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Discovery and optimization of novel fatty acid transport protein 1 (FATP1) inhibitors

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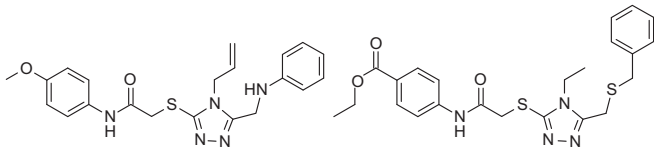
ABSTRACT

The discovery, optimization and structure–activity relationship of novel FATP1 inhibitors have been described. The detailed SAR studies of each moiety of the inhibitors combined with metabolite analysis led to the identification of the potent inhibitors **11p** and **11q** with improved blood stability.

Fatty acid transport protein 1 (FATP1) is known as a transmembrane protein with Acyl-CoA synthase activity that is highly expressed in a skeletal muscle as well as in adipose tissue, brown adipose tissue and heart.[1] One of the important roles of FATP1 is thought to be the uptake and metabolism of a fatty acid by converting corresponding Acyl-CoA with an insulin signal. The previous report of a FATP1 deficient mouse showed the improvement of high fat-induced insulin resistance as well as the reduction of intramuscular accumulation of fatty acyl-CoA, suggesting that the inhibition of FATP1 is an attractive therapeutic target for insulin resistance.[2] Herein we report the first discovery and structure–activity relationship of novel triazole derivatives as FATP1 inhibitors with improved blood stability.[3]

By high-throughput screening of our corporate library, several FATP1 inhibitors were identified. Since the transportation of a fatty acid by FATP1 is known to involve acyl-CoA synthesis, measuring the inhibition of acyl-CoA synthetase (ACS) led to the identification of structural related FATP1 inhibitors, triazole **1** and **2** (Fig. 1) with moderate human in vitro activity. Unfortunately, both compounds lack in vitro activity against mouse, and high blood stability cannot be expected because of the existence of several metabolically unstable functional groups. Thus, we initiated the derivatization of **1** and **2** to improve in vitro activities against human and mouse FATP1s avoiding metabolically labile functional groups.

Replacement of benzylthio group of **2** with aniline moiety of **1** led to the identification of **3a** with improved human and mouse in vitro activities by over ten times (Table 1). The inhibitory activities against human and mouse FATP1s are evaluated by measuring acyl-CoA synthetase activities of human and mouse FATP1s. As metabolically unstable ester moiety still remained in **3a**, our efforts were focused on modifying the substituent of the left phenyl ring in compound **3a** as indicated in Table 1. Meta derivative **3b** proved to decrease in vitro activities against both species. Methyl ester **3c** showed the substantial loss of in vitro activity against mouse, whereas *t*-butyl ester **3d** and carboxylic acid **3e** lost the potencies against both species. These results suggest that the proper size of



1
humanFATP1 IC_{50} = 6.6 μM
mouseFATP1 IC_{50} >10 μM

2
humanFATP1 IC_{50} = 7.6 μM
mouseFATP1 IC_{50} >10 μM

Figure 1. HTS hits **1** and **2** with FATP1 IC_{50} values.

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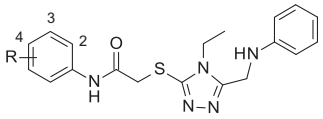
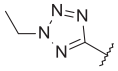
Table 1
IC_{50} values of FATP1 inhibitors **1–3**



Compd	R	Human FATP1 IC_{50}[a] (μM)	Mouse FATP1 IC_{50}[a] (μM)
1		6.6	>10
2		7.6	>10
3a	4-CO_2Et	0.33	1.1
3b	3-CO_2Et	>1	2.9
3c	4-CO_2Me	0.54	ND[b]
3d	4-CO_2*t*-Bu	>1	>10
3e	4-CO_2H	>1	>10
3f	4-CONHMe	>1	>10
3g	4-CONMe$_2$	>1	>10
3h	4-CONHEt	>1	>10
3i	H	>1	>10
3j	4-Cl	>1	>10
3k	4-OMe	>1	>10
3l	4-SO_2Me	>1	>10
3m	4-CN	>1	5.1
3n		>1	>10
3o	4-COMe	>1	ND[c]
3p	4-COEt	0.40	1.0
3q	4-CO*n*-Pr	0.23	1.1
3r	4-COPh	>1	>10

[a] Inhibition of recombinant human or mouse acyl-CoA synthetase activity of FATP1 in an assay using 10 μM [1-^{14}C] oleic acid as a substrate in 100 mM Tris–HCl (pH 7.4), 30 mM NaCl, 0.5 mM ATP, 5 mM $MgCl_2$, 0.5 mM coenzyme A, 0.05% Triton-X 100, 2 mM dithiothreitol. The IC_{50} values represent the average of at least $n = 2$.
[b] Not determined. **3c** Inhibits 56% of FATP1 ACS activity at 10 μM.
[c] Not determined. **3o** Inhibits 63% of FATP1 ACS activity at 10 μM.

lipophilic substituent is required in this position to retain in vitro potencies against human and mouse FATP1s. In addition, other kinds of carbonyl derivatives such as amides **3f–h** lost the activity. The replacement of ester function to a hydrogen atom, chloride, methoxy and methylsulfonyl groups resulted in the loss of in vitro activities against both species. Ethyl tetorazole **3n**, known as ester bioisostere, is not active at all. Therefore, synthesis of several ketone derivatives was decided after the identification of methyl ketone **3o** with a moderate mouse in vitro activity. Among the ketone derivatives, benzophenone **3r** lost the activities, whereas ethyl ketone **3p** and *n*-propyl ketone **3q** retained fair human and mouse in vitro activities.

Exceedingly high in vivo clearance of ethyl ketone **3p** was observed after intravenous administration to mouse, and high clearance found to be mediated in large part by blood instability: the blood stability of ethyl ketone **3p** was 28%. The blood stability was evaluated by measuring the remaining (%) of the parent compound after 2 h incubation with rat blood. To understand the cause of instability, the metabolites were analyzed. Ethyl ketone **3p** decomposed within 5 min after intravenous administration to mouse. LC–MS/MS analysis of the blood sample indicated the existence of two main metabolites, carboxylic acid **4** and secondary

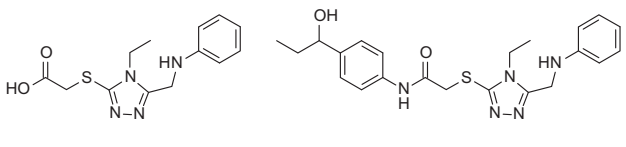
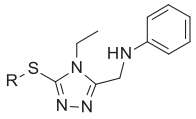
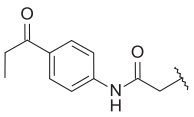
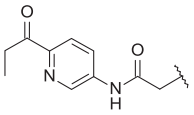
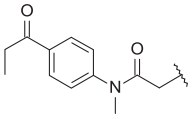
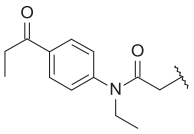
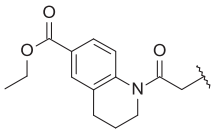
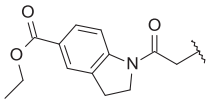
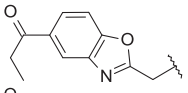
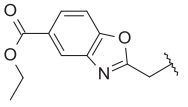


Figure 2. Two main metabolites of triazole **3p** in rodent.

Table 2
IC_{50} values of FATP1 inhibitors **3p**, **6–11**



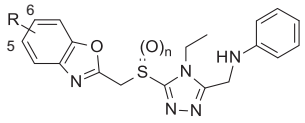
Compd	R	Human FATP1 IC_{50}[a] (μM)	Mouse FATP1 IC_{50}[a] (μM)	Blood stability[b] (%)
3p		0.40	1.0	28
6		0.40	1.5	ND[c]
7		0.58	2.0	1.9
8		>1	>10	ND[c]
9		>1	2.9	ND[c]
10		>1	>10	ND[c]
11a		0.19	0.36	8.9
11b		0.68	0.84	0

[a] Inhibition of recombinant human or mouse acyl-CoA synthetase activity of FATP1 in an assay using 10 μM [1-^{14}C] oleic acid as a substrate in 100 mM Tris–HCl (pH 7.4), 30 mM NaCl, 0.5 mM ATP, 5 mM $MgCl_2$, 0.5 mM coenzyme A, 0.05% Triton-X 100, 2 mM dithiothreitol. The IC_{50} values represent the average of at least $n = 2$.
[b] Remaining (%) of the test compound after 2 h incubation with rat blood.
[c] Not determined.

alcohol **5** (Fig. 2). This analysis clearly indicated that amide and ketone moieties are metabolically unstable. Then we focused on the replacement of these moieties as indicated in Table 2 and 3.

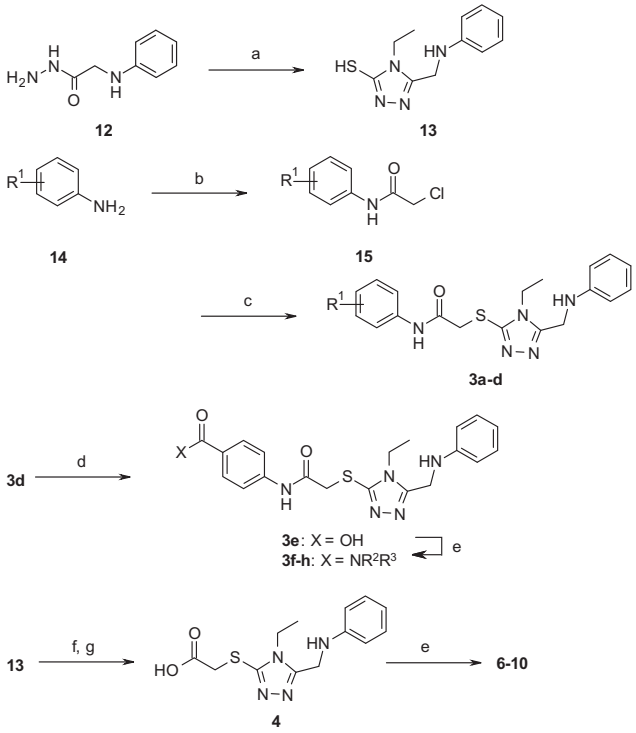
The replacement of amide moiety is summarized in Table 2. Pyridine **6** retained in vitro potencies. Although, *N*-methyl amide **7** retained in vitro activities without the improvement of blood stability, *N*-ethyl derivative **8** lost the potencies. As *N*-methyl derivative retained in vitro activities, tetrahydroquinoline **9** and dihydroindole **10** with ester functional group were prepared because of the synthetic feasibility, and these compounds showed reduced activities. Another fused pattern was then examined to modify the amide bond in triazole **3p**. This modification led to the identification of benzoxazoles **11**. In benzoxazole **11a**, we achieved not only replacement of the amide bond, but also an increase in in vitro potencies against both species by several times. Still the blood stability of benzoxazoles **11a** is moderate, and

Table 3
IC_{50} values of FATP1 inhibitors **11**



Compd	R	*n*	Human FATP1 IC_{50}[a] (μM)	Mouse FATP1 IC_{50}[a] (μM)	Blood stability[b] (%)
11a	5-COEt	0	0.19	0.36	8.9
11b	5-CO_2Et	0	0.68	0.84	0
11c	6-COEt	0	0.90	1.2	35
11d	5-CO*i*-Pr	0	0.60	0.52	42
11e	5-COEt	2	1.67	11.9	44
11f	5-CH(OH)Et	0	>1	3.7	80
11g	5-C(=NHOH)Et	0	0.26	0.20	100
11h	5-C(=NHOMe)Et	0	>1	>10	ND[c]
11i	H	0	>1	>10	ND[c]
11j	5-OMe	0	>1	2.7	ND[c]
11k	5-Me	0	>1	>10	ND[c]
11l	5-Et	0	>1	1.8	ND[c]
11m	5-F	0	>1	7.8	ND[c]
11n	5-Cl	0	>1	2.3	92
11o	6-Cl	0	>1	5.4	ND[c]
11p	5-CF_3	0	0.36	0.36	ND[c]
11q	5-OCF_3	0	0.10	0.091	100

[a] Inhibition of recombinant human or mouse acyl-CoA synthetase activity of FATP1 in an assay using 10 μM [1-^{14}C] oleic acid as a substrate in 100 mM Tris–HCl (pH 7.4), 30 mM NaCl, 0.5 mM ATP, 5 mM $MgCl_2$, 0.5 mM coenzyme A, 0.05% Triton-X 100, 2 mM dithiothreitol. The IC_{50} values represent the average of at least $n = 2$.
[b] Remaining (%) of the test compound after 2 h incubation with rat blood.
[c] Not determined.



Scheme 1. Reagents and conditions: (a) EtNCS, NaOH, THF, H_2O, reflux, 57%; (b) chloroacetyl chloride, KI, Et_3N, THF, reflux; (c) **13**, NaOAc, EtOH, reflux; (d) HCl, EtOAc; (e) corresponding amine, WSCD·HCl, HOBt·H_2O, Et_3N, CH_2Cl_2; (f) *t*-butyl bromoacetate, NaOAc, EtOH, reflux, 68%; (g) TFA, CH_2Cl_2, 53%.

obviously ethyl ketone moiety causes this blood liability. Therefore further optimization of this position was required.

The synthesis of triazoles **3–10** is shown in Scheme 1. After synthesizing thiol **13** in the usual manner,[4] S-alkylation of versatile thiol **13** with various chlorides **15**, which were prepared from corresponding aniline and chloroacetyl chloride provided triazoles **3a–d**. Amides **3f–h** were synthesized by amidation of carboxylic acid **3f** prepared from *t*-butyl ester **3d**. Triazoles **6–10** were synthesized by amidation of carboxylic acid **4**.

The replacement of the carbonyl group on benzoxazole ring in **11** was examined as shown in Table 3. Regioisomer **11c** and isopropyl ketone **11d** retained human and mouse in vitro activities without improving blood stability. The blood stability of sulfone **11e** was not improved. The metabolite analysis of **11a** indicated the existence of metabolite **11f** (data not shown). This racemic alcohol **11f** decreased in vitro potencies by over ten times with the improvement of blood stability. Oxime **11g** retained in vitro potencies without blood instability, whereas methyl oxime **11h** lost in vitro activities. In addition, methoxy or alkyl derivatives lost the activities. Although the introduction of fluoro substituent resulted in the reduction of mouse in vitro activity, a moderate in vitro activity against mouse was observed in chloro derivative **11n**. This encouraged us to synthesize and evaluate trifluoromethyl derivative **11p**, which possesses a good in vitro activities against human and mouse FATP1s. Similarly, with retaining the excellent blood stability, trifluoromethoxy derivative **11q** showed good human and mouse in vitro activities of 0.10 and 0.091 μM, respectively Table 4.

Benzoxazole derivatives **11** were synthesized by S-alkylation of versatile thiol **13** with benzylchlorides as shown in Scheme 2. Regio selective syntheses of 5-acyl benzoxazole **11a** and 6-acyl benzoxazole **11e** were accomplished using the Henichart method.[5] Friedel-Cfafts acylation of amide **16**, followed by ring closure provided benzylchloride **18**, whereas benzylchloride **22** was prepared from 2-benzoxazolinone **19**. Other benzoxazole derivatives except for **11e–h** were prepared in a similar manner.[6,7] Sulfone **11e** and alcohol **11f** were synthesized via *N*-Boc protected intermediate **25**, which was prepared from *N*-Boc aniline **23** in a similar manner as described in Scheme 1. Then, oxidation of sulfur or reduction of ketone in **25** followed by deprotection of the Boc group provided sulfone **11e** and alcohol **11f**, respectively. Oxime **11g** and **11h** were prepared using the usual manner.

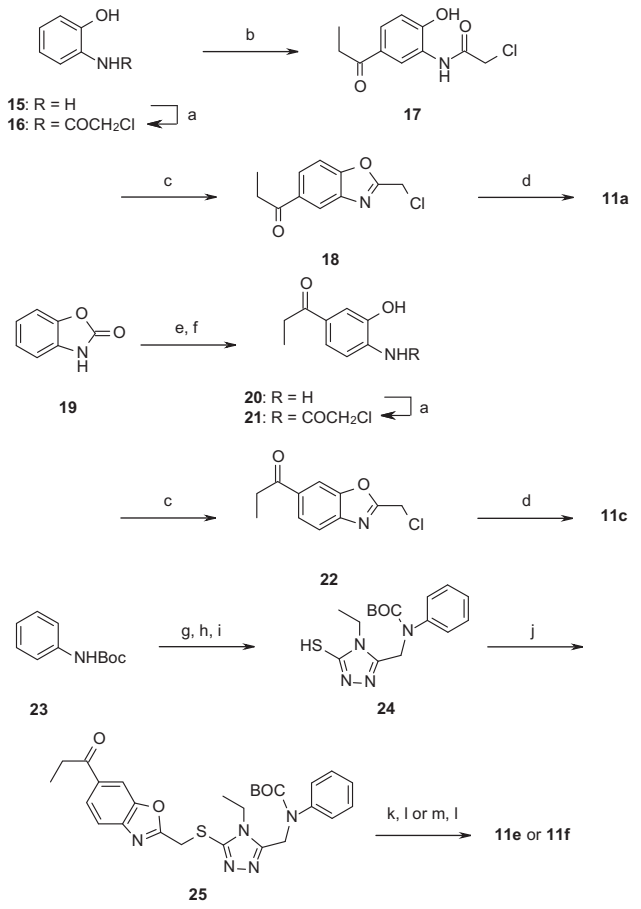
Trifluoromethyl derivatives **11p** and trifluoromethoxy derivative **11q** were selected for the evaluation of pharmacokinetics in rat. Pharmacokinetic (PK) parameters of **11a**, **11p** and **11q** were determined by orally (10 mg/kg) and intravenously (1 mg/kg) administered rats. As expected, the very high clearance (378 mL/min/kg) of ethyl ketone **11a** was improved by the replacement of the ketone group; and the clearance of **11p** and **11q** is 56.0, 52.0 mL/min/kg, respectively. Similarly, Cmax and AUC values were improved: ethyl ketone **11a** was not observed in rat plasma, whereas a moderate amount of **11p** and **11q** was observed in rat plasma.

In summary, we described the discovery and optimization of triazole derivatives as the first FATP1 inhibitors. By the

Table 4
Rat PK parameters of **11a**, **11p** and **11q**

Compd	T_{max}[a] (h)	C_{max}[a] (μg/mL)	AUC[a] (hr ∗ μg/mL)	$T_{1/2}$[a] (h)	Cl[b] (mL/min/kg)	Vd[b] (L/kg)	%*F*
11a	LOQ[c]	LOQ[c]	LOQ[c]	LOQ[c]	378	2.69	NA
11p	0.33	0.22	0.28	1.23	56.0	1.86	9.2
11q	0.33	0.19	0.20	1.02	52.0	1.73	5.4

[a] Average of two rats dosed at 10 mg/kg po in DMA/PG/(20%HPβCD/saline): 10/10/80.
[b] Average of two rats dosed at 1 mg/kg iv in DMA/PG/(20%HPβCD/saline): 10/10/80.
[c] Under limit of quantification.



Scheme 2. Reagents and conditions: (a) Chloroacetyl chloride, Et_3N, CH_2Cl_2; (b) propionyl chloride, $AlCl_3$, DMF; (c) PPTS, xylene, 150 °C; (d) **13**, AcONa, EtOH, reflux; (e) propionic acid, polyphospholic acid, 14%; (f) NaOH, H_2O, reflux, 50%; (g) methyl bromoacetate, NaO*t*-Bu, THF, reflux, 61%; (h) hydrazine monohydrate, EtOH, reflux, 91%; (i) EtNCS, NaOH, THF, H_2O, reflux, 52%; (j) **22**, NaOAc, EtOH, reflux; (k) mCPBA, CH_2Cl_2, 73%; (l) HCl, EtOAc; (m) $NaBH_4$, MeOH, 0 °C, 98%.

optimization of several positions, benzoxazoles **11p** and **11q** were identified with the improvement of both human and mouse in vitro potencies as well as blood stability. Our strategy includes removing each metabolic site which resulted in highly metabolically stable compounds. The in vivo evaluation with insulin resistance model mice is underway and to be reported in the near future.

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6. The synthesis of **11p** was performed as follows: Step 1. Synthesis of 4-ethyl-5-[(phenylamino)methyl]-4*H*-1,2,4-triazole-3-thiol (**13**) A mixture of phenylamino-acetic acid hydrazide (3.56 g, 21.6 mmol), ethyl isothiocyanate (1.88 mL, 21.6 mmol) and 2 M NaOH (5.0 mL, 10.0 mmol) in THF(10 mL) was stirred at reflux for 2 h. 1 M HCl was added to the cooled reaction mixture until the mixture became pH 6. The mixture was extracted with CH_2Cl_2 (10 mL). The organic layers were dried (Na_2SO_4), concentrated and purified by column chromatography (hexane/EtOAc = 1:1) to provide **13** (4.23 g, 84%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.39 (3H, t, J = 6.8 Hz), 4.08 (1H, brs), 4.15 (2H, q, J = 7.3 Hz), 4.39 (2H, d, J = 5.9 Hz), 6.72 (2H, d, J = 8.8 Hz), 6.84 (1H, t, J = 7.3 Hz), 7.23 (2H, t, J = 8.3 Hz), 10.28 (1H, brs). Step 2. Synthesis of *N*-{[4-ethyl-5-({[5-(trifluoromethyl)-1,3-benzoxazol-2-yl]methyl}sulfanyl)-4*H*-1,2,4-triazol-3-yl]methyl}aniline (**11p**) A mixture of **13** (0.026 g, 0.11 mmol), 2-chloromethyl-5-trifluoromethyl-1,3-benzoxazole (0.024 g, 0.10 mmol), sodium acetate (0.018 g, 0.31 mmol) in ethanol (5.0 mL) was stirred at reflux for 1 h. The cooled reaction mixture was concentrated and purified by column chromatography (hexane/EtOAc = 1:1) to provide **11p** (0.033 g, 74%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.28 (3H, t, J = 7.4 Hz), 3.97 (2H, q, J = 7.2 Hz), 4.44 (2H, s), 4.71 (2H, s), 6.71 (2H, d, J = 8.2 Hz), 6.78 (1H, t, J = 7.0 Hz), 7.19 (2H, dd, J = 8.6, 7.4 Hz), 7.50 (1H, d, J = 8.6 Hz), 7.58 (1H, d, J = 8.6 Hz), 7.92 (1H, s).; Anal. Calcd for $C_{20}H_{18}F_3N_5OS$: C, 55.42; H, 4.19; N, 16.16. Found: C, 55.20; H, 4.29; N, 15.92.
7. The synthesis of *N*-{[4-ethyl-5-({[5-(trifluoromethoxy)-1,3-benzoxazol-2-yl]methyl}sulfanyl)-4*H*-1,2,4-triazol-3-yl]methyl}aniline (**11q**): A mixture of **13** (0.276 g, 1.18 mmol), 2-chloromethyl-5-trifluoromethoxy-1,3-benzoxazole (0.270 g, 1.07 mmol), sodium acetate (0.187 g, 3.22 mmol) in ethanol (10 mL) was stirred at reflux for 1 h. The cooled reaction mixture was concentrated and purified by column chromatography (hexane/EtOAc = 1:1) to provide **11q** (0.44 g, 57%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.35 (3H, t, J = 7.3 Hz), 4.04 (2H, q, J = 7.2 Hz), 4.51 (2H, s), 4.76 (2H, s), 6.78 (2H, d, J = 8.3 Hz), 6.85 (1H, t, J = 7.3 Hz), 7.25 (3H, t, J = 7.8 Hz), 7.46 (1H, d, J = 9.3 Hz), 7.57 (1H, s); Anal. Calcd for $C_{20}H_{18}N_5O_2S$: C, 53.45; H, 4.04; N, 15.58. Found: C, 53.37; H, 4.05; N, 15.56.