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Discovery of novel (4-piperidinyl)-piperazines as potent and orally active acetyl-CoA carboxylase 1/2 non-selective inhibitors: F-Boc and triF-Boc groups are acid-stable bioisosteres for the Boc group

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ABSTRACT

Novel (4-piperidinyl)-piperazine derivatives were synthesized and evaluated as ACC1/2 non-selective inhibitors. Optimization of the substituents on the nitrogen of the piperidine ring led to the identification of the fluorine substituted *tert*-butoxycarbonyl group. Advanced analog, 1,1,1-trifluoro-2-methylpropan-2-yl 4-{4-[(2-amino-6-methyl-1-benzothiophen-3-yl)carbonyl]piperazin-1-yl}piperidine-1-carboxylate (**12c**) showed potent inhibitory activities in enzyme-assay and cell-based assays. Compound **12c** also exhibited reduction of hepatic de novo fatty acid synthesis in rats after oral administration.

1. Introduction

Acetyl-CoA carboxylase (ACC) is a biotin-dependent homo oligomeric protein composed of a carboxyltransferase (CT), a biotin carboxyl carrier protein and biotin carboxylase (BC) domains. ACC is involved in the synthesis of malonyl-CoA from acetyl-CoA in an ATP-dependent manner. Malonyl-CoA works not only as a substrate for de novo fatty acid synthesis, but also as an allosteric inhibitor of carnitine palmitoyl transferase (CPT-1), a key enzyme that positively regulates mitochondrial β-oxidation. Therefore, inhibition of ACC is expected to reduce de novo fatty acid synthesis (FAS) and to enhance fatty acid β-oxidation (FAO) through disinhibition of CPT-1, which might benefit treatment of metabolic disorders such as obesity and type 2 diabetes.[1]

Two ACC isoforms, ACC1 and ACC2, have been cloned in rodents and humans. ACC1 is predominantly expressed in lipogenic tissues such as liver and adipose tissue, whereas ACC2 is predominantly expressed in oxidative tissues such as liver, skeletal muscle and heart.[2] Recent studies reported by Harada et al. indicate that hepatic ACC2 could partially cover ACC1 function as a backup system.[3] Consequently, reduction of malonyl-CoA levels in these tissues by ACC1/2 non-selective inhibitors is expected to reduce de novo fatty acid synthesis and triglyceride (TG)-rich lipoprotein secretions in liver while increasing fatty acid β-oxidation in liver and skeletal muscle. Therefore, an ACC1/2 non-selective inhibitor might provide a novel therapeutic approach for treating various metabolic disorders.

Several classes of small molecule ACC1/2 non-selective inhibitors have been reported to date (Fig. 1).[4] A piperidinylpiperidine class of ACC1/2 non-selective inhibitors was disclosed by the Pfizer group where compound **1** (CP-640186) was shown to significantly inhibit fatty acid synthesis in diabetic animal models.[1a] A spirochromanones class of ACC inhibitors **2**, **3**, and, **4** was disclosed by Merck-Banyu,[5] Takeda,[6] and Pfizer,[7,8] respectively. The Abbott group disclosed the aryl-ether derivative **5** as an ACC2 selective inhibitor.[9]

We have recently reported the structure–activity relationships of a (4-piperidinyl)-piperazine class of ACC1/2 non-selective inhibitors, as represented by compound **6a** (Fig. 2).[10] Compounds **6a** and **6b** showed potent inhibitory activity in the enzyme assay, whereas their activity in the HepG2 cell-based assay was considerably lower. Hence, we decided to further optimize compound **6** for improved cell activity and in vivo activity. Our efforts were focused on replacing the 2,6-diphenylpyridine and 1-acetyl-piperidine moieties of compound **6a** to improve the intrinsic potency and cell

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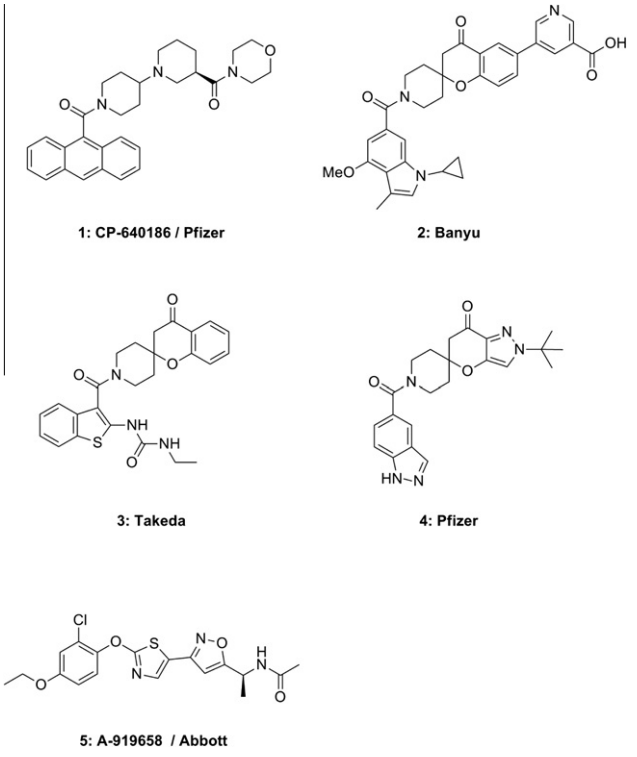


Figure 1. Structures of representative ACC inhibitors.

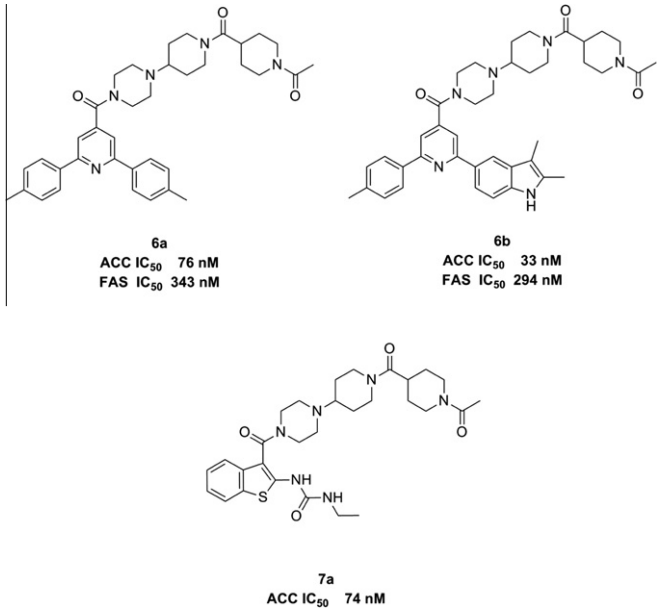


Figure 2. Compound **6a**, **6b** and novel potent ACC1/2 inhibitor **7a**.

permeability by reducing the molecular weight and molecular complexity such as the number of rings. By the evaluation of a variety of heterocycles for the replacement of the 2,6-diphenylpyridine moiety of compound **6a**, the 2-ureido benzothiophene group as in **7a** was found to be a suitable surrogate structure in terms of potency and molecular weight.

Further modification efforts were centered on compound **7a** and the identification of smaller and more potent substituents than the original 1-acetylpiperidin-4-yl group was attempted. Herein, we report the identification of potent and orally active ACC1/2 non-selective inhibitor **12c**, which features, a novel fluorine-substituted *tert*-butoxycarbonyl (F-Boc) group. The F-Boc group was found to be an acid-stable bioisostere for the Boc group.

2. Chemistry

The synthetic route for the target compounds is outlined in Scheme 1. Commercially available 2-amino-benzo[*b*]thiophene-3-carboxylic acid ethyl ester (**21**) was hydrolyzed to form **22**. The carboxylic acid **22** was coupled with *tert*-butoxy piperazine-1-carboxylate in the presence of EDCI, followed by removal of the *tert*-butoxycarbonyl (Boc) group to give **23**. The piperazine group of **23** was reductively alkylated with *tert*-butyl 4-oxopiperidine-1-carboxylate followed by cleavage of the Boc group to furnish **24**. The resulting diamine **24** was coupled with the desired acid chloride or chloroformate to afford **25**, which was treated with triphosgene and ethylamine to give the target compounds **7a–j**, **8a–f** and **9**.

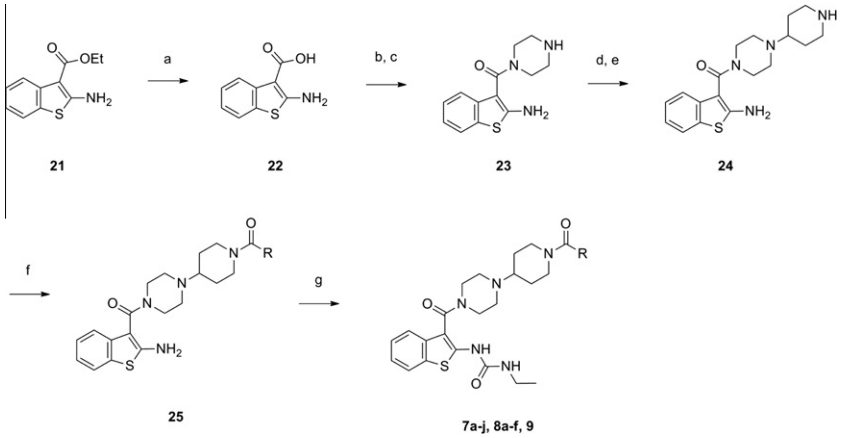
The synthetic route for the F-Boc intermediates is described in Scheme 2. Carbamoylimidazole **28a** was readily prepared from ethyl fluoroacetate (**26**) in two steps. Ethyl fluoroacetate (**26**) was reacted with excess methylmagnesium bromide to give **27a**, which was coupled with 1,1′-carbonyldiimidazole and treated with methyl iodide to furnish carbamoylimidazolium salt **28a**.[11] Addition of 4-piperidone to the carbamoylimidazolium salt **28a** yielded carbamate **29a**, which was reductively aminated with *tert*-butoxy piperazine-1-carboxylate to give **30a**. Removal of the Boc group of **30a** gave the key intermediate **31a**. The trifluoro-Boc intermediate **31b** was prepared from commercially available 2-trifluoromethyl-2-propanol (**27b**) in the same manner.

The preparation of the desired benzothiphene and thienopyridine derivatives is illustrated in Scheme 3. The corresponding benzothiophene carboxylic acids **22** and **32a–c** or thienopyridine carboxylic acids **33** and **34a–c**[12] were coupled with the intermediates **31a** and **31b** to afford **35**, **36**, and **37**. For compound **37**, the *p*-methoxy benzyl group was cleaved prior to the final urea formation. Treatment of **35**, **36**, and **38** with triphosgene and ethylamine gave the target compounds **10a–b**, **12b–c**, **13–20**.

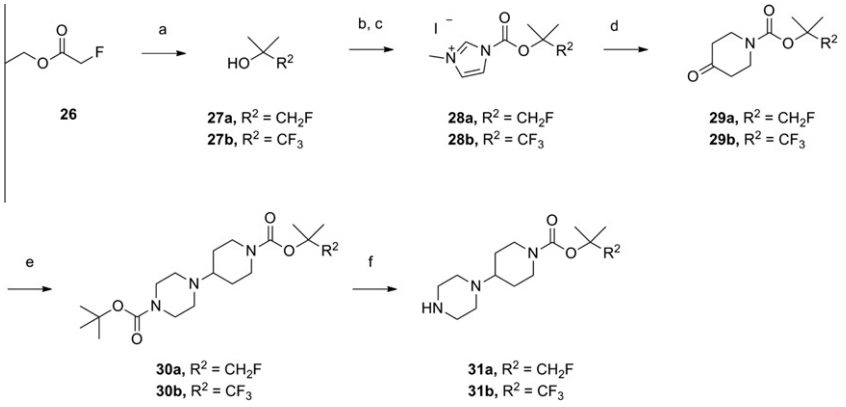
3. Results and discussion

The derivatives were screened against partially purified human liver ACC enzymes, and the subtype selectivity of the optimized compounds was confirmed using recombinant human ACC1 and ACC2 enzymes. Among the tested compounds, selected potent compounds were further evaluated for their ability to decrease fatty acid synthesis (FAS) and increase fatty acid oxidation (FAO) in HepG2 cells. FAS inhibition was assessed by measuring the decrease in [^{14}C] acetate incorporation into cellular lipids,[1a] and the effects on FAO were evaluated by measuring the generation of T_2O in the culture media.[13]

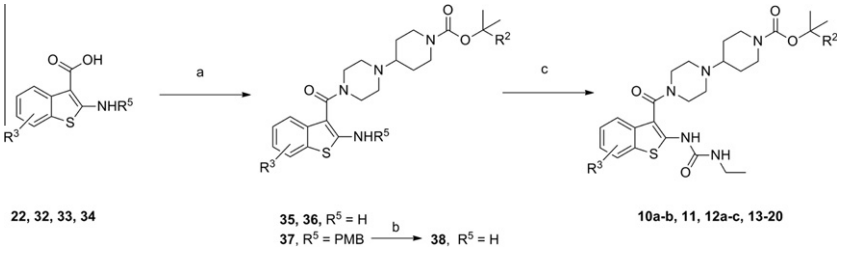
Activities resulting from modification of the right hand moiety of **6a** are shown in Table 1. Replacement of the 1-acetylpiperidine moiety with small alkyl groups such as methyl and ethyl groups resulted in a reduction of potency. Of the amide derivatives **7b–h**, lipophilic bulky groups showed an increase in potency. The cyclopentyl derivative **7h** was 38-fold more potent than the methyl derivative **7b**. The cyclohexyl derivative **7i** was equipotent to compound **7a**. The *tert*-butyl derivative **7j** showed slightly reduced potency compared to **7a**. We were glad to find that the carbamate derivatives **8a–f** had good inhibitory activity. Of them the *tert*-butoxy derivative **8f** was the most potent derivative. The corresponding urea derivative **9** exhibited decreased potency relative to **8f**. The potent analog **8f**, which has an IC_{50} value of 24 nM in the enzyme assay, displayed potent inhibitory activity against fatty



Scheme 1. Reagents and conditions: (a) NaOH, EtOH–THF, rt; (b) *tert*-butoxy piperazine-1-carboxylate, EDCI, HOBt, $CHCl_3$, rt; (c) 4 M HCl–AcOEt, AcOEt, rt; (d) *tert*-butyl 4-piperidone-1-carboxylate, rt, $CHCl_3$, then $NaBH(OAc)_3$, rt; (e) 4 M HCl–AcOEt, AcOEt, rt; (f) (i) R^1COCl, Et_3N, $CHCl_3$, 0 °C; (g) (i) triphosgene, Et_3N, 70% ethylamine aq, $CHCl_3$, 0 °C; (ii) 4 M HCl–AcOEt, or 1 M maleic acid in AcOEt.



Scheme 2. Reagents and conditions: (a) MeMgBr, THF, −60 to 0 °C; (b) CDI, $CHCl_3$, rt; (c) MeI, CH_3CN, rt; (d) 4-piperidone trifluoroacetate, Et_3N, CH_3CN, rt; (e) *tert*-butoxy piperazine-1-carboxylate, $NaBH(OAc)_3$, rt; (f) 4 M HCl–AcOEt, AcOEt, rt.



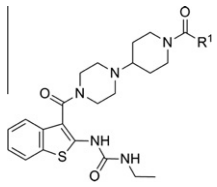
Scheme 3. Reagents and conditions: (a) **31a** or **31b**, EDCI, HOBt, Et_3N, DMF; (b) TFA, $CHCl_3$, rt; (c) (i) ethyl isocyanate, pyridine, 60 °C; (ii) 4 M HCl–AcOEt.

acid synthesis in HepG2 cells with an IC_{50} value of 79 nM. Although compound **8f** displayed attractive activity, **8f** utilizes the *tert*-butoxycarbonyl (Boc) group as a pharmacophore, which is readily cleaved from an amino group under acidic conditions. In fact, only 23% of the parent was recovered after incubation of compound **8f** in simulated gastric media (pH 1.2) for 3 h (Table 2). Because of the stability of the *tert*-butyl cation, the Boc group is labile to acidic conditions. We thought that introduction of electron-withdrawing groups to the *tert*-butyl group would destabilize the corresponding cation structure, thus making the group stable under acidic conditions. Hence, fluorine-substituted derivatives **10a** and **10b** were prepared and evaluated (Table 2.). The F-Boc and triF-Boc derivatives **10a** and **10b** retained potency in both the enzyme and cell assays. As anticipated, compounds **10a** and **10b** were stable after incubation in the simulated gastric media. Monofluoro substitution was shown to have a significant stabilizing effect.

The metabolic stabilities of compounds **10a** and **10b** were assessed using a human microsomal incubation assay. Only 3% and 5% of the intact compounds were recovered for **10a** and **10b**, respectively, indicating that compounds **10a** and **10b** are metabolically unstable (Table 3). Similarly, the isopropyl carbamate derivative **8c** showed very poor metabolic turnover (3%). The metabolite identification studies of **8c** revealed that the main metabolic pathway is the oxidation of the benzothiophene moiety.

Consequently, we evaluated the substituent effect on the benzothiophene moiety of compounds **10a** and **10b** (Table 3). The 5-methyl derivative **11** was equipotent to the parent **8c** but metabolically unstable, whereas the 6-methyl derivatives **12a–c**

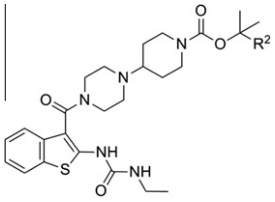
Table 1
SAR of benzothiophene derivatives: variation of the amide, carbamate and urea moieties



Compd	R^1	ACC IC_{50}[a] (nM)	FAS IC_{50}[b] (nM)
6a		76	343
6b		33	294
7a	1-Acetylpiperidin-4-yl	74	ND[c]
7b	Me	1864	ND[c]
7c	Et	597	ND[c]
7d	*n*-Pr	242	ND[c]
7e	*n*-Bu	207	ND[c]
7f	*iso*-Bu	144	ND[c]
7g	*iso*-Pr	131	ND[c]
7h	*c*-Pentyl	49	309
7i	*c*-Hex	81	146
7j	*tert*-Bu	109	450
8a	MeO	175	ND[c]
8b	EtO	55	228
8c	*iso*-PrO	28	208
8d	*c*-PeO	52	512
8e	*c*-HexO	76	635
8f	*tert*-BuO	24	79
9	*tert*-BuNH	119	775

[a] Inhibitory activity of compounds on the malonyl-CoA synthesis of human ACC1/2.
[b] Inhibitory activity of fatty acid synthesis in HepG2 cells.
[c] ND = No data.

Table 2
In vitro activities of the disubstituted 4-(4-piperidinyl)-piperazines **8f**, **10a** and **10b**

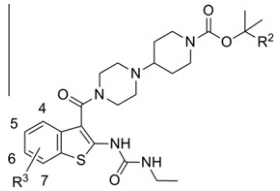


Compd	R^2	ACC IC_{50}[a] (nM)	FAS IC_{50}[b] (nM)	Acid stability[c] (%)
8f	CH_3	24	79	23
10a	CH_2F	17	61	98
10b	CF_3	31	81	100

[a] Inhibitory activity of compounds on the malonyl-CoA synthesis of human ACC1/2. The IC_{50} value represents the mean from at least two independent experiments.
[b] Inhibitory activity of fatty acid synthesis in HepG2 cells.
[c] HPLC determination after 3 h incubation at 37 °C in aqueous solution (pH 1.2).

exhibited a 2-fold decrease in potency relative to the compounds **8c**, **10a**, and **10b**. However, the 6-methyl derivatives showed a significant improvement in metabolic stability. The 6-trifluoromethyl derivative **13** and 6-fluoro derivative **14** displayed decreased potency and poor metabolic stability. The 6-chloro and 6-methoxy derivatives **15** and **16** were equipotent to the parent **10b**. Among the thienopyridine derivatives **17**–**20**, compound **17** was significantly potent, but its metabolic stability was poor. In the cell-based FAS assay, the isopropoxy derivatives **8c**, **11**, and **12a** exhibited an 8–10-fold decrease in inhibitory activity relative to the enzyme activities. In contrast, the F-Boc and triF-Boc derivatives **10a**, **10b**, **12b**, **12c**, and **13**–**20** showed small discrepancies between the enzyme and cell activities. In particular, compound **12c** displayed no discrepancy between these two assays.

Table 3
In vitro activities of the disubstituted 4-(4-piperidinyl)-piperazines **10a**–**b**, **8c**, **11**–**20**



Compd	R^2	R^3	ACC IC_{50}[a] (nM)	FAS IC_{50}[b] (nM)	hMS[c] (%)
10a	CH_2F	H	17	61	3
10b	CF_3	H	31	81	5
8c	H	H	28	208	3
11	H	5-Me	32	254	3
12a	H	6-Me	60	628	77
12b	CH_2F	6-Me	32	73	84
12c	CF_3	6-Me	58	58	87
13	CF_3	6-CF_3	98	ND[d]	7
14	CF_3	6-F	62	ND[d]	17
15	CF_3	6-Cl	38	ND[d]	51
16	CF_3	6-MeO	27	67	29
17	CF_3	4-Aza	12	49	13
18	CF_3	5-Aza	117	ND[d]	ND[d]
19	CF_3	6-Aza	41	152	67
20	CF_3	7-Aza	45	67	3

[a] Inhibitory activity of compounds on the malonyl-CoA synthesis of human ACC1/2. The IC_{50} value represents the mean from at least two independent experiments.
[b] Inhibitory activity of fatty acid synthesis in HepG2 cells.
[c] Fraction (%) remaining after 15-min incubation with human liver microsomes (1 mg protein/mL).
[d] ND = No data.

The pharmacological profiles of **12c** are summarized in Table 4 those of **6**. Compound **12c** similarly inhibited human and rat liver cytosolic ACC activities. Regarding the isoform selectivity, compound **12c** favorably inhibited human recombinant ACC2 (rhACC2) preferentially over human recombinant ACC1 (rhACC1).[14] In HepG2 cell assays, compound **12c** showed dose-dependent inhibition of fatty acid synthesis, with an IC_{50} of 0.06 μM, and increased fatty acid oxidation, with an EC_{50} of 0.37 μM. The pharmacokinetic profile of **12c** was examined in Sprague–Dawley (SD) rats. Compound **12c** had excellent plasma exposure owing to good bioavailability. Notably, the concentration of **12c** in liver was quite high (10300 ng/g tissue) compared to plasma (435 ng/mL) at 1 h

Table 4
Pharmacological profiles and plasma exposure of **6a** and **12c**

Pharmacological profiles	**6a**	**12c**
Enzyme assay[a]		
Human ACC1/2	76 nM	58 nM
Rat ACC1/2	70 nM	32 nM
Recombinant human ACC1	101 nM	192 nM
Recombinant human ACC2	23 nM	95 nM
Cell-based assay		
HepG2 cell FAS (IC_{50})	0.34 μM	0.06 μM
HepG2 cell FAO (EC_{50})	0.58 μM	0.37 μM
Plasma exposure after po[b]		
C_{max} (ng/mL)	107	603
T_{max} (h)	4.00	5.33
AUC (ng/mL h)	1000	7480

[a] Inhibitory activity of compounds on the malonyl-CoA synthesis of human ACC1/2, rat ACC1/2, recombinant hACC1 and hACC2. The IC_{50} value represents the mean from at least two independent experiments.
[b] Plasma exposure after single oral administration of **6a** and **12c** at a dose of 10 mg/kg to male Sprague–Dawley rats.

after 10 mg/kg oral administration. Therefore, we selected the compound **12c** as a test compound for efficacy studies in animal models of hyperlipidemia.

Acute effects of compound **12c** on the fatty acid synthesis were examined in SD rats as described in Section 5.1. Consistent with its potent intrinsic activity and high exposures in livers, compound **12c** (10 mg/kg, po) potently suppressed de novo fatty acid synthesis of livers (by 74.6%) at 1 h post administration.

Encouraged by the potent inhibitory effects found in the acute model, we evaluated the chronic efficacy of **12c** in fructose-drinking rats. Fructose feeding provides a dietary model of hypertriglycemia because fructose stimulates hepatic de novo lipogenesis and VLDL production in rats.[15,16] Compound **12c** reduced plasma and liver triglyceride levels of fructose-drinking rats in a dose-dependent manner with a minimum effective dose of 3–10 mg/kg (Fig. 3). In contrast, Benzafibrate, a PPAR-α agonist, reduced plasma triglyceride levels but not liver triglyceride levels. These observations suggest compound **12c**, a potent non-selective ACC1/2 inhibitor, effectively ameliorates hypertriglycemia and steatosis by dual actions: blockage of de novo lipid synthesis and increase in fat burning. At the same time, considerable skin irritation was observed around the nose and all toes of the rats due to systemic distribution. Considering that lipids are essential components of skin barriers and several transgenic animals lacking lipid synthesis enzymes (i.e., SCD-1,[17] DGAT-1[18]) develop skin damage, we could not exclude the possibility that the skin irritations observed here

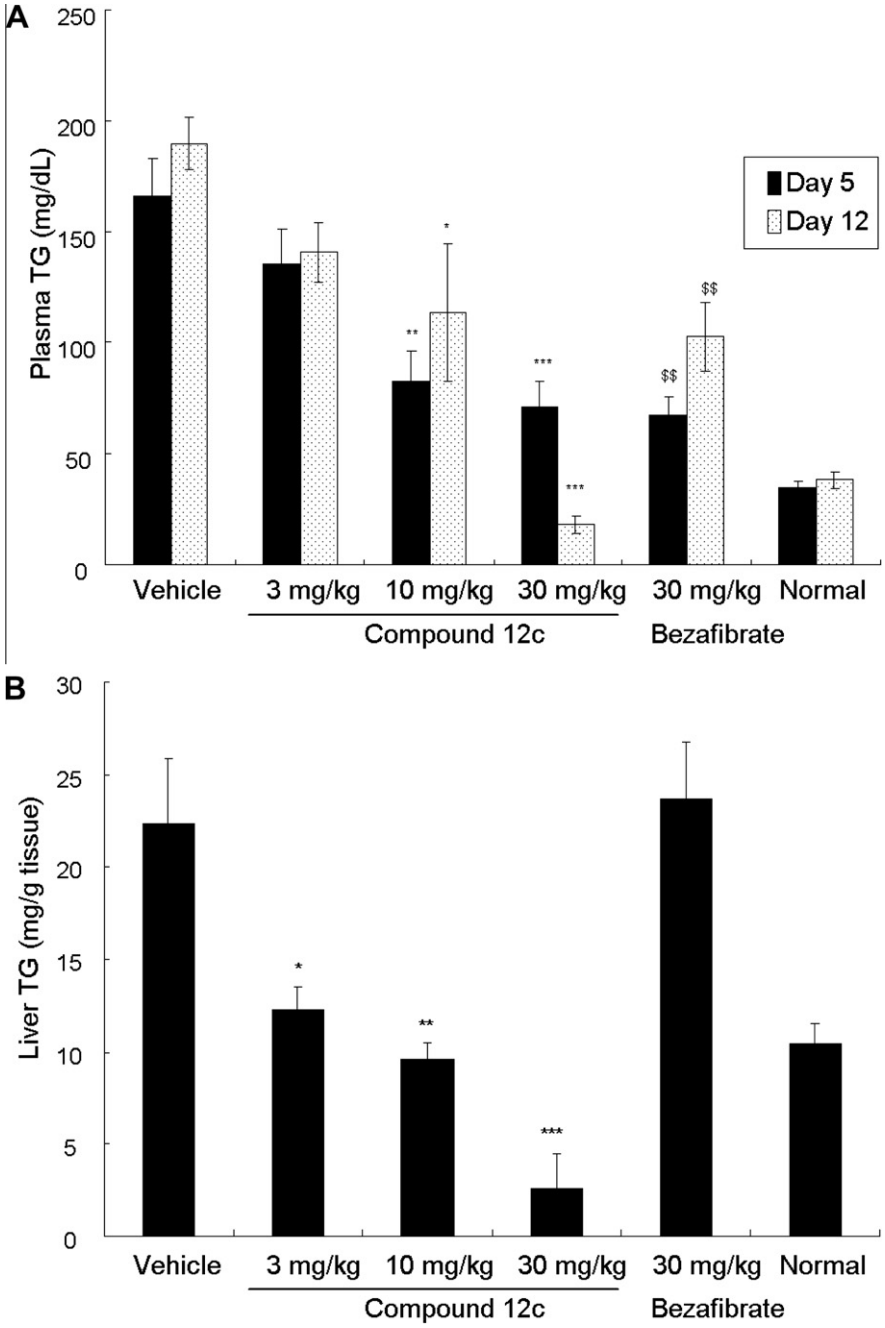


Figure 3. Effect of **12c** and Bezafibrate on the reduction of (A) plasma and (B) liver triglyceride levels in fructose-drinking rats. Plasma triglyceride levels were measured on days 5 and 12. Liver triglyceride levels were measured on day 12. *Values significantly different from Vehicle (p <0.05). **Values significantly different from Vehicle (p <0.01). ***Values significantly different from Vehicle (p <0.001). Data are means ± S.E.

were mechanism-based. For further understanding of the therapeutic potential ACC inhibitors, an examination of the elaboration of this study would be required.

4. Conclusion

In summary, a novel (4-piperidinyl)-piperazine class of compounds as ACC1/2 non-selective inhibitors was designed, synthesized and evaluated. Replacement of both substituents of the (4-piperidinyl)-piperazine core of parent compound **6a** gave novel derivative **12c** with a unique triF-Boc group that exhibited potent ACC1/2 non-selective inhibitory activity and good oral activity. Compound **12c** potently reduced liver triglyceride levels and ameliorated hypertriglycemia of fructose-drinking rats. These results suggest that ACC1/2 non-selective inhibitors might have potential in the treatment of metabolic disorders such as obesity and type 2 diabetes.

5. Experimental

5.1. Materials and methods

Unless otherwise noted, all solvents, chemicals, and reagents were obtained commercially and used without purification. Reactions were monitored by TLC analysis using Merck Silica Gel 60 F_{254} thin-layer plates. Column chromatography was carried out on a silica gel KANTO Reagents 60 N (spherical, neutral) or Fuji silysia chemical Chromatorex NH. The ^{1}H NMR spectra were obtained at 300 MHz on a Varian Instruments INOVA 300, 500 MHz on a JEOL ECA500, or 600 MHz on a JEOL JNM-ECA600 with chemical shift (δ, ppm) reported relative to tetramethylsilane as an internal standard. Electrospray Ionization (ESI) mass spectra were taken on a micromass Platform-LC mass spectrometer or Shimadzu LCMS-2010EV. Elemental analysis was performed using Perkin–Elmer 2400II or Yanaco MT-6 and are within ±0.4% of theory unless otherwise noted.

5.2. Chemistry

5.2.1. 2-Amino-1-benzothiophene-3-carboxylic acid (22)

To a solution of 2-amino-benzo[*b*]thiophene-3-carboxylic acid ethyl ester (5.24 g, 23.6 mmol) in ethanol–tetrahydrofuran (1:1, 60.0 mL) was added 2 M aqueous sodium hydroxide solution (60.0 mL) at room temperature, and the mixture was refluxed for 8 h. After the removing solvents in vacuo, the residue was added water and 2 M aqueous hydrochloric acid solution to adjusted pH 5.0. The resulting precipitate was obtained by filtration, washed with water and dried in vacuo to give **22** (4.34 g, 95%) as a pink powder. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.00–7.07 (1H, m), 7.18–7.25 (1H, m), 7.58 (1H, d, J = 7.3 Hz), 7.92 (2H, br s), 7.95–8.02 (1H, m), 12.28 (1H, br s); MS (ESI): m/z 191 $[M-H]^+$.

5.2.2. (2-Amino-1-benzothiophen-3-yl)(piperazin-1-yl)methanone (23)

A mixture of **22** (4.00 g, 20.7 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (4.75 g, 24.8 mmol), *tert*-butoxy piperazine-1-carboxylate (3.86 g, 20.7 mmol) and 1-hydroxybenzotriazole monohydrate (HOBt) (3.36 g, 24.8 mmol) in chloroform (60.0 mL) was stirred for 16 h. The reaction mixture was washed with brine, the organic layer was dried over magnesium sulfate and concentration in vacuo. The residue was dissolved in ethyl acetate (30.0 mL). To the mixture was added 4 M hydrogen chloride in ethyl acetate (30.0 mL, 120 mmol) and the mixture was stirred for 4 h. The resulting precipitate was obtained by filtration and dissolved in water. The aqueous solution was basified by addition of 2 M aqueous sodium hydroxide solution and extracted with chloroform. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to afford **23** (3.87 g, 73%) as a colorless amorphous. Compound **23** was used for the next step without further purification. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.78–3.02 (4H, m), 3.44–3.69 (4H, m), 5.22 (2H, br s), 7.05–7.16 (1H, m), 7.23–7.43 (2H, m), 7.50–7.59 (1H, m).

5.2.3. (2-Amino-1-benzothiophen-3-yl)[4-(piperidin-4-yl)piperazin-1-yl]methanone (24)

A mixture of **23** (3.84 g, 14.6 mmol), *tert*-butyl 4-oxopiperidine-1-carboxylate (2.93 g, 14.6 mmol) and sodium tri-acetoxy borohidride (6.23 g, 29.4 mmol) in chloroform (40.0 mL) was stirred for 16 h at room temperature and quenched by addition of 2 M aqueous sodium hydroxide solution in ice bath. The mixture was diluted with water and extracted with chloroform twice. The combined organics were washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified by NH silica gel column chromatography (50% ethyl acetate/*n*-hexane) to afford *tert*-butyl 4-{4-[(2-amino-1-benzothiophen-3-yl)carbonyl]piperazin-1-yl}piperidine-1-carboxylate (5.97 g, 91%) as a colorless amorphous. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.36–1.48 (11H, m), 1.75–1.81 (2H, m), 2.38–2.45 (1H, m), 2.49–2.55 (2H, m), 2.60 –2.75 (4H, m), 3.52–3.58 (2H, m), 3.60–3.67 (2H, m), 4.05–4.24 (2H, m), 5.23 (2H, s), 7.09–7.13 (1H, m), 7.26–7.29 (1H, m), 7.37 (1H, d, J = 7.3 Hz), 7.54 (1H, d, J = 7.8 Hz).

To a solution of the carbamate (5.97 g, 13.4 mmol) in ethyl acetate (30.0 mL) was added 4 M hydrogen chloride in ethyl acetate (30.0 mL, 120 mmol). The reaction mixture was stirred for 2 days. The resulting precipitate was obtained by filtration and dissolved in water the aqueous solution was basified by addition of 2 M aqueous sodium hydroxide solution and extracted with chloroform. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to afford **24** (4.24 g, 92%) as an off-white amorphous. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.36–1.46 (2H, m), 1.78–1.84 (2H, m), 2.35–2.42 (1H, m), 2.49–2.69 (6H, m), 3.12–3.18 (2H, m), 3.52–3.69 (4H, m), 5.22 (2H, s), 7.08–7.14 (1H, m), 7.25–7.30 (1H, m), 7.37 (1H, d, J = 7.3 Hz), 7.54 (1H, d, J = 7.8 Hz); MS (ESI): m/z 345 $[M+H]^+$.

5.2.4. 1-(4-{4-[(2-Amino-1-benzothiophen-3-yl)carbonyl]-piperazin-1-yl}piperidin-1-yl)propan-1-one (25c)

To a solution of **24** (150 mg, 0.44 mmol) in chloroform (3.0 mL) were added triethylamine (0.06 mL, 0.44 mmol) and isobutyryl chloride (0.05 ml, 0.44 mmol) at 0 °C and the reaction was stirred for 1 h. The reaction mixture was added 1 M aqueous sodium hydroxide solution (3.0 ml) and the organic layer was washed with brine, dried over anhydrous magnesium sulfate. After concentration in vacuo, the residue was purified by NH silica gel column chromatography (10% methanol/chloroform) to afford **25c** (123 mg, 71%) as a colorless powder. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.14 (3H, t, J = 7.4 Hz), 1.36–1.46 (2H, m), 1.81–1.89 (2H, m), 2.34 (2H, q, J = 7.4 Hz), 2.47–2.67 (6H, m), 2.97–3.04 (1H, m), 3.52–3.58 (2H, m), 3.60–3.67 (2H, m), 3.87–3.93 (1H, m), 4.63–4.68 (1H, m), 5.24 (2H, s), 7.09–7.13 (1H, m), 7.25–7.30 (1H, m), 7.36 (1H, d, J = 8.3 Hz), 7.54 (1H, d, J = 7.8 Hz); MS (ESI): m/z 401 $[M+H]^+$.

The following compounds **25a**, **25d**–**p** were prepared from the corresponding starting materials in a similar manner to that described for **25c**.

5.2.5. 1-{4-[(4-{4-[(2-Amino-1-benzothiophen-3-yl)carbonyl]-piperazin-1-yl}piperidin-1-yl)carbonyl]piperidin-1-yl}-ethanone (25a)

Pale yellow amorphous (yield 51%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.35–1.47 (2H, m), 1.60–1.96 (6H, m), 2.09 (3H, s), 2.47–2.76

(8H, m), 3.01–3.14 (2H, m), 3.51–3.69 (4H, m), 3.84–3.99 (2H, m), 4.54–4.67 (2H, m), 5.21–5.28 (2H, m), 7.08–7.14 (1H, m), 7.26–7.30 (1H, m), 7.36 (1H, d, J = 8.3 Hz), 7.55 (1H, d, J = 7.8 Hz); MS (ESI): m/z 496 $[M-H]^-$.

5.2.6. 1-(4-{4-[(2-Amino-1-benzothiophen-3-yl)carbonyl]-piperazin-1-yl}piperidin-1-yl)butan-1-one (25d)

Colorless amorphous (yield 65%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.96 (3H, t, J = 7.3 Hz), 1.35–1.46 (2H, m), 1.61–1.69 (2H, m), 1.81–1.90 (2H, m), 2.28–2.32 (2H, m), 2.46–2.59 (4H, m), 2.60–2.66 (2H, m), 2.97–3.04 (1H, m), 3.52–3.58 (2H, m), 3.59–3.68 (2H, m), 3.88–3.94 (1H, m), 4.63–4.69 (1H, m), 5.24 (2H, s), 7.09–7.13 (1H, m), 7.25–7.30 (1H, m), 7.36 (1H, d, J = 7.8 Hz), 7.54 (1H, d, J = 7.8 Hz); MS (ESI): m/z 415 $[M+H]^+$.

5.2.7. 1-(4-{4-[(2-Amino-1-benzothiophen-3-yl)carbonyl]-piperazin-1-yl}piperidin-1-yl)pentan-1-one (25e)

Colorless amorphous (yield 44%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.92 (3H, t, J = 7.57 Hz), 1.32–1.46 (4H, m), 1.56–1.63 (2H, m), 1.81–1.90 (2H, m), 2.29–2.35 (2H, m), 2.46–2.68 (6H, m), 2.97–3.04 (1H, m), 3.51–3.68 (4H, m), 3.88–3.94 (1H, m), 4.63–4.70 (1H, m), 5.24 (2H, s), 7.09–7.13 (1H, m), 7.26–7.29 (1H, m), 7.36 (1H, d, J = 8.3 Hz), 7.55 (1H, d, J = 8.7 Hz); MS (ESI): m/z 429 $[M+H]^+$.

5.2.8. 1-(4-{4-[(2-Amino-1-benzothiophen-3-yl)carbonyl]-piperazin-1-yl}piperidin-1-yl)-3-methylbutan-1-one (25f)

Colorless amorphous (yield 65%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.96 (6H, d, J = 6.9 Hz), 1.34–1.45 (2H, m), 1.82–1.90 (2H, m), 2.05–2.15 (1H, m), 2.17–2.25 (2H, m), 2.46–2.67 (6H, m), 2.98–3.04 (1H, m), 3.52–3.68 (4H, m), 3.90–3.95 (1H, m), 4.66–4.70 (1H, m), 5.25 (2H, s), 7.09–7.13 (1H, m), 7.26–7.30 (1H, m), 7.36 (1H, d, J = 8.3 Hz), 7.54 (1H, d, J = 7.8 Hz); MS (ESI): m/z 429 $[M+H]^+$.

5.2.9. 1-(4-{4-[(2-Amino-1-benzothiophen-3-yl)carbonyl]-piperazin-1-yl}piperidin-1-yl)-2-methylpropan-1-one (25g)

Colorless amorphous (yield 67%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.08–1.15 (6H, m), 1.35–1.47 (2H, m), 1.82–1.92 (2H, m), 2.46–2.59 (4H, m), 2.60–2.67 (2H, m), 2.75–2.84 (1H, m), 2.98–3.06 (1H, m), 3.52–3.58 (2H, m), 3.60–3.68 (2H, m), 3.95–4.02 (1H, m), 4.64–4.71 (1H, m), 5.24 (2H, s), 7.08–7.13 (1H, m), 7.24–7.30 (1H, m), 7.35–7.38 (1H, m), 7.53–7.56 (1H, m); MS (ESI): m/z 415 $[M+H]^+$.

5.2.10. (2-Amino-1-benzothiophen-3-yl){4-[1-(cyclopentyl-carbonyl)piperidin-4-yl]piperazin-1-yl}methanone (25h)

Colorless amorphous (yield 67%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.33–1.47 (2H, m), 1.51–1.65 (2H, m), 1.68–1.92 (8H, m), 2.45–2.69 (6H, m), 2.83–2.94 (1H, m), 2.96–3.05 (1H, m), 3.48–3.71 (4H, m), 3.97–4.08 (1H, m), 4.63–4.71 (1H, m), 5.25 (2H, s), 7.07–7.15 (1H, m), 7.24–7.30 (1H, m), 7.36 (1H, d, J = 7.3 Hz), 7.54 (1H, d, J = 7.8 Hz); MS (ESI): m/z 441 $[M+H]^+$.

5.2.11. (2-Amino-1-benzothiophen-3-yl){4-[1-(cyclohexyl-carbonyl)piperidin-4-yl]piperazin-1-yl}methanone (25i)

Colorless amorphous (yield 63%); ^{1}H NMR (600 MHz, $CDCl_3$): δ1.21–1.31 (3H, m), 1.33–1.58 (4H, m), 1.65–1.73 (3H, m), 1.76–1.92 (4H, m), 2.43–2.57 (5H, m), 2.60–2.67 (2H, m), 2.96–3.04 (1H, m), 3.52–3.58 (2H, m), 3.60–3.68 (2H, m), 3.93–4.00 (1H, m), 4.63–4.70 (1H, m), 5.23 (2H, s), 7.09–7.13 (1H, m), 7.25–7.30 (1H, m), 7.36 (1H, d, J = 7.8 Hz), 7.54 (1H, d, J = 7.8 Hz); MS (ESI); m/z 455 $[M+H]^+$.

5.2.12. 1-(4-{4-[(2-Amino-1-benzothiophen-3-yl)carbonyl]-piperazin-1-yl}piperidin-1-yl)-2,2-dimethylpropan-1-one (25j)

Colorless amorphous (yield 64%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.27 (9H, s), 1.37–1.46 (2H, m), 1.83–1.89 (2H, m), 2.45–2.54 (3H, m), 2.61–2.67 (2H, m), 2.75–2.82 (2H, m), 3.52–3.68 (4H, m), 4.44–4.50 (2H, m), 5.24 (2H, s), 7.09–7.13 (1H, m), 7.25–7.30 (1H, m), 7.37 (1H, d, J = 8.3 Hz), 7.54 (1H, d, J = 7.9 Hz); MS (ESI): m/z 429 $[M+H]^+$.

5.2.13. Methyl 4-{4-[(2-amino-1-benzothiophen-3-yl)carbonyl]-piperazin-1-yl}piperidine-1-carboxylate (25k)

Pale yellow amorphous (yield 59%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.38–1.47 (2H, m), 1.76–1.83 (2H, m), 2.41–2.47 (1H, m), 2.48–2.55 (2H, m), 2.59–2.66 (2H, m), 2.72–2.82 (2H, m), 3.52–3.58 (2H, m), 3.60–3.70 (5H, m), 4.05–4.31 (2H, m), 5.23 (2H, s), 7.09–7.12 (1H, m), 7.25–7.29 (1H, m), 7.36 (1H, d, J = 7.8 Hz), 7.54 (1H, d, J = 7.8 Hz).

5.2.14. Ethyl 4-{4-[(2-amino-1-benzothiophen-3-yl)carbonyl]-piperazin-1-yl}piperidine-1-carboxylate (25l)

Colorless amorphous (yield 86%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.26 (3H, t, J = 7.1 Hz), 1.37–1.49 (2H, m), 1.75–1.85 (2H, m), 2.41–2.49 (1H, m), 2.49–2.56 (2H, m), 2.60–2.68 (2H, m), 2.71–2.83 (2H, m), 3.51–3.70 (4H, m), 4.07–4.30 (4H, m), 5.19–5.27 (2H, m), 7.12 (1H, t, J = 6.9 Hz), 7.23–7.31 (1H, m), 7.37 (1H, d, J = 7.8 Hz), 7.55 (1H, d, J = 7.8 Hz); MS (ESI): m/z 417 $[M+H]^+$.

5.2.15. Propan-2-yl 4-{4-[(2-amino-1-benzothiophen-3-yl)-carbonyl]piperazin-1-yl}piperidine-1-carboxylate (25m)

Colorless amorphous (yield 56%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.24 (6H, d, J = 6.0 Hz), 1.37–1.48 (2H, m), 1.75–1.85 (2H, m), 2.40–2.48 (1H, m), 2.49–2.57 (2H, m), 2.61–2.68 (2H, m), 2.69–2.81 (2H, m), 3.51–3.70 (4H, m), 4.07–4.31 (2H, m), 4.87–4.95 (1H, m), 5.19–5.27 (2H, m), 7.12 (1H, t, J = 7.6 Hz), 7.24–7.31 (1H, m), 7.37 (1H, d, J = 8.3 Hz), 7.55 (1H, d, J = 7.8 Hz); MS (ESI): m/z 431 $[M+H]^+$.

5.2.16. Cyclopentyl 4-{4-[(2-amino-1-benzothiophen-3-yl)-carbonyl]piperazin-1-yl}piperidine-1-carboxylate (25n)

Pale yellow amorphous (yield 73%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.35–1.46 (2H, m), 1.52–1.74 (6H, m), 1.74–1.89 (4H, m), 2.39–2.47 (1H, m), 2.47–2.55 (2H, m), 2.59–2.67 (2H, m), 2.68–2.79 (2H, m), 3.50–3.58 (2H, m), 3.59–3.68 (2H, m), 3.96–4.34 (2H, m), 5.06–5.11 (1H, m), 5.25 (2H, br s), 7.08–7.12 (1H, m), 7.25–7.29 (1H, m), 7.36 (1H, d, J = 7.8 Hz), 7.54 (1H, d, J = 7.8 Hz); MS (ESI): m/z 457 $[M+H]^+$.

5.2.17. Cyclohexyl 4-{4-[(2-amino-1-benzothiophen-3-yl)-carbonyl]piperazin-1-yl}piperidine-1-carboxylate (25o)

Pale yellow amorphous (yield 80%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.22–1.32 (1H, m), 1.33–1.47 (6H, m), 1.48–1.55 (1H, m), 1.65–1.72 (2H, m), 1.75–1.87 (4H, m), 2.40–2.47 (1H, m), 2.48–2.56 (2H, m), 2.60–2.67 (2H, m), 2.69–2.81 (2H, m), 3.51–3.58 (2H, m), 3.59–3.68 (2H, m), 4.11–4.27 (2H, m), 4.63–4.69 (1H, m), 5.23 (2H, br s), 7.09–7.13 (1H, m), 7.25–7.30 (1H, m), 7.36 (1H, d, J = 8.3 Hz), 7.54 (1H, d, J = 7.8 Hz); MS (ESI): m/z 471 $[M+H]^+$.

5.2.18. 4-{4-[(2-Amino-1-benzothiophen-3-yl)- carbonyl]piperazin-1-yl}-N-*tert*-butylpiperidine-1-carboxamide (25p)

Colorless amorphous (yield 99%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.35 (9H, s), 1.40–1.52 (2H, m), 1.77–1.86 (2H, m), 2.38–2.46 (1H, m), 2.48–2.57 (2H, m), 2.60–2.69 (2H, m), 2.70–2.78 (2H, m), 3.50–3.70 (4H, m), 3.89–3.97 (2H, m), 4.32 (1H, br s), 5.24 (2H, br s), 7.12 (1H, t, J = 7.6 Hz), 7.25–7.31 (1H, m), 7.37 (1H, d, J = 7.3 Hz), 7.55 (1H, d, J = 7.8 Hz); MS (ESI): m/z 444 $[M+H]^+$.

5.2.19. 1-Ethyl-3-(3-{[4-(1-propanoylpiperidin-4-yl)piperazin-1-yl]carbonyl}-1-benzothiophen-2-yl)urea (7c)

To a solution of **25c** (120 mg, 0.29 mmol) in chloroform (5.0 mL) were added triethylamine (0.08 mL, 0.58 mmol) and triphosgene (28 mg, 0.096 mmol) at 0 °C. The reaction mixture was stirred for 5 min and added 70% aqueous ethylamine solution (0.07 mL, 0.87 mmol) at room temperature. After stirring for 10 min, the solvent was removed in vacuo and the residue was purified by NH silica gel column chromatography (ethyl acetate) to afford **7c** (66 mg, 47%) as a colorless powder. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.07–1.17 (6H, m), 1.36–1.46 (2H, m), 1.81–1.89 (2H, m), 2.34 (2H, q, J = 7.5 Hz), 2.46–2.60 (4H, m), 2.61–2.69 (2H, m), 2.97–3.04 (1H, m), 3.24–3.30 (2H, m), 3.51–3.73 (4H, m), 3.87–3.94 (1H, m), 4.63–4.70 (1H, m), 5.02–5.08 (1H, m), 7.19–7.23 (1H, m), 7.31–7.35 (1H, m), 7.43 (1H, d, J = 7.8 Hz), 7.72 (1H, d, J = 7.8 Hz), 9.42 (1H, br s).; MS (ESI): m/z 472 $[M+H]^+$. Anal. Calcd for $C_{24}H_{33}N_5O_3S{\cdot}0.2H_2O$: C, 60.66; H, 7.08; N, 14.74. Found: C, 60.94; H, 6.95; N, 14.44.

The following compounds **7a**, **7b**, **7d**–**j**, **8a**–**f**, and **9** were prepared from the corresponding starting materials in a similar manner to that described for **7c**. Compounds **7a**, **7h**, **8d** and **8f** were obtained as maleate.

5.2.20. 1-{3-[(4-{1-[(1-Acetylpiperidin-4-yl)carbonyl]piperidin-4-yl}piperazin-1-yl)carbonyl]-1-benzothiophen-2-yl}-3-ethylurea maleate (7a)

The corresponding free amine was obtained as a pale yellow amorphous (yield 82%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.99–1.07 (3H, m), 1.34–1.47 (2H, m), 1.58–1.96 (6H, m), 2.10 (3H, s), 2.46–2.77 (8H, m), 3.02–3.15 (2H, m), 3.17–3.26 (2H, m), 3.53–3.67 (4H, m), 3.85–3.99 (2H, m), 4.54–4.68 (2H, m), 5.27 (1H, br s), 7.17–7.24 (1H, m), 7.30–7.36 (1H, m), 7.42 (1H, d, J = 8.3 Hz), 7.72 (1H, d, J = 8.3 Hz), 9.40 (1H, br s); MS (ESI): m/z 569 $[M+H]^+$. To a solution of the free amine (549 mg, 0.97 mmol) in ethylacetate (4.0 mL) and ethanol (1.0 ml)was added 0.1 M ethylacetate maleic acid solution (9.7 mL, 0.97 mmol) at room temperature. After the stirring overnight, the resultant precipitate was collected by filtration (562 mg) as a colorless powder. Anal. Calcd for $C_{29}H_{40}N_6O_4S{\cdot}1.0C_4H_4O_4{\cdot}3.0H_2O$: C, 53.65; H, 6.82; N, 11.37. Found: C, 53.76; H, 6.45; N, 10.98.

5.2.21. 1-(3-{[4-(1-Acetylpiperidin-4-yl)piperazin-1-yl]carbonyl}-1-benzothiophen-2-yl)-3-ethylurea (7b)

Colorless powder (yield 38%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.93–1.00 (3H, m), 1.35–1.49 (2H, m), 1.79–1.89 (2H, m), 2.09 (s, 3 H), 2.47–2.68 (6H, m), 3.02–3.09 (1H, m), 3.14–3.21 (2H, m), 3.54–3.67 (4H, m), 3.82–3.89 (1H, m), 4.61–4.67 (1H, m), 5.43 (1H, br s), 7.19–7.24 (1H, m), 7.30–7.35 (1H, m), 7.41 (1H, d, J = 7.8 Hz), 7.72 (1H, d, J = 8.3 Hz), 9.39 (1H, br s); MS (ESI): m/z 458 $[M+H]^+$. Anal. Calcd for $C_{23}H_{31}N_5O_3S{\cdot}0.2H_2O$: C, 59.90; H, 6.86; N, 15.19. Found: C, 59.91; H, 6.86; N, 15.03.

5.2.22. 1-(3-{[4-(1-Butanoylpiperidin-4-yl)piperazin-1-yl]-carbonyl}-1-benzothiophen-2-yl)-3-ethylurea (7d)

Colorless powder (yield 14%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.93–0.99 (3H, m), 1.04–1.15 (3H, m), 1.34–1.46 (2H, m), 1.60–1.69 (2H, m), 1.78–1.91 (2H, m), 2.24–2.33 (2H, m), 2.42–2.59 (4H, m), 2.61–2.69 (2H, m), 2.98–3.04 (1H, m), 3.24–3.30 (2H, m), 3.52–3.78 (4H, m), 3.88–3.94 (1H, m), 4.64–4.69 (1H, m), 5.04–5.09 (1H, m), 7.19–7.23 (1H, m), 7.31–7.35 (1H, m), 7.43 (1H, d, J = 7.8 Hz), 7.72 (1H, d, J = 7.8 Hz), 9.41(1H, br s); MS (ESI): m/z 486 $[M+H]^+$. Anal. Calcd for $C_{25}H_{35}N_5O_3S{\cdot}0.4H_2O$: C, 60.93; H, 7.32; N, 14.21. Found: C, 60.95; H, 7.34; N, 14.33.

5.2.23. 1-Ethyl-3-(3-{[4-(1-pentanoylpiperidin-4-yl)piperazin-1-yl]carbonyl}-1-benzothiophen-2-yl)urea (7e)

Colorless powder (yield 54%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.93 (3H, t, J = 7.3 Hz), 1.03 (3H, t, J = 7.1 Hz), 1.32–1.47 (4H, m), 1.56–1.66 (2H, m), 1.80–1.90 (2H, m), 2.30–2.36 (2H, m), 2.47–2.70 (6H, m), 2.97–3.06 (1H, m), 3.18–3.26 (2H, m), 3.54–3.72 (4H, m), 3.88–3.95 (1H, m), 4.64–4.70 (1H, m), 5.20–5.26 (1H, m), 7.17–7.23 (1H, m), 7.30–7.36 (1H, m), 7.42 (1H, d, J = 8.3 Hz), 7.72 (1H, d, J = 7.8 Hz), 9.41 (1H, br s); MS (ESI): m/z 500 $[M+H]^+$. Anal. Calcd for $C_{26}H_{37}N_5O_3S$: C, 62.50; H, 7.46; N, 14.02. Found C, 62.58; H, 7.53; N, 13.96.

5.2.24. 1-Ethyl-3-[3-({4-[1-(3-methylbutanoyl)piperidin-4-yl]piperazin-1-yl}carbonyl)-1-benzothiophen-2-yl]urea (7f)

Colorless powder (yield 47%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.96 (6H, d, J = 6.4 Hz), 1.02 (3H t, J = 7.1 Hz), 1.34–1.47 (2H, m), 1.81–1.89 (2H, m), 2.06–2.15 (1H, m), 2.18–2.24 (2H, m), 2.46–2.69 (6H, m), 2.97–3.05 (1H, m), 3.17–3.25 (2H, m), 3.51–3.71 (4H, m), 3.89–3.96 (1H, m), 4.64–4.72 (1H, m), 5.23–5.29 (1H, m), 7.17–7.24 (1H, m), 7.30–7.35 (1H, m), 7.42 (1H, d, J = 7.8 Hz), 7.72 (1H, d, J = 7.8 Hz), 9.40 (1H, br s); MS (ESI): m/z 500 $[M+H]^+$. Anal. Calcd for $C_{26}H_{37}N_5O_3S$: C, 62.50; H, 7.46; N, 14.02. Found: C, 62.50; H, 7.61; N, 13.84.

5.2.25. 1-Ethyl-3-[3-({4-[1-(2-methylpropanoyl)piperidin-4-yl]piperazin-1-yl}carbonyl)-1-benzothiophen-2-yl]urea (7g)

Colorless powder (yield 67%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.06–1.16 (9H, m), 1.34–1.47 (2H, m), 1.81–1.92 (2H, m), 2.46–2.59 (4H, m), 2.62–2.71 (2H, m), 2.76–2.84 (1H, m), 2.98–3.07 (1H, m), 3.24–3.31 (2H, m), 3.50–3.73 (4H, m), 3.94–4.03 (1H, m), 4.64–4.72 (1H, m), 5.03–5.09 (1H, m), 7.19–7.23 (1H, m), 7.31–7.36 (1H, m), 7.44 (1H, d, J = 7.8 Hz), 7.72 (1H, d, J = 7.8 Hz), 9.41 (1H, br s); MS (ESI): m/z 486 $[M+H]^+$. Anal. Calcd for $C_{25}H_{35}N_5O_3S{\cdot}0.2H_2O$: C, 61.37; H, 7.29; N, 14.31. Found: C, 61.66; H, 7.19; N, 14.04.

5.2.26. 1-[3-({4-[1-(Cyclopentylcarbonyl)piperidin-4-yl]piperazin-1-yl}carbonyl)-1-benzothiophen-2-yl]-3-ethylurea maleate (7h)

The corresponding free amine was obtained as a colorless powder (yield 84%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.07 (3H, t, J = 7.1 Hz), 1.35–1.47 (2H, m), 1.52–1.63 (2H, m), 1.69–1.92 (8H, m), 2.48–2.60 (4H, m), 2.62–2.71 (2H, m), 2.90 (1H, q, J = 8.0 Hz), 3.02 (1H, t, J = 11.9 Hz), 3.22–3.29 (2H, m), 3.56–3.74 (4H, m), 4.03 (1H, d, J = 13.8 Hz), 4.69 (1H, d, J = 11.9 Hz), 5.13 (1H, br s), 7.22 (1H, t, J = 7.6 Hz), 7.34 (1H, t, J = 7.6 Hz), 7.44 (1H, d, J = 8.3 Hz), 7.73 (1H, d, J = 8.3 Hz), 9.43 (1H, br s); MS (ESI): m/z 512 $[M+H]^+$. The maleate was prepared from the free amine in a similar manner to that described for **7a**. Anal. Calcd for $C_{27}H_{37}N_5O_3S{\cdot}1.0C_4H_4O_4{\cdot}2.0H_2O$: C, 56.09; H, 6.83; N, 10.55. Found: C, 56.09; H, 6.76; N, 10.52.

5.2.27. 1-[3-({4-[1-(Cyclohexylcarbonyl)piperidin-4-yl]piperazin-1-yl}carbonyl)-1-benzothiophen-2-yl]-3-ethylurea (7i)

Colorless powder (yield 76%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.03–1.10 (2H, m), 1.21–1.32 (4H, m), 1.33–1.61 (5H, m), 1.62–1.73 (3H, m), 1.76–1.91 (4H, m), 2.41–2.57 (4H, m), 2.61–2.70 (2H, m), 2.97–3.04 (1H, m), 3.21–3.28 (2H, m), 3.51–3.73 (4H, m), 3.94–4.00 (1H, m), 4.64–4.71 (1H, m), 5.08–5.15 (1H, m), 7.19–7.23 (1H, m), 7.31–7.35 (1H, m), 7.43 (1H, d, J = 7.8 Hz), 7.72 (1H, d, J = 7.8 Hz), 9.41 (1H, br s); MS (ESI): m/z 526 $[M+H]^+$. Anal. Calcd for $C_{28}H_{39}N_5O_3S{\cdot}0.7H_2O$: C, 62.47; H, 7.56; N, 13.01. Found: C, 62.68; H, 7.52; N, 12.52.

5.2.28. 1-[3-({4-[1-(2,2-Dimethylpropanoyl)piperidin-4-yl]piperazin-1-yl}carbonyl)-1-benzothiophen-2-yl]-3-ethylurea (7j)

Pale yellow amorphous (yield 84%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.07 (3H, t, J = 7.1 Hz), 1.28 (9H, s), 1.36–1.46 (2H, m), 1.83–1.89 (2H, m), 2.47–2.56 (3H, m), 2.63–2.70 (2H, m), 2.74–2.83 (2H, m), 3.21–3.29 (2H, m), 3.55–3.73 (4H, m), 4.44–4.51 (2H, m), 5.13 (1H, br s), 7.18–7.25 (1H, m), 7.30–7.37 (1H, m), 7.43 (1H, d, J = 8.3 Hz), 7.72 (1H, d, J = 7.8 Hz), 9.42 (1H, br s); MS (ESI): m/z 500 $[M+H]^+$. Anal. Calcd for $C_{26}H_{37}N_5O_3S{\cdot}0.3H_2O$: C, 61.83; H, 7.50; N, 13.87. Found: C, 61.86; H, 7.43; N, 13.86.

5.2.29. Methyl 4-[4-({2-[(ethylcarbamoyl)amino]-1-benzothiophen-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate (8a)

Colorless powder (yield 37%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.07–1.13 (3H, m), 1.37–1.47 (2H, m), 1.75–1.83 (2H, m), 2.42–2.56 (3H, m), 2.62–2.69 (2H, m), 2.72–2.83 (2H, m), 3.24–3.31 (2H, m), 3.52–3.71 (7H, m), 4.06–4.31 (2H, m), 5.01–5.06 (1H, m), 7.19–7.23 (1H, m), 7.31–7.35 (1H, m), 7.42–7.45 (1H, m), 7.71–7.73 (1H, m), 9.41 (1H, br s); MS (ESI): m/z 474 $[M+H]^+$. Anal. Calcd for $C_{23}H_{31}N_5O_4S{\cdot}0.2H_2O$: C, 57.89; H, 6.63; N, 14.68. Found: C, 57.93; H, 6.54; N, 14.38.

5.2.30. Ethyl 4-[4-({2-[(ethylcarbamoyl)amino]-1-benzothiophen-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate (8b)

Colorless powder (yield 17%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.97–1.06 (3H, m), 1.26 (3H, t, J = 7.1 Hz), 1.37–1.48 (2H, m), 1.60 (2H, s), 1.74–1.85 (2H, m), 2.42–2.58 (3H, m), 2.61–2.70 (2H, m), 2.72–2.84 (2H, m), 3.17–3.25 (2H, m), 3.52–3.73 (2H, m), 4.07–4.31 (4H, m), 5.20–5.27 (1H, m), 7.22 (1H, t, J = 8.0 Hz), 7.34 (1H, t, J = 7.6 Hz), 7.43 (1H, d, J = 8.3 Hz), 7.73 (1H, d, J = 7.8 Hz), 9.41 (1H, br s); MS (ESI): m/z 488 $[M+H]^+$. Anal. Calcd for $C_{24}H_{33}N_5O_4S{\cdot}0.8H_2O$: C, 57.42; H, 6.95; N, 13.95. Found: C, 57.63; H, 6.87; N, 13.69.

5.2.31. Propan-2-yl 4-[4-({2-[(ethylcarbamoyl)amino]-1-benzothiophen-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate (8c)

Colorless powder (yield 69%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.99–1.06 (3H, m), 1.24 (6H, d, J = 6.0 Hz), 1.37–1.47 (2H, m), 1.55–1.64 (2H, m), 1.75–1.84 (2H, m), 2.42–2.49 (1H, m), 2.49–2.58 (2H, m), 2.61–2.69 (2H, m), 2.70–2.81 (2H, m), 3.18–3.25 (2H, m), 3.54–3.72 (2H, m), 4.09–4.30 (2H, m), 4.88–4.95 (1H, m), 5.20–5.26 (1H, m), 7.22 (1H, t, J = 7.6 Hz), 7.34 (1H, t, J = 6.9 Hz), 7.43 (1H, d, J = 7.8 Hz), 7.73 (1H, d, J = 7.8 Hz), 9.42 (1H, br s); MS (ESI): m/z 502 $[M+H]^+$. Anal. Calcd for $C_{25}H_{35}N_5O_4S{\cdot}0.8H_2O$: C, 58.19; H, 7.15; N, 13.57. Found: C, 58.45; H, 7.05; N, 13.32.

5.2.32. Cyclopentyl 4-[4-({2-[(ethylcarbamoyl)amino]-1-benzothiophen-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate (8d)

Pale yellow amorpous (yield 54%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.98–1.08 (3H, m), 1.22–1.32 (2H, m), 1.34–1.46 (2H, m), 1.64–1.74 (4H, m), 1.74–1.89 (4H, m), 2.37–2.58 (3H, m), 2.59–2.69 (2H, m), 2.69–2.80 (2H, m), 3.18–3.27 (2H, m), 3.40–3.78 (4H, m), 3.98–4.33 (2H, m), 5.06–5.11 (1H, m), 5.11–5.18 (1H, m), 7.18–7.23 (1H, m), 7.31–7.35 (1H, m), 7.43 (1H, d, J = 7.8 Hz), 7.72 (1H, d, J = 7.8 Hz), 9.41 (1H, br s); MS (ESI): m/z 528 $[M+H]^+$; The maleate was prepared from the free amine in a similar manner to that described for **7a**. Anal. Calcd for $C_{27}H_{37}N_5O_4S{\cdot}1.0H_2O$: C, 59.43; H, 7.20; N, 12.83. Found: C, 59.70; H, 7.05; N, 12.75.

5.2.33. Cyclohexyl 4-[4-({2-[(ethylcarbamoyl)amino]-1-benzothiophen-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate (8e)

Colorless amorphous (yield 80%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.99–1.07 (3H, m), 1.22–1.32 (2H, m), 1.33–1.47 (5H, m), 1.48–1.55 (1H, m), 1.64–1.73 (2H, m), 1.74–1.88 (4H, m), 2.40–2.59 (3H, m), 2.60–2.69 (2H, m), 2.69–2.82 (2H, m), 3.18–3.26 (2H, m), 3.48–3.76 (4H, m), 4.10–4.30 (2H, m), 4.63–4.70 (1H, m), 5.14–5.21 (1H, m), 7.18–7.23 (1H, m), 7.30–7.35 (1H, m), 7.43 (1H, d, J = 7.8 Hz), 7.72 (1H, d, J = 7.8 Hz), 9.41 (1H, br s); MS (ESI): m/z 542 $[M+H]^+$. Anal. Calcd for $C_{28}H_{39}N_5O_4S{\cdot}0.5H_2O$: C, 61.07; H, 7.32; N, 12.72. Found: C, 60.80; H, 7.30; N, 12.51.

5.2.34. *tert*-Butyl 4-[4-({2-[(ethylcarbamoyl)amino]-1-benzothiophen-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate (8f)

The corresponding free amine was obtained as a colorless powder (yield 51%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.01 (3H, t, J = 7.1 Hz), 1.37–1.45 (2H, m), 1.46 (9H, s), 1.76–1.81 (2H, m), 2.41–2.47 (1H, m), 2.50–2.59 (2H, m), 2.63–2.76 (4H, m), 3.17–3.25 (2H, m), 3.57–3.66 (4H, m), 4.10–4.29 (2H, m), 5.35 (1H, br s), 7.21 (1H, t, J = 7.1 Hz), 7.33 (1H, t, J = 7.3 Hz), 7.43 (1H, d, J = 7.8 Hz), 7.73 (1H, d, J = 7.8 Hz), 9.43 (1H, br s); MS (ESI): m/z 516 $[M+H]^+$. The maleate was prepared from the free amine in a similar manner to that described for **7a**. Anal. Calcd for $C_{26}H_{37}N_5O_4S{\cdot}1.0C_4H_4O_4{\cdot}1.7H_2O$: C, 54.40; H, 6.76; N, 10.57. Found: C, 54.42; H, 6.73; N, 10.72.

5.2.35. *N-tert*-Butyl-4-[4-({2-[(ethylcarbamoyl)amino]-1-benzothiophen-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxamide (9)

Colorless amorphous (yield 81%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.93–1.01 (3H, m), 1.34 (9H, s), 1.40–1.50 (2H, m), 1.55–1.67 (2H, m), 1.74–1.84 (2H, m), 2.39–2.46 (1H, m), 2.47–2.59 (2H, m), 2.60–2.68 (2H, m), 2.69–2.77 (2H, m), 3.12–3.23 (2H, m), 3.53–3.75 (2H, m), 3.88–3.96 (2H, m), 4.33 (1H, br s), 5.30–5.38 (1H, m), 7.20 (1H, t, J = 7.6 Hz), 7.33 (1H, t, J = 7.6 Hz), 7.42 (1H, d, J = 7.8 Hz), 7.72 (1H, d, J = 7.8 Hz), 9.41 (1H, br s); MS (ESI): m/z 515 $[M+H]^+$: Anal. Calcd for $C_{26}H_{38}N_6O_3S{\cdot}0.2H_2O$: C, 60.25; H, 7.47; N, 16.04. Found; C, 60.15; H, 7.44; N, 16.04.

5.2.36. 1-Fluoro-2-methylpropan-2-ol (27a)

To a solution of ethyl fluoroacetate (21.2 g, 200 mmol) in tetrahydrofuran (200 mL) was added dropwise 3.0 M diethylether methyl magnesium bromide solution (128 ml, 414 mmol) at −60 °C and the reaction was stirred for 1 h under argon atmosphere. The mixture was allowed to warm to 0 °C and stirred for 4 h. To this was added water (100 mL), concd hydrochloride (36.0 mL) and sodium chloride (20.0 g), then extracted with Ether (200 mL). The organic layer was dried over sodium sulfate and distilled under normal pressure at 71–102 °C to afford a solution of **27a** in tetrahydrofuran (123 mmol, 62%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.25 (3H, s), 1.26 (3H, s), 4.20 (2H, d, J = 47.5 Hz).

5.2.37. 1-{[(1-Fluoro-2-methylpropan-2-yl)oxy]carbonyl}-3-methyl-1H-imidazol-3-ium iodide (28a)

A mixture of **27a** (10.1 g, 110 mmol) and 1,1′-carbonyldiimidazole (CDI) (35.7 g, 220 mmol) in chloroform (220 mL) was stirred at room temperature for 13 h under nitrogen atmosphere. The reaction mixture was poured into water (200 mL) and the organic layer was washed with brine (100 mL). The aqueous layer was extracted with chloroform (100 mL). The combined organics were dried over anhydrous magnesium sulfate and concentrated in vacuo to afford 1-fluoro-2-methylpropan-2-yl-1*H*-imidazole-1-carboxylate (21.7

g, 99.9 mmol) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.64 (3H, s), 1.65 (3H, s), 4.56 (2H, d, J = 47.5 Hz), 7.06 (1H, s), 7.38 (1H, s), 8.09 (1H, s).

A mixture of 1-fluoro-2-methylpropan-2-yl-1*H*-imidazole-1-carboxylate (21.7 g, 99.9 mmol) and iodomethane (29.0 mL, 466 mmol) in acetonitrile (132 mL) was stirred at room temperature for 32 h. The solvent and excess iodomethane were removed in vacuo to give **28a** (30.1 g, 91.7 mmol) as a brown amorphous. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.75 (3H, s), 1.76 (3H, s), 4.29 (3H, s), 4.68 (2H, d, J = 47.0 Hz), 7.46 (1H, m), 7.71 (1H, m), 10.66 (1H, s).

5.2.38. 3-Methyl-1-{[(1,1,1-trifluoro-2-methylpropan-2-yl)oxy]-carbonyl}-1*H*-imidazol-3-ium iodide (28b)

Compound **28b** was prepared from commercially available 1,1,1-trifluoro-2-methylpropan-2-ol using the procedure described for **28a** as a colorless powder. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.92 (6H, s), 4.31 (3H, s), 7.65–7.68 (2H, m), 10.22 (1H, s).

5.2.39. 1-Fluoro-2-methylpropan-2-yl 4-oxopiperidine-1-carboxylate (29a)

Compound **28a** (30.1 g, 91.7 mmol) was dissolved in chloroform (164 mL) and added to a solution of 4-piperidone trifluoroacetate (24.7 g, 116 mmol) and triethylamine (32.3 mL, 232 mmol) in chloroform (300 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature for 2 h and poured into water (200 mL). The organic layer was washed with water (100 mL) and brine (100 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography (10% ethyl acetate/*n*-hexane) to afford **29a** (14.5 g, 73%) as a colorless powder. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.50 (3H, s), 1.51 (3H, s), 2.45 (4H, t, J = 6.2 Hz), 3.73 (4H, t, J = 6.4 Hz), 4.50 (2H, d, J = 47.4 Hz); MS (ESI): m/z 218 $[M+H]^+$.

5.2.40. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-oxopiperidine-1-carboxylate (29b)

Compound **29b** was prepared from **28b** using the procedure described for **29a** as a colorless powder. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.73 (6H, s), 2.49 (4H, br s), 3.75 (4H, br s); MS (ESI): m/z 254 $[M+H]^+$.

5.2.41. *tert*-Butyl 4-(1-{[(1-fluoro-2-methylpropan-2-yl)oxy]-carbonyl}piperidin-4-yl)piperazine-1-carboxylate (30a)

A mixture of **29a** (14.0 g, 64.4 mmol) and 1-(*tert*-butoxycarbonyl)piperidone (12.1 g, 65.0 mmol) in $CHCl_3$ (322 mL) was stirred at room temperature for 1.5 h. To this was added sodium tri-acetoxyborohidride (27.6 g, 130 mmol) and the reaction mixture was stirred for 5 h. The reaction mixture was added 3 M aqueous sodium hydroxide solution (70 mL) to basify. The organic layer was washed with brine (100 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography (5% methanol/chloroform) to afford **30a** (19.1 g, 49.6 mmol) as a colorless powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30–1.53 (16H, m), 1.70–1.86 (3H, m), 2.31–2.55 (5H, m), 2.61–2.86 (2H, m), 3.36–3.48 (4H, m), 4.04–4.23 (2H, m), 4.47 (2H, d, J = 47.7 Hz); MS (ESI): m/z 388 $[M+H]^+$.

5.2.42. *tert*-Butyl 4-(1-{[(1,1,1-trifluoro-2-methylpropan-2-yl)oxy]carbonyl}piperidin-4-yl)piperazine-1-carboxylate (30b)

Compound **30b** was prepared from **29b** using the procedure described for **30a** as a colorless powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.37–1.50 (11H, m), 1.69 (6H, s), 1.76–1.87 (2H, m), 2.37–2.56 (5H, m), 2.66–2.88 (2H, m), 3.43 (4H, br s), 3.98–4.23 (2H, m); MS (ESI): m/z 424 $[M+H]^+$.

5.2.43. 1-Fluoro-2-methylpropan-2-yl 4-(piperazin-1-yl)piperidine-1-carboxylate dihydrochloride (31a)

To a solution of **30a** (18.0 g, 46.5 mmol) in ethyl acetate (210 mL) was added 4 M hydrogen chloride in ethyl acetate (70 mL, 279 mmol) and the mixture was stirred for 2 h. The solvent was removed in vacuo and the residue was added Ether (200 mL). The resulting precipitate was obtained by filtration and dried in vacuo to afford **31a** (14.2 g, 85%) as an colorless powder. ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.40 (3H, s), 1.40 (3H, s), 1.56 (2H, br s), 1.99–2.18 (2H, m), 2.64–2.88 (2H, m), 3.20–3.75 (10H, m), 3.96–4.16 (2H, m) 4.49 (2H, d, J = 47.7 Hz); MS (ESI): m/z 288 $[M+H]^+$. Anal. Calcd for $C_{14}H_{26}FN_3O_2{\cdot}2.0HCl{\cdot}0.2H_2O$: C, 46.21; H, 7.87; N, 11.55. Found: C, 46.14; H, 7.84; N, 11.52.

5.2.44. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-(piperazin-1-yl)piperidine-1-carboxylate (31b)

To a solution of **30b** (11.5 g, 26.3 mmol) in methanol (160 mL) was added 4 M hydrogen chloride in ethyl acetate (80 mL, 320 mmol) and the mixture was stirred for 5 h. The solvent was removed in vacuo and the residue was dissolved in water the aqueous solution was basified by addition of 2 M aqueous sodium hydroxide solution and extracted with chloroform. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to afford **31a** as a colorless powder (7.73 g, 91%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.36–1.51 (2H, m), 1.67 (6H, m), 1.76–1.91 (2H, m), 2.33 –2.41 (1H, m), 2.54 (4H, br s), 2.66–2.86 (2H, m), 2.91 (4H, t, J = 4.81 Hz), 3.97–4.24 (2H, m); MS (ESI): m/z 324 $[M+H]^+$.

5.2.45. 1-Fluoro-2-methylpropan-2-yl 4-{4-[(2-amino-1-benzothiophen-3-yl)carbonyl]piperazin-1-yl}piperidine-1-carboxylate (35a)

A mixture of **22** (120 mg, 0.60 mmol), EDCI (138 mg, 0.72 mmol), **31a** (216 mg, 0.60 mmol) and HOBt (97 mg, 0.72 mmol) in *N*,*N*-dimethylformamide (6.0 mL) was stirred for 20 h. The reaction mixture was diluted with ethyl acetate and washed with brine, the organic layer was dried over magnesium sulfate and concentration in vacuo. The residue was purified by silica gel column chromatography (5% methanol/chloroform) to afford **35a** (228 mg, 82%) as a pale yellow amorphous. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.38–1.48 (8H, m), 1.72–1.86 (2H, m), 2.38–2.83 (7H, m), 3.49–3.71 (4H, m), 4.03–4.25 (2H, m), 4.47 (2H, d, J = 47.7 Hz), 5.18–5.27 (2H, m), 7.11 (1H, t, J = 7.1 Hz), 7.25–7.30 (1H, m), 7.36 (1H, d, J = 7.8 Hz), 7.54 (1H, d, J = 7.8 Hz); MS (ESI): m/z 463 $[M+H]^+$.

The following compounds **35b**, **36a–h**, **37a–d** were prepared from the corresponding starting materials in a similar manner to that described for **35a**.

5.2.46. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-{4-[(2-amino-1-benzothiophen-3-yl)carbonyl]piperazin-1-yl}piperidine-1-carboxylate (35b)

Colorless amorphous (87%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.37–1.49 (2H, m), 1.68 (6H, s), 1.76–1.87 (2H, m), 2.39–2.48 (1H, m), 2.48–2.57 (2H, m), 2.58–2.68 (2H, m), 2.68–2.87 (2H, m), 3.50–3.70 (4H, m), 3.98–4.23 (2H, m), 5.24 (2H, s), 7.09–7.13 (1H, m), 7.25–7.30 (1H, m), 7.36 (1H, d, J = 7.8 Hz), 7.54 (1H, d, J = 7.8 Hz); MS (ESI): m/z 499 $[M+H]^+$.

5.2.47. Propan-2-yl 4-{4-[(2-amino-5-methyl-1-benzothiophen-3-yl)carbonyl]piperazin-1-yl}piperidine-1-carboxylate (36a)

Pale yellow amorphous (51%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.23 (6H, d, J = 6.0 Hz), 1.38–1.47 (2H, m), 1.77–1.82 (2H, m), 2.39–2.54

(6H, m), 2.61–2.78 (4H, m), 3.51–3.70 (4H, m), 4.11–4.27 (2H, m), 4.87–4.94 (1H, m), 5.14–5.19 (2H, m), 6.94 (d, J = 7.79 Hz, 1H), 7.18 (1H, s), 7.42 (1H, d, *J* = 7.8 Hz); MS (ESI): *m*/*z* 445 $[M+H]^+$.

5.2.48. Propan-2-yl 4-{4-[(2-amino-6-methyl-1-benzothiophen-3-yl)carbonyl]piperazin-1-yl}piperidine-1-carboxylate (36b)

Colorless amorphous (51%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.23 (6H, d, *J* = 6.0 Hz), 1.37–1.45 (2H, m), 1.76–1.82 (2H, m), 2.39 (3H, s), 2.40–2.54 (3H, m), 2.60–2.78 (4H, m), 3.51–3.66 (4H, m), 4.12–4.25 (2H, m), 4.87–4.93 (1H, m), 5.12–5.17 (2H, m), 7.10 (1H, d, *J* = 9.7 Hz), 7.24–7.27 (1H, m), 7.35 (1H, s); MS (ESI): *m*/*z* 445 $[M+H]^+$.

5.2.49. 1-Fluoro-2-methylpropan-2-yl 4-{4-[(2-amino-6-methyl-1-benzothiophen-3-yl)carbonyl]piperazin-1-yl}piperidine-1-carboxylate (36c)

Colorless amorphous (73%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.37–1.50 (8H, m), 1.75– 1.83 (2H, m), 2.38–2.54 (6H, m), 2.59–2.82 (4H, m), 3.51–3.67 (4H, m), 4.04–4.22 (2H, m), 4.47 (2H, d, *J* = 47.2 Hz), 5.12–5.17 (2H, m), 7.08–7.11 (1H, m), 7.24–7.27 (1H, m), 7.35 (1H, s); MS (ESI): *m*/*z* 477 $[M+H]^+$.

5.2.50. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-{4-[(2-amino-6-methyl-1-benzothiophen-3-yl)carbonyl]piperazin-1-yl}piperidine-1-carboxylate (36d)

Colorless amorphous (64%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.43 (2H, br s), 1.68 (6H, s), 1.76–1.85 (2H, m), 2.39 (3H, s), 2.40–2.46 (1H, m), 2.48–2.54 (2H, m), 2.58–2.66 (2H, m), 2.68–2.85 (2H, m), 3.50–3.57 (2H, m), 3.59–3.68 (2H, m), 4.00–4.21 (2H, m), 5.13–5.18 (2H, s), 7.10 (1H, d, *J* = 7.3 Hz), 7.23–7.27 (1H, m), 7.35 (1H, s); MS (ESI): *m*/*z* 513 $[M+H]^+$.

5.2.51. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-(4-{[2-amino-6-(trifluoromethyl)-1-benzothiophen-3-yl]carbonyl}piperazin-1-yl)piperidine-1-carboxylate (36e)

Pale yellow amorphous (76%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.37–1.50 (2H, m), 1.68 (6H, s), 1.75–1.88 (2H, m), 2.40–2.48 (1H, m), 2.49–2.58 (2H, m), 2.60–2.88 (4H, m), 3.47–3.71 (4H, m), 3.99–4.22 (2H, m), 5.48 (2H, br s), 7.42 (1H, d, *J* = 8.7 Hz), 7.51 (1H, d, *J* = 7.3 Hz), 7.80 (1H, s); MS (ESI): *m*/*z* 567 $[M+H]^+$.

5.2.52. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-{4-[(2-aminothieno[2,3-*c*]pyridin-3-yl)carbonyl]piperazin-1-yl}piperidine-1-carboxylate (36f)

Pale yellow amorphous (69%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.38–1.49 (2H, m), 1.68 (6H, s), 1.77–1.86 (2H, m), 2.42–2.48 (1H, m), 2.48–2.57 (2H, m), 2.60–2.69 (2H, m), 2.69–2.87 (2H, m), 3.47–3.67 (4H, m), 4.00–4.23 (2H, m), 5.75–5.80 (2H, m), 7.20 (1H, d, *J* = 5.5 Hz), 8.38 (1H, d, *J* = 6.0 Hz), 8.71 (1H, s); MS (ESI): *m*/*z* 500 $[M+H]^+$.

5.2.53. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-{4-[(2-amino-6-chloro-1-benzothiophen-3-yl)carbonyl]piperazin-1-yl}piperidine-1-carboxylate (36g)

Colorless amorphous (87%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.37–1.49 (2H, m), 1.68 (6H, s), 1.76–1.87 (2H, m), 2.40–2.56 (3H, m), 2.58–2.67 (2H, m), 2.68–2.87 (2H, m), 3.45–3.69 (4H, m), 3.98–4.23 (2H, m), 5.27 (2H, br s), 7.22–7.29 (2H, m), 7.52 (1H, d, *J* = 1.8 Hz); MS (ESI): *m*/*z* 533 $[M+H]^+$.

5.2.54. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-{4-[(2-amino-6-methoxy-1-benzothiophen-3-yl)carbonyl]piperazin-1-yl}piperidine-1-carboxylate (36h)

Colorless amorphous (87%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.43 (2H, br s), 1.64–1.85 (8H, m), 2.42–2.48 (1H, m), 2.49–2.57 (2H, m), 2.59–2.67 (2H, m), 2.69–2.85 (2H, m), 3.50–3.58 (2H, m), 3.59–3.67 (2H, m), 3.83 (3H, s), 4.00–4.20 (2H, m), 5.03–5.10 (2H, m), 6.90 (1H, dd, *J* = 8.9, 2.5 Hz), 7.09 (1H, d, *J* = 2.3 Hz), 7.24–7.28 (1H, m); MS (ESI): *m*/*z* 529 $[M+H]^+$.

5.2.55. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-[4-({6-fluoro-2-[(4-methoxybenzyl)amino]-1-benzothiophen-3-yl}carbonyl)-piperazin-1-yl]piperidine-1-carboxylate (37a)

Pale yellow amorphous (80%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.35–1.50 (2H, m), 1.68 (6H, s), 1.74–1.87 (2H, m), 2.38–2.53 (3H, m), 2.56–2.66 (2H, m), 2.67–2.86 (2H, m), 3.45–3.62 (4H, m), 3.80 (3H, s), 4.00–4.21 (2H, m), 4.38 (2H, d, *J* = 6.0 Hz), 6.86–6.91 (3H, m), 6.98–7.04 (1H, m), 7.22–7.32 (4H, m); MS (ESI): *m*/*z* 637 $[M+H]^+$.

5.2.56. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-[4-({2-[(4-methoxybenzyl)amino]thieno[3,2-*b*]pyridin-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate (37b)

Yellow amorphous (94%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.39–1.51 (2H, m), 1.69 (6H, s), 1.77–1.91 (2H, m), 2.40–2.49 (1H, m), 2.58–2.91 (6H, m), 3.56–3.74 (4H, m), 3.81 (3H, s), 3.99–4.24 (2H, m), 4.44 (2H, d, *J* = 5.5 Hz), 6.87–6.95 (3H, m), 7.32 (2H, d, *J* = 8.7 Hz), 7.61–7.68 (1H, m), 7.75–7.80 (1H, m), 8.44–8.47 (1H, m); MS (ESI): *m*/*z* 620 $[M+H]^+$.

5.2.57. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-[4-({2-[(4-methoxybenzyl)amino]thieno[3,2-*c*]pyridin-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate (37c)

Pale yellow amorphous (82%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.36–1.50 (2H, m), 1.69 (6H, s), 1.76–1.88 (2H, m), 2.39–2.48 (1H, m), 2.50–2.87 (6H, m), 3.57 (4H, br s), 3.81 (3H, s), 4.00–4.24 (2H, m), 4.43 (2H, d, *J* = 6.0 Hz), 6.90 (2H, d, *J* = 8.7 Hz), 7.28–7.34 (3H, m), 7.49 (1H, d, *J* = 5.5 Hz), 8.22 (1H, d, *J* = 5.0 Hz), 8.66 (1H, s); MS (ESI): *m*/*z* 620 $[M+H]^+$.

5.2.58. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-[4-({2-[(4-methoxybenzyl)amino]thieno[2,3-*b*]pyridin-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate (37d)

Pale yellow amorphous (63%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.36–1.50 (2H, m), 1.69 (6H, s), 1.76–1.87 (2H, m), 2.39–2.55 (3H, m), 2.58–2.67 (2H, m), 2.69–2.88 (2H, m), 3.42–3.64 (4H, m), 3.81 (3H, s), 4.00–4.23 (2H, m), 4.44 (2H, d, *J* = 6.0 Hz), 6.89 (2H, d, *J* = 8.71 Hz),, 7.16–7.21 (1H, m), 7.28–7.35 (3H, m), 7.53–7.58 (1H, m), 8.17–8.22 (1H, m); MS (ESI): *m*/*z* 620 $[M+H]^+$.

5.2.59. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-{4-[(2-amino-6-fluoro-1-benzothiophen-3-yl)carbonyl]piperazin-1-yl}piperidine-1-carboxylate (38a)

To a solution of **37a** (590 mg, 0.927 mmol) in chloroform (3.00 mL) was added trifluoroacetic acid (3.00 mL). The reaction mixture was stirred at room temperature for 1 h, and poured 3 M sodium hydroxide aqueous solution. The organic layer was washed with brine and dried over anhydrous magnesium sulfate, concentrated. The residue was purified by NH silica gel column chromatography (20% ethyl acetate/*n*-hexane) to afford **38a** as a pale yellow amorphous (40%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.36–1.51 (2H, m), 1.67 (6H, s), 1.76–1.88 (2H, m), 2.38–2.57 (3H, m), 2.58–2.87 (4H, m), 3.47–3.68 (4H, m), 3.98–4.24 (2H, m), 5.15 (2H, br s), 6.99–7.06 (1H, m), 7.23–7.33 (2H, m); MS (ESI): *m*/*z* 517 $[M+H]^+$.

The following compounds **38b**–**d** were prepared from the corresponding starting materials in a similar manner to that described for **38a.**

5.2.60. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-{4-[(2-aminothieno[3,2-*b*]pyridin-3-yl)carbonyl]piperazin-1-yl}piperidine-1-carboxylate (38b)

Pale yellow amorphous (92%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.37–1.52 (2H, m), 1.69 (6H, s), 1.76–1.91 (2H, m), 2.40–2.50 (1H, m), 2.52–2.90 (6H, m), 3.32–3.94 (4H, m), 3.99–4.25 (2H, m), 5.73 (2H, br s), 6.98 (1H, dd, J = 8.0, 4.8 Hz), 7.80 (1H, d, J = 7.8 Hz), 8.42–8.50 (1H, m); MS (ESI): m/z 500 $[M+H]^+$.

5.2.61. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-{4-[(2-aminothieno[3,2-*c*]pyridin-3-yl)carbonyl]piperazin-1-yl}piperidine-1-carboxylate (38c)

Colorless amorphous (95%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.38–1.50 (2H, m), 1.68 (6H, s), 1.76–1.88 (2H, m), 2.41–2.49 (1H, m), 2.52–2.88 (6H, m), 3.61 (4H, br s), 3.99–4.24 (2H, m), 5.51 (2H, br s), 7.50 (1H, d, J = 6.0 Hz), 8.28 (1H, d, J = 5.0 Hz), 8.66 (1H, s); MS (ESI): m/z 500 $[M+H]^+$.

5.2.62. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-{4-[(2-aminothieno[2,3-*b*]pyridin-3-yl)carbonyl]piperazin-1-yl}piperidine-1-carboxylate (38d)

Pale yellow amorphous (92%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.38–1.51 (2H, m), 1.68 (6H, s), 1.76–1.88 (2H, m), 2.41–2.57 (3H, m), 2.59–2.88 (4H, m), 3.46–3.55 (2H, m), 3.60–3.71 (2H, m), 4.01–4.23 (2H, m), 5.53 (2H, br s), 7.17–7.24 (1H, m), 7.58 (1H, d, J = 6.4 Hz), 8.25 (1H, d, J = 6.4 Hz); MS (ESI): m/z 500 $[M+H]^+$.

5.2.63. 1-Fluoro-2-methylpropan-2-yl 4-[4-({2-[(ethylcarbamoyl)amino]-1-benzothiophen-3-yl}carbonyl)piperazin-1-yl]-piperidine-1-carboxylate hydrochloride (10a)

To a solution of **35a** (228 mg, 0.493 mmol) in pyridine (6.00 mL) was added ethyl isocyanate (0.195 mL, 2.47 mmol). The reaction mixture was heated at 60 °C for 17 h and concentrated. To this was added chloroform and washed with water (5 times). The organic layer was washed with brine and dried over anhydrous magnesium sulfate, then concentrated. The residue was purified by NH silica gel column chromatography (ethyl acetate) to afford the free amine of **7a** as a colorless amorphous (80%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.06 (3H, t, J = 7.1 Hz), 1.37–1.49 (8H, m), 1.74–1.83 (2H, m), 2.40–2.57 (3H, m), 2.59–2.83 (4H, m), 3.20–3.27 (2H, m), 3.51–3.71 (4H, m), 4.05–4.23 (2H, m), 4.47 (2H, d, J = 47.2 Hz), 5.14 (1H, s), 7.21 (1H, t, J = 7.6 Hz), 7.33 (1H, t, J = 7.6 Hz), 7.43 (1H, d, J = 8.3 Hz), 7.72 (1H, d, J = 8.0 Hz), 9.41 (1H, s); MS (ESI): m/z 534 $[M+H]^+$.

To a solution of the free amine (210 mg, 0.394 mmol) in ethyl acetate (10.0 mL) was added 4.0 M ethylacetate hydro chloride solution (0.108 mL, 0.433 mmol) at room temperature. After the stirring for 2 h, the resultant precipitate was collected by filtration (184 mg) to afford **10a** as a colorless powder. Anal. Calcd for $C_{26}H_{36}FN_4O_4S{\cdot}1.0HCl{\cdot}1.0H_2O$: C, 53.10; H, 6.68; N, 11.91. Found: C, 52.94; H, 6.39; N, 11.70.

The following compounds **10b**, **11**, **12a–c**, **13–20** were prepared from the corresponding starting materials in a similar manner to that described for **10a**.

5.2.64. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-[4-({2-[(ethylcarbamoyl)amino]-1-benzothiophen-3-yl}carbonyl)piperazin-1-yl]-piperidine-1-carboxylate hydrochloride hydrochloride (10b)

Colorless powder (84%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.10 (3H, t, J = 7.3 Hz), 1.38–1.48 (2H, m), 1.56 (6H, s), 1.76–1.86 (2H, m), 2.40–2.56 (3H, m), 2.60–2.87 (4H, m), 3.24–3.31 (2H, m), 3.49–3.74 (4H, m), 3.98–4.22 (2H, m), 5.04 (1H, t, J = 5.3 Hz), 7.21 (1H, t, J = 7.6 Hz), 7.33 (1H, t, J = 7.6 Hz), 7.44 (1H, d, J = 7.8 Hz), 7.72 (1H, d, J = 7.8 Hz), 9.41 (1H, s); MS (ESI): 570 $[M+H]^+$. Anal. Calcd for $C_{26}H_{34}F_3N_5O_4S{\cdot}1.0HCl{\cdot}0.75H_2O$: C, 50.40; H, 5.94; N, 11.30. Found: C, 50.62; H, 5.71; N, 11.25.

5.2.65. Propan-2-yl 4-[4-({2-[(ethylcarbamoyl)amino]-5-methyl-1-benzothiophen-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate maleate (11)

Colorless powder (69%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.06 (3H, t, J = 7.1 Hz), 1.24 (6H, d, J = 6.4 Hz), 1.37–1.46 (2H, m), 1.76–1.82 (2H, m), 2.42–2.55 (6H, m), 2.64–2.79 (4H, m), 3.20–3.28 (2H, m), 3.54–3.74 (4H, m), 4.13–4.26 (2H, m), 4.87–4.94 (1H, m), 5.16 (1H, s), 7.02–7.06 (1H, m), 7.22 (1H, s), 7.58–7.61 (1H, m), 9.36 (1H, s); MS (ESI): 516 $[M+H]^+$. Anal. Calcd for $C_{26}H_{37}N_5O_4S{\cdot}$-$C_4H_4O_4{\cdot}1.5H_2O$: C, 54.70; H, 6.73; N, 10.63. Found: C, 54.52; H, 6.25; N, 10.32.

5.2.66. Propan-2-yl 4-[4-({2-[(ethylcarbamoyl)amino]-6-methyl-1-benzothiophen-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate (12a)

Colorless amorphous (37%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.08 (3H, t, J = 7.1 Hz), 1.23 (6H, d, J = 6.4 Hz), 1.36–1.46 (2H, m), 1.76–1.82 (2H, m), 2.40–2.55 (7H, m), 2.62–2.78 (4H, m), 3.21–3.30 (2H, m), 3.53–3.70 (3H, m), 4.14–4.26 (2H, m), 4.87–4.94 (1H, m), 5.13 (1H, s), 7.15 (1H, d, J = 8.3 Hz), 7.32 (1H, d, J = 8.3 Hz), 7.52 (1H, s), 9.36 (1H, s); MS (ESI): 516 $[M+H]^+$. Anal. Calcd for $C_{26}H_{37}N_5O_4S{\cdot}0.9H_2O$: C, 58.71; H, 7.35; N, 13.17. Found C, 58.81; H, 7.39; N, 13.02.

5.2.67. 1-Fluoro-2-methylpropan-2-yl 4-[4-({2-[(ethylcarbamoyl)amino]-6-methyl-1-benzothiophen-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate hydrochloride (12b)

Pale red powder (77%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.06 (3H, t, J = 7.1 Hz), 1.38–1.50 (8H, m), 1.74–1.82 (2H, m), 2.39–2.55 (6H, m), 2.61–2.84 (4H, m), 3.20–3.29 (2H, m), 3.53–3.71 (4H, m), 4.05–4.24 (2H, m), 4.47 (2H, d, J = 47.2 Hz), 5.17 (1H, s), 7.15 (1H, d, J = 8.3 Hz), 7.31 (1H, d, J = 8.3 Hz), 7.52 (1H, s), 9.35 (1H, s); MS (ESI): 548 $[M+H]^+$. Anal. Calcd for $C_{27}H_{38}FN_5O_4S{\cdot}1.0HCl{\cdot}1.0H_2O$: C, 53.85; H, 6.86; N, 11.63. Found: C, 53.69; H, 6.71; N, 11.40.

5.2.68. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-[4-({2-[(ethylcarbamoyl)amino]-6-methyl-1-benzothiophen-3-yl}carbonyl)-piperazin-1-yl]piperidine-1-carboxylate (12c)

Colorless powder (76%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.00 (3H, t, J = 6.9 Hz), 1.36–1.48 (2H, m), 1.68 (6H, s), 1.75–1.85 (2H, m), 2.39–2.47 (4H, m), 2.48–2.57 (2H, m), 2.59–2.67 (2H, m), 2.68–2.86 (2H, m), 3.15–3.23 (2H, m), 3.51–3.72 (4H, m), 3.99–4.22 (2H, m), 5.29 (1H, t, J = 5.3 Hz), 7.15 (1H, d, J = 8.3 Hz), 7.28–7.32 (1H, m), 7.52 (1H, s), 9.36 (1H, s); MS (ESI): 584 $[M+H]^+$. Anal. Calcd for $C_{27}H_{36}F_3N_5O_4S{\cdot}1.0HCl{\cdot}1.0H_2O$: C, 50.82; H, 6.16; N, 10.97. Found: C, 50.93; H, 5.93; N, 10.76.

5.2.69. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-(4-{[2-amino-6-(trifluoromethyl)-1-benzothiophen-3-yl]carbonyl}piperazin-1-yl}piperidine-1-carboxylatehydrochloride (13)

Pale yellow powder (70%); ^{1}H NMR (600 MHz, $CDCl_3$): δ ppm 0.99–1.10 (3H, m), 1.37–1.50 (2H, m), 1.68 (6H, s), 1.75–1.89 (2H, m), 2.40–2.60 (3H, m), 2.61–2.89 (4H, m), 3.20–3.29 (2H, m), 3.44–3.80 (4H, m), 3.98–4.25 (2H, m), 5.23 (1H, s), 7.50 (1H, d, J = 8.3 Hz), 7.56 (1H, d, J = 8.3 Hz), 7.99 (1H, br s), 9.53 (1H, s); MS (ESI): 638 $[M+H]^+$. Anal. Calcd for $C_{27}H_{33}F_6N_5O_4S{\cdot}$ $1.0HCl{\cdot}0.5H_2O$: C, 47.47; H, 5.16; N, 10.25. Found: C, 47.40; H, 4.94; N, 10.13.

5.2.70. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-[4-({2-[(ethylcarbamoyl)amino]-6-fluoro-1-benzothiophen-3-yl}carbonyl)-piperazin-1-yl]piperidine-1-carboxylate (14)

Pale yellow powder (74%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.03–1.09 (3H, m), 1.36–1.50 (2H, m), 1.68 (6H, s), 1.74–1.87 (2H, m), 2.40–2.58 (3H, m), 2.59–2.89 (4H, m), 3.19–3.28 (2H, m),

3.44–3.76 (4H, m), 3.98–4.23 (2H, m), 5.11 (1H, br s), 7.04–7.12 (1H, m), 7.33–7.39 (1H, m), 7.40–7.44 (1H, m), 9.35 (1H, s); MS (ESI): 588 $[M+H]^+$. Anal. Calcd for $C_{26}H_{33}F_4N_5O_4S{\cdot}1.0HCl{\cdot}1.0H_2O$: C, 48.63; H, 5.65; N, 10.91. Found: C, 48.84; H, 5.35; N, 10.84.

5.2.71. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-[4-({6-chloro-2-[(ethylcarbamoyl)amino]-1-benzothiophen-3-yl}carbonyl)-piperazin-1-yl]piperidine-1-carboxylate hydrochloride (15)

Pale yellow powder (87%); ^1H NMR (600 MHz, $CDCl_3$): δ 1.04–1.11 (3H, m), 1.36–1.50 (2H, m), 1.68 (6H, s), 1.75–1.86 (2H, m), 2.41–2.57 (3H, m), 2.60–2.87 (4H, m), 3.22–3.30 (2H, m), 3.47–3.75 (4H, m), 3.98–4.23 (2H, m), 5.10 (1H, br s), 7.28–7.31 (1H, m), 7.32–7.36 (1H, m), 7.70 (1H, d, J = 8.3 Hz), 9.40 (1H, s); MS (ESI): 604 $[M+H]^+$. Anal. Calcd for $C_{26}H_{33}ClF_3N_5O_4S{\cdot}1.0HCl{\cdot}1.0H_2O$: C, 47.42; H, 5.51; N, 10.63. Found: C, 47.77; H, 5.33; N, 10.65.

5.2.72. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-[4-({2-[(ethylcarbamoyl)amino]-6-methoxy-1-benzothiophen-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate hydrocloride (16)

^1H NMR (600 MHz, $CDCl_3$): δ 1.09 (3H, t, J = 7.3 Hz), 1.37–1.48 (2H, m), 1.68 (6H, s), 1.75–1.85 (2H, m), 2.40–2.57 (3H, m), 2.59–2.68 (2H, m), 2.68–2.86 (2H, m), 3.23–3.30 (2H, m), 3.51–3.71 (4H, m), 3.86 (3H, s), 4.00–4.21 (2H, m), 5.04 (1H, t, J = 5.5 Hz), 6.96 (1H, dd, J = 8.7, 2.3 Hz), 7.22 (1H, d, J = 2.29 Hz), 7.33 (1H, d, J = 8.7 Hz), 9.29 (1H, s); MS (ESI): 600 $[M+H]^+$. Anal. Calcd for $C_{27}H_{36}F_3N_5O_5S{\cdot}1.0HCl{\cdot}0.5H_2O$: C, 50.27; H, 5.94; N, 10.86. Found: C, 50.04; H, 5.90; N, 10.53.

5.2.73. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-[4-({2-[(ethylcarbamoyl)amino]thieno[3,2-*b*]pyridin-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate hydrochloride (17)

Pale brown powder (78%); ^1H NMR (600 MHz, $CDCl_3$): δ 0.89–1.03 (3H, m), 1.40–1.52 (2H, m), 1.69 (6H, s), 1.78–1.93 (2H, m), 2.43–2.90 (7H, m), 3.13–3.26 (2H, m), 3.42–3.58 (2H, m), 3.80–3.93 (2H, m), 4.00–4.26 (2H, m), 5.48 (1H, s), 7.07–7.12 (1H, m), 7.96–8.01 (1H, m), 8.52–8.57 (1H, m), 9.87 (1H, s); MS (ESI): 571 $[M+H]^+$. Anal. Calcd for $C_{25}H_{33}F_3N_6O_4S{\cdot}2.0HCl{\cdot}2.0H_2O$: C, 44.18; H, 5.78; N, 12.37. Found: C, 44.49; H, 5.53; N, 12.36.

5.2.74. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-[4-({2-[(ethylcarbamoyl)amino]thieno[3,2-*c*]pyridin-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate hydrochloride (18)

Pale yellow powder (84%); ^1H NMR (600 MHz, $CDCl_3$): δ 1.03–1.13 (3H, m), 1.36–1.51 (2H, m), 1.69 (6H, s), 1.76–1.86 (2H, m), 2.42–2.50 (1H, m), 2.51–2.90 (6H, m), 3.27 (2H, dd, J = 7.3, 5.5 Hz), 3.52–3.72 (4H, m), 4.00–4.24 (2H, m), 5.30 (1H, s), 7.67 (1H, d, J = 4.6 Hz), 8.36 (1H, t, J = 5.5 Hz), 8.77 (1H, s), 9.56 (1H, s); MS (ESI): 571 $[M+H]^+$. Anal. Calcd for $C_{25}H_{33}F_3N_6O_4S{\cdot}2.0HCl{\cdot}2.0H_2O$: C, 44.18; H, 5.78; N, 12.37. Found: C, 44.27; H, 5.71; N, 12.27.

5.2.75. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-[4-({2-[(ethylcarbamoyl)amino]thieno[2,3-*c*]pyridin-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate hydrochloride (19)

Colorless powder (86%); ^1H NMR (600 MHz, $CDCl_3$): δ 1.00–1.08 (3H, m), 1.38–1.48 (2H, m), 1.68 (6H, s), 1.76–1.85 (2H, m), 2.42–2.59 (3H, m), 2.61–2.87 (4H, m), 3.22–3.29 (2H, m), 3.49–3.69 (4H, m), 4.00–4.22 (2H, m), 5.51 (1H, s), 7.29 (1H, t, J = 5.5 Hz), 8.46 (1H, d, J = 5.5 Hz), 8.93 (1H, s), 9.70 (1H, s); MS (ESI): 571 $[M+H]^+$. Anal. Calcd for $C_{25}H_{33}F_3N_6O_4S{\cdot}2.0HCl{\cdot}4.0H_2O$: C, 41.96; H, 6.06; N, 11.74. Found: C, 42.12; H, 5.74; N, 11.75.

5.2.76. 1,1,1-Trifluoro-2-methylpropan-2-yl 4-[4-({2-[(ethylcarbamoyl)amino]thieno[2,3-*b*]pyridin-3-yl}carbonyl)piperazin-1-yl]piperidine-1-carboxylate hydrochloride (20)

Pale yellow powder (62%); ^1H NMR (600 MHz, $CDCl_3$): δ 1.17 (t, J = 7.1 Hz, 3H), 1.37–1.50 (2H, m), 1.68 (6H, s), 1.75–1.88 (2H, m), 2.40–2.56 (3H, m), 2.62–2.88 (4H, m), 3.34 (2H, dd, J = 7.3, 5.5 Hz), 3.47–3.56 (2H, m), 3.60–3.75 (2H, m), 4.00–4.23 (2H, m), 5.15–5.23 (1H, m), 7.24–7.29 (1H, m), 7.64–7.71 (1H, m), 8.39–8.46 (1H, m), 9.63 (1H, s); MS (ESI): 571 $[M+H]^+$. Anal. Calcd for $C_{25}H_{33}F_3N_6O_4S{\cdot}2.0HCl{\cdot}2.0H_2O$: C, 44.18; H, 5.78; N, 12.37. Found: C, 44.45; H, 5.57; N, 12.33.

5.2.77. HPLC analysis

For the chemical stability test, the residual amount was analyzed using reverse-phase HPLC with a CAPCELL PAK C18 UG120 (5 μm particle size, ϕ 4.6 × 150 mm; SHISEIDO), then eluted at 1.0 mL/min with acetonitrile–H_2O buffer solution and monitored using UV absorbance at 240 nm.

5.3. Biology

5.3.1. Preparation of human liver ACC

Human liver cytosol was obtained from KAC Co. The cytosol was saturated with ammonium sulfate (final 30%). The resulting precipitate was dissolved in 50 mM MOPS buffer (pH 7.5, 250 mM sucrose, 2.0 mM EDTA, 2.0 mM DTT, 5% glycerol), and then loaded onto a RESOURCE Q anion-exchange column (GE Healthcare) that was pre-equilibrated with 50 mM MOPS buffer. The ACC fractions were eluted with 50 mM MOPS buffer containing 250 mM NaCl and pooled as partially purified human ACCs.

5.3.2. ACCs enzyme assay

ACCs were preincubated with a test compound or vehicle (DMSO) in the assay buffer (60.6 mM Tris acetate, pH 7.5, 1.32 μM β-mercaptoethanol, 5 mM magnesium acetate, 8 mM magnesium sulfate, 10 mM sodium citrate and 1 mg/mL BSA) at 37 °C for 30 min. The enzyme reaction was started by adding the substrate mixture; 2.12 mM ATP, $NaHCO_3$ (18.2, 12 or 0.35 mM for liver ACC, rhACC1 or rhACC2, respectively) and [^{14}C] acetyl-CoA (25, 35 or 10 μM for liver ACC, rhACC1 or rhACC2, respectively). The enzyme reaction was terminated by adding 10 mM carnitin and 1.88 U/mL carnitine acetyltransferase (SIGMA). Under these conditions, the residual [^{14}C] acetyl-CoA was converted to [^{14}C] acetyl-carnitine. The reaction mixture (40 μL) was then mixed with 90 μL of 50% (w/v) cation-exchage resin in a 96-well filtration plate. In this procedure, [^{14}C] acetyl-carnitine, a positively charged substrate, was absorbed to the resin. Radioactivity of the eluate containing a negatively charged [^{14}C] malonyl-CoA was measured as ACCs activities.

5.3.3. Fatty acid synthesis in HepG2 cells

HepG2 cells were seeded into a 24-well plate at 3×10^5 cells/well in DMEM containing 10% FBS and maintained for 48 h. Cells were starved in serum-free DMEM containing 1.5% BSA for 1 h with or without a test compound. 4 μCi of [^3H] acetate was added to each well. After 2-h incubation, the medium was removed and the cells were washed with PBS two times. The cell were saponified by addition of 0.5 mL of 0.5 M KOH in H_2O and 0.5 mL of 5 M KOH in MeOH, followed by incubation for 2 h at 70 °C. Following saponification, the reaction mixture was washed three times with hexane, and the organic phase was discarded. The aqueous phase was acidified to pH <2 by HCl, and then fatty acids were extracted with hexane. The radioactivity of the fatty acids was measured using a liquid scintillation counter.

5.3.4. Fatty acid oxidation in HepG2 cells

HepG2 cells were seeded into a 24-well plate at 1.5×10^5 cells/well in DMEM containing 10% FBS and maintained for 48 h. The medium was removed and replaced with 20 mM HEPES (pH 7.4) buffer containing 140 mM NaCl, 5.0 mM KCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$ and 0.2% BSA with or without a test compound. After 1 h, 1.5 μCi of [^{3}H] palmitic acid was added to each well. After a 1-h incubation, the medium was transferred to a tube and then 10% TCA was added. After centrifugation, the supernatants were removed and mixed with 1% (w/v) charcoal in a 96-well filtration plate. The radioactivity of [^{3}H] H_2O in the eluate was measured using a MicroBeta (Perkin Elmer).

5.3.5. Expression and purification of rhACC1 and rhACC2

N-terminally c-Myc tagged recombinant human ACC1 (1–2346 a.a.) and ACC2 (149–2458 a.a. deleted N-terminal mitochondrial anchoring domain [1–148 a.a.]) were expressed in baculovirus/Sf9 systems. The tagged ACCs were purified using a c-Myc tagged protein mild purification kit (MBL).

5.3.6. Metabolic stability in liver microsomes

A test compound (final concentration; 5.0 μM) was incubated in the assay buffer containing 1 mg/mL human or rat microsomes, 1.5 mM glucose-6-phosphate, 0.16 mM β-nicotinamide-adenine dinucleotide phosphate, 1 U/mL glucose-6-phosphate dehydrogenase, 250 mM phosphate buffer, 2.4 mM magnesium chloride and 69 mM potassium chloride at 37 °C. Concentrations of the test compounds were determined by LC–MS/MS. Metabolic stability was calculated from the ratio of the test compound concentration at 0 min to its concentration after 15-min incubation.

5.4. Animals and experimental design

5.4.1. De novo fatty acid synthesis studies in SD rats

Six week-old SD rats were used for de novo fatty acid synthesis (FAS) studies. Rats were fasted overnight and then were administrated a test compound orally. One hour after compound administration, rats received an intraperitoneal injection of 0.5 mL of [^{14}C] acetate (64 μCi/mL). One hour later, rats were sacrificed and livers were removed. Liver pieces (0.75 g) were saponified in 1.5 mL of 2.5 M NaOH, followed by the addition of 2 mL of EtOH. Next day, the saponified samples were washed with petroleum ether. The remaining aqueous phase was acidified to pH <2 by HCl, and then fatty acids were extracted with petroleum ether. Radioactivities of the fatty acid fractions were measured by liquid scintillation counter.

5.4.2. Triglyceride lowering effect studies in fructose-drinking rats

Six week-old SD rats were maintained with free access to standard rodent chow and water containing 10% fructose for 3 weeks. Thereafter, the fructose-drinking rats were randomized to four groups as follows; vehicle (0.5% methylcellulose, po, b.i.d.), compound **12c** (3, 10 or 30 mg/kg, po, b.i.d.), possessing matched plasma triglyceride levels (ca. 170 mg/dL). Normal rats were fed plane water and treated with vehicle. On day 5 and 12, blood samples were taken from tail vein for the measurement of plasma triglyceride levels. On day 12, rats were sacrificed and livers were removed and frozen until use. Livers were homogenized with isopropanol and then centrifuged at 600 g for 5 min. The supernatants were used for the measurement of hepatic triglyceride levels. All experimental procedures involving animal were approved by the Institutional Animal Care and Use Committee of Taisho Pharmaceutical Co., Ltd and were in accordance with the Guidelines for Propre Conduct of Animal Experiments (Science Council of Japan, 2006).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.01.041.

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