H, d, J = 8.2 Hz), 7.00 (2H, d, J = 8.2 Hz); MS (EI) m/z: 290 [M^+].

5.1.6. *tert*-Butyl 4-(4-fluorophenyl)-1,4-diazepane-1-carboxylate (4e)

The title compound was prepared in a similar manner described for **4c**. Yield: 45%; white solid; mp 88–89 °C; IR (KBr) 2968, 2921,1682, 1515, 1418, 1244, 828 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.36 (4.5H, s), 1.43 (4.5H, s), 1.90–2.01 (2H, m), 3.21 (1H, t, J = 5.9 Hz), 3.32 (1H, t, J = 5.9 Hz), 3.46–3.63 (6H, m), 6.58–6.64 (2H, m), 6.92 (2H, t, J = 8.6 Hz); MS (EI) m/z: 294 [M^+]; Anal. Calcd for $C_{16}H_{23}FN_2O_2{\cdot}0.14H_2O$: C, 64.73; H, 7.90; N, 9.44; F, 6.40. Found: C, 64.77; H, 7.92; N, 9.36; F, 6.37.

5.1.7. *tert*-Butyl 4-(4-*tert*-butylphenyl)-1,4-diazepane-1-carboxylate (4f)

The title compound was prepared in a similar manner described for **4c**. Yield: 9%; white solid; mp 62–64 °C; IR (KBr) 2962, 1686, 1520, 1420, 1364, 1246, 1170 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.27 (9H, s), 1.35 (4.5H, s), 1.43 (4.5H, s), 1.91–2.00 (2H, m), 3.21 (1H, t, J = 5.9 Hz), 3.32 (1H, t, J = 5.9 Hz), 3.48–3.59 (6H, m), 6.62 (2H, d, J = 8.8 Hz), 7.20 (2H, d, J = 8.8 Hz); MS (EI) m/z: 332 [M^+].

5.1.8. *tert*-Butyl 4-(4-methoxyphenyl)-1,4-diazepane-1-carboxylate (4g)

The title compound was prepared in a similar manner described for **4c**. Yield: 41%; brown liquid; IR (film) 2974, 1693, 1513, 1415, 1242, 1169, 1041, 930, 815 cm^{-1}; ^1H NMR ($CDC1_3$, 500 MHz) δ 1.37 (4.5H, s), 1.44 (4.5H, s), 1.92–1.99 (2H, m), 3.20 (1H, t, J = 5.9 Hz), 3.31 (1H, t, J = 5.9 Hz), 3.47–3.57 (6H, m), 3.74 (3H, s), 6.65 (2H, d, J = 9.3 Hz), 6.81 (2H, d, J = 9.3 Hz); HRMS m/z calcd for $C_{17}H_{26}N_2O_3$ 306.1943, found 306.1935; Anal. Calcd for $C_{17}H_{26}N_2O_3{\cdot}0.36H_2O$: C, 65.26; H, 8.61; N, 8.95. Found: C, 64.92; H, 8.29; N, 8.87.

5.1.9. *tert*-Butyl 4-(4-isopropoxyphenyl)-1,4-diazepane-1-carboxylate (4h)

The title compound was prepared in a similar manner described for **4c**. Yield: 14%; colorless oil; IR (film) 2974, 1694, 1511, 1415, 1366, 1237, 1168, 1122 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.28 (6H, d, J = 5.9 Hz), 1.37 (4.5H, s), 1.43 (4.5H, s), 1.90–2.00 (2H, m), 3.20 (1H, t, J = 5.9 Hz), 3.31 (1H, t, J = 5.5 Hz), 3.40–3.61 (6H, m), 4.32–4.38 (1H, m), 6.61 (2H, d, J = 9.0 Hz), 6.78 (2H, d, J = 9.0 Hz); MS (EI) m/z: 334 [M^+].

5.1.10. *tert*-Butyl 4-(4-cyanophenyl)-1,4-diazepane-1-carboxylate (4i)

4-Fluorobenzonitrile (1.21 g, 10.0 mmol), 1-(*tert*-butoxycarbonyl)homopiperazine (1.96 mL, 10.0 mmol) and triethylamine

(1.53 mL, 11.0 mmol) were dissolved in dimethylsulfoxide (20 mL) and the mixture was stirred at 100 °C for 16 h. After cooling to room temperature, the mixture was diluted with ethyl acetate, washed successively with water and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate, 2:1) to give the title compound (1.90 g, 63%) as pale yellow oil. IR (film) 2975, 2214, 1691, 1606, 1521, 1417, 1365, 1240, 1178, 929, 819, 544 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.16 (4.5H, s), 1.28 (4.5H, s), 1.68–1.85 (2H, m), 3.17 (1H, t, J = 5.9 Hz), 3.25 (1H, t, J = 5.5 Hz), 3.44–3.68 (6H, m), 6.81 (2H, d, J = 9.4 Hz), 7.48 (2H, dd, J = 9.4, 3.5 Hz); HRMS m/z calcd for $C_{17}H_{23}N_3O_2$ 301.1790, found 301.1784.

5.1.11. *tert*-Butyl 4-(4-ethoxycarbonylphenyl)-1,4-diazepane-1-carboxylate (4j)

The title compound was prepared in a similar manner described for **4a**. Yield: 44%; brown oil; IR (film) 2976, 1697, 1605, 1522, 1415, 1364, 1281, 1183, 1108, 770 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.36 (3H, t, J = 7.0 Hz), 1.36 (4.5H, s), 1.43 (4.5H, s), 1.91–2.02 (2H, m), 3.20 (1H, t, J = 5.9 Hz), 3.30 (1H, t, J = 5.9 Hz), 3.51–3.66 (6H, m), 4.30 (2H, q, J = 7.0 Hz), 6.65 (2H, d, J = 9.0 Hz), 7.87 (2H, d, J = 9.0 Hz); MS (FAB) m/z: 348 [M^+].

5.1.12. Benzyl 4-{4-[2-(dimethylamino)-2-oxoethyl]phenyl}-1,4-diazepane-1-carboxylate (4k)

The reaction was performed following the method described for **4a** using 2-(4-bromophenyl)-*N*,*N*-dimethyl acetamide[15] and benzyl 1-homopiperazinecarboxylate. Yield: 80%; yellow oil; IR (film) 3481, 2939, 1699, 1642, 1520, 1423 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.90–2.06 (2H, m), 2.95 (3H, s), 3.00 (3H, s), 3.27–3.33 (1H, m), 3.34–3.40 (1H, m), 3.50–3.67 (8H, m), 5.08 (1H, s), 5.13 (1H, s), 6.60–6.68 (2H, m), 7.10 (2H, d, J = 8.2 Hz), 7.27–7.40 (5H, m); MS (FAB) m/z: 396 [M+H]$^+$.

5.1.13. *tert*-Butyl 4-(2-pyridyl)-1,4-diazepane-1-carboxylate (4l)

Potassium *tert*-butoxide (1.68 g, 15.0 mmol), tris(dibenzylideneacetone)dipalladium(0) (91.6 mg, 0.100 mmol) and 1,3-bis-(2,6-diisopropylphenyl)-imidazolium chloride (85.0 mg, 0.200 mmol) were mixed in 1,4-dioxane (20 mL) under N_2 atmosphere, and a 1,4-dioxane (10 mL) solution of 2-bromopyridine (2.37 g, 15.0 mmol) and 1-(*tert*-butoxycarbonyl)homopiperazine (2.00 g, 10.0 mmol) was added dropwise. After stirring at room temperature for 12 h, the mixture was diluted with water, and the insolubles were removed by filtration. The filtrate was extracted with ethyl acetate, and the extract was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 4:1) to give the title compound (2.27 g, 82%) as pale yellow oil. IR (neat) 1694, 1597, 1494, 1240, 1169, 928, 770 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.38, (4.5H, s), 1.43, (4.5H, s), 1.92–1.99 (2H, m), 3.21–3.34 (2H, m), 3.54–3.78 (6H,m), 6.47–6.52 (2H, m), 7.38–7.42 (1H, m), 8.10–8.12 (1H, m); MS (FAB) m/z: 278 [M+H]$^+$.

5.1.14. *tert*-Butyl 4-(4-pyridyl)-1,4-diazepane-1-carboxylate (4m)

The title compound was prepared in a similar manner described for **4l**. Yield: 34%; pale yellow oil; mp 124–129 °C; IR (KBr) 1674, 1597, 1167, 987 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.36 (4.5H, s), 1.42 (4.5H, s), 1.92–1.99 (2H, m), 3.21–3.34 (2H, m), 3.52–3.69 (6H, m), 6.50 (2H, d, J = 6.5 Hz), 8.18–8.20 (2H, m); MS (EI) m/z: 277 [M^+]; Anal. Calcd for $C_{15}H_{23}N_3O_2$: C, 64.95; H, 8.36; N, 15.15. Found: C, 64.78; H, 8.46; N, 15.03.

5.1.15. 2-(1,4-Diazepan-1-yl)thiazole (4n)

2-Bromothiazole (8.20 g, 50.0 mmol) and homopiperazine (10.0 g, 100 mmol) were stirred in 1-butanol (100 mL) under reflux for 24 h. After cooling to room temperature, the insolubles were removed by filtration, and the filtrate was concentrated in vacuo. The residue was diluted with 1 M aqueous solution of NaOH (30 mL), and the mixture was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were dried over Na_2SO_4, and concentrated in vacuo. The residue in methanol (30 mL) was added 4 M hydrogen chloride in 1,4-dioxane (30 mL) was added. The deposited hydrochloride salt was separated by filtration and washed with 1,4-dioxane. The obtained solid was mixed with 1 M aqueous solution of NaOH (60 mL), and the mixture was extracted with CH_2Cl_2 (3 × 50 mL). The extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the title compound (3.60 g, 73%) as pale yellow oil. IR (neat) 3304, 1534, 1139, 614 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.90–1.96 (2H, m), 2.89–2.91 (2H, m), 3.04–3.07 (2H, m), 3.66–3.70 (4H, m), 6.44 (1H, d, J = 3.5 Hz), 7.15 (1H, d, J = 3.5 Hz); MS (EI) m/z: 183 [M^+].

5.1.16. 1-Phenyl-1,4-diazepane (5a)

To a methanol (5.0 mL) solution of **4a** (2.76 g, 10.0 mmol) was added 4 M hydrogen chloride in 1,4-dioxane (15 mL), and the mixture was stirred at room temperature for 1 h and concentrated in vacuo. The residue was treated with aqueous solution of potassium carbonate (100 mL), and the mixture was extracted with a mixed solvent of CH_2Cl_2/2-propanol (4:1, 3 × 100 mL). The extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the title compound (1.44 g, 82%) as yellow liquid. IR (film) 2931, 1598, 1506, 1394, 1245, 1035, 748, 692 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.87–1.92 (2H, m), 2.83 (2H, t, J = 5.9 Hz), 3.03 (2H, t, J = 5.5 Hz), 3.54–3.59 (4H, m), 6.65 (1H, t, J = 8.3 Hz), 6.70 (2H, d, J = 8.3 Hz), 7.21 (2H, t, J = 8.3 Hz); HRMS m/z calcd for $C_{11}H_{16}N_2$ 176.1313, found 176.1318.

5.1.17. 1-(*o*-Tolyl)-1,4-diazepane (5b)

The title compound was prepared from **4b** in a similar manner described for **5a**. Yield: 100%; yellow liquid; IR (film) 2938, 2831, 1598, 1492, 1458, 1213, 1163, 1114, 759, 724 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.87–1.92 (2H, m), 2.32 (3H, s), 3.02 (2H, t, J = 5.5 Hz), 3.07 (2H, t, J = 5.9 Hz), 3.12–3.17 (4H, m), 6.94 (1H, t, J = 7.8 Hz), 7.07 (1H, d, J = 7.8 Hz), 7.12–7.17 (2H, m); HRMS m/z calcd for $C_{12}H_{18}N_2$ 190.1470, found 190.1443.

5.1.18. 1-(*m*-Tolyl)-1,4-diazepane (5c)

The title compound was prepared from **4c** in a similar manner described for **5a**. Yield: 100%; yellow liquid; IR (film) 2928, 1601, 1498, 1363, 1178, 765, 692 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.86–1.91 (2H, m), 2.29 (3H, s), 2.82 (2H, t, J = 5.9 Hz), 3.02 (2H, t, J = 5.5 Hz), 3.52–3.57 (4H, m), 6.47–6.51 (2H, m), 7.09 (2H, dd, J = 9.4, 1.9 Hz); HRMS m/z calcd for $C_{12}H_{18}N_2$ 190.1470, found 190.1456.

5.1.19. 1-(*p*-Tolyl)-1,4-diazepane (5d)

The title compound was prepared from **4d** in a similar manner described for **5a**. Yield: 100%; pale yellow oil; IR (film) 3318, 2923, 1618, 1520, 1394, 1363, 1189, 802 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.87–1.92 (2H, m), 2.23 (3H, s), 2.79–2.85 (2H, m), 2.99–3.05 (2H, m), 3.49–3.58 (4H, m), 6.60 (2H, d, J = 8.2 Hz), 7.00 (2H, d, J = 8.2 Hz); MS (EI) m/z: 190 [M^+].

5.1.20. 1-(4-Fluorophenyl)-1,4-diazepane (5e)

The title compound was prepared from **4e** in a similar manner described for **5a**. Yield: 81%; pale yellow oil; IR (film) 3322, 2935, 1611, 1513, 1228, 814 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.87–1.92 (2H, m), 2.83 (2H, t, J = 5.9 Hz), 3.02 (2H, t, J = 5.5 Hz), 3.51 (2H, t, J = 5.5 Hz), 3.54 (2H, t, J = 5.9 Hz), 6.61 (2H, dd, J = 9.3, 4.4 Hz), 6.91 (2H, t, J = 9.3 Hz); MS (EI) m/z: 194 [M^+].

5.1.21. 1-(4-*tert*-Butylphenyl)-1,4-diazepane (5f)

The title compound was prepared from **4f** in a similar manner described for **5a**. Yield: 97%; pale brown oil; IR (film) 2959, 1614, 1520, 1363, 1201, 812, 552 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.28 (9H, s), 1.87–1.92 (2H, m), 2.84 (2H, t, J = 5.9 Hz), 3.02 (2H, t, J = 5.5 Hz), 3.52 (2H, t, J = 5.5 Hz), 3.54 (2H, t, J = 5.9 Hz), 6.63 (2H, d, J = 8.4 Hz), 7.21 (2H, d, J = 8.4 Hz); MS (EI) m/z: 232 [M^+].

5.1.22. 1-(4-Methoxyphenyl)-1,4-diazepane (5g)

The title compound was prepared from **4g** in a similar manner described for **5a**. Yield: 100%; yellow liquid; IR (film) 2934, 1513, 1241, 1040, 814 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.87–1.92 (2H, m), 2.89 (2H, t, J = 5.9 Hz), 3.02 (2H, t, J = 5.5 Hz), 3.48–3.54 (4H, m), 3.75 (3H, s), 6.65 (2H, d, J = 9.0 Hz), 6.82 (2H, d, J = 9.0 Hz); HRMS m/z calcd for $C_{12}H_{18}N_2O$ 206.1419, found 206.1424.

5.1.23. 1-(4-Isopropoxyphenyl)-1,4-diazepane (5h)

The title compound was prepared from **4h** in a similar manner described for **5a**. Yield: 89%; pale orange prism crystal; mp 62–64 °C; IR (film) 2974, 2932, 1511, 1237, 1114, 957, 815 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.29 (6H, d, J = 5.9 Hz), 1.86–1.91 (2H, m), 2.83 (2H, t, J = 5.9 Hz), 3.01 (2H, t, J = 5.5 Hz), 3.49 (2H, t, J = 5.5 Hz), 3.52 (2H, t, J = 5.9 Hz), 4.33–4.37 (1H, m), 6.61 (2H, d, J = 9.0 Hz), 6.79 (2H, d, J = 9.0 Hz); MS (EI) m/z: 234 [M^+].

5.1.24. 4-(1,4-Diazepan-1-yl)benzonitrile (5i)

The title compound was prepared from **4i** in a similar manner described for **5a**. Yield: 95%; pale yellow oil; IR (film) 2935, 2211, 1606, 1521, 1404, 1178, 817, 544 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.85–1.90 (2H, m), 2.81 (2H, t, J = 5.9 Hz), 3.01 (2H, t, J = 5.5 Hz), 3.55 (2H, t, J = 5.5 Hz), 3.61 (2H, t, J = 5.9 Hz), 6.65 (2H, t, J = 9.0 Hz), 8.43 (2H, d, J = 9.0 Hz); HRMS m/z calcd for $C_{12}H_{15}N_3$ 201.1266, found 201.1268.

5.1.25. Ethyl 4-(1,4-diazepan-1-yl)benzoate (5j)

The title compound was prepared from **4j** in a similar manner described for **5a**. Yield: 74%; pale yellow oil; IR (film) 2976, 1697, 1605, 1522, 1415, 1364, 1281, 1183, 1108, 770 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.36 (3H, t, J = 7.0 Hz), 1.88–1.96 (2H, m), 2.79–2.87 (2H, m), 3.05 (2H, t, J = 5.5 Hz), 3.61 (2H, t, J = 5.5 Hz), 3.65 (2H, t, J = 5.9 Hz), 4.32 (2H, q, J = 7.0 Hz), 6.66 (2H, d, J = 9.0 Hz), 7.89 (2H, d, J = 9.0 Hz); MS (EI) m/z: 248 [M^+].

5.1.26. 2-[4-(1,4-Diazepan-1-yl)phenyl]-*N*,*N*-dimethylacetamide (5k)

A mixture of **4k** (2.94 g, 7.43 mmol) and 10% Pd/C (H_2O content: 50 wt %, 2.50 g) in ethanol (30 mL) was stirred at room temperature under hydrogen atmosphere for 1 h. After removal of Pd/C by filtration, the filtrate was concentrated in vacuo to give the title compound (1.83 g, 94%) as pale yellow oil. IR (film) 3430, 2934, 1629, 1520, 1398 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.86–1.90 (2H, m), 2.79–2.84 (2H, m), 2.94 (3H, s), 2.97–3.04 (5H, m), 3.47 (2H, s), 3.49–3.57 (4H, m), 6.62 (2H, d, J = 9.0 Hz), 7.07 (2H, d, J = 9.0 Hz); MS (FAB) m/z: 262 [M+H]$^+$.

5.1.27. 1-(2-Pyridyl)-1,4-diazepane (5l)

To a CH_2Cl_2 (8.0 mL) solution of **4l** (2.17 g, 7.80 mmol) was added trifluoroacetic acid (8.0 mL) under ice-cooling. The reaction mixture was stirred at room temperature for 3 h and concentrated in vacuo. The residue was treated with 1 M aqueous solution of NaOH and extracted with CH_2Cl_2. The extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the title compound (1.34 g, 97%) as pale yellow oil. IR (neat) 3308, 1597, 1496, 1440, 769 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.86–1.92 (2H, m), 2.83–2.86 (2H, m), 3.01–3.04 (2H, m), 3.69–3.74 (4H, m), 6.46–6.50 (2H, m), 7.38–7.42 (1H, m), 8.10–8.14 (1H, m); MS (EI) m/z: 177 [M^+].

5.1.28. 1-(4-Pyridyl)-1,4-diazepane (5m)

The title compound was prepared from **4m** in a similar manner described for **5a**. Yield: 97%; pale yellow oil; IR (neat) 3270, 1600, 1518, 804 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.86–1.92 (2H, m), 2.83 (2H, t, J = 5.9 Hz), 3.02 (2H, t, J = 5.5 Hz), 3.53–3.61 (4H, m), 6.51 (2H, d, J = 6.6 Hz), 8.20 (2H, d, J = 6.6 Hz); MS (FAB) m/z: 178 [M+H]$^+$.

5.1.29. Benzyl 4-[4-(2,2,2-trifluoroacetyl)phenyl]-1,4-diazepane-1-carboxylate (7)

The reaction was performed following the method described for **4i** using 2,2,2,4′-tetrafluoroacetophenone and benzyl 1-homopiperazinecarboxylate. Yield: 100%; pale yellow oil; IR (film) 2959, 1697, 1595, 1528, 1423, 1166 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.93–2.06 (2H, m), 3.33–3.38 (1H, m), 3.40–3.46 (1H, m), 3.60–3.74 (6H, m), 5.07 (1H, s), 5.13 (1H, s), 6.67–6.74 (2H, m), 7.25–7.37 (5H, m), 7.91–7.98 (2H, m); MS (FAB) m/z: 407 [M+H]$^+$.

5.1.30. 4-{4-[(Benzyloxy)carbonyl]-1,4-diazepan-1-yl}benzoic acid (8)

To a DMF (35 mL) solution of **7** (7.18 g, 17.7 mmol) was added 8 M aqueous solution of NaOH (4.40 mL, 35.2 mmol), and the mixture was stirred at 80 °C for 1 h. After cooling to room temperature, the mixture was diluted with water and washed with ethyl acetate. The aqueous layer was made acidic with 1 M hydrochloric acid and extracted with ethyl acetate. The extract was dried over $MgSO_4$ and concentrated in vacuo. The residue was triturated with diethyl ether, and the deposited solid was filtered to give the title compound (5.83 g, 93%) as colorless prism crystal. Mp 135–137 °C; IR (KBr) 3063, 2947, 2667, 2562, 1699, 1667, 1600, 1417 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.92–2.07 (2H, m), 3.30–3.36 (1H, m), 3.41 (1H, t, J = 5.9 Hz), 3.58–3.71 (6H, m), 5.08 (1H, s), 5.14 (1H, s), 6.63–6.72 (2H, m), 7.24–7.38 (5H, m), 7.91–7.99 (2H, m); MS (EI) m/z: 354 [M^+].

5.1.31. Benzyl 4-[4-(dimethylcarbamoyl)phenyl]-1,4-diazepane-1-carboxylate (9a)

To a THF (80 mL) solution of **8** (7.09 g, 20.0 mmol) was added 1,1′-carbonyldiimidazole (3.89 g, 24.0 mmol) under ice-cooling and the mixture was stirred for 30 min. The reaction mixture was warmed to room temperature, and a THF solution (2.0 M, 15.0 mL, 30.0 mmol) of dimethylamine was added. The mixture was stirred for 30 min and diluted with saturated sodium hydrogen carbonate aqueous solution and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate) to give the title compound (7.63 g, 100%) as pale yellow oil. IR (film) 3475, 2939, 1699, 1608, 1493, 1423, 1391 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.90–2.06 (2H, m), 3.07 (6H, s), 3.26–3.33 (1H, m), 3.34–3.41 (1H, m), 3.52–3.70 (6H, m), 5.10 (1H, s), 5.14 (1H, s), 6.59–6.71 (2H, m), 7.22–7.42 (7H, m); MS (FAB) m/z: 382 [M+H]$^+$.

5.1.32. Benzyl 4-[4-(methylcarbamoyl)phenyl]-1,4-diazepane-1-carboxylate (9b)

The title compound was prepared from **8** in a similar manner described for **9a**. Yield: 100%; pale yellow oil; IR (film) 3336, 2944, 1694, 1607, 1513, 1423, 1231, 1120, 767 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.91–2.06 (2H, m), 2.99 (3H, d, J = 5.1 Hz), 3.28–3.34 (1H, m), 3.36–3.41 (1H, m), 3.55–3.70 (6H, m), 5.07 (1H, s), 5.13 (1H, s), 5.90–6.00 (1H, m), 6.63–6.71 (1H, m), 7.26–7.39 (5H, m), 7.61–7.68 (2H, m), 7.91–7.96 (1H, m); MS (FAB) m/z: 368 [M+H]$^+$.

5.1.33. 4-(1,4-Diazepan-1-yl)-*N*,*N*-dimethylbenzamide (10a)

The title compound was prepared from **9a** in a similar manner described for **5k**. Yield: 94%; colorless oil; IR (film) 3444, 3321, 2931, 1672, 1608, 1493, 1390 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.85–1.94 (2H, m), 2.79–2.85 (2H, m), 3.00–3.05 (2H, m), 3.03–3.10 (6H, m), 3.54–3.64 (4H, m), 6.66 (2H, d, J = 8.6 Hz), 7.36 (2H, d, J = 8.6 Hz); MS (EI) m/z: 247 [M^+].

5.1.34. 4-(1,4-Diazepan-1-yl)-*N*-methyl-benzamide (10b)

The title compound was prepared from **9b** in a similar manner described for **5k**. Yield: 100%; colorless oil; IR (film) 3311, 2936, 1606, 1514, 1403, 1327, 1204, 829, 767 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.85–1.94 (2H, m), 2.82 (2H, t, J = 5.9 Hz), 2.98 (3H, d, J = 4.7 Hz), 3.00–3.06 (2H, m), 3.53–3.67 (4H, m), 5.90–6.03 (1H, m), 6.67 (2H, d, J = 9.0 Hz), 7.64 (2H, d, J = 9.0 Hz); MS (EI) m/z: 233 [M^+].

5.1.35. (*Z*)-2-Cyano-3-(dimethylamino)but-2-enethioamide (12a)

2-Cyanothioacetamide (1.00 g, 10.0 mmol) and *N*,*N*-dimethylacetamide dimethylacetal (1.73 g, 13.0 mmol) were stirred in acetonitrile (5.0 mL) at room temperature for 1 h. The deposited solid was filtered and washed with acetonitrile to give the title compound (1.05 g, 62%) as pale yellow solid. Mp 155–158 °C; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.27 (3H, s), 3.03 (6H, s), 8.08 (1H, br s), 8.83 (1H, br s).

5.1.36. (*Z*)-2-Cyano-3-(propylamino)but-2-enethioamide (12b)

(*Z*)-2-Cyano-3-ethoxybut-2-enethioamide[12] (0.340 g, 2.00 mmol) and propylamine (0.158 g, 2.50 mmol) were suspended in ethanol (5.0 mL) and the mixture was stirred at room temperature for 15 h. The deposited solid was filtered and washed with ethanol to give the title compound (0.289 g, 79%) as pale yellow solid. Mp 149–151 °C; IR (KBr) 3400, 3287, 3187, 2190, 1612 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.97 (3H, t, J = 7.4 Hz), 1.55–1.64 (2H, m), 2.30 (3H, s), 3.35–3.40 (2H, m), 7.65 (1H, br s), 8.45 (1H, br s), 12.74 (1H, br s); MS (FAB) m/z: 184 [M+H]$^+$; Anal. Calcd for $C_8H_{13}N_3S$: C, 52.43; H, 7.15; N, 22.93; S, 17.49. Found: C, 52.59; H, 7.25; N, 22.83; S, 17.51.

5.1.37. (*Z*)-2-Cyano-3-(cyclohexylamino)but-2-enethioamide (12c)

The title compound was prepared in a similar manner described for **12b**. Yield: 76%; pale yellow solid; mp 142–144 °C; IR (KBr) 3412, 3297, 3188, 2187, 1613, 1493, 1410 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.33–1.61 (6H, m), 1.81–1.93 (4H, m), 2.37 (3H, s), 3.55–3.64 (1H, m), 6.38 (1H, br s), 6.69 (1H, br s), 12.92 (1H, br s); MS (EI) m/z: 223 [M^+].

5.1.38. (*Z*)-2-Cyano-3-(dipropylamino)but-2-enethioamide (12d)

The title compound was prepared in a similar manner described for **12b**. Yield: 44%; pale yellow solid; IR (KBr) 3370, 3290, 3201, 2172, 1627, 1544, 1500, 867 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.85 (6H, t, J = 7.4 Hz), 1.55–1.64 (4H, m), 2.30 (3H, s), 3.37–3.40 (4H, m), 8.36 (1H, br s), 8.96 (1H, br s); MS (EI) m/z: 225 [M^+]; Anal. Calcd for $C_{11}H_{19}N_3S$: C, 58.63; H, 8.50; N, 18.65; S, 14.23. Found: C, 58.58; H, 8.44; N, 18.62; S, 13.96.

5.1.39. (*Z*)-2-Cyano-3-piperidin-1-yl-but-2-enethioamide (12f)

The title compound was prepared in a similar manner described for **12b**. Yield: 65%; pale yellow solid; mp 161–166 °C; IR (KBr) 3381, 3268, 3170, 2182, 1600, 1534, 873, 838, 636 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.58–1.63 (6H, m), 2.27 (3H, s), 3.31–3.36 (4H, m), 8.15 (1H, br s), 8.86 (1H, br s); MS (EI) m/z: 209 [M^+]; Anal. Calcd for $C_{10}H_{15}N_3S$: C, 57.38; H, 7.22; N, 20.08; S, 15.32. Found: C, 57.27; H, 7.16; N, 20.03; S, 15.47.

5.1.40. (*Z*)-2-Cyano-3-morpholino-but-2-enethioamide (12g)

The title compound was prepared in a similar manner described for **12b**. Yield: 85%; pale yellow solid; mp 156–160 °C; IR (KBr) 3374, 3255, 3165, 2177, 1605, 1540, 1119, 985, 884 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.26 (3H, s), 3.36–3.39 (4H, m), 3.64–3.67 (4H, m), 8.38 (1H, br s), 9.06 (1H, br s); MS (FAB) m/z: 212 [M+H]$^+$; Anal. Calcd for $C_9H_{13}N_3OS$: C, 51.16; H, 6.20; N, 19.89; S, 15.18. Found: C, 51.15; H, 6.14; N, 19.73; S, 15.17.

5.1.41. (*Z*)-2-Cyano-3-(4-phenyl-1,4-diazepan-1-yl)but-2-enethioamide (12k)

The title compound was prepared from **5a** in a similar manner described for **12b**. Yield: 85%; pale yellow solid; mp 151–153 °C; IR (KBr) 3290, 2185, 1599, 1538, 1503, 1397, 1369, 1011, 873, 754 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.91–1.96 (2H, m), 2.23 (3H, s), 3.50–3.55 (4H, m), 3.61–3.63 (2H, m), 3.71–3.73 (2H, m), 6.61 (1H, t, J = 7.3 Hz), 6.76 (2H, d, J = 7.3 Hz), 7.16 (2H, t, J = 7.3 Hz), 8.33 (1H, br s), 9.00 (1H, br s); HRMS m/z calcd for $C_{16}H_{21}N_4S$ 301.1487, found 301.1465; Anal. Calcd for $C_{16}H_{20}N_4S$: C, 63.97; H, 6.71; N, 18.65; S, 10.67. Found: C, 63.76; H, 6.47; N, 18.75; S, 10.62.

5.1.42. (*Z*)-2-Cyano-3-[4-(*o*-tolyl)-1,4-diazepan-1-yl]but-2-enethioamide (12l)

The title compound was prepared from **5b** in a similar manner described for **12b**. Yield: 55%; pale yellow solid; mp 99–100 °C; IR (KBr) 3286, 3173, 2184, 1599, 1521, 1410, 1294, 1220, 881, 765 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 2.00–2.04 (2H, m), 2.24 (3H, s), 2.36 (3H, s), 3.01 (2H, t, J = 5.4 Hz), 3.16–3.17 (2H, m), 3.66 (2H, t, J = 5.4 Hz), 3.70–3.72 (2H, m), 6.95 (1H, t, J = 6.8 Hz), 7.07 (1H, d, J = 7.3 Hz), 7.12–7.17 (2H, m), 8.27 (1H, br s), 8.93 (1H, br s); HRMS m/z calcd for $C_{17}H_{23}N_4S$ 315.1644, found 315.1643; Anal. Calcd for $C_{17}H_{22}N_4S{\cdot}0.56H_2O$: C, 62.92; H, 7.18; N, 17.26; S, 9.88. Found: C, 62.65; H, 6.88; N, 17.11; S, 9.13.

5.1.43. (*Z*)-2-Cyano-3-[4-(*m*-tolyl)-1,4-diazepan-1-yl]but-2-enethioamide (12m)

The title compound was prepared from **5c** in a similar manner described for **12b**. Yield: 62%; pale yellow solid; mp 144–148 °C; IR (KBr) 3151, 2188, 1601, 1542, 1345, 1174, 912, 766 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.91–1.95 (2H, m), 2.22 (3H, s), 2.23 (3H, s), 3.48–3.54 (4H, m), 3.60–3.63 (2H, m), 3.68–3.71 (2H, m), 6.44 (1H, d, J = 7.0 Hz), 6.55–6.57 (2H, m), 7.03 (1H, t, J = 7.0 Hz), 8.34 (1H, br s), 9.01 (1H, br s); HRMS m/z calcd for $C_{17}H_{23}N_4S$ 315.1644, found 315.1645; Anal. Calcd for $C_{17}H_{22}N_4S$: C, 64.93; H, 7.05; N, 17.82; S, 10.20. Found: C, 64.65; H, 7.05; N, 17.73; S, 10.13.

5.1.44. (*Z*)-2-Cyano-3-[4-(4-fluorophenyl)-1,4-diazepan-1-yl]but-2-enethioamide (12o)

The title compound was prepared from **5e** in a similar manner described for **12b**. Yield: 30%; pale yellow solid; mp 150–152 °C; IR (KBr) 3356, 3269, 3178, 2177, 1615, 1537, 1507 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.89–1.98 (2H, m), 2.23 (3H, s), 3.47–3.54 (4H, m), 3.57–3.63 (2H, m), 3.66–3.72 (2H, m), 6.74 (2H, dd, J = 9.0, 4.3 Hz), 6.97 (2H, t, J = 9.0 Hz), 8.31 (1H, br s), 8.96 (1H, br s); MS (FAB) m/z: 319 [M+H]$^+$; Anal. Calcd for $C_{16}H_{19}FN_4S{\cdot}0.4H_2O$: C, 59.02; H, 6.13; N, 17.21. Found: C, 58.73; H, 5.84; N, 17.20.

5.1.45. (*Z*)-2-Cyano-3-[4-(4-methoxyphenyl)-1,4-diazepan-1-yl]but-2-enethioamide (12q)

The title compound was prepared from **5g** in a similar manner described for **12b**. Yield: 64%; pale yellow solid; mp 157–158 °C;

IR (KBr) 3284, 3154, 2179, 1512, 1361, 1243, 1037, 821 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.89–1.96 (2H, m), 2.24 (3H, s), 3.44 (2H, t, J = 5.9 Hz), 3.49 (2H, t, J = 5.9 Hz), 3.57–3.63 (4H, m), 3.64 (3H, s), 6.70 (2H, d, J = 9.0 Hz), 6.76 (2H, d, J = 9.0 Hz), 8.27 (1H, br s), 8.95 (1H, br s); HRMS m/z calcd for $C_{17}H_{23}N_4OS$ 331.1593, found 331.1589; Anal. Calcd for $C_{17}H_{22}N_4OS$: C, 61.79; H, 6.71; N, 16.95; S, 9.70. Found: C, 61.51; H, 6.69; N, 16.99; S, 9.71.

5.1.46. (*Z*)-2-Cyano-3-[4-(4-cyanophenyl)-1,4-diazepan-1-yl]but-2-enethioamide (12s)

The title compound was prepared from **5i** in a similar manner described for **12b**. Yield: 58%; pale yellow solid; mp 169–171 °C; IR (KBr) 3290, 2214, 217, 1604, 1519, 1408, 1179, 819, 543 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.88–1.94 (2H, m), 2.21 (3H, s), 3.52 (2H, t, J = 5.5 Hz), 3.58–3.63 (4H, m), 3.82 (2H, t, J = 5.5 Hz), 6.85 (2H, d, J = 9.0 Hz), 7.51 (2H, d, J = 9.0 Hz), 8.38 (1H, br s), 9.02 (1H, br s); HRMS m/z calcd for $C_{17}H_{20}N_5S$ 326.1440, found 326.1436.

5.1.47. (*Z*)-2-Cyano-3-[4-(2-pyridyl)-1,4-diazepan-1-yl]but-2-enethioamide (12x)

The title compound was prepared from **5l** in a similar manner described for **12b**. Yield: 79%; pale yellow solid; mp 155–157 °C; IR (KBr) 3398, 3288, 3184, 2185, 1598, 1540, 1494, 1439, 773 cm^{-1}, ^1H NMR (DMSO-d_6, 400 MHz) δ 1.88–1.94 (2H, m), 2.24 (3H, s), 3.55–3.70 (6H, m), 3.89–3.92 (2H, m), 6.57 (1H, dd, J = 7.0, 5.0 Hz), 6.72 (1H, d, J = 8.6 Hz), 7.50 (1H, ddd, J = 8.6, 7.0, 2.0 Hz), 8.07 (1H, dd, J = 5.0, 2.0 Hz), 8.34 (1H, br s), 9.01 (1H, br s); MS (FAB) m/z: 302 $[M+H]^+$; Anal. Calcd for $C_{15}H_{19}N_5S$: C, 59.77; H, 6.35; N, 23.24; S, 10.64. Found: C, 59.38; H, 6.34; N, 22.99; S, 10.53.

5.1.48. (*Z*)-2-Cyano-3-[4-(4-pyridyl)-1,4-diazepan-1-yl]but-2-enethioamide (12y)

The title compound was prepared from **5m** in a similar manner described for **12b**. Yield: 57%; pale yellow solid; IR (KBr) 3172, 2185, 1600, 1538, 1520, 1411 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.89–1.94 (2H, m), 2.23 (3H, s), 3.52–3.62 (6H, m), 3.78–3.80 (2H, m), 6.70 (2H, d, J = 6.7 Hz), 8.09 (2H, d, J = 6.7 Hz), 8.41 (1H, br s), 9.04 (1H, br s); MS (FAB) m/z: 302 $[M+H]^+$.

5.1.49. (*Z*)-2-Cyano-3-(4-thiazol-2-yl-1,4-diazepan-1-yl)but-2-enethioamide (12z)

The title compound was prepared from **4n** in a similar manner described for **12b**. Yield: 91%; pale yellow solid; mp 149–152 °C; IR (KBr) 3276, 3130, 2182, 1531, 885, 615 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.95–2.00 (2H, m), 2.27 (3H, s), 3.57–3.69 (6H, m), 3.80–3.84 (2H, m), 6.77 (1H, d, J = 3.5 Hz), 7.12 (1H, d, J = 3.5 Hz), 8.42 (1H, br s), 9.06 (1H, br s); MS (FAB) m/z: 308 $[M+H]^+$; Anal. Calcd for $C_{13}H_{17}N_5S_2$: C, 50.79; H, 5.57; N, 22.78; S, 20.86. Found: C, 50.52; H, 5.64; N, 22.44; S, 21.10.

5.1.50. 4-(Dimethylamino)-2-thioxo-1,2-dihydropyridine-3-carbonitrile (14a)

Compound **12a** (1.05 g, 6.20 mmol) and *N*,*N*-dimethylformamide dimethylacetal (2.22 g, 18.6 mmol) were stirred in toluene (10 mL) under reflux for 2 h, and then cooled to room temperature. The mixture was concentrated in vacuo. The residue was suspended in 1 M aqueous solution of NaOH (10 mL) and stirred under reflux for 30 min. After cooling to room temperature, 1 M hydrochloric acid (15 mL) was added. The deposited solid was filtered and washed with water and ethanol to give the title compound (0.871 g, 78%) as pale yellow solid. Mp 246–250 °C; ^1H NMR (DMSO-d_6, 400 MHz) δ 3.12 (6H, s), 6.25 (1H, d, J = 7.3 Hz), 7.29–7.33 (1H, m), 12.40 (1H, br s).

5.1.51. 4-(4-Phenyl-1,4-diazepan-1-yl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile (14k)

The title compound was prepared from **12k** in a similar manner described for **14a**. Yield: 84%; pale yellow solid; mp 128–132 °C; IR (KBr) 2954, 2205, 1626, 1504, 1248, 1136, 928, 750, 617 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.90–1.96 (2H, m), 3.51 (2H, t, J = 5.9 Hz), 3.71 (4H, q, J = 5.9 Hz), 3.93 (2H, t, J = 5.9 Hz), 6.40 (1H, d, J = 7.4 Hz), 6.56 (1H, t, J = 7.1 Hz), 6.74 (2H, d, J = 7.1 Hz), 7.12 (2H, t, J = 7.1 Hz), 7.83 (1H, d, J = 7.4 Hz), 12.47 (1H, br s); HRMS m/z calcd for $C_{17}H_{18}N_4S$ 310.1252, found 310.1224; Anal. Calcd for $C_{17}H_{18}N_4S$: C, 65.78; H, 5.84; N, 18.05; S, 10.33. Found: C, 65.58; H, 5.51; N, 18.03; S, 10.24.

5.1.52. 4-[4-(*p*-Tolyl)-1,4-diazepan-1-yl]-2-thioxo-1,2-dihydropyridine-3-carbonitrile (14n)

Compound **5d** (463 mg, 2.39 mmol) and (2*Z*)-2-cyano-3-ethoxybut-2-enethioamide (386 mg, 2.27 mmol) were stirred in DMF (4.8 mL) at room temperature for 30 min. Subsequently, *N*,*N*-dimethylformamide dimethylacetal (0.302 mL, 2.27 mmol) was added to the mixture. The mixture was stirred at room temperature for 30 min, and then warmed to 60 °C and stirred for 1 h. After cooling to room temperature, ethyl acetate (10 mL) and water (50 mL) were added to the mixture, and the deposited solid was filtered and washed with ethyl acetate and water to give the title compound (125 mg, 28%) as a pale brown solid. Mp 220–223 °C; IR (KBr) 3115, 2940, 2205, 1626, 1518, 1250, 928, 798, 775 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.08–2.13 (2H, m), 2.25 (3H, s), 3.56 (2H, t, J = 5.9 Hz), 3.71–3.76 (2H, m), 3.76–3.80 (2H, m), 4.10–4.15 (2H, m), 6.19 (1H, d, J = 7.8 Hz), 6.65 (2H, d, J = 8.2 Hz), 7.05 (2H, d, J = 8.2 Hz), 7.20 (1H, d, J = 7.8 Hz), 11.22 (1H, br s); MS (EI) m/z: 324 $[M^+]$; Anal. Calcd for $C_{18}H_{20}N_4S{\cdot}0.1H_2O$: C, 66.27; H, 6.24; N, 17.17; S, 9.83. Found: C, 66.07; H, 6.19; N, 17.10; S, 9.60.

5.1.53. 4-[4-(4-*tert*-Butylphenyl)-1,4-diazepan-1-yl]-2-thioxo-1,2-dihydropyridine-3-carbonitrile (14p)

The title compound was prepared from **5f** in a similar manner described for **14n**. Yield: 21%; pale brown solid; mp 257–258 °C; IR (KBr) 3121, 3041, 2958, 2205, 1625, 1519, 1459, 1247 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.28 (9H, s), 2.12 (2H, m), 3.56 (2H, t, J = 5.9 Hz), 3.71–3.81 (4H, m), 4.13 (2H, t, J = 5.1 Hz), 6.20 (1H, d, J = 7.6 Hz), 6.66 (2H, d, J = 8.6 Hz), 7.22 (1H, d, J = 7.6 Hz), 7.24 (2H, d, J = 8.6 Hz), 11.94 (1H, br s); MS (EI) m/z: 366 $[M^+]$.

5.1.54. 4-[4-(4-Isopropoxyphenyl)-1,4-diazepan-1-yl]-2-thioxo-1,2-dihydropyridine-3-carbonitrile (14r)

The title compound was prepared from **5h** in a similar manner described for **14n**. Yield: 32%; pale brown solid; mp 216–218 °C; IR (KBr) 3440, 3128, 3046, 2974, 2205, 1625, 1511, 1241 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.30 (6H, d, J = 5.9 Hz), 2.08–2.14 (2H, m), 3.51 (2H, t, J = 5.9 Hz), 3.73 (2H, t, J = 5.4 Hz), 3.76 (2H, t, J = 5.9 Hz), 4.12 (2H, t, J = 5.4 Hz), 4.37–4.42 (1H, m), 6.21 (1H, d, J = 7.8 Hz), 6.67 (2H, d, J = 8.8 Hz), 6.82 (2H, d, J = 8.8 Hz), 7.23 (1H, d, J = 7.8 Hz), 11.84 (1H, br s); MS (EI) m/z: 368 $[M^+]$; Anal. Calcd for $C_{20}H_{24}N_4OS{\cdot}0.15H_2O$: C, 64.71; H, 6.60; N, 15.09; S, 8.64. Found: C, 64.59; H, 6.46; N, 15.09; S, 8.47.

5.1.55. Ethyl 4-[4-(3-cyano-2-thioxo-1,2-dihydropyridin-4-yl)-1,4-diazepan-1-yl]benzoate (14t)

The title compound was prepared from **5j** in a similar manner described for **14n**. Yield: 28%; pale brown solid; mp 202–204 °C; IR (KBr) 3120, 2972, 2205, 1697, 1623, 1604, 1519, 1285, 1240, 1186, 768 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.27 (3H, t, J = 7.0 Hz), 1.88–1.99 (2H, m), 3.57–3.64 (2H, m), 3.70–3.77 (2H, m), 3.79–3.85 (2H, m), 3.93–3.99 (2H, m), 4.20 (2H, q, J = 7.0 Hz), 6.42 (1H, d, J = 7.8 Hz), 6.83 (2H, d, J = 9.0 Hz), 7.34 (1H, d,

J = 7.8 Hz), 7.72 (2H, d, J = 9.0 Hz), 12.53 (1H, br s); MS (FAB) m/z: 383 $[M+H]^+$; Anal. Calcd for $C_{20}H_{22}N_4O_2S{\cdot}0.12H_2O$: C, 62.45; H, 5.83; N, 14.57; S, 8.34. Found: C, 62.22; H, 5.72; N, 14.65; S, 8.51.

5.1.56. 4-[4-(3-Cyano-2-thioxo-1,2-dihydropyridin-4-yl)-1,4-diazepan-1-yl]-*N*,*N*-dimethyl benzamide (14u)

The title compound was prepared from **10a** in a similar manner described for **14n**. Yield: 24%; pale brown solid; mp 239–241 °C; IR (KBr) 3180, 3147, 3045, 2960, 2918, 2208, 1605, 1525 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.92–2.00 (2H, m), 2.94 (6H, s), 3.54–3.61 (2H, m), 3.71–3.81 (4H, m), 3.95–4.01 (2H, m), 6.44 (1H, d, J = 7.8 Hz), 6.79 (2H, d, J = 8.8 Hz), 7.26 (2H, d, J = 8.8 Hz), 7.36 (1H, d, J = 7.8 Hz), 12.50 (1H, br s); MS (FAB) m/z: 382 $[M+H]^+$; Anal. Calcd for $C_{20}H_{23}N_5OS{\cdot}0.6H_2O$: C, 61.23; H, 6.22; N, 17.85; S, 8.17. Found: C, 61.32; H, 6.18; N, 17.95; S, 8.12.

5.1.57. 4-[4-(3-Cyano-2-thioxo-1,2-dihydropyridin-4-yl)-1,4-diazepan-1-yl]-*N*-methyl-benzamide (14v)

The title compound was prepared from **10b** in a similar manner described for **14n**. Yield: 30%; pale brown solid; IR (KBr) 3340, 2940, 2206, 1606, 1510, 1301, 1241, 766 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.90–1.99 (2H, m), 2.72 (3H, d, J = 3.5 Hz), 3.55–3.62 (2H, m), 3.70–3.77 (2H, m), 3.78–3.84 (2H, m), 3.93–4.00 (2H, m), 6.44 (1H, d, J = 7.8 Hz), 6.80 (2H, d, J = 8.2 Hz), 7.36 (1H, d, J = 7.8 Hz), 7.66 (2H, d, J = 8.2 Hz), 8.01 (1H, br s), 12.55 (1H, br s); MS (FAB) m/z: 368 $[M+H]^+$.

5.1.58. 2-{4-[4-(3-Cyano-2-thioxo-1,2-dihydropyridin-4-yl)-1,4-diazepan-1-yl]phenyl}-*N*,*N*-dimethylacetamide (14w)

The title compound was prepared from **5k** in a similar manner described for **14n**. Yield: 47%; pale brown solid; IR (KBr) 3124, 3034, 2938, 2203, 1616, 1519 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.04–2.16 (2H, m), 2.96 (3H, s), 3.02 (3H, s), 3.52–3.63 (4H, m), 3.69–3.82 (4H, m), 4.08–4.16 (2H, m), 6.19 (1H, d, J = 7.8 Hz), 6.66 (2H, d, J = 8.6 Hz), 7.12 (2H, d, J = 8.6 Hz), 7.22 (1H, d, J = 7.8 Hz), 11.94 (1H, br s); MS (FAB) m/z: 396 $[M+H]^+$.

5.1.59. 3-Amino-4-(dimethylamino)thieno[2,3-*b*]pyridine-2-carboxamide (1)

To a DMF (10 mL) solution of **14a** (870 mg, 4.86 mmol) were added 8 M aqueous solution of NaOH (2.00 mL) and 2-chloroacetamide (542 mg, 5.80 mmol), and the mixture was stirred at room temperature for 1 h. Water (10 mL) was added to the mixture, and the deposited solid was filtered and washed with water and ethanol to give the title compound (790 mg, 69%) as pale yellow solid. Mp 208–211 °C; IR (KBr) 3430, 3296, 3132, 1673, 1582, 1372, 979 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.80 (6H, s), 6.96 (1H, d, J = 5.5 Hz), 6.97 (2H, br s), 7.04 (2H, br s), 8.36 (1H, d, J = 5.5 Hz); MS (FAB) m/z: 237 $[M+H]^+$; Anal. Calcd for $C_{10}H_{12}N_4OS$: C, 50.83; H, 5.12; N, 23.71; S, 13.57. Found: C, 50.70; H, 4.98; N, 23.53; S, 13.38.

5.1.60. 3-Amino-4-(propylamino)thieno[2,3-*b*]pyridine-2-carboxamide (15b)

Compound **12b** (290 mg, 1.58 mmol) and *N*,*N*-dimethylformamide dimethylacetal (0.630 mL, 4.74 mmol) were stirred in toluene (3.0 mL) under reflux for 2 h. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was suspended in 1 M aqueous solution of NaOH (10 mL) and stirred under reflux for 30 min. After cooling to room temperature, 1 M hydrochloric acid (15 mL) was added, and the deposited solid was filtered and washed with water and ethanol to give a crude solid (0.17 g) of 4-(propylamino)-2-thioxo-1*H*-pyridine-3-carbonitrile (**14b**). The obtained solid was dissolved in DMF (3.0 mL), and 8 M aqueous solution of NaOH (0.50 mL) and 2-chloroacetamide (103 mg, 1.10 mmol) were added. The mixture was stirred at room temperature for 1 h, and water (3.0 mL) was added. The deposited solid was filtered and washed with water and ethanol to give the title compound (0.106 g, 27%) as pale yellow solid. Mp 214–215 °C; IR (KBr) 3348, 3319, 3189, 1650, 1592, 1504, 1364 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.95 (3H, t, J = 7.4 Hz), 1.59–1.68 (2H, m), 3.16–3.21 (2H, m), 6.41 (1H, d, J = 5.6 Hz), 6.44 (1H, t, J = 5.4 Hz), 6.81 (2H, br s), 7.02 (2H, br s), 8.05 (1H, d, J = 5.6 Hz); HRMS m/z calcd for $C_{11}H_{15}N_4OS$ 251.0967, found 251.0968; Anal. Calcd for $C_{11}H_{14}N_4OS{\cdot}1.1H_2O$: C, 48.91; H, 6.04; N, 20.74; S, 11.87. Found: C, 49.06; H, 5.92; N, 20.71; S, 11.90.

5.1.61. 3-Amino-4-(cyclohexylamino)thieno[2,3-*b*]pyridine-2-carboxamide (15c)

The title compound was prepared from **12c** in a similar manner described for **15b**. Yield: 14%; pale yellow solid; mp 244–247 °C; IR (KBr) 3141, 1664, 1598, 1513, 1497, 1108 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.20–1.98 (10H, m), 3.43–3.50 (1H, m), 6.16 (1H, d, J = 7.5 Hz), 6.46 (1H, d, J = 5.7 Hz), 6.71 (2H, br s), 7.08 (2H, br s), 8.06 (1H, d, J = 5.7 Hz); HRMS m/z calcd for $C_{14}H_{19}N_4OS$ 291.1280, found 291.1284; Anal. Calcd for $C_{14}H_{18}N_4OS{\cdot}0.3H_2O$: C, 56.85; H, 6.34; N, 18.94; S, 10.85. Found: C, 57.00; H, 6.27; N, 18.95; S, 10.61.

5.1.62. 3-Amino-4-(dipropylamino)thieno[2,3-*b*]pyridine-2-carboxamide (15d)

The title compound was prepared from **12d** in a similar manner described for **15b**. Yield: 23%; pale yellow solid; mp 126–128 °C; IR (KBr) 3419, 3320, 3160, 2960, 1650, 1578, 1499, 1459, 1372, 993 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.79 (6H, t, J = 7.4 Hz), 1.39–1.48 (4H, m), 3.10 (4H, t, J = 7.4 Hz), 7.06 (4H, br s), 7.11 (1H, d, J = 5.5 Hz), 8.39 (1H, d, J = 5.5 Hz); HRMS m/z calcd for $C_{14}H_{21}N_4OS$ 293.1436, found 293.1440; Anal. Calcd for $C_{14}H_{20}N_4OS{\cdot}0.1H_2O$: C, 57.16; H, 6.92; N, 19.04; S, 10.90. Found: C, 57.13; H, 6.60; N, 19.17; S, 11.10.

5.1.63. 3-Amino-4-pyrrolidin-1-yl thieno[2,3-*b*]pyridine-2-carboxamide (15e)

(*Z*)-2-Cyano-3-ethoxybut-2-enethioamide (0.170 g, 1.00 mmol) and pyrrolidine (0.085 g, 1.20 mmol) in ethanol (3.0 mL) were stirred at room temperature for 18 h. The deposited solid was filtered and washed with ethanol to give a crude solid (0.18 g) of (*Z*)-2-cyano-3-pyrrolidin-1-yl-but-2-enethioamide (**12e**). The obtained solid and *N*,*N*-dimethylformamide dimethylacetal (0.358 g, 3.00 mmol) were stirred in toluene (2 mL) under reflux for 2 h, and then cooled to room temperature. The mixture was concentrated in vacuo. The residue was suspended in 1 M aqueous solution of NaOH (2 mL) and stirred under reflux for 30 min. After cooling to room temperature, 1 M hydrochloric acid (3 mL) was added. The deposited solid was filtered and washed with water and ethanol to give a crude solid (0.15 g) of 4-pyrrolidin-1-yl-2-thioxo-1,2-dihydropyridine-3-carbonitrile (**14e**). The obtained solid was dissolved in DMF (2.0 mL), and 8 M aqueous solution of NaOH (0.41 mL) and 2-chloroacetamide (93 mg, 1.00 mmol) were added. The mixture was stirred at room temperature for 1 h, and water (2.0 mL) was added. The deposited solid was filtered and washed with water and ethanol to give the title compound (0.150 g, 58%) as pale yellow solid. Mp 275–283 °C; IR (KBr) 3429, 3297, 3138, 2999, 2877, 1673, 1611, 1584, 1503, 1372, 1341, 1273, 1113, 1056, 1002, 618, 486 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.91 (4H, s), 3.30 (4H, s), 6.87 (1H, d, J = 5.8 Hz), 6.88 (2H, s), 7.02 (2H, br s), 8.26 (1H, d, J = 5.8 Hz); MS (FAB) m/z: 263 $[M+H]^+$; Anal. Calcd for $C_{12}H_{14}N_4OS$: C, 54.94; H, 5.38; N, 21.36; S, 12.22. Found: C, 54.54; H, 5.15; N, 21.10; S, 12.03.

5.1.64. 3-Amino-4-(1-piperidyl)thieno[2,3-*b*]pyridine-2-carboxamide (15f)

The title compound was prepared from **12f** in a similar manner described for **15b**. Yield: 38%; pale yellow solid; mp 191–192 °C; IR (KBr) 3460, 3329, 3176, 1651, 1589, 1501, 1378, 963 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.72–1.79 (6H, m), 2.55–3.40 (4H, m), 6.93 (2H, br s), 6.99 (1H, d, J = 5.1 Hz), 7.07 (2H, br s), 8.40 (1H, d, J = 5.1 Hz); HRMS m/z calcd for $C_{13}H_{17}N_4OS$ 277.1123, found 277.1123; Anal. Calcd for $C_{13}H_{16}N_4OS{\cdot}0.5H_2O$: C, 54.72; H, 6.00; N, 19.63; S, 11.24. Found: C, 54.68; H, 6.03; N, 19.67; S, 11.05.

5.1.65. 3-Amino-4-morpholino-thieno[2,3-*b*]pyridine-2-carboxamide (15g)

4-(Dimethoxymethyl)morpholine[16] was used in place of *N*,*N*-dimethylformamide dimethylacetal and the title compound was prepared from **12g** in a similar manner described for **15b**. Yield: 28%; pale yellow solid; mp 232–234 °C; IR (KBr) 3427, 3377, 3170, 1670, 1579, 1501, 1373, 1112, 969 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.90–3.15 (4H, m), 3.84–3.86 (4H, m), 6.95 (2H, br s), 7.05 (1H, d, J = 5.3 Hz), 7.13 (2H, br s), 8.46 (1H, d, J = 5.3 Hz); HRMS m/z calcd for $C_{12}H_{15}N_4O_2S$ 279.0916, found 279.0924; Anal. Calcd for $C_{12}H_{14}N_4O_2S{\cdot}0.3H_2O$: C, 50.80; H, 5.19; N, 19.75; S, 11.30. Found: C, 50.95; H, 4.86; N, 19.72; S, 11.28.

5.1.66. *tert*-Butyl 4-[3-amino-2-(aminocarbonyl)thieno[2,3-*b*]pyridin-4-yl]piperazine-1-carboxylate (15h)

The title compound was prepared in a similar manner described for **15e**. Yield: 25%; pale yellow solid; mp 198–203 °C; IR (KBr) 3428, 3323, 3176, 2974, 1693, 1649, 1584, 1501, 1367, 1241, 1169, 1124, 976, 959, 825, 770 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.42 (9H, s), 3.25 (4H, s), 3.29 (4H, s), 6.95 (2H, br s), 7.03 (1H, d, J = 5.4 Hz), 7.12 (2H, br s), 8.45 (1H, d, J = 5.4 Hz); MS (FAB) m/z: 378 $[M+H]^+$; Anal. Calcd for $C_{17}H_{23}N_5O_3S{\cdot}0.3H_2O$: C, 53.33; H, 6.21; N, 18.29; S, 8.37. Found: C, 53.14; H, 5.86; N, 18.06; S, 8.41.

5.1.67. 3-Amino-4-(4-methylpiperazin-1-yl)thieno[2,3-*b*]pyridine-2-carboxamide (15i)

The title compound was prepared in a similar manner described for **15e**. Yield: 22%; pale yellow solid; mp 260–263 °C; IR (KBr) 3502, 3424, 3322, 3161, 2939, 2801, 1653, 1588, 1502, 1451, 1371, 1344, 1288, 1246, 1199, 973, 819, 737, 683, 625, 476 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.26 (3H, s), 2.40–2.72 (4H, br s), 2.85–3.22 (4H, br s), 6.89 (2H, br s), 7.00 (1H, d, J = 5.5 Hz), 7.07 (2H, s), 8.41 (1H, d, J = 5.5 Hz); MS (FAB) m/z: 292 $[M+H]^+$; Anal. Calcd for $C_{13}H_{17}N_5OS{\cdot}0.3H_2O$: C, 52.61; H, 5.98; N, 23.60; S, 10.80. Found: C, 52.62; H, 5.79; N, 23.63; S, 10.92.

5.1.68. 3-Amino-4-(4-phenylpiperazin-1-yl)thieno[2,3-*b*]pyridine-2-carboxamide (15j)

The title compound was prepared in a similar manner described for **15e**. Yield: 35%; pale yellow solid; mp 250–252 °C; IR (KBr) 3438, 3318, 3176, 2832, 1645, 1596, 1579, 1447, 1377, 1343, 1238, 1136, 978, 914, 831, 762, 733, 693, 626, 484 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.89–3.71 (8H, br s), 6.70 (1H, t, J = 7.1 Hz), 6.93 (2H, br s), 6.99 (2H, d, J = 9.0 Hz), 7.07 (1H, d, J = 5.5 Hz), 7.10 (2H, br s), 7.22 (2H, dd, J = 9.0, 7.1 Hz), 8.44 (1H, d, J = 5.5 Hz); MS (FAB) m/z: 354 $[M+H]^+$; Anal. Calcd for $C_{18}H_{19}N_5OS{\cdot}0.26H_2O$: C, 60.37; H, 5.49; N, 19.56; S, 8.95. Found: C, 60.18; H, 5.33; N, 19.55; S, 9.02.

5.1.69. 3-Amino-4-(4-phenyl-1,4-diazepan-1-yl)thieno[2,3-*b*]pyridine-2-carboxamide (15k)

The title compound was prepared from **14k** in a similar manner described for **1**. Yield: 87%; pale yellow solid; mp 215–218 °C; IR (KBr) 3340, 3316, 3143, 1645, 1598, 1504, 1369, 1233, 939, 752, 694 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.10–2.15 (2H, m), 3.16–3.19 (2H, m), 3.26–3.29 (2H, m), 3.52 (2H, t, J = 6.3 Hz), 3.75 (2H, t, J = 6.3 Hz), 6.58 (1H, t, J = 8.2 Hz), 6.74 (2H, d, J = 8.2 Hz), 6.95 (2H, br s), 7.05 (1H, d, J = 5.5 Hz), 7.05 (2H, br s), 7.14 (2H, t, J = 8.2 Hz), 8.36 (1H, d, J = 5.5 Hz); HRMS m/z calcd for $C_{19}H_{21}N_5OS$ 367.1467, found 367.1462; Anal. Calcd for $C_{19}H_{21}N_5OS{\cdot}0.24H_2O$: C, 61.38; H, 5.82; N, 18.84; S, 8.62. Found: C, 61.22; H, 5.64; N, 18.94; S, 8.49.

5.1.70. 3-Amino-4-[4-(*o*-tolyl)-1,4-diazepan-1-yl]thieno[2,3-*b*]pyridine-2-carboxamide (15l)

Compound **12l** (0.220 g, 0.700 mmol) and *N*,*N*-dimethylformamide dimethylacetal (0.186 mL, 1.40 mmol) were stirred in ethanol (5.0 mL) at room temperature for 1 h, and the mixture was concentrated in vacuo. The residue was dissolved in *N*,*N*-dimethylacetamide (1.4 mL), and the mixture was stirred at 80 °C for 1 h. After cooling to room temperature, 8 M aqueous solution of NaOH (0.30 mL) and 2-chloroacetamide (80.0 mg, 0.856 mmol) were added. After the mixture was stirred at room temperature for 1 h, water was added to the mixture, and the deposited solid was filtered and washed with water and ethanol to give the title compound (0.184 g, 69%) as pale yellow solid. Mp 206–209 °C (decomposition); IR (KBr) 3427, 3308, 3142, 1583, 1493, 1374, 1228, 1053, 944, 767 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.97–2.04 (2H, m), 2.23 (3H, s), 3.11 (2H, t, J = 5.9 Hz), 3.22–3.25 (2H, m), 3.38–3.42 (4H, m), 6.86–7.15 (9H, m), 8.34 (1H, d, J = 5.5 Hz); HRMS m/z calcd for $C_{20}H_{24}N_5OS$ 382.1702, found 382.1701; Anal. Calcd for $C_{20}H_{23}N_5OS$: C, 62.97; H, 6.08; N, 18.36. Found: C, 62.79; H, 6.27; N, 18.20.

5.1.71. 3-Amino-4-[4-(*m*-tolyl)-1,4-diazepan-1-yl]thieno[2,3-*b*]pyridine-2-carboxamide (15m)

The title compound was prepared from **12m** in a similar manner described for **15l**. Yield: 31%; pale yellow solid; mp 209–212 °C; IR (KBr) 3327, 3169, 2830, 1637, 1579, 1498, 1373, 1234, 1182, 942, 767 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.10–2.15 (2H, m), 2.22 (3H, s), 3.14–3.18 (2H, m), 3.26–3.28 (2H, m), 3.52 (2H, t, J = 6.3 Hz), 3.73 (2H, t, J = 4.7 Hz), 6.41 (1H, d, J = 7.8 Hz), 6.53–6.56 (2H, m), 6.97 (2H, br s), 7.01 (1H, t, J = 7.8 Hz), 7.05 (1H, d, J = 5.5 Hz), 7.06 (2H, br s), 8.37 (1H, d, J = 5.5 Hz); HRMS m/z calcd for $C_{20}H_{24}N_5OS$ 382.1702, found 382.1699; Anal. Calcd for $C_{20}H_{23}N_5OS{\cdot}0.08H_2O$: C, 62.73; H, 6.10; N, 18.29; S, 8.37. Found: C, 62.56; H, 6.08; N, 18.14; S, 8.32.

5.1.72. 3-Amino-4-[4-(*p*-tolyl)-1,4-diazepan-1-yl]thieno[2,3-*b*]pyridine-2-carboxamide (15n)

The title compound was prepared from **14n** in a similar manner described for **1**. Yield: 89%; pale yellow solid; mp 175–176 °C; IR (KBr) 3429, 3317, 3170, 3093, 2942, 2833, 1635, 1579, 1520, 1372 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.07–2.16 (2H, m), 2.18 (3H, s), 3.15–3.22 (2H, m), 3.23–3.30 (2H, m), 3.51 (2H, t, J = 6.3 Hz), 3.72 (2H, t, J = 4.7 Hz), 6.66 (2H, d, J = 8.2 Hz), 6.92–6.99 (4H, m), 7.02–7.09 (3H, m), 8.37 (1H, d, J = 5.1 Hz); MS (FAB) m/z: 382 $[M+H]^+$; Anal. Calcd for $C_{20}H_{23}N_5OS$: C, 62.97; H, 6.08; N, 18.36; S, 8.41. Found: C, 62.58; H, 6.02; N, 18.23; S, 8.29.

5.1.73. 3-Amino-4-[4-(4-fluorophenyl)-1,4-diazepan-1-yl]thieno[2,3-*b*]pyridine-2-carboxamide (15o)

The title compound was prepared from **12o** in a similar manner described for **15l**. Yield: 47%; pale yellow solid; mp 203–205 °C; IR (KBr) 3453, 3324, 3179, 2948, 2838, 1646, 1579, 1510, 1369 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 2.10–2.19 (2H, m), 3.16–3.24 (2H, m), 3.30 (2H, s), 3.49–3.56 (2H, m), 3.71–3.78 (2H, m), 6.76 (2H, dd, J = 9.0, 4.4 Hz), 6.96–7.05 (4H, m), 7.06–7.12 (3H, m), 8.40 (1H, d, J = 5.1 Hz); MS (FAB) m/z: 386 $[M+H]^+$; Anal. Calcd for $C_{19}H_{20}FN_5OS{\cdot}1.35H_2O$: C, 57.95; H, 5.81; N, 17.78; F, 4.82; S, 8.14. Found: C, 57.58; H, 5.41; N, 17.76; F, 4.73; S, 7.99.

5.1.74. 3-Amino-4-[4-(4-*tert*-butylphenyl)-1,4-diazepan-1-yl]thieno[2,3-*b*]pyridine-2-carboxamide (15p)

The title compound was prepared from **14p** in a similar manner described for **1**. Yield: 72%; pale yellow solid; mp 231–232 °C; IR (KBr) 3440, 3324, 3182, 2957, 1645, 1579, 1519, 1364 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.21 (9H, s), 2.06–2.17 (2H, m), 3.16–3.22 (2H, m), 3.23–3.30 (2H, m), 3.52 (2H, t, J = 6.3), 3.74 (2H, t, J = 4.7), 6.69 (2H, d, J = 9.0), 6.89 (2H, br s), 7.03–7.09 (3H, m), 7.17 (2H, d, J = 9.0 Hz), 8.38 (1H, d, J = 5.5 Hz); MS (EI) m/z: 423 [M^+]; Anal. Calcd for $C_{23}H_{29}N_sOS{\cdot}0.27H_2O$: C, 64.48; H, 6.95; N, 16.35; S, 7.48. Found: C, 64.17; H, 6.64; N, 16.31; S, 7.43.

5.1.75. 3-Amino-4-[4-(4-methoxyphenyl)-1,4-diazepan-1-yl]thieno[2,3-*b*]pyridine-2-carboxamide (15q)

The title compound was prepared from **12q** in a similar manner described for **15l**. Yield: 67%; pale yellow solid; mp 206–208 °C; IR (KBr) 3446, 3328, 3168, 1578, 1511, 1370, 1240, 1037, 937, 816 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.78–1.86 (2H, m), 2.89–2.95 (2H, m), 2.97–3.01 (2H, m), 3.19 (2H, t, J = 5.9 Hz), 3.37 (3H, s), 3.37–3.42 (2H, m), 6.43 (2H, d, J = 9.4 Hz), 6.51 (2H, d, J = 9.4 Hz), 6.69 (2H, br s), 6.77–6.79 (3H, m), 8.10 (1H, d, J = 5.5 Hz); HRMS m/z calcd for $C_{20}H_{24}N_5O_2S$ 398.1651, found 398.1635; Anal. Calcd for $C_{20}H_{23}N_5O_2S{\cdot}0.52H_2O$: C, 59.04; H, 5.96; N, 17.21. Found: C, 58.73; H, 6.09; N, 17.52.

5.1.76. 3-Amino-4-[4-(4-isopropoxyphenyl)-1,4-diazepan-1-yl]thieno[2,3-*b*]pyridine-2-carboxamide (15r)

The title compound was prepared from **14r** in a similar manner described for **1**. Yield: 79%; pale yellow solid; mp 173–175 °C; IR (KBr) 3441, 3324, 2973, 1644, 1579, 1510, 1370, 1235 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.21 (6H, d, J = 5.9 Hz), 2.07–2.16 (2H, m), 3.16–3.24 (2H, m), 3.25–3.33 (2H, m), 3.47 (2H, t, J = 5.9 Hz), 3.69 (2H, t, J = 4.7 Hz), 4.34–4.40 (1H, m), 6.68 (2H, d, J = 9.0 Hz), 6.76 (2H, d, J = 9.0 Hz), 6.97 (2H, br s), 7.03–7.11 (3H, m), 8.37 (1H, d, J = 5.5 Hz); MS (EI) m/z: 425 [M^+]; Anal. Calcd for $C_{22}H_{27}N_5O_2S$: C, 62.09; H, 6.40; N, 16.46; S, 7.54. Found: C, 61.83; H, 6.23; N, 16.32; S, 7.38.

5.1.77. 3-Amino-4-[4-(4-cyanophenyl)-1,4-diazepan-1-yl]thieno[2,3-*b*]pyridine-2-carboxamide (15s)

The title compound was prepared from **12s** in a similar manner described for **15l**. Yield: 33%; pale yellow solid; mp 151–153 °C; IR (KBr) 3439, 3327, 2211, 1605, 1519, 1366, 1178, 938, 818, 544 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.13–2.18 (2H, m), 3.17–3.19 (2H, m), 3.22–3.30 (2H, m), 3.61 (2H, t, J = 6.3 Hz), 3.84 (2H, t, J = 5.1 Hz), 6.86 (2H, d, J = 9.0 Hz), 6.96 (2H, br s), 7.05 (1H, d, J = 5.5 Hz), 7.07 (2H, br s), 7.52 (2H, t, J = 9.0 Hz), 8.37 (1H, d, J = 5.5 Hz); HRMS m/z calcd for $C_{20}H_{21}N_6OS$ 393.1497, found 393.1501; Anal. Calcd for $C_{20}H_{20}N_6OS{\cdot}0.94H_2O$: C, 58.67; H, 5.39; N, 20.53. Found: C, 58.99; H, 5.51; N, 20.22.

5.1.78. Ethyl 4-[4-(3-amino-2-carbamoyl-thieno[2,3-*b*]pyridin-4-yl)-1,4-diazepan-1-yl]benzoate (15t)

To a DMF (1.3 mL) solution of **14t** (0.250 g, 0.654 mmol) were added 2-chloroacetamide (73.0 mg, 0.781 mmol) and 20% EtOH solution of NaOEt (0.760 mL, 1.94 mmol), and the mixture was stirred at room temperature for 30 min. 1 M Hydrochloric acid was added to the mixture, and the deposited solid was filtered and washed with water and EtOH to give the title compound (0.207 g, 72%) as pale yellow solid. Mp 124–126 °C; IR (KBr) 3440, 3324, 1695, 1646, 1603, 1365, 1280, 1186 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.28 (3H, t, J = 7.0 Hz), 2.13–2.22 (2H, m), 3.14–3.24 (2H, m), 3.27–3.36 (2H, m), 3.64 (2H, t, J = 6.4 Hz), 3.82–3.89 (2H, m), 4.23 (2H, q, J = 7.0 Hz), 6.84 (2H, d, J = 9.3 Hz), 6.99 (2H, br s), 7.08 (1H, d, J = 5.4 Hz), 7.10 (2H, br s), 7.77 (2H, d, J = 9.3 Hz), 8.40 (1H, d, J = 5.4 Hz); MS (FAB) m/z: 440 [M+H]$^+$; Anal. Calcd for $C_{22}H_{25}N_5O_3S{\cdot}1.1H_2O$: C, 57.53; H, 5.97; N, 15.25; S, 6.98. Found: C, 57.65; H, 5.91; N, 15.04; S, 6.97.

5.1.79. 3-Amino-4-(4-{4-[(dimethylamino)carbonyl]phenyl}-1,4-diazepan-1-yl)thieno[2,3-*b*]pyridine-2-carboxamide (15u)

The title compound was prepared from **14u** in a similar manner described for **1**. Yield: 83%; pale brown solid; mp 159–161 °C; IR (KBr) 3437, 3327, 3187, 2930, 2847, 1606, 1497, 1387 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.11–2.20 (2H, m), 2.97 (6H, s), 3.16–3.24 (2H, m), 3.27–3.30 (2H, m), 3.58 (2H, t, J = 6.3 Hz), 3.78–3.84 (2H, m), 6.76 (2H, d, J = 9.0 Hz), 6.97 (2H, br s), 7.04–7.12 (3H, m), 7.28 (2H, d, J = 9.0 Hz), 8.38 (1H, d, J = 5.1 Hz); MS (FAB) m/z: 439 [M+H]$^+$; Anal. Calcd for $C_{22}H_{26}N_6O_2S{\cdot}1.2H_2O$: C, 57.42; H, 6.22; N, 18.26; S, 6.97. Found: C, 57.17; H, 6.03; N, 18.45; S, 6.83.

5.1.80. 3-Amino-4-[4-[4-(methylcarbamoyl)phenyl]-1,4-diazepan-1-yl]thieno[2,3-*b*]pyridine-2-carboxamide (15v)

The title compound was prepared from **14v** in a similar manner described for **1**. Yield: 46%; pale yellow solid; mp 132–134 °C; IR (KBr) 3440, 3324, 1642, 1605, 1508, 1368, 1203 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 2.13–2.20 (2H, m), 2.74 (3H, d, J = 4.4 Hz), 3.15–3.23 (2H, m), 3.27–3.35 (2H, m), 3.61 (2H, t, J = 6.4 Hz), 3.80–3.86 (2H, m), 6.79 (2H, d, J = 8.8 Hz), 7.01 (2H, br s), 7.07 (1H, d, J = 5.4 Hz), 7.10 (2H, br s), 7.70 (2H, d, J = 8.8 Hz), 8.06 (1H, q, J = 4.4 Hz), 8.40 (1H, d, J = 5.4 Hz); MS (FAB) m/z: 425 [M+H]$^+$; Anal. Calcd for $C_{21}H_{24}N_6O_2S{\cdot}2.19H_2O$: C, 54.36; H, 6.17; N, 18.11; S, 6.91. Found: C, 54.54; H, 5.99; N, 18.04; S, 6.65.

5.1.81. 3-Amino-4-(4-{4-[2-(dimethylamino)-2-oxoethyl]phenyl}-1,4-diazepan-1-yl)thieno[2,3-*b*]pyridine-2-carboxamide (15w)

The title compound was prepared from **14w** in a similar manner described for **1**. Yield: 56%; pale yellow solid; mp 133–135 °C; IR (KBr) 3435, 3326, 3189, 2935, 2839, 1634, 1579, 1519 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 2.10–2.18 (2H, m), 2.82 (3H, s), 2.98 (3H, s), 3.15–3.23 (2H, m), 3.25–3.33 (2H, m), 3.49–3.56 (4H, m), 3.72–3.78 (2H, m), 6.71 (2H, d, J = 8.8 Hz), 7.00 (2H, br s), 7.03 (2H, d, J = 8.8 Hz), 7.07 (1H, d, J = 5.4 Hz), 7.09 (2H, br s), 8.39 (1H, d, J = 5.4 Hz); MS (FAB) m/z: 453 [M+H]$^+$; Anal. Calcd for $C_{23}H_{28}N_6O_2S{\cdot}1.16H_2O$: C, 58.35; H, 6.45; N, 17.75. Found: C, 58.10; H, 6.19; N, 18.03.

5.1.82. 3-Amino-4-[4-(2-pyridyl)-1,4-diazepan-1-yl]thieno[2,3-*b*]pyridine-2-carboxamide (15x)

The title compound was prepared from **12x** in a similar manner described for **15l**. Yield: 27%; pale yellow solid; mp 237–239 °C; IR (KBr) 3444, 3325, 3167, 1650, 1596, 1496, 1371, 942, 770 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.11–2.17 (2H, m), 3.17–3.32 (4H, m), 3.70 (2H, t, J = 6.3 Hz), 3.96–4.03 (2H, m), 6.57 (1H, dd, J = 7.0, 5.1 Hz), 6.69 (1H, d, J = 8.6 Hz), 7.01 (2H, br s), 7.07 (1H, d, J = 5.1 Hz), 7.10 (2H, br s), 7.51 (1H, ddd, J = 8.6, 7.0, 2.0 Hz), 8.09 (1H, dd, J = 5.1, 2.0 Hz), 8.39 (1H, d, J = 5.1 Hz); HRMS m/z calcd for $C_{18}H_{21}N_6OS$ 369.1498, found 369.1497; Anal. Calcd for $C_{18}H_{20}N_6SO{\cdot}0.3H_2O$: C, 57.83; H, 5.55; N, 22.48; S, 8.58. Found: C, 57.99; H, 5.35; N, 22.43; S, 8.59.

5.1.83. 3-Amino-4-[4-(4-pyridyl)-1,4-diazepan-1-yl]thieno[2,3-*b*]pyridine-2-carboxamide (15y)

The title compound was prepared from **12y** in a similar manner described for **15l**. Yield: 12%; pale yellow solid; mp 288–291 °C (decomposition); IR (KBr) 3334, 1650, 1597, 1573, 1510, 1367 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.13–2.18 (2H, m), 3.15–3.30 (4H, m), 3.56–3.59 (2H, m), 3.79–3.81 (2H, m), 6.68

(2H, d, J = 6.7 Hz), 6.98 (2H, br s), 7.06 (1H, d, J = 5.1 Hz), 7.09 (2H, br s), 8.09 (2H, d, J = 6.7 Hz), 8.38 (1H, d, J = 5.1 Hz); HRMS m/z calcd for $C_{18}H_{21}N_6OS$ 369.1498, found 369.1497; Anal. Calcd for $C_{18}H_{20}N_6OS{\cdot}0.3H_2O$: C, 57.83; H, 5.55; N, 22.48; S, 8.58. Found: C, 57.76; H, 5.48; N, 22.46; S, 8.50.

5.1.84. 3-Amino-4-(4-thiazol-2-yl-1,4-diazepan-1-yl)thieno[2,3-*b*]pyridine-2-carboxamide (15z)

The title compound was prepared from **12z** in a similar manner described for **15l**. Yield: 31%; pale yellow solid; mp 243–245 °C; IR (KBr) 3432, 3310, 3154, 1648, 1580, 1509, 1375, 933, 614 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.48–2.50 (2H, m), 3.19–3.37 (4H, m), 3.62 (2H, t, J = 5 9 Hz), 3.93–3.95 (2H, m), 6.73 (1H, d, J = 3.5 Hz), 7.00 (2H, br s), 7.06 (1H, d, J = 5.5 Hz), 7.10 (2H, br s), 7.13 (1H, d, J = 3.5 Hz), 8.39 (1H, d, J = 5.5 Hz); HRMS m/z calcd for $C_{16}H_{19}N_6OS_2$ 375.1062, found 375.1064; Anal. Calcd for $C_{16}H_{18}N_6OS_2{\cdot}1.1H_2O$: C, 48.74; H, 5.16; N, 21.31; S, 16.26. Found: C, 48.98; H, 5.03; N, 21.22; S, 15.89.

5.1.85. 3-Amino-4-piperazin-1-ylthieno[2,3-*b*]pyridine-2-carboxamide dihydrochloride (15aa)

Compound **15h** (340 mg, 0.901 mmol) was suspended in 1,4-dioxane (10 mL) and was added 4 M hydrogen chloride in 1,4-dioxane (4.0 mL), and the mixture was stirred at room temperature for 2 h. The solvent was removed in vacuo to give the title compound (316 mg, 100%) as yellow solid. Mp 270–280 °C; IR (KBr) 3320, 3180, 2925, 2770, 2717, 1648, 1604, 1446, 1395, 1259, 1059, 973, 906, 797, 556, 540, 516 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 3.58–4.28 (8H, br s), 7.13 (1H, d, J = 5.1 Hz), 7.24 (2H, br s), 8.53 (1H, d, J = 5.1 Hz), 9.45 (2H, br s); MS (FAB) m/z: 278 $[M+H]^+$; Anal. Calcd for $C_{12}H_{15}N_5OS{\cdot}2HCl{\cdot}H_2O$: C, 39.14; H, 5.20; N, 19.02. Found: C, 38.99; H, 5.13; N, 18.77.

5.1.86. 4-[4-(3-Amino-2-carbamoyl-thieno[2,3-*b*]pyridin-4-yl)-1,4-diazepan-1-yl]benzoic acid (15ab)

Compound **15t** (130 mg, 0.296 mmol) was suspended in ethanol (6.0 mL) and 1 M aqueous solution of NaOH (3.0 mL) was added, and the mixture was stirred under reflux for 10 h. After cooling to room temperature, 1 M hydrochloric acid (3.0 mL) was added to the mixture, and the deposited solid was filtered and washed with water and ethanol to give the title compound (96.0 mg, 79%) as pale yellow solid. IR (KBr) 3444, 3327, 3181, 2494, 1645, 1600, 1368, 1270, 1184, 939, 772 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.07–2.12 (2H, m), 3.15–3.22 (2H, m), 3.26–3.36 (2H, m), 3.62 (2H, t, J = 6.4 Hz), 3.76–3.88 (2H, m), 6.79 (2H, d, J = 9.0 Hz), 6.97 (2H, br s), 7.06 (1H, d, J = 5.4 Hz), 7.08 (2H, br s), 7.73 (2H, d, J = 9.0 Hz), 8.38 (1H, d, J = 5.4 Hz); MS (FAB) m/z: 412 $[M+H]^+$.

5.1.87. 3-Amino-4-[4-(4-carbamoylphenyl)-1,4-diazepan-1-yl]thieno[2,3-*b*]pyridine-2-carboxamide (15ac)

To a DMSO (2.0 mL) solution of **15s** (52.0 mg, 0.133 mmol) were added K_2CO_3 (13.0 mg, 0.0941 mmol) and dropwise 30% H_2O_2 (0.400 mL, 3.92 mmol), and the reaction mixture was stirred at room temperature for 1 h. Water was added to the mixture, and the deposited solid was filtered and washed with water and ethanol to give the title compound (38.7 mg, 71%) as pale yellow solid. Mp 165–167 °C; IR (KBr) 3442, 3325, 3188, 1645, 1601, 1367 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.11–2.22 (2H, m), 3.13–3.24 (2H, m), 3.26–3.35 (2H, m), 3.56–3.65 (2H, m), 3.79–3.87 (2H, m), 6.78 (2H, d, J = 9.0 Hz), 6.91 (1H, br s), 7.01 (2H, br s), 7.08 (1H, d, J = 5.5 Hz), 7.10 (2H, br s), 7.63 (1H, br s), 7.74 (2H, t, J = 9.0 Hz), 8.40 (1H, d, J = 5.5 Hz); MS (EI) m/z: 411 $[M+H]^+$; Anal. Calcd for $C_{20}H_{22}N_6O_2S{\cdot}1.2H_2O$: C, 55.59; H, 5.69; N, 19.45; S, 7.42. Found: C, 55.63; H, 5.57; N, 19.50; S, 7.49.

5.2. Biology

5.2.1. ALP assay

ST2 cells were seeded at 4000 cells/well in a 96 well plate (Falcon #3072) and incubated at 37 °C with or without test compound for 4 days. After the incubation period, the plate was washed by PBS twice. The ALP activity was assayed by the method of Lowry et al.[8]

5.2.2. Calcification assay

Bone marrow cells were prepared and cultured according to the method of Maniatopoulos et al.[10] Briefly, rat bone marrow cells were isolated from the femora of Wistar rats and cultured in a tissue culture flask. After 7 days, the cells were harvested, plated in dishes, and cultured with or without test compound for 10 days.

5.2.3. OVX rat study

All experimental procedures were performed in accordance with the in-house guideline of the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd. Eight-week-old F344 rats (Charles River) were either sham-operated or ovariectomized (OVX). OVX rats were orally administered with the test compound for 6 weeks. Sham-operated rats were also treated with vehicle (0.5% methylcellulose solution). Areal Bone mineral density (aBMD) of femurs and tibiae was measured by dual energy X-ray absorptiometry (DXA; DCS-600R, Aloka Co. Ltd., Tokyo, Japan). Serum osteocalcin was analyzed by the commercial ELISA kit (Biotrak, RPNJ404).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.01.071. These data include MOL files and InChiKeys of the most important compounds described in this article.

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