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Novel acyl coenzyme A (CoA): Diacylglycerol acyltransferase-1 inhibitors: Synthesis and biological activities of diacylethylenediamine derivatives

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ABSTRACT

A series of diacylethylenediamine derivatives were synthesized and evaluated for their inhibitory activity against DGAT-1 and pharmacokinetic profile to discover new small molecule DGAT-1 inhibitors. Among the compounds, *N*-[2-({[1-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl]carbonyl}amino)ethyl]-6-(2,2,2-trifluoroethoxy)pyridine-3-carboxamide **3x** showed potent inhibitory activity and excellent PK profile. Oral administration of **3x** to mice with dietary-induced obesity resulted in reduced body weight gain and white adipose tissue weight.

1. Introduction

The concurrent epidemics of obesity, diabetes and cardiovascular disease have become a rapidly increasing health problem facing the world's industrialized countries. Obesity is one of the main risk factors in cardiovascular disease. The high unmet need for treatments to control obesity has encouraged the identification of many new mechanistic targets, one of which is triacylglycerol (TG) synthesis inhibition. One of the key enzymes in TG synthesis is acyl coenzyme A (CoA): diacylglycerol acyltransferase (DGAT), which catalyzes the final and only committed step of the TG synthesis pathway. Two DGAT enzymes, DGAT-1 and DGAT-2, have been identified, and both are ubiquitously expressed. The highest levels of expression of these enzymes are found in tissues that are active in TG synthesis, such as white adipose tissue, small intestine, and liver.[1,2] The phenotype of DGAT-1 deficient mice has already been described.[3–5] These mice are viable, resistant to diet-induced obesity (DIO) and show increased sensitivity to insulin and leptin. These findings suggest that pharmacological inhibition of DGAT-1 may be a feasible therapeutic strategy for human obesity and type 2 diabetes. Recent studies[6,7] have demonstrated that treatment of rodents with small molecule DGAT-1 inhibitors (**I**–**II**) showed some of the pharmacological features of DGAT-1 deficient mice. We have also reported the discovery of the potent DGAT-1 inhibitor *N*-(5-benzyl-4-phenyl-1,3-thiazol-2-yl)-4-(4,5-diethoxy-2-methylphenyl)-4-oxobutanamide (**1**).[8] Administration of **1** to KKA^{y} mice as a food admixture, exhibited anti-obesity effects in a four-week study. However, after administration by methylcellulose suspension **1** did not show sufficient in vivo efficacy due to its poor pharmacokinetic (PK) profile, which might be caused by poor aqueous solubility and metabolic stability. Compound **1** was also suspected of having potential bioactivation-related toxicities as a consequence of oxidative ring-opening of the thiazole moiety to yield potentially toxic thioureas.[9–11] It also had the potential to give rise to aromatic amine metabolites, which have the risk of mutagenicity.[12] Therefore we started to research a new type of compound to address these concerns.

As a modification of compound **1**, the reverse amide compound **2**, which avoids the risk of giving rise to aromatic amine metabolites, was found to have DGAT-1 inhibitory activity; however it still exhibited poor solubility and poor metabolic stability (Fig. 1). To improve the potency of compound **2**, and to replace the thiazole moiety, we first synthesized a lead library **3**, focused on the acyl group (R^1CO) and the five-membered heteroaromatic ring bearing the phenyl group. From this library we discovered compound **3a**, which possessed potent inhibitory activity and improved aqueous solubility. However **3a** still exhibited a poor PK profile that may be

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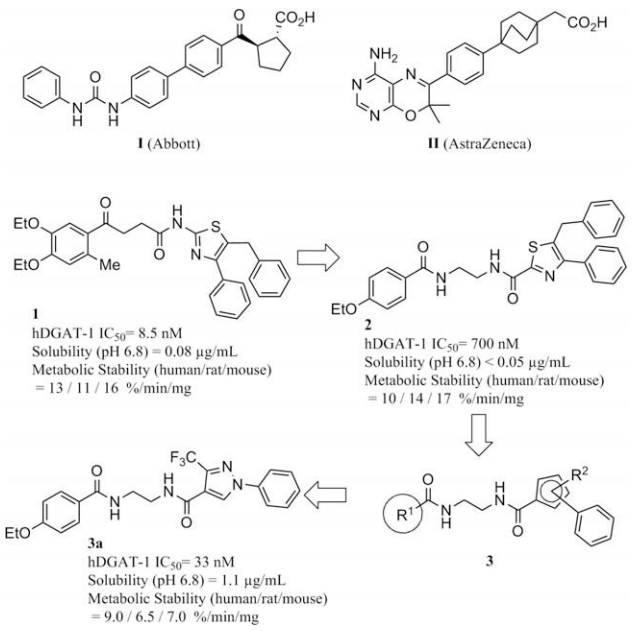


Figure 1. Known compounds, general design **3** and novel DGAT-1 inhibitor **3a**.

attributed to insufficient metabolic stability (Table 3). To achieve good in vivo efficacy, we started the optimization of **3a**, focusing on the pyrazole ring substituents, the linker portion and the terminal benzoyl group, with the goal of improving the PK profile while maintaining good potency. At each position we sought to decrease or maintain the overall lipophilic nature of the compound, and to utilize substituents which we believed would alleviate oxidative metabolism. In this paper, we describe the synthesis, structure–activity relationships (SAR) and biological properties of this novel series of diacylethylenediamine derivatives.

2. Chemistry

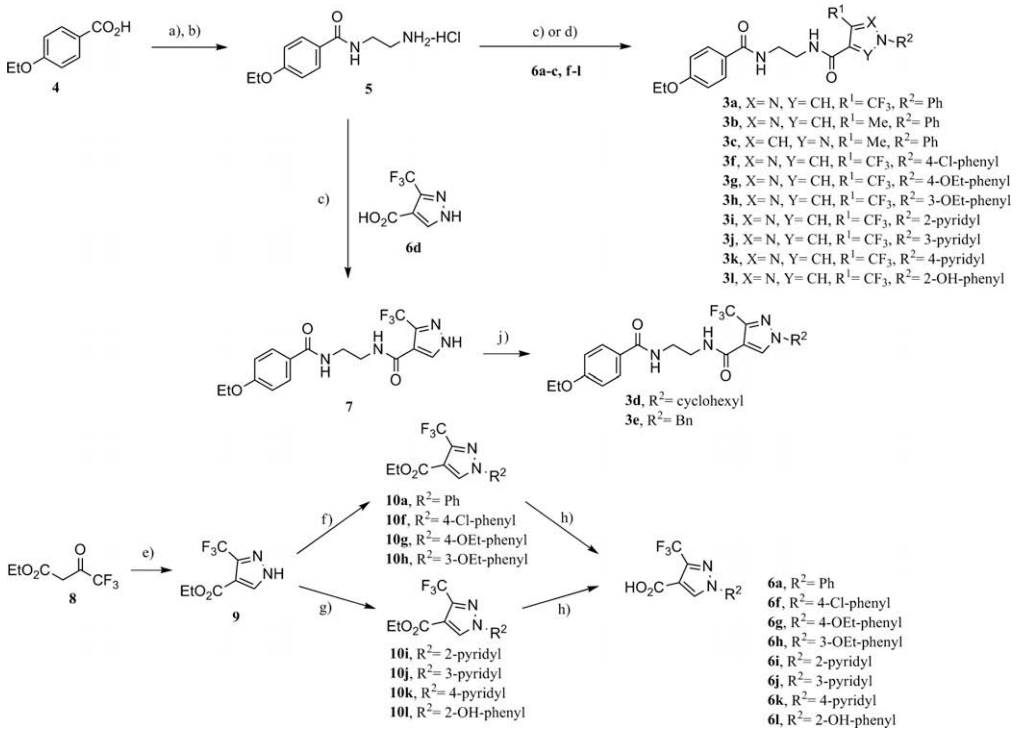
The target compounds **3a–l**, designed for optimization of the substituted pyrazole moiety, were synthesized as illustrated in Scheme 1. The key intermediate **5** was prepared from 4-ethoxybenzoic acid **4** by condensation with mono-protected ethylenediamine and subsequent removal of the *tert*-butyl carbamate group. Compounds **3a–c**, **f–l** and the intermediate **7** were obtained from **5** by a second condensation with pyrazole carboxylic acids **6a–d**,[13–15] **f–l**. Compounds **3d** and **3e** were prepared from **7** by regioselective alkylation with corresponding alkyl bromides. Pyrazole carboxylic acids **6a**, **f–l** were obtained from pyrazole ester **9** by regioselective arylation[16,17] and subsequent hydrolysis.

The synthesis of target compounds **3m–x**, designed for optimization of the linker and benzoyl moieties, is shown in Scheme 2. Use of the mono-protected ethylenediamine derivatives **11i–iv** led to compounds **3q–v** and the intermediate esters **14m–p**. Hydroxymethyl derivatives **3m–p** were obtained by reduction of the esters **14m–p**. The 2-alkoxypyridine derivatives **3w** and **3x** were prepared from the 2-chloropyridine derivative **3v** by etherification with the corresponding alcohols.

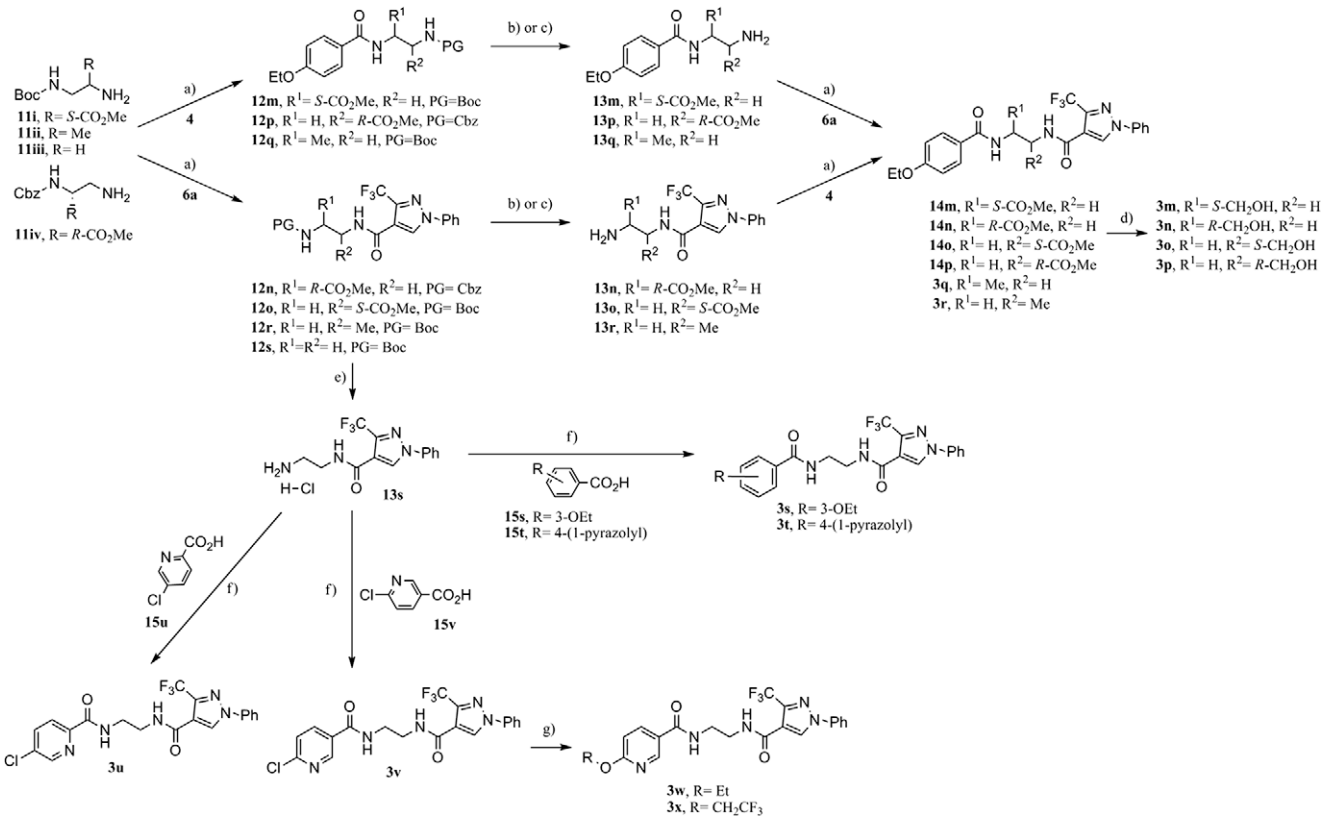
3. Results and discussion

The compounds **3a–x** were evaluated for their inhibitory activities against human DGAT-1 enzyme and TG synthesis in the C_2C_{12} cell line, their in vitro metabolic stability (human, rat, and mouse microsomes), and their PK profile in rats via cassette dosing. The results are summarized in Tables 1–3.

We initially examined the effect of optimizing the substituents on the pyrazole ring, as shown in Table 1. The reduced activity of



Scheme 1. Modification of the pyrazole moiety. Reagents and conditions: (a) *tert*-butyl (2-aminoethyl)carbamate, EDCl, HOBt, CH_3CN; (b) 4 N HCl in AcOEt; (c) EDCl, HOBt, Et_3N, DMF; (d) HATU, DIEPA, THF; (e) (i) $CH(OEt)_3$, Ac_2O; (ii) NH_2NH_2–H_2O, CH_3OH; (f) R^2-$B(OH)_2$, $Cu(OAc)_2$, pyridine, DMF, air; (g) R^2-Br, CuI, (1*S*,2*S*)-*N*1,*N*2-dimethylcyclohexane-1,2-diamine, toluene; (h) 2 N NaOH, EtOH; (j) alkyl bromide, K_2CO_3.



Scheme 2. Modification of Linker fragment and benzyl moiety. Reagents and conditions: EDCl, HOBt, Et_3N, DCM; (b) TFA; (c) 10% Pd/C, THF, H_2 gas; (d) LiCl, $NaBH_4$, THF–EtOH; (e) 4 N HCl AcOEt; (f) HATU, DIEPA, THF; (g) ROH, 60% NaH, THF.

compounds **3b** and **3c** suggested that the existence of a trifluoromethyl group and the position of the pyrazole ring nitrogen atoms are important to give high potency. Replacement of the phenyl ring by an alkyl or an arylalkyl group exhibited decreased potency and insufficient metabolic stability (**3a** vs **3d**, **e**). Incorporating substituents in the *para*-position (**3f** and **3g**) and in the *meta*-position (**3h**) did not improve metabolic stability versus the parent (**3a**).

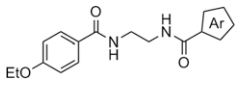
Replacement of the phenyl ring with a heteroaromatic group (to decrease the overall lipophilicity) afforded the 2-pyridyl derivative **3i** (IC_{50} = 28 nM). This compound exhibited good potency, however its metabolic stability was not significantly improved. Isomeric pyridyl derivatives **3j** and **3k** exhibited drastically decreased potency. Based on the reasonable potency of **3i**, we introduced polar groups at the 2-position of the phenyl ring. However, the 2-hydroxyphenyl derivative **3l** exhibited decreased potency, insufficient metabolic stability and a poor PK profile. Overall, we believed the 1-phenyl-3-trifluoromethylpyrrazol-4-carbamoyl group was an acceptable pyrazole moiety to use for the remaining SAR, as it provided potent inhibitory activity and moderate metabolic stability.

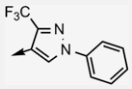
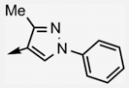
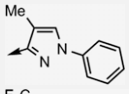
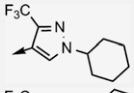
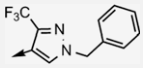
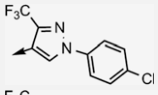
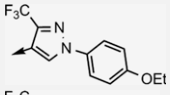
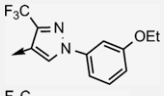
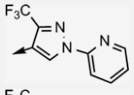
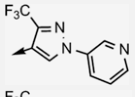
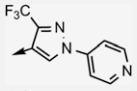
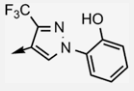
Concurrently, we examined substituents on the ethylene linker (R^1 or R^2) as shown in Table 2. Our design concept was based on a belief that the ethylene linker of **3a** serves as a mimic of 1,2-diacylglycerol, the substrate of the DGAT-1 enzyme. With this modification, we aimed to enhance potency with the insertion of a hydroxymethyl group. Alternatively, even if the 1,2-diacylglycerol hypothesis was incorrect, it could lead to an improvement of metabolic stability by affording an overall decrease in lipophilicity. However, the hydroxymethyl derivatives **3m**–**p** showed drastically reduced potency. Methyl derivatives **3q** and **3r** also decreased enzyme activities, and these results suggested that substitution on the ethylene linker would be disfavored.

The third concurrent focus of SAR effort was modification of the benzoyl moiety in **3a** to improve the metabolic stability (Table 3). Moving the ethoxy group from *para*- to *meta*-position decreased potency significantly (**3s** vs **3a**). The 4-pyrazol-1-ylphenyl derivative **3t** showed a slightly decreased potency. These results suggested that an alkoxy substituent at the *para*-position of the phenyl ring was desirable. Subsequently, we examined the replacement of the phenyl ring by pyridyl, in an attempt to improve metabolic stability by reducing the lipophilicity. In order to compare the insertion position of the nitrogen atom we synthesized two types of chloropyridines **3u** and **3v**. 2-Chloro-5-pyridine derivative **3v** showed more potent DGAT-1 inhibition and better in vitro metabolic stability than that of the 5-chloro-2-pyridine derivative **3u**. Several of these analogs were evaluated for rat PK, as shown in Table 3. Chloropyridyl amide **3v** exhibited a great improvement of the PK profile compared to **3a**, with much lower clearance and a large increase in area under the blood concentration-time curve (AUC_{po}) and bioavailability. We desired to replace the potential metabolically labile halogen, and consequently we introduced alkoxy groups at the *para*-(2-) position. The 2-ethoxy-5-pyridine derivative **3w** (IC_{50} = 25 nM) exhibited potent DGAT-1 inhibitory activity. Compound **3w** showed improved metabolic stability as compared to **3a**; however it still showed low plasma concentration. In our further optimization of the substituent at the 2-position of the pyridine ring, 2-(2,2,2-trifluoroethoxy) derivative **3x** exhibited potent DGAT-1 inhibitory activity in both enzyme (IC_{50} = 22 nM) and cell-based (IC_{50} = 44 nM) assays. Compound **3x** also showed a significant improvement of the metabolic stability and >17,000-fold higher AUC_{po} than that of **3a**.

Encouraged by the potency and excellent PK profile of compound **3x**, we tested it for efficacy in animal models of obesity. In one study, C57BL/6 mice on a high-fat diet were dosed with

Table 1
SAR around the pyrazole ring substituent



Compd	Ar	Enzyme IC_{50} (nM)	Cell IC_{50} (nM)	Metabolic stability[a] (human/rat/mouse)	Rats cassette dosing PK	
					AUCpo[b]	BA[c]
3a		33	77	9.0 / 6.5 / 7.0	0.3	0.1
3b		650	260	5.0 / 5.8 / 2.5	NT*	NT*
3c		2000	1200	12 / 12 / 14	NT*	NT*
3d		220	650	8.7 / 11 / 7.8	ND**	ND**
3e		790	770	5.5 / 18 / 7.5	NT*	NT*
3f		94	1500	4.5 / 7.2 / 3.4	22	3.3
3g		140	380	7.5 / 8.7 / 14	NT*	NT*
3h		44	600	7.4 / 7.1 / 6.5	54	7.4
3i		28	91	14 / 10 / 7.0	28	8.1
3j		1900	3400	NT*	NT*	NT*
3k		5500	>10,000	NT*	NT*	NT*
3l		98	610	5.7 / 18 / 0.5	0.9	1.4

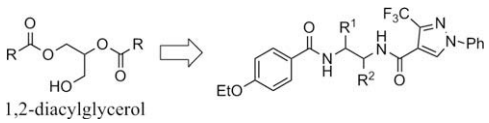
[a] %/min/mg.
[b] Area under the blood concentration-time curve (ng h/mL).
[c] Bioavailability (%).
* Not tested.
** Not detected.

compound **3x** (3 mg/kg, b.i.d., 0.5% MC suspension) for four weeks. The treated animals in the study had a significant and sustainable reduction in body weight gain (−75%) and white adipose tissue weight (mesenteric: −25%, perirenal: −12%, subcutaneous: −2.8%) without affecting total food intake. The details of this study and other advanced pharmacology experiments with this compound will be reported separately.

4. Conclusion

In this study, we designed the lead library **3** from compounds **1** and **2**, leading to the discovery of lead compound **3a**. We optimized substituents on the pyrazole ring, the linker portion, and benzoyl group of compound **3a**, with the goal of improving the PK profile while maintaining good potency. Replacement of the 4-ethoxybenzoyl group of **3a** with a 2-(2,2,2-trifluoroethoxy)pyrid-5-yl group gave compound **3x**, which exhibited both potent DGAT-1 inhibitory activity and an excellent PK profile due to good metabolic stability. Compound **3x** has a unique structure, and in contrast to many known DGAT-1 inhibitors, does not possess carboxylic acid or amino functionalities. Furthermore, **3x** exhibited superior efficacy in the reduction of body weight gain and white adipose tissue weight in DIO mice. These results suggest that the small molecule DGAT-1 inhibitor **3x** might have potential in the treatment of obesity and metabolic syndrome.

Table 2
Effect of insertion into ethylene Linker



Compd	R^1	R^2	Enzyme IC_{50} (nM)
3a	H	H	33
3m	*S*-CH_2OH	H	>10,000
3n	*R*-CH_2OH	H	>10,000
3o	H	*S*-CH_2OH	>10,000
3p	H	*R*-CH_2OH	>10,000
3q	Me	H	5300
3r	H	Me	2800

5. Experimental section

5.1. Chemistry

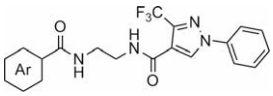
All melting points were determined on a Yanagimoto micro-melting point apparatus or Stanford Research Systems OptiMelt automated melting point system and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian Mercury-300 (300 MHz), Bruker DPX-300 (300 MHz) or Bruker-400 (400 MHz) with tetramethylsilane as internal standard. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad. Coupling constants (*J* values) are given in hertz (Hz). LC/MS (ESI-MS) analyses were carried out using a Waters Open-Lynx system. Optical rotations were determined with a JASCO P-1030 digital polarimeter. Elemental analyses were carried out by Takeda Analytical Laboratories Ltd and were within ±0.4% of the theoretical values for the elements indicated unless otherwise noted.

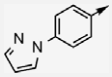
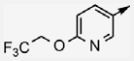
Reactions were carried out at room temperature unless otherwise noted and followed by thin layer chromatography (TLC) on silica gel 60 F254 precoated TLC plates (E. Merck) or by HPLC using an octadecyl silica (ODS) column (A-303, 4.6 mm id × 250 mm, YMC Co., Ltd). Standard workup procedures were as follows. The reaction mixture was partitioned between the indicated solvent and water. Organic extracts were combined and washed in the indicated order using the following aqueous solutions: water, 5% aqueous sodium carbonate solution (aqueous $NaHCO_3$), saturated sodium chloride (NaCl) solution (brine), 1 N aqueous sodium hydroxide solution (1 N NaOH) and 1 N hydrochloric acid (1 N HCl). Extracts were dried over anhydrous magnesium sulfate (MgSO4), filtered, and evaporated in vacuo. Chromatographic separations were carried out on Silica gel 60 (0.063–0.200 mm, E. Merck) on Purif-Pack (SI 60 μm or NH 60 μm, Fuji Silysia, Ltd) or ODS (CPO-273L, prepacked column, 22–300 mm, Kusano Kagaku Kikai Co.) using the indicated eluents. Yields were not maximized.

5.1.1. *N*-(2-Aminoethyl)-4-ethoxybenzamide hydrochloride (5)

A mixture of 4-ethoxybenzoic acid **4** (3.00 g, 18.0 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (5.19 g, 27.0 mmol), *tert*-butyl (2-aminoethyl)carbamate (3.08 g, 19.9 mmol) and 1-hydroxybenzotriazole monohydrate (HOBt) (4.15 g, 27.0 mmol) in acetonitrile (30 ml) was stirred for 4 h. The reaction mixture was concentrated in vacuo. The residue was diluted with water and extracted with ethyl acetate. The extracts were washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was dissolved in ethyl acetate (50 ml). To the mixture was added 4 N hydrogen chloride in ethyl acetate (19.0 ml, 76.2 mmol) and the mixture was stirred for 24 h. The reaction mixture was concentrated in vacuo and the residue was collected by filtration to give **5** (3.12 g, 71%) as a white solid. Compound **5** was used for next step without

Table 3
SAR around benzoyl moiety



Compd	Ar	Enzyme IC_{50} (nM)	Cell IC_{50} (nM)	Metabolic stability[a] (human/rat/mouse)	Rats cassette dosing PK test[b]					
					V_{dss}[c]	CL_{total}[d]	C_{max}[e]	AUC_{po}[f]	MRT_{po}[g]	BA[h]
3a		33	77	9.0 / 6.5 / 7.0	1453	5535	0.4	0.3	0.38	0.1
3s		3600	5100	14 / 8.3 / 19	NT*	NT*	NT*	NT*	NT*	NT*
3t		79	400	5.9 / 6.5 / 3.5	1346	1838	6.4	25	3.2	4.5
3u		1300	1200	4.8 / 2.6 / 1.8	NT*	NT*	NT*	NT*	NT*	NT*
3v		49	370	2.2 / 1.1 / 0	728	343	182	964	3.3	33
3w		25	87	5.1 / 3.4 / 2.5	399	1098	103	74	0.73	7.6
3x		22	44	2.1 / 0.4 / 0	325	95	800	5292	4.2	50

[a] %/min/mg.
[b] iv: 0.1 mg/0.5 mL/kg, po: 1 mg/5 mL/kg, *n* = 3.
[c] Distribution volume (mL/kg).
[d] Total clearance (mL/h/kg).
[e] Maximum blood concentration (ng/mL).
[f] Area under the blood concentration-time curve (ng h/mL).
[g] Mean residence time (h).
[h] Bioavailability (%).

further purification. mp 179–180 °C; ^{1}H NMR (DMSO-d_6) δ: 1.34 (3H, t, J = 7.0 Hz), 2.97 (2H, t, J = 6.0 Hz), 3.51 (2H, q, J = 5.8 Hz), 4.08 (2H, q, J = 6.8 Hz), 6.94–7.02 (2H, m), 7.84–7.93 (2H, m), 8.11 (2H, br s.), 8.64 (1H, t, J = 5.3 Hz); LC/MS m/z: 209 (MH^+); Anal. Calcd for $C_{11}H_{17}N_2O_2Cl$: C, 53.99; H, 7.00; N, 11.45. Found: C, 53.88; H, 7.04; N, 11.41.

5.1.2. *N*-(2-{[(4-Ethoxyphenyl)carbonyl]amino}ethyl)-1-phenyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3a)

A mixture of **5** (98 mg, 0.40 mmol), EDCI (115 mg, 0.60 mmol), **6a** (102 mg, 0.40 mmol), HOBt (92 mg, 0.60 mmol) and triethylamine (0.112 ml, 0.80 mmol) in *N*,*N*-dimethylformamide (DMF) (5 ml) was stirred for 24 h. The reaction mixture was diluted with aqueous $NaHCO_3$ and extracted with ethyl acetate. The extracts were washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was recrystallized from ethyl acetate–hexane to give **3a** (148 mg, 83%) as a white solid. mp 202–203 °C; ^{1}H NMR ($CDCl_3$) δ: 1.42 (3H, t, J = 7.0 Hz), 3.57–3.77 (4H, m), 4.06 (2H, q, J = 6.9 Hz), 6.85–6.98 (2H, m), 7.36–7.45 (1H, m), 7.46–7.61 (3H, m), 7.66–7.74 (2H, m), 7.74–7.89 (3H, m), 8.55 (1H, s); LC/MS m/z: 447 (MH^+); Anal. Calcd for $C_{22}H_{21}N_4O_3F_3$: C, 59.19; H, 4.74; N, 12.55. Found: C, 59.23; H, 4.78; N, 12.60.

The following compounds **3f**–**l**, **q**–**s**, **7**, **14m**–**p** were prepared from the corresponding starting materials in a similar manner to that described for **3a**.

5.1.3. 1-(4-Chlorophenyl)-*N*-(2-{[(4-ethoxyphenyl)carbonyl]amino}ethyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3f)

Solid (yield 24%); mp 192–194 °C; ^{1}H NMR (DMSO-d_6) δ: 1.34 (3H, t, J = 7.0 Hz), 3.40 (4H, m), 4.07 (2H, q, J = 7.0 Hz), 6.97 (2H, m), 7.68 (2H, m), 7.83 (4H, m), 8.43 (1H, m), 8.48 (1H, m), 9.08 (1H, m); LC/MS m/z: 481 (MH^+); Anal. Calcd for $C_{22}H_{20}N_4O_3ClF_3$: C, 54.95; H, 4.19; N, 11.65. Found: C, 54.85; H, 4.03; N, 11.77.

5.1.4. 1-(4-Ethoxyphenyl)-*N*-(2-{[(4-ethoxyphenyl)carbonyl]amino}ethyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3g)

White solid (yield 91%); mp 199–200 °C; ^{1}H NMR (DMSO-d_6) δ: 1.29–1.40 (6H, m), 3.35–3.44 (4H, m), 4.02–4.14 (4H, m), 6.93–7.01 (2H, m), 7.08–7.16 (2H, m), 7.65–7.73 (2H, m), 7.77–7.85 (2H, m), 8.44 (2H, d, J = 2.3 Hz), 8.93 (1H, d, J = 1.1 Hz); LC/MS m/z: 491 (MH^+); Anal. Calcd for $C_{24}H_{25}N_4O_4F_3$: C, 58.77; H, 5.14; N, 11.42. Found: C, 58.60; H, 5.24; N, 11.39.

5.1.5. 1-(3-Ethoxyphenyl)-*N*-(2-{[(4-ethoxyphenyl)carbonyl]amino}ethyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3h)

Solid (yield 32%); mp 174–176 °C; ^{1}H NMR (DMSO-d_6) δ: 1.35 (6H, m), 3.40 (4H, m), 4.10 (4H, m), 6.96 (2H, m), 7.02 (1H, m), 7.35 (2H, m), 7.49 (1H, t, J = 7.8 Hz), 7.81 (2H, m), 8.44 (2H, m), 9.07 (1H, m); LC/MS m/z: 491 (MH^+); Anal. Calcd for $C_{24}H_{25}N_4O_4F_3 \cdot 0.1H_2O$: C, 58.56; H, 5.16; N, 11.38. Found: C, 58.30; H, 5.21; N, 11.42.

5.1.6. *N*-(2-{[(4-Ethoxyphenyl)carbonyl]amino}ethyl)-1-pyridin-2-yl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3i)

White solid (yield 88%); mp 223–224 °C; ^{1}H NMR (DMSO-d_6) δ: 1.33 (3H, t, J = 7.0 Hz), 3.40 (4H, br s), 4.08 (2H, q, J = 6.8 Hz), 6.94–7.00 (2H, m), 7.53 (1H, ddd, J = 7.4, 4.9, 0.9 Hz), 7.78–7.85 (2H, m), 7.99 (1H, d, J = 8.3 Hz), 8.07–8.14 (1H, m), 8.44 (1H, br s), 8.56–8.60 (1H, m), 8.63–8.70 (1H, m), 9.38 (1H, d, J = 0.8 Hz); LC/MS m/z: 448 (MH^+); Anal. Calcd for $C_{21}H_{20}N_5O_3F_3$: C, 56.37; H, 4.51; N, 15.65. Found: C, 56.29; H, 4.60; N, 15.64.

5.1.7. *N*-(2-{[(4-Ethoxyphenyl)carbonyl]amino}ethyl)-1-pyridin-3-yl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3j)

Solid (yield 39%); mp 199–201 °C; ^{1}H NMR (DMSO-d_6) δ: 1.33 (3H, t, J = 7.0 Hz), 3.41 (4H, m), 4.08 (2H, q, J = 7.0 Hz), 6.97 (2H, d, J = 9.0 Hz), 7.65 (1H, m), 7.81 (2H, d, J = 9.0 Hz), 8.23 (1H, m), 8.43 (1H, m), 8.51 (1H, m), 8.68 (1H, m), 9.06 (1H, m), 9.13 (1H, m); LC/MS m/z: 448 (MH^+); Anal. Calcd for $C_{21}H_{20}N_5O_3F_3$: C, 56.37; H, 4.51; N, 15.65. Found: C, 56.26; H, 4.57; N, 15.60.

5.1.8. *N*-(2-{[(4-Ethoxyphenyl)carbonyl]amino}ethyl)-1-pyridin-4-yl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3k)

Solid (yield 55%); mp 228–230 °C; ^{1}H NMR (DMSO-d_6) δ: 1.33 (3H, t, J = 7.0 Hz), 3.41 (4H, m), 4.08 (2H, q, J = 7.0 Hz), 6.97 (2H, d, J = 9.0 Hz), 7.82 (2H, d, J = 9.0 Hz), 7.85 (2H, m), 8.43 (1H, s), 8.55 (1H, s), 8.78 (2H, m), 9.26 (1H, s); LC/MS m/z: 448 (MH^+); Anal. Calcd for $C_{21}H_{20}N_5O_3F_3$: C, 56.37; H, 4.51; N, 15.65. Found: C, 56.39; H, 4.63; N, 15.59.

5.1.9. *N*-(2-{[(4-Ethoxyphenyl)carbonyl]amino}ethyl)-1-(2-hydroxyphenyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3l)

White solid (yield 83%); mp 220–223 °C; ^{1}H NMR (DMSO-d_6) δ: 1.33 (3H, t, J = 7.0 Hz), 3.39 (4H, br s), 4.07 (2H, q, J = 6.8 Hz), 6.91–7.00 (3H, m), 7.11 (1H, dd, J = 8.3, 1.1 Hz), 7.26–7.35 (1H, m), 7.57 (1H, dd, J = 7.9, 1.5 Hz), 7.77–7.84 (2H, m), 8.43 (1H, br s), 8.52 (1H, br s), 8.85 (1H, s), 10.73 (1H, br s); LC/MS m/z: 463 (MH^+); Anal. Calcd for $C_{22}H_{21}N_4O_4F_3$: C, 57.14; H, 4.58; N, 12.12. Found: C, 56.93; H, 4.81; N, 12.04.

5.1.10. *N*-(2-{[(4-Ethoxyphenyl)carbonyl]amino}propyl)-1-phenyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3q)

White solid (yield 44%); mp 191–193 °C; ^{1}H NMR (DMSO-d_6) δ: 1.18 (3H, d, J = 6.6 Hz), 1.34 (3H, t, J = 7.0 Hz), 3.35–3.41 (2H, m), 4.07 (2H, q, J = 7.0 Hz), 4.15–4.25 (1H, m), 6.94–6.99 (2H, m), 7.45–7.50 (1H, m), 7.57–7.64 (2H, m), 7.78–7.84 (4H, m), 8.12 (1H, d, J = 8.2 Hz), 8.45 (1H, t, J = 5.9 Hz), 9.05 (1H, s); LC/MS m/z: 461 (MH^+); Anal. Calcd for $C_{23}H_{23}N_4O_3F_3$: C, 59.99; H, 5.03; N, 12.17. Found: C, 59.91; H, 5.08; N, 12.12.

5.1.11. *N*-(2-{[(4-Ethoxyphenyl)carbonyl]amino}-1-methylethyl)-1-phenyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3r)

White solid (yield 42%); mp 174–176 °C; ^{1}H NMR (DMSO-d_6) δ: 1.17 (3H, t, J = 8.6 Hz), 1.32 (3H, t, J = 7.0 Hz), 3.34–3.45 (2H, m), 4.07 (2H, q, J = 7.0 Hz), 4.15–4.24 (1H, m), 6.94–6.98 (2H, m), 7.45–7.51 (1H, m), 7.57–7.65 (2H, m), 7.78–7.85 (4H, m), 8.17 (1H, d, J = 8.2 Hz), 8.38 (1H, t, J = 5.9 Hz), 9.07 (1H, s); LC/MS m/z: 461 (MH^+); Anal. Calcd for $C_{23}H_{23}N_4O_3F_3 \cdot 0.4H_2O$: C, 59.07; H, 5.13; N, 11.98. Found: C, 59.43; H, 5.28; N, 11.54.

5.1.12. *N*-(2-{[(4-Ethoxyphenyl)carbonyl]amino}ethyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (7)

Solid (yield 34%); mp 160–162 °C; ^{1}H NMR ($CDCl_3$) δ: 1.34 (3H, t, J = 7.0 Hz), 3.36 (4H, m), 4.07 (2H, q, J = 7.0 Hz), 6.96 (2H, m), 7.80 (2H, m), 8.34 (2H, m), 8.41 (1H, m), 13.74 (1H, br s); Anal. Calcd for $C_{16}H_{17}N_4O_3$: C, 51.89; H, 4.63; N, 15.13. Found: C, 51.71; H, 4.64; N, 15.15.

5.1.13. (*S*)-Methyl *N*-[(4-ethoxyphenyl)carbonyl]-3-({[1-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl]carbonyl}amino)alaninate (14m)

White solid (yield 63%); mp 77–79 °C; ^{1}H NMR ($CDCl_3$) δ: 1.41 (3H, t, J = 7.0 Hz), 3.77 (3H, s), 3.86–4.00 (2H, m), 4.03

(2H, q, J = 7.0 Hz), 4.88 (1H, m), 6.85 (2H, m), 7.25 (1H, m), 7.34–7.44 (3H, m), 7.54–7.62 (3H, m), 7.26 (2H, m), 8.43 (1H, s); LC/MS m/z: 505 (MH^+); Anal. Calcd for $C_{24}H_{23}N_4O_5F_3 \cdot 0.2H_2O$: C, 56.74; H, 4.64; N, 11.03. Found: C, 56.55; H, 4.65; N, 10.98.

5.1.14. (*R*)-Methyl *N*-[(4-ethoxyphenyl)carbonyl]-3-({[1-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl]carbonyl}amino)alaninate (14n)

White solid (yield 73%); mp 73–75 °C; ^{1}H NMR (DMSO-d_6) δ: 1.34 (3H, t, J = 7.0 Hz), 3.60–3.70 (1H, m), 3.66 (3H, s), 3.82 (1H, dt, J = 13.7, 5.8 Hz), 4.09 (2H, q, J = 7.0 Hz), 4.62 (1H, dt, J = 7.4, 5.5 Hz), 7.00 (2H, d, J = 9.0 Hz), 7.48 (1H, tt, J = 7.4, 1.5 Hz), 7.56–7.63 (2H, m), 7.78–7.88 (4H, m), 8.61 (1H, t, J = 5.9 Hz), 8.67 (1H, d, J = 7.4 Hz), 9.04 (1H, s); LC/MS m/z: 505 (MH^+); Anal. Calcd for $C_{24}H_{23}N_4O_5F_3$: C, 57.14; H, 4.60; N, 11.11. Found: C, 56.84; H, 4.64; N, 10.98.

5.1.15. (*S*)-Methyl 3-{[(4-ethoxyphenyl)carbonyl]amino}-*N*-{[1-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl]carbonyl}alaninate (14o)

White solid (yield 84%); mp 73–75 °C; ^{1}H NMR ($CDCl_3$) δ: 1.41 (3H, t, J = 7.0 Hz), 3.80 (3H, m), 3.95 (2H, m), 4.04 (2H, q, J = 7.0 Hz), 4.89 (1H, m), 6.88 (3H, m), 7.40 (1H, m), 7.49 (2H, m), 7.64–7.74 (5H, m), 8.43 (1H, m); LC/MS m/z: 505 (MH^+); Anal. Calcd for $C_{24}H_{23}N_4O_5F_3 \cdot 0.2H_2O$: C, 56.74; H, 4.64; N, 11.03. Found: C, 56.60; H, 4.69; N, 11.06.

5.1.16. (*R*)-Methyl 3-{[(4-ethoxyphenyl)carbonyl]amino}-*N*-{[1-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl]carbonyl}alaninate (14p)

White solid (yield 76%); mp 73–75 °C; ^{1}H NMR (DMSO-d_6) δ: 1.27 (3H, t, J = 7.0 Hz), 3.51–3.61 (1H, m), 3.60 (3H, s), 3.65–3.75 (1H, m), 4.02 (2H, q, J = 7.0 Hz), 4.61 (1H, dd, J = 13.6, 6.6 Hz), 6.92 (2H, d, J = 9.0 Hz), 7.44 (1H, tt, J = 7.4, 1.6 Hz), 7.53–7.61 (2H, m), 7.72 (2H, d, J = 9.0 Hz), 7.75–7.80 (2H, m), 8.45 (1H, t, J = 5.9 Hz), 8.68 (1H, d, J = 7.4 Hz), 9.08 (1H, s); LC/MS m/z: 505 (MH^+); Anal. Calcd for $C_{24}H_{23}N_4O_5F_3 \cdot 0.2H_2O$: C, 56.74; H, 4.64; N, 11.03. Found: C, 56.60; H, 4.68; N, 10.97.

5.1.17. *N*-(2-(4-Ethoxybenzamido)ethyl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxamide (3b)

A mixture of **5** (200 mg, 0.981 mmol), *N*,*N*-diisopropylethylamine (DIPEA) (349 mg, 2.697 mmol), 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) (373 mg, 0.981 mmol) and 3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid **6b** (165 mg, 0.817 mmol) in THF (15 ml) was stirred at room temperature overnight. The reaction mixture was quenched by adding water (150 ml). The obtained solid were filtered and washed with water and dried under high vacuum to give **3b** (146 mg, 43%) as a tan solid. mp 158–160 °C; ^{1}H NMR (DMSO-d_6) δ: 1.33 (3H, t, J = 6.9 Hz), 2.43 (3H, s), 3.39 (4H, s), 4.05–4.10 (2H, m), 6.97 (2H, d, J = 8.8 Hz), 7.33 (1H, t, J = 7.4 Hz), 7.52 (2H, t, J = 7.9 Hz), 7.73 (2H, d, J = 7.8 Hz), 7.82 (2H, d, J = 8.8 Hz), 8.12 (1H, s), 8.44 (1H, s), 8.83 (1H, s); LC/MS m/z: 393 (MH^+); Anal. Calcd for $C_{22}H_{24}N_4O_3 \cdot 0.4H_2O$: C, 64.42; H, 6.04; N, 13.76. Found: C, 64.67; H, 6.20; N, 13.88.

The following compounds **3c**, **s**–**v** were prepared from the corresponding starting materials in a similar manner to that described for **3b**.

5.1.18. *N*-(2-{[(4-Ethoxyphenyl)carbonyl]amino}ethyl)-4-methyl-1-phenyl-1*H*-pyrazole-3-carboxamide (3c)

Solid (yield 15%); mp 195–198 °C; ^{1}H NMR (DMSO-d_6) δ: 1.33 (3H, t, J = 6.9 Hz), 2.27 (3H, s), 3.40–3.44 (4H, m), 4.02–4.10 (2H, m), 6.97 (2H, d, J = 8.8 Hz), 7.34 (1H, t, J = 7.3 Hz), 7.52 (2H, t, J = 7.9 Hz), 7.82 (2H, d, J = 8.8 Hz), 7.89 (2H, d, J = 7.8 Hz), 8.37 (2H, br s), 8.44 (1H, s); LC/MS m/z: 393 (MH^+); Anal. Calcd for $C_{22}H_{24}N_4O_3$: C, 67.33; H, 6.16; N, 14.28. Found: C, 67.10; H, 6.22; N, 14.05.

5.1.19. *N*-(2-{[(3-Ethoxyphenyl)carbonyl]amino}ethyl)-1-phenyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3s)

Solid (yield 18%); mp 157–158 °C; ^{1}H NMR (DMSO-d_6) δ: 1.32 (3H, t, J = 6.9 Hz), 3.42–3.43 (4H, m), 4.03–4.08 (2H, m), 7.05–7.08 (1H, m), 7.34–7.43 (3H, m), 7.48 (1H, t, J = 7.4 Hz), 7.61 (2H, t, J = 8.0 Hz), 7.80 (1H, s), 7.82 (1H, s), 8.49 (1H, s), 8.56 (1H, s), 9.06 (1H, s); LC/MS m/z: 447 (MH^+); Anal. Calcd for $C_{22}H_{21}N_4O_3F_3 \cdot 0.5H_2O$: C, 58.02; H, 4.87; N, 12.30. Found: C, 57.74; H, 4.74; N, 12.04.

5.1.20. 1-Phenyl-*N*-[2-({[4-(1*H*-pyrazol-1-yl)phenyl]carbonyl}amino)ethyl]-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide hydrochloride (3t)

White solid (yield 65%); mp 235–236 °C; ^{1}H NMR (DMSO-d_6) δ: 3.39–3.51 (4H, m), 6.59 (1H, dd, J = 2.6, 1.9 Hz), 7.44–7.51 (1H, m), 7.57–7.65 (2H, m), 7.77–7.85 (3H, m), 7.92–8.03 (4H, m), 8.52 (1H, br s.), 8.61 (1H, d, J = 2.6 Hz), 8.67 (1H, br s.), 9.08 (1H, d, J = 1.1 Hz); LC/MS m/z: 469 (MH^+); Anal. Calcd for $C_{23}H_{19}N_6O_2F_3$: C, 58.97; H, 4.09; N, 17.94. Found: C, 58.97; H, 4.13; N, 17.89.

5.1.21. 5-Chloro-*N*-[2-({[1-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl]carbonyl}amino)ethyl]pyridine-2-carboxamide (3u)

Solid (yield 15%); mp 166–168 °C; ^{1}H NMR ($CDCl_3$) δ: 3.71 (4H, br s), 6.83 (1H, br s), 7.41 (1H, t, J = 7.2 Hz), 7.51 (2H, t, J = 8.4 Hz), 7.68–7.71 (2H, m), 7.82 (1H, dd, J = 2.4, 8.4 Hz), 8.13 (1H, d, J = 8.4 Hz), 8.31 (1H, br s), 8.42 (1H, s), 8.52 (1H, d, J = 1.6 Hz); LC/MS m/z: 438 (MH^+); Anal. Calcd for $C_{19}H_{15}N_5O_2ClF_3 \cdot 0.6H_2O$: C, 49.64; H, 3.42; N, 15.24. Found: C, 49.59; H, 3.41; N, 15.02.

5.1.22. 6-Chloro-*N*-[2-({[1-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl]carbonyl}amino)ethyl]pyridine-3-carboxamide (3v)

Solid (yield 73%); mp 199–200 °C ^{1}H NMR (DMSO-d_6) δ: 3.39–3.49 (4H, m), 7.44–7.52 (1H, m), 7.57–7.68 (3H, m), 7.78–7.84 (2H, m), 8.24 (1H, dd, J = 8.3, 2.3 Hz), 8.48–8.56 (1H, m), 8.82–8.86 (1H, m), 8.88 (1H, br s.), 9.09 (1H, d, J = 1.1 Hz); LC/MS m/z: 438 (MH^+); Anal. Calcd for $C_{19}H_{15}N_5O_2ClF_3 \cdot 0.2H_2O$: C, 51.70; H, 3.52; N, 15.87. Found: C, 51.58; H, 3.41; N, 15.86.

5.1.23. 1-Cyclohexyl-*N*-(2-{[(4-ethoxyphenyl)carbonyl]amino}-ethyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3d)

To a solution of **7** (0.1 g, 0.27 mmol) in bromocyclohexane (0.44 g, 2.70 mmol) was added potassium carbonate (0.11 g, 0.81 mmol). The mixture was stirred at 120 °C for 16 h. The mixture was diluted with EtOAc and filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (9:0.5:0.5 DCM/EtOAc/MeOH) then recrystallized from ethyl acetate to give **3d** (0.030 g, 25%) as a solid. mp 185–187 °C; ^{1}H NMR (DMSO-d_6) δ: 1.23 (1H, m), 1.34 (3H, t, J = 7.0 Hz), 1.43 (2H, m), 1.65 (2.5H, m), 1.77–1.90 (2.5H, m), 2.05 (2H, m), 3.36 (4H, m), 4.08 (2H, q, J = 7.0 Hz), 4.24 (1H, m), 6.96 (2H, d, J = 8.9 Hz), 7.80 (2H, d, J = 8.9 Hz), 8.31 (1H, s), 8.39 (1H, s), 8.40 (1H, s); LC/MS m/z: 453 (MH^+); Anal. Calcd for $C_{22}H_{27}N_4O_3F_3$: C, 58.40; H, 6.01; N, 12.38. Found: C, 58.36; H, 6.08; N, 12.44.

5.1.24. 1-Benzyl-*N*-(2-{[(4-ethoxyphenyl)carbonyl]amino}-ethyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3e)

The compound **3e** was prepared from compound **7** and benzyl bromide in a similar manner to that described for **3d**. White solid

(yield 74%); mp 186 °C; ^{1}H NMR ($CDCl_3$) δ: 1.43 (3H, t, J = 7.0 Hz), 3.55–3.65 (4H, m), 4.07 (2H, q, J = 7.2 Hz), 5.30 (2H, s), 6.81–6.98 (4H, m), 7.22–7.31 (2H, m), 7.34–7.42 (3H, m), 7.66–7.73 (2H, m), 7.84 (1H, s); LC/MS m/z: 461 (MH^+); Anal. Calcd for $C_{23}H_{23}N_4O_3F_3$: C, 59.99; H, 5.03; N, 12.17. Found: C, 60.09; H, 5.01; N, 12.23.

5.1.25. Ethyl 3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (9)

A mixture of ethyl 3,3,3-trifluoro-2-oxo-butanoate **8** (64.8 g, 0.352 mol), triethyl orthoformate (117 ml, 0.703 mol) and acetic anhydride (100 ml, 1.06 mol) was stirred at 120 °C for 2 h and at 140 °C for 5 h. The reaction mixture was concentrated in vacuo. The residue was dissolved in methanol (500 ml), hydrazine hydrate (25.6 ml, 0.528 mol) added, and the reaction heated at reflux for 3 h. The reaction mixture was concentrated in vacuo. The residue was extracted with ethyl acetate, washed with water and brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was recrystallized from ethyl acetate–hexane to give **9** (50 g, 68%) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 1.28 (4H, t, J = 7.1 Hz), 4.25 (2H, q, J = 7.0 Hz), 8.59 (1H, s), 14.13 (1H, br s); LC/MS m/z: 209 (MH^+).

5.1.26. Ethyl 1-phenyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (10a)

To a solution of **9** (15.2 g, 73.0 mmol) in DMF were added phenylboronic acid (17.8 g, 146 mmol), copper(II) acetate (19.9 g, 110 mmol) and pyridine (11.8 ml, 146 mmol) and the reaction stirred for 24 h in air. The precipitate was removed by filtration with Celite. The filtrate was concentrated in vacuo. The residue was extracted with ethyl acetate, washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography to give **10a** (15.5 g, 75%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.39 (3H, t, J = 7.0 Hz), 4.37 (2H, q, J = 7.0 Hz), 7.42 (1H, m), 7.52 (2H, m), 7.72 (2H, m), 8.48 (1H, m).

The following compounds **10f**–**h** were prepared from the corresponding starting materials in a similar manner to that described for **10a**.

5.1.27. Ethyl 1-(4-chlorophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (10f)

Solid (yield 87%); mp 54–55 °C; ^{1}H NMR ($CDCl_3$) δ: 1.39 (3H, t, J = 7.0 Hz), 4.37 (2H, q, J = 7.0 Hz), 7.49 (2H, m), 7.68 (2H, m), 8.47 (1H, m); Anal. Calcd for $C_{13}H_{10}N_2O_2ClF_3$: C, 49.00; H, 3.16; N, 8.79. Found: C, 49.05; H, 3.11; N, 8.87.

5.1.28. Ethyl 1-(4-ethoxyphenyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (10g)

Solid (yield 82%); mp 102 °C; ^{1}H NMR ($CDCl_3$) δ: 1.38 (3H, t, J = 7.2 Hz), 1.44 (3H, t, J = 7.0 Hz), 4.08 (2H, q, J = 7.2 Hz), 4.36 (2H, q, J = 7.2 Hz), 6.94–7.03 (2H, m), 7.55–7.64 (2H, m), 8.37 (1 H, s); LC/MS m/z: 329 (MH^+); Anal. Calcd for $C_{15}H_{15}N_2O_3F_3$: C, 54.88; H, 4.61; N, 8.53. Found: C, 54.94; H, 4.57; N, 8.66.

5.1.29. Ethyl 1-(3-ethoxyphenyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (10h)

Solid (yield 72%); ^{1}H NMR ($CDCl_3$) δ: 1.38 (3H, t, J = 7.0 Hz), 1.45 (3H, t, J = 7.0 Hz), 4.10 (2H, q, J = 7.0 Hz), 4.36 (2H, q, J = 7.0 Hz), 6.93 (1H, m), 7.23 (1H, m), 7.28 (1H, m), 7.38 (1H, t, J = 8.2 Hz), 8.45 (1H, m).

5.1.30. Ethyl 1-(2-hydroxyphenyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (10l)

To a 50 mL sealed tube flushed with argon were added copper(I) iodide (0.0229 g, 0.120 mmol), potassium carbonate (0.697 g, 5.05 mmol), and **9** (0.500 g, 2.40 mmol). (1*S*,2*S*)-*N*1,*N*2-dimethylcyclohexane-1,2-diamine (0.0683 g, 0.481 mmol) and 2-bromophenol (0.334 ml, 2.88 mmol) were then added along with toluene (3 ml). The tube was sealed and heated to 110 °C overnight, then cooled to room temperature. The mixture was filtered through celite to remove the solid. The filtrate was concentrated under vacuum and the residue was purified by silica gel column chromatography (10% Et_2O/DCM) to give **10l** (0.393 g, 55%) as a white solid. mp 122–123 °C; ^{1}H NMR ($CDCl_3$) δ: 1.40 (3H, t, J = 7.2 Hz), 4.39 (2H, q, J = 7.2 Hz), 6.99 (1H, ddd, J = 8.3, 7.2, 1.5 Hz), 7.15 (1H, dd, J = 8.3, 1.1 Hz), 7.30 (1H, ddd, J = 8.5, 7.4, 1.5 Hz), 7.43 (1H, dd, J = 8.3, 1.5 Hz), 8.57 (1H, d, J = 0.8 Hz), 9.69 (1H, s); LC/MS m/z: 301 (MH^+); Anal. Calcd for $C_{13}H_{11}N_2O_3F_3$: C, 52.01; H, 3.69; N, 9.33. Found: C, 51.97; H, 3.71; N, 9.33.

The following compounds **10i**–**k** were prepared from the corresponding starting materials in a similar manner to that described for **10l**.

5.1.31. Ethyl 1-(pyridin-2-yl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (10i)

Solid (yield 87%); ^{1}H NMR ($CDCl_3$) δ 1.39 (3H, t, J = 7.4 Hz), 4.37 (2H, q, J = 7.0 Hz), 7.34 (1H, m), 7.90 (1H, m), 8.06 (1H, m), 8.48 (1H, m), 9.13 (1H, m).

5.1.32. Ethyl 1-(pyridin-3-yl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (10j)

Solid (yield 74%); ^{1}H NMR ($CDCl_3$) δ: 1.40 (3H, t, J = 7.4 Hz), 4.38 (2H, q, J = 7.4 Hz), 7.49 (1H, m), 8.11 (1H, m), 8.54 (1H, m), 8.69 (1H, m), 9.02 (1H, m).

5.1.33. Ethyl 1-(pyridin-4-yl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (10k)

Solid (yield 20%); ^{1}H NMR ($CDCl_3$) δ: 1.39 (3H, t, J = 7.2 Hz), 4.39 (2H, q, J = 7.2 Hz), 7.68–7.73 (2H, m), 8.63 (1H, d, J = 1.1 Hz), 8.74–8.81 (2H, m); LC/MS m/z: 286 (MH^+).

5.1.34. 1-Phenyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (6a)

To a solution of **10a** (15.5 g, 54.5 mmol) in ethanol (150 ml) and THF (50 ml) was added 2 N NaOH (110 ml, 220 mmol). The mixture was stirred at 50 °C for 1 h. The reaction mixture was concentrated in vacuo. The residue was extracted with water and washed with diethyl ether. The water layer was acidified by 6 N hydrochloric acid (36.7 ml, 220 mmol) and extracted with ethyl acetate. The extracts were washed with brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The obtained solid was collected and washed with water to give **6a** (11.8 g, 84%) as a white solid. mp 182–184 °C; ^{1}H NMR (DMSO-d_6) δ: 7.47 (1H, t, J = 7.3 Hz), 7.54–7.61 (2H, m), 7.91–7.98 (2H, m), 9.23 (1H, d, J = 0.9 Hz), 13.23 (1H, s); LC/MS m/z: 279 ($M+Na^+$); Anal. Calcd for $C_{11}H_7N_2O_2F_3 \cdot 0.2H_2O$: C, 50.86; H, 2.87; N, 10.78. Found: C, 50.81; H, 2.57; N, 10.81.

The following compounds **6f**–**l** were prepared from the corresponding starting materials in a similar manner to that described for **6a**.

5.1.35. 1-(4-Chlorophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (6f)

Solid (yield 29%); mp 192–193 °C; ^{1}H NMR ($CDCl_3$) δ: 7.64 (2H, m), 7.98 (2H, m), 9.26 (1H, s), 13.27 (1H, br s); Anal. Calcd for $C_{11}H_6N_2O_2ClF_3 \cdot 0.3H_2O$: C, 44.63; H, 2.25; N, 9.46. Found: C, 44.47; H, 2.22; N, 9.50.

5.1.36. 1-(4-Ethoxyphenyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (6g)

White solid (yield 78%); mp 176–177 °C; ^{1}H NMR ($CDCl_3$) δ: 1.35 (3H, t, J = 7.0 Hz), 4.09 (2H, q, J = 7.1 Hz), 7.05–7.12 (2H, m),

7.78–7.86 (2H, m), 9.10 (1H, d, J = 0.8 Hz), 13.15 (1H, br s); LC/MS m/z: 301 (MH^+); Anal. Calcd for $C_{13}H_{11}N_2O_3F_3 \cdot 0.1H_2O$: C, 51.38; H, 3.68; N, 9.22. Found: C, 51.13; H, 3.69; N, 8.91.

5.1.37. 1-(3-Ethoxyphenyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (6h)

Solid (yield 75%); mp 177–178 °C; ^{1}H NMR ($CDCl_3$) δ: 1.36 (3H, t, J = 7.0 Hz), 4.13 (2H, q, J = 7.0 Hz), 7.00 (1H, m), 7.47 (3H, m), 9.27 (1H, m); Anal. Calcd for $C_{13}H_{11}N_2O_3F_3$: C, 52.01; H, 3.69; N, 9.33. Found: C, 51.83; H, 3.63; N, 9.34.

5.1.38. 1-(Pyridin-2-yl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (6i)

Solid (yield 95%); mp 231–232 °C; ^{1}H NMR ($CDCl_3$) δ: 7.55 (1H, m), 7.99 (1H, m), 8.11 (1H, m), 8.58 (1H, m), 9.10 (1H, m), 13.33 (1H, br s); Anal. Calcd for $C_{10}H_6N_3O_2F_3$: C, 46.70; H, 2.35; N, 16.34. Found: C, 46.46; H, 2.49; N, 16.15.

5.1.39. 1-(Pyridin-3-yl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (6j)

Solid (yield 96%); mp 216–217 °C; ^{1}H NMR ($CDCl_3$) δ: 7.62 (1H, m), 8.34 (1H, m), 8.66 (1H, m), 9.17 (1H, br s), 9.33 (1H, m); Anal. Calcd for $C_{10}H_6N_3O_2F_3$: C, 46.70; H, 2.35; N, 16.34. Found: C, 46.68; H, 2.35; N, 16.34.

5.1.40. 1-(Pyridin-4-yl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (6k)

Solid (yield 70%); mp 310–311 °C; ^{1}H NMR ($CDCl_3$) δ: 8.01 (2H, m), 8.75 (2H, m), 9.47 (1H, m); Anal. Calcd for $C_{10}H_6N_3O_2F_3$: C, 46.70; H, 2.35; N, 16.34. Found: C, 46.35; H, 2.34; N, 16.26.

5.1.41. 1-(2-Hydroxyphenyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (6l)

White solid (yield 73%); mp 223–224 °C; ^{1}H NMR (DMSO-d_6) δ: 6.98 (1H, td, J = 7.6, 1.3 Hz), 7.11 (1H, dd, J = 8.3, 1.1 Hz), 7.28–7.37 (1H, m), 7.58 (1H, dd, J = 7.9, 1.9 Hz), 8.78 (1H, d, J = 1.1 Hz), 10.64 (1H, br s.), 13.10 (1H, br s.); LC/MS m/z: 273 (MH^+); Anal. Calcd for $C_{11}H_7N_2O_3F_3$: C, 48.54; H, 2.59; N, 10.29. Found: C, 48.51; H, 2.59; N, 10.29.

5.1.42. (*S*)-Methyl 3-[(*tert*-butoxycarbonyl)amino]-*N*-{[1-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl]carbonyl}alaninate (12o)

To a mixture of **11i** (1.00 g, 3.90 mmol) in DCM (50 mL) were added successively **6a** (1.0 g, 3.90 mmol), EDCI (0.9 g, 4.71 mmol), HOBt·H_2O (0.690 g, 5.10 mmol) and triethylamine (0.800 g, 7.90 mmol). The mixture was stirred at room temperature for 12 h and 2 N HCl was added (100 mL). The organic layer was isolated and washed with 10% aqueous K_2CO_3 and brine. The combined organic layers were dried over $MgSO_4$, concentrated and purified by silica gel column chromatography (DCM/MeOH 99:1) to give **12o** (1.2 g, 67%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.42 (9H, s), 3.64–3.68 (2H, m), 3.80 (3H, s), 4.74–4.82 (1H, m), 4.91–4.97 (1H, m), 7.32–7.38 (1H, m), 7.39–7.44 (1H, m), 7.48–7.54 (2H, m), 7.69–7.72 (2H, m), 8.44 (1H, br); LC/MS m/z: 357 (MH^+−Boc).

The following compounds **12m**, **n**, **p**–**s** were prepared from the corresponding starting materials in a similar manner to that described for **12o**.

5.1.43. (*S*)-Methyl 3-[(*tert*-butoxycarbonyl)amino]-*N*-[(4-ethoxyphenyl)carbonyl]alaninate (12m)

White solid (yield 60%); ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, t, J = 7.0 Hz), 1.42 (9H, s), 3.60–3.70 (2H, m), 3.78 (3H, s), 4.12 (2H, q, J = 7.0 Hz), 4.70–4.76 (1H, m), 6.89–6.94 (2H, m), 7.70–7.83 (2H, m); LC/MS m/z: 267 (MH^+−Boc).

5.1.44. (*R*)-Methyl *N*-[(benzyloxy)carbonyl]-3-({[1-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl]carbonyl}amino)alaninate (12n)

White solid (yield 90%); ^{1}H NMR (DMSO-d_6) δ: 3.50–3.70 (2H, m), 3.64 (3H, s), 4.28–4.35 (1H, m), 5.05 (2H, s), 7.25–7.37 (5H, m), 7.45–7.51 (1H, m), 7.58–7.63 (2H, m), 7.70–7.75 (1H, m), 7.78–7.83 (2H, m), 8.48 (1H, t, J = 5.9 Hz), 9.03 (1H, s).

5.1.45. (*R*)-Methyl *N*-[(benzyloxy)carbonyl]-3-{[(4-ethoxyphenyl)-carbonyl]amino}alaninate (12p)

^{1}H NMR (DMSO-d_6) δ: 1.34 (3H, t, J = 7.1 Hz), 3.61–3.54 (2H, m), 3.61 (3H, s), 4.08 (2H, q, J = 7.1 Hz), 4.33–4.26 (1H, m), 5.07–4.98 (2H, m), 7.00–6.95 (2H, m), 7.37–7.29 (5H, m), 7.79–7.71 (3H, m), 8.41–8.35 (1H, m).

5.1.46. *tert*-Butyl (2-{[(4-ethoxyphenyl)carbonyl]amino}-propyl)carbamate (12q)

White solid (yield, 93%); ^{1}H NMR ($CDCl_3$) δ: 1.23 (3H, d, J = 6.2 Hz), 1.39 (9H, s), 1.42 (3H, t, J = 7.0 Hz), 3.15–3.23 (1H, m), 3.30–3.40 (1H, m), 4.06 (2H, q, J = 7.0 Hz), 4.14–4.20 (1H, m), 5.04 (1H, t, J = 6.6 Hz), 6.86–6.90 (2H, m), 6.99 (1H, d, J = 6.6 Hz), 7.75–7.80 (2H, m); LC/MS m/z: 223 (MH^+−Boc).

5.1.47. *tert*-Butyl [2-({[1-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl]carbonyl}amino)propyl]carbamate (12r)

White solid (yield 88%); ^{1}H NMR ($CDCl_3$) δ: 1.25 (3H, d, J = 6.6 Hz), 1.40 (9H, s), 3.25–3.30 (2H, m), 4.16–4.24 (1H, m), 4.95–5.01 (1H, m), 6.74–6.79 (1H, m), 7.38–7.43 (1H, m), 7.47–7.52 (2H, m), 7.66–7.71 (2H, m), 8.39 (1H, s); LC/MS m/z: 313 (MH^+−Boc).

5.1.48. *tert*-Butyl [2-({[1-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl]carbonyl}amino)ethyl]carbamate (12s)

White solid (4.1 g, 86%); ^{1}H NMR ($CDCl_3$) δ: 1.43 (9H, s), 3.37 (2H, q, J = 5.8 Hz), 3.56 (2H, q, J = 5.4 Hz), 4.98 (1H, s), 6.84 (1H, s), 7.37–7.44 (1H, m), 7.47–7.54 (2H, m), 7.69 (2H, d, J = 7.9 Hz), 8.41 (1H, s). LC/MS m/z: 421 (MNa^+).

5.1.49. (*R*)-Methyl 2-amino-3-(1-phenyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamido)propanoate (13n)

To a solution of **12n** (4.50 g, 9.20 mmol) in dry THF (300 mL) was added 10%wt palladium on charcoal (dry) (1.00 g) under argon atmosphere. The mixture was shaken under hydrogen (45 psi) for 4 days at room temperature. The palladium was filtered off and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (DCM/MeOH = 95:5) to give **13n** (2.57 g, 84%) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 3.17 (1H, s), 3.18 (1H, s), 3.40–3.45 (2H, m), 3.62 (3H, s), 4.06–4.12 (1H, m), 7.45–7.50 (1H, m), 7.58–7.63 (2H, m), 7.81–7.85 (2H, m), 8.37 (1H, t, J = 5.9 Hz), 9.09 (1H, s).

5.1.50. (*R*)-Methyl 3-{[(4-ethoxyphenyl)carbonyl]amino}-alaninate (13p)

Compound **13p** was prepared from **12p** in a similar manner to that described for **13n**. ^{1}H NMR (DMSO-d_6) δ: 1.34 (3H, t, J = 7.1 Hz), 3.33 (1H, br s), 3.45–3.35 (2H, m), 3.56–3.51 (1H, m), 3.59 (3H, s), 4.08 (2H, q, J = 7.1 Hz), 6.99–6.94 (2H, m), 7.70–7.77 (2H, m), 8.35–8.30 (1H, m).

5.1.51. (*S*)-Methyl 3-amino-*N*-{[1-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl]carbonyl}alaninate (13o)

To **12o** (1.0 g, 2.2 mmol) was added TFA (10 mL). The mixture was stirred at room temperature for 1 h and concentrated in vacuo.

The residue was dissolved in water (50 mL) and sodium carbonate was added until alkaline. After stirring 1 h at room temperature, the aqueous layer was extracted with DCM. The combined organic layers were dried over $MgSO_4$ and concentrated to give **13o** (780 mg, quant.): ^{1}H NMR ($CDCl_3$) δ: 3.17 (1H, dd, J = 13.3, 4.7 Hz), 3.25 (1H, dd, J = 13.3, 3.9 Hz), 3.81 (3H, s), 4.78 (1H, dt, J = 7.0, 4.3 Hz), 7.17 (1H, br s), 7.39–7.44 (1H, m), 7.48–7.54 (2H, m), 7.69–7.73 (2H, m), 8.49 (1H, s).

The following compounds **13m**, **q**, **r** were prepared from the corresponding starting materials in a similar manner to that described for **13o**.

5.1.52. (*S*)-Methyl 3-amino-*N*-[(4-ethoxyphenyl)carbonyl]alaninate (13m)

Clear paste (quant.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.35 (3H, t, J = 7.0 Hz), 3.19–3.40 (2H, m), 3.69 (3H, s), 4.10 (2H, q, J = 7.0 Hz), 4.74–4.80 (1H, m), 7.00–7.05 (2H, m), 7.85–7.90 (2H, m), 8.01 (2H, br s), 8.20 (1H, d, J = 8.2 Hz).

5.1.53. *N*-(2-Amino-1-methylethyl)-4-ethoxybenzamide (13q)

Clear oil (quant.); ^{1}H NMR ($CDCl_3$) δ: 1.22 (3H, d, J = 7.0 Hz), 1.39 (3H, t, J = 7.0 Hz), 3.02–3.11 (2H, m), 3.99 (2H, q, J = 7.0 Hz), 4.35–4.43 (1H, m), 6.77–6.82 (2H, m), 7.39 (1H, d, J = 8.6 Hz), 7.64–7.69 (2H, m), 8.03 (2H, br s); LC/MS m/z: 223 (MH^+).

5.1.54. *N*-(2-Amino-1-methylethyl)-1-phenyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (13r)

White solid (quant.); ^{1}H NMR ($CDCl_3$) δ: 1.22 (3H, d, J = 6.6 Hz), 3.10–3.20 (2H, m), 4.40–4.50 (1H, m), 6.35 (2H, br s), 7.30–7.43 (3H, m), 7.47 (1H, d, J = 7.8 Hz), 7.58–7.63 (2H, m), 8.67 (1H, s); LC/MS m/z: 313 (MH^+).

5.1.55. (*S*)-*N*-[2-{[(4-Ethoxyphenyl)carbonyl]amino}-1-(hydroxymethyl)ethyl]-1-phenyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3o)

To a solution of **14o** (647 mg, 1.30 mmol) in anhydrous THF/EtOH (2:1, 10 mL) was added lithium chloride (109 mg, 2.60 mmol). Upon dissolution, sodium borohydride (97 mg, 2.60 mmol) was added and the mixture was stirred at room temperature for 18 h. The mixture was concentrated to dryness, water (50 mL) was added and the pH was brought to 2–3 by addition of 2 N HCl. The aqueous layer was extracted with DCM (3 × 50 mL) and the combined organic layers were dried over $MgSO_4$. Concentration yielded **3o** (450 mg, 74%) as a white solid. mp 220–222 °C; $[\alpha]_D^{25}$ −82.4° (c 0.965, methanol); ^{1}H NMR (DMSO-d_6) δ 1.32 (3H, t, J = 7.0 Hz), 3.40 (1H, m), 3.45–3.68 (3H, m), 4.07 (2H, q, J = 7.0 Hz), 4.13 (1H, m), 6.96 (2H, m), 7.48 (1H, m), 7.61 (2H, m), 7.78–7.82 (4H, m), 8.08 (1H, d, J = 8.2 Hz), 8.40 (1H, t, J = 5.9 Hz), 9.13 (1H, s); LC/MS m/z: 477 (MH^+); Anal. Calcd for $C_{23}H_{23}N_4O_4F_3{\cdot}0.2H_2O$: C, 57.55; H, 4.91; N, 11.67. Found: C, 57.36; H, 4.93; N, 11.69.

The following compounds **3m**, **n**, **p** were prepared from the corresponding starting materials in a similar manner to that described for **3o**.

5.1.56. (*S*)-*N*-(2-{[(4-Ethoxyphenyl)carbonyl]amino}-3-hydroxypropyl)-1-phenyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3m)

White solid (yield 63%); mp 211–212 °C; $[\alpha]_D^{25}$ −88.5° (c 0.986, methanol); ^{1}H NMR (DMSO-d_6) δ: 1.33 (3H, t, J = 7.0 Hz), 3.40–3.60 (4H, m), 4.07 (2H, q, J = 7.0 Hz), 4.13 (1H, m), 4.60 (1H, br s), 6.96 (2H, m), 7.48 (1H, m), 7.61 (2H, m), 7.78–7.82 (4H, m), 8.00 (1H, d, J = 7.4 Hz), 8.48 (1H, t, J = 5.9 Hz), 9.08 (1H, s); LC/MS m/z: 459 (M^+-H_2O); Anal. Calcd for $C_{23}H_{23}N_4O_4F_3{\cdot}0.4H_2O$: C, 57.12; H, 4.96; N, 11.58. Found: C, 56.83; H, 4.94; N, 11.55.

5.1.57. (*R*)-*N*-(2-{[(4-Ethoxyphenyl)carbonyl]amino}-3-hydroxypropyl)-1-phenyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3n)

White solid (yield 85%); mp 209–212 °C; $[\alpha]_D^{25}$ +87.9° (c 0.951, methanol); ^{1}H NMR (DMSO-d_6) δ: 1.33 (3H, t, J = 7.0 Hz), 3.39–3.60 (4H, m), 4.07 (2H, q, J = 7.0 Hz), 4.08–4.16 (1H, m), 4.79 (1H, t, J = 5.8 Hz), 6.96 (2H, d, J = 9.0 Hz), 7.47 (1H, tt, J = 7.4, 1.5 Hz), 7.56–7.63 (2H, m), 7.77–7.84 (4H, m), 7.97 (1H, d, J = 7.4 Hz), 8.44 (1H, t, J = 5.9 Hz), 9.04 (1H, s); LC/MS m/z: 459 (M^+-H_2O); Anal. Calcd for $C_{23}H_{23}N_4O_4F_3$: C, 57.98; H, 4.87; N, 11.76. Found: C, 58.11; H, 4.76; N, 11.76.

5.1.58. (*R*)-*N*-[2-{[(4-Ethoxyphenyl)carbonyl]amino}-1-(hydroxymethyl)ethyl]-1-phenyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (3p)

White solid (yield 88%); mp 223–225 °C; $[\alpha]_D^{25}$ +88.6° (c 0.957, methanol); ^{1}H NMR (DMSO-d_6) δ: 1.33 (3H, t, J = 7.0 Hz), 3.35–3.57 (4H, m), 4.06 (2H, q, J = 7.0 Hz), 4.10–4.18 (1H, m), 4.83 (1H, t, J = 5.8 Hz), 6.96 (2H, d, J = 9.0 Hz), 7.48 (1H, tt, J = 7.4, 1.6 Hz), 7.57–7.64 (2H, m), 7.76–7.84 (4H, m), 8.06 (1H, d, J = 8.2 Hz), 8.38 (1H, t, J = 5.9 Hz), 9.11 (1H, s); LC/MS m/z: 477 (MH^+), 459 (M^+-H_2O); Anal. Calcd for $C_{23}H_{23}N_4O_4F_3$: C, 57.98; H, 4.87; N, 11.76. Found: C, 58.06; H, 4.70; N, 11.84.

5.1.59. *N*-(2-Aminoethyl)-1-phenyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide hydrochloride (13s)

To a solution of **12s** (4.10 g, 10.3 mmol) in ethyl acetate (40 ml) was added 4 N hydrogen chloride in ethyl acetate (25.7 ml, 103 mmol) and stirring continued for 24 h. The reaction mixture was concentrated in vacuo and the residue was collected by filtration to give **13s** (3.40 g, 99%) as a white solid. Compound **13s** was used for next step without further purification. ^{1}H NMR (DMSO-d_6) δ: 3.02 (2H, d, J = 5.3 Hz), 3.52 (2H, q, J = 5.8 Hz), 7.48 (1H, t, J = 7.4 Hz), 7.58–7.65 (2H, m), 7.82–7.88 (2H, m), 8.21 (3H, s), 8.95 (1H, t, J = 5.4 Hz), 9.59 (1H, d, J = 0.9 Hz). LC/MS m/z: 321 (MNa^+).

5.1.60. *N*-[2-({[1-Phenyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl]carbonyl}amino)ethyl]-6-(2,2,2-trifluoroethoxy)pyridine-3-carboxamide (3x)

To a solution of 2,2,2-trifluoroethanol (1.00 g, 10.0 mmol) in dry THF (8 ml) was added 60% NaH in oil (320 mg, 8.00 mmol) and the mixture was stirred for 30 min at room temperature. **3v** (876 mg, 2.00 mmol) was added and the mixture was stirred at 75 °C for 4 h. The solution was then cooled and concentrated. The residue was diluted with water and extracted with ethyl acetate. The organic layer was then dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was recrystallized from ethyl acetate–hexane to give **3x** (968 mg, 97%) as a white solid. mp 155–156 °C; ^{1}H NMR (DMSO-d_6) δ: 3.36–3.49 (4H, m), 5.06 (2H, q, J = 9.1 Hz), 7.04–7.11 (1H, m), 7.44–7.52 (1H, m), 7.56–7.65 (2H, m), 7.75–7.86 (2H, m), 8.21 (1H, dd, J = 8.7, 2.3 Hz), 8.50 (1H, br s.), 8.63–8.76 (2H, m), 9.07 (1H, d, J = 0.8 Hz); LC/MS m/z: 502 (MH^+); Anal. Calcd for $C_{21}H_{17}N_5O_3F_6$: C, 50.31; H, 3.42; N, 13.97. Found: C, 50.55; H, 3.45; N, 13.86.

The compound **3w** was prepared from the corresponding starting materials in a similar manner to that described for **3x**.

5.1.61. 6-Ethoxy-*N*-[2-({[1-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl]carbonyl}amino)ethyl]pyridine-3-carboxamide (3w)

White solid (yield 48%); mp 179–181 °C; ^{1}H NMR (DMSO-d_6) δ: 1.32 (3H, t, J = 7.0 Hz), 3.42 (4H, br s), 4.33–4.38 (2H, m), 6.85 (1H, d, J = 8.8 Hz), 7.48 (1H, t, J = 7.3 Hz), 7.61 (2H, t, J = 7.9 Hz), 7.81 (2H, d, J = 7.8 Hz), 8.09–8.12 (1H, m), 8.48 (1H, br s), 8.59 (1H, br s), 8.64 (1H, s), 9.06 (1H, s); LC/MS m/z: 448 (MH^+); Anal. Calcd

for $C_{21}H_{20}N_5O_3F_3$: C, 56.37; H, 4.51; N, 15.65. Found: C, 56.45; H, 4.55; N, 15.67.

5.2. Biology

5.2.1. Assay of hDGAT-1 inhibitory activity in vitro

Human DGAT-1 (hDGAT-1) was expressed in Sf9 insect cells using a baculovirus expression system. The microsome of the Sf9 cells was obtained as an enzyme source.

The hDGAT-1 reaction mixtures contained 100 mM Tris–HCl (pH 7.5), 250 mM sucrose, 150 mM $MgCl_2$, 0.01% bovine serum albumin (BSA fatty acid free), 1% acetone, 5 μg/mL the microsome of Sf9 expressing hDGAT-1 and 25 μM 1,2-dioleoyl-*sn*-glycerol at the final concentration. For inhibitor testing, serial dilutions of the compounds were added to the reaction mixture in the final concentration of 0.1% DMSO. The reaction was initiated by adding 25 μM [^{14}C]-Oleoyl-CoA at the final concentration. The reactions were performed in a 96-well plate in a final volume of 100 μL and were terminated after 15 min at 32 °C by addition of 300 μL of chloroform/methanol (1:2 by volume) mixed solvent. After mixing the solution, 200 μL of phosphate buffer saline (PBS) was added to facilitate phase separation. After centrifugation (3000 rpm, 3 min), 50 μL of the chloroform layer was spotted on a thin layer chromatography (TLC) plate. Lipids were separated by TLC with a solvent system of *n*-hexane/diethyl ether/ethyl acetate/acetic acid (74:15:15:1 by volume). The radioactivity of [^{14}C]-triglyceride was counted with BAS-2500 imaging system (Fujifilm) to calculate the hDGAT-1 activity or inhibition rates of the compounds.

5.2.2. Assay of intracellular DGAT inhibitory activity in mouse myoblast C_2C_{12} cell line in vitro

C_2C_{12} cells were cultured with Dulbecco's Modified Eagle Medium (D-MEM) supplemented with 10% FBS, 50 U/mL of penicillin and 50 μg/mL of streptomycin for two days as myoblast cells without differentiation to myotube and were treated by trypsine-EDTA, following were suspended in phosphate buffer saline(PBS). The serial dilutions of compound were added in the C_2C_{12} cells suspension and incubated at 37 °C for 20 min. Then, [^{14}C]-Oleic acid was added as a substrate of DGAT reaction. The reactions were performed in a 96-well plate in a final volume of 100 μL at the concentration of 10^6 C_2C_{12} cells/mL, 5 μM [^{14}C]-Oleic acid, 0.001% BSA (fatty acid free) and 0.2% ethanol and 0.1% DMSO. The reactions were terminated after 20 min at 37 °C by addition of 300 μL of chloroform/methanol (1:2 by volume) mixed solvent. After mixing the solution, 200 μL of DW was added to facilitate phase separation. After centrifugation (2000 rpm, 2 min), 45 μL of the chloroform layer was spotted on a thin layer chromatography (TLC) plate. Lipids were separated by TLC with a solvent system of *n*-hexane/diethyl ether/ethyl acetate/acetic acid (255:30:15:0.6 by volume). The radioactivity of [^{14}C]-triglyceride was counted with BAS-2500 imaging system (Fujifilm) to calculate intracellular DGAT inhibitory activity of the compounds.

5.2.3. Metabolic stability assay

Hepatic microsomes from mice, rats and humans were purchased from Xenotech, LLC (Lenexa, KS). An incubation mixture with a final volume of 0.1 mL consisted of microsomal protein in 50 mmol/L KH_2PO_4–K_2HPO_4 phosphate buffer (pH 7.4) and 1 μmol/L test compound. The concentration of hepatic microsomal protein was 0.2 mg/mL. An NADPH-generating system containing 50 mmol/L $MgCl_2$, 50 mmol/L glucose-6-phosphate, 5 mmol/L beta-$NADP^+$ and 15 unit/mL glucose-6-phosphate dehydrogenase was prepared and added to the incubation mixture with a 10% volume of the reaction mixture. After the addition of the NADPH-generating system, the mixture was incubated at 37 °C for 0 and 20 min. The reaction was terminated by the addition of acetonitrile equivalent to the volume of the reaction mixture. All incubations were made in duplicate. Test compound in the reaction mixture was measured by HPLC system equipped with a UV detector. For metabolic stability determinations, chromatograms were analyzed for parent compound disappearance from the reaction mixtures.

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References and notes

1. Cases, S.; Smith, S. J.; Zheng, Y.; Myers, H. M.; Lear, S. R.; Sande, E.; Novak, S.; Collins, C.; Welch, C. B.; Lusis, A. J.; Erickson, S. K.; Farese, R. V., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 13018.
2. Cases, S.; Stone, S. J.; Zhou, P.; Yen, E.; Tow, B.; Lardizabal, K. D.; Voelker, T.; Farese, R. V., Jr. *J. Biol. Chem.* **2001**, *276*, 38870.
3. Smith, S. J.; Cases, S.; Jensen, D. R.; Chen, H. C.; Sande, E.; Tow, B.; Sanan, D. A.; Raber, J.; Eckel, R. H.; Farese, R. V., Jr. *Nat. Genet.* **2000**, *25*, 87.
4. Chen, H. C.; Smith, S. J.; Ladha, Z.; Jensen, D. R.; Ferreira, L. D.; Pulawa, L. K.; McGuire, J. G.; Pitas, R. E.; Eckel, R. H.; Farese, R. V., Jr. *J. Clin. Invest.* **2002**, *109*, 1049.
5. Chen, H. C.; Stone, S. J.; Zhou, P.; Buhman, K. K.; Farese, R. V., Jr. *Diabetes* **2002**, *51*, 3189.
6. Zhao, G.; Souers, A. J.; Voorbach, M.; Falls, H. D.; Droz, B.; Brodjian, S.; Lau, Y. Y.; Iyengar, R. R.; Gao, J.; Judd, A. S.; Wagaw, S. H.; Ravn, M. M.; Engstrom, K. M.; Lynch, J. K.; Mulhern, M. M.; Freeman, J.; Dayton, B. D.; Wang, X.; Grihalde, N.; Fry, D.; Beno, D. W. A.; Marsh, K. C.; Su, Z.; Diaz, G. J.; Collins, C. A.; Sham, H.; Reilly, R. M.; Brune, M. E.; Kym, P. R. *J. Med. Chem.* **2008**, *51*, 380.
7. Birch, A. M.; Birtles, S.; Buckett, L. K.; Kemmitt, P. D.; Smith, G. J.; Smith, T. J. D.; Turnbull, A. V.; Wang, S. J. Y. *J. Med. Chem.* **2009**, *52*, 1558.
8. Nakada, Y.; Ogino, M.; Asano, K.; Aoki, K.; Miki, H.; Yamamoto, T.; Kato, K.; Masago, M.; Tamura, N.; Shimada, M. *Chem. Pharm. Bull.* **2010**, *58*, in press.
9. Guengerich, F. P.; MacDonald, J. S. *Chem. Res. Toxicol.* **2007**, *20*, 344.
10. Hobbs, D. C. *Drug Metab. Dispos.* **1977**, *5*, 75.
11. Onderwater, R. C.; Commandeur, J. N.; Menge, W. M.; Vermeulen, N. P. *Chem. Res. Toxicol.* **1999**, *12*, 396.
12. Schwab, C. E.; Huber, W. W.; Parzefall, W.; Hietsch, G.; Kassie, F.; Schulte, -H. R.; Knasmuller, S. *Crit. Rev. Toxicol.* **2000**, *30*, 1.
13. 3-Methyl-1-phenylpyrazole-4-carboxylic acid **6b** was prepared as described in Abdelilah, B.; Michel, H.-H. *Bull. Soc. Chim. Fr.* **1988**, *3*, 540.
14. 4-Methyl-1-phenylpyrazole-3-carboxylic acid **6c** was prepared as described in Martins, M. A. P.; Freitag, R. A.; Rosa, A. da.; Flores, A. F. C.; Zanatte, N.; Bonacorso, H. G. *J. Hetrocycl. Chem.* **1999**, *36*, 217.
15. 3-(Trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid **6d** was prepared as described in Kazmierski, W. M.; Aquino, C. J.; Bifulco, N.; Boros, E. E.; Chauder, B. A.; Chong, P. Y.; Duan, M.; Deanda, Jr. F.; Koble, C. S.; Mclean, E. W. Patent WO 2004/054974.
16. Compounds **10a**, **f**, **g**, **h**: Lam, P. Y. S.; Clarkt, C. G.; Saubernt, S.; Adamst, J.; Winters, M. P.; Chan, D. M. T.; Combst, A. *Tetrahedron Lett.* **1998**, *39*, 2941.
17. Compounds **10i–l**: Antilla, J. C.; Baskin, J. M.; Barder, T. E.; Buchwald, S. L. *J. Org. Chem.* **2004**, *69*, 5578.