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Original article

Evaluation of thiazole containing biaryl analogs as diacylglycerol acyltransferase 1 (DGAT1) inhibitors

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ABSTRACT

Biphenyl carboxylic acids, exemplified by compound **5**, are known potent inhibitors of diacylglycerol acyltransferase, DGAT1, an enzyme involved in the final committed step of triglyceride biosynthesis. We have synthesized and evaluated 2-phenylthiazole, 4-phenylthiazole, and 5-phenylthiazole analogs as DGAT1 inhibitors. The 5-phenylthiazole series exhibited potent DGAT1 inhibition when evaluated using an in vitro enzymatic assay and an in vivo fat tolerance test in mice. Compound **33** (IC_{50} = 23 nM) exhibiting promising oral pharmacokinetic parameters (AUC_{inf} = 7058 ng*h/ml, $T_{1/2}$ = 0.83 h) coupled with 87 percent reduction of plasma triglycerides in vivo may serve as a lead for developing newer anti-obesity agents.

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1. Introduction

Obesity, the oldest known metabolic disorder, is characterized by an accumulation of triacylglycerols within the adipocytes. An enhanced risk of several pathologic conditions such as hypertension [1], stroke [2], coronary heart disease [3], cancer [4], inflammatory disease [5] including arthritis [6], and Type 2 diabetes [7] have been widely documented amongst the obese. Presently orlistat is the only marketed anti-obesity drug that is known to act by preventing the absorption of fat from human diet [8]. However adverse effects such as steatorrhea, fecal incontinence, and urgent bowel movements are commonly observed with the use of orlistat thereby reducing its appeal within the target population [9]. The lack of therapeutic alternatives to orlistat presents a pressing need for the identification of newer chemical scaffolds, devoid of side-effects, to be developed as anti-obesity agents.

In humans the biosynthesis of triacylglycerols is catalyzed by the enzyme Acyl CoA:diacylglycerol acyltransferase (DGAT) [10]. Two isoforms of the DGAT enzyme are presently known namely, DGAT1 and DGAT2. In mammals, DGAT1 is widely expressed in the skeletal muscle, intestine, and testis while DGAT2 is expressed particularly in the liver and adipose tissues. DGAT2 deficiency in mice knockout models led to death soon after birth apparently from profound reductions in substrates required for energy metabolism coupled with an impaired permeability barrier function of the skin Ref. [11]. On the other hand, DGAT1 knockouts were viable, resistant to weight gain when fed a high-fat diet, and exhibited increased insulin and leptin sensitivity [12]. Consequently inhibition of DGAT1 has emerged as a plausible strategy for the treatment of obesity. Of late, several potent DGAT1 inhibitors have been reported and a few compounds are being clinically evaluated (Fig. 1) [13–16].

A series of DGAT1 inhibitors belonging to an amino biphenyl carboxylic acid scaffold, exemplified by compound **5** [hDGAT1 IC_{50} = 35 nM, in vitro solubility (pH 7.4) = 0.01 mg/ml] have been

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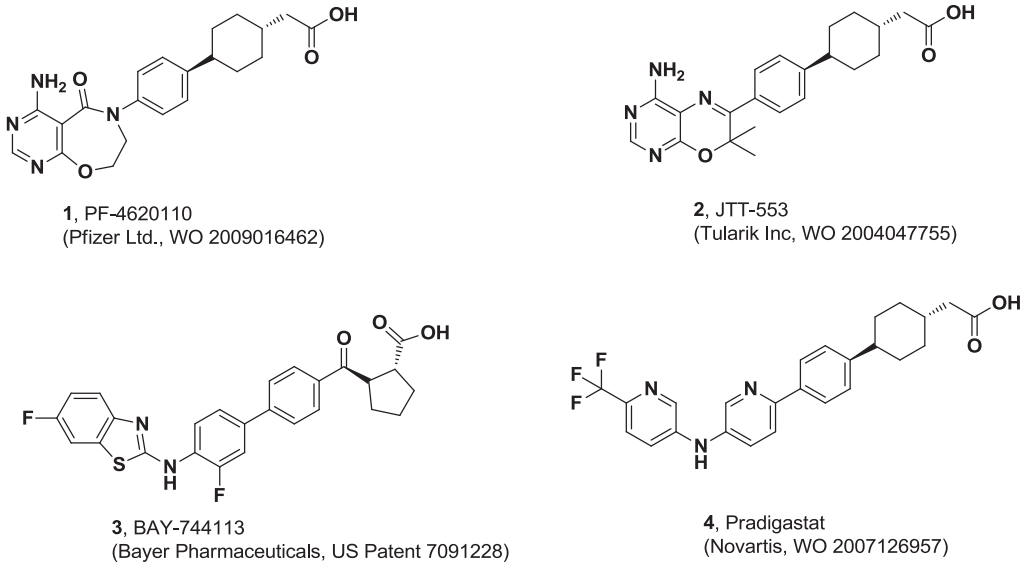


Fig. 1. Clinically studied hDGAT1 inhibitors.

disclosed by Bayer Pharmaceuticals [15,17]. These efforts prompted us to explore for orally active inhibitors of the DGAT1 enzyme. We have recently reported a 3-phenylisoxazole scaffold that retains DGAT1 inhibition and displays a marked improvement in solubility over compound **5** [18]. In an attempt to identify additional pharmacophores for DGAT1 inhibition we evaluated the effect of a thiazole in place of the phenyl ring 'B' of compound **5** (Fig. 2). The thiazole moiety has been positioned either in a 2-phenylthiazole orientation represented by compound **6**, a 5-phenylthiazole orientation represented by compound **7**, or a 4-phenylthiazole orientation represented by compound **8**. Of these three analogs, compounds **6** (DGAT1 Inhibition [1 μM] = 31%) and **8** (DGAT1 Inhibition [1 μM] = 25%) did not exhibit substantial in vitro DGAT1 potency. On the other hand, compound **7** (DGAT1 Inhibition [1 μM] = 81%, IC_{50} = 10 nM) belonging to the 5-phenylthiazole scaffold exhibits potent DGAT1 inhibition comparable to that of compound **5**. We hence directed our efforts toward the synthesis of compounds belonging to the 5-phenylthiazole scaffold. Our earlier efforts have highlighted the advantage of replacing the benzothiazole substituent with a phenyl urea as exemplified by structure **9**

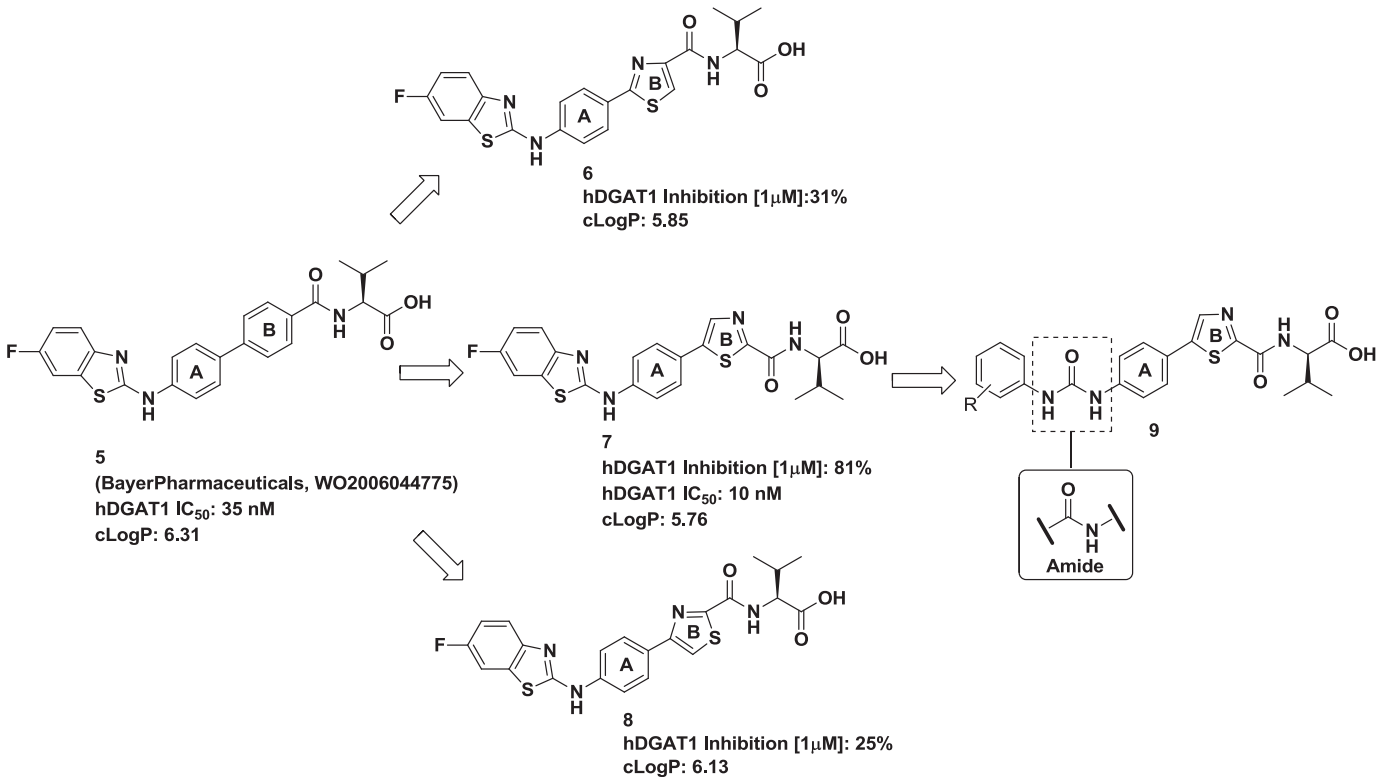


Fig. 2. Stepwise development of the 5-phenylthiazole scaffold.

(Fig. 2) [18,19]. With such a modification we were able to retain the DGAT1 activity and improve on the cLogP and solubility. In this study, we have evaluated the effects of incorporating a phenyl urea in combination with a 5-phenylthiazole scaffold. Subsequently the effects of replacing the urea linker with an amide linker have also been studied. Apart from their effect on the in vitro and in vivo DGAT1 activity oral absorption pharmacokinetic properties were also evaluated for selected compounds. Our efforts in this study were focused toward the development of a novel heteroaryl scaffold that retains DGAT1 activity and exhibits an acceptable oral pharmacokinetic profile comparable to that of compound **5**.

2. Chemistry

Ethyl 2-(4-nitrophenyl)thiazole-4-carboxylate (**12**), a key intermediate for the synthesis of the 2-phenylthiazole analog, 2-(2-(4-((6-fluorobenzo[*d*]thiazol-2-yl)amino)phenyl)thiazole-4-carboxamido)-3-methylbutanoic acid (**6**), was synthesized in two steps starting from 4-nitrobenzamide (**10**) as depicted in Scheme 1. Thiolation of compound **9** to 4-nitrobenzothioamide (**11**) followed by its cyclization in the presence of ethyl bromopyruvate in ethanol yielded compound **12**. Similarly ethyl 4-(4-nitrophenyl)thiazole-2-carboxylate (**15**), a key intermediate for the synthesis of the 4-phenylthiazole analog, 2-(4-(4-((6-fluorobenzo[*d*]thiazol-2-yl)amino)phenyl)thiazole-2-carboxamido)-3-methylbutanoic acid (**8**), was synthesized by bromination of 4-nitroacetophenone (**13**) to 2-bromo-1-(4-nitrophenyl)ethanone (**14**) and its subsequent cyclization to compound **15** on refluxing with ethyl 2-amino-2-thioxoacetate in methanol (Scheme 2).

Synthesis of compound **6** (Scheme 3) involved an hydrolysis of the key intermediate **12** to its corresponding acid, 2-(4-nitrophenyl)thiazole-4-carboxylic acid (**16**). Coupling of **16** with L-valine methyl ester hydrochloride using isobutyl chloroformate and *N*-methyl morpholine yielded methyl 3-methyl-2-(2-(4-nitrophenyl)thiazole-4-carboxamido)butanoate (**18**). Reduction of compound **18** using iron-ammonium chloride yielded the corresponding amine, methyl 2-(2-(4-aminophenyl)thiazole-4-carboxamido)-3-methylbutanoate (**20**). Coupling of **20** with 2-chloro-6-fluoro benzothiazole under acidic conditions followed by a subsequent hydrolysis with 1.0 M lithium hydroxide at room temperature afforded compound **6**, the desired 2-phenylthiazole analog. Compound **8** was similarly synthesized from its key intermediate **15** (Scheme 3) by following a reaction sequence analogous to that of compound **6**.

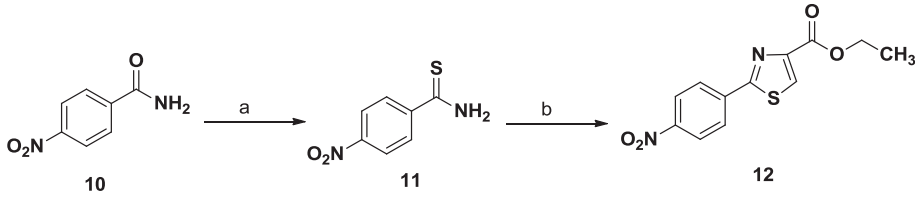
The synthesis of 2-(5-(4-((6-fluorobenzo[*d*]thiazol-2-yl)amino)phenyl)thiazole-2-carboxamido)-3-methylbutanoic acid (**7**), 5-phenylthiazole analogs possessing a urea linker (**28**–**32**), and the 5-phenylthiazole analogs possessing an amide linker (**33**–**38**) has been depicted in Scheme 4. This synthetic sequence diversifies at a key amine intermediate, methyl 2-(5-(4-aminophenyl)thiazole-2-carboxamido)-3-methylbutanoate (**27**) to yield these different analogs. Compound **27** was synthesized over six steps starting from 2-bromo-1-(4-nitrophenyl)ethanone (**14**). Compound **14** was converted to 2-amino-1-(4-nitrophenyl)ethanone hydrochloride (**22**) following a treatment with hexamethylenetetramine. Coupling of compound **22** with ethyl 2-chloro-2-oxoacetate yielded ethyl 2-((2-(4-nitrophenyl)-2-oxoethyl)amino)-2-oxoacetate (**23**) that was subsequently cyclized to ethyl 5-(4-nitrophenyl) thiazole-2-carboxylate (**24**) on treatment with Lawesson's reagent. Hydrolysis of compound **24** yielded its free acid, 5-(4-nitrophenyl) thiazole-2-carboxylic acid (**25**). Compound **25** when coupled with L-valine methyl ester hydrochloride using isobutyl chloroformate and *N*-methyl morpholine yielded methyl 3-methyl-2-(5-(4-nitrophenyl)thiazole-2-carboxamido)butanoate (**26**). Reduction of compound **26** using iron-ammonium chloride in ethanol yielded the desired amine intermediate, methyl 2-(5-(4-aminophenyl) thiazole-2-carboxamido)-3-methylbutanoate (**27**). Coupling of compound **27** with 2-chloro-6-fluoro benzothiazole and a subsequent hydrolysis with 1.0 M lithium hydroxide yielded compound **7**. Compounds **28**–**32** possessing the urea linker were synthesized by coupling compound **27** with substituted phenyl isocyanates and subsequent hydrolysis with 1.0 M lithium hydroxide. Similarly compounds possessing the amide linker (**33**–**36**) were synthesized by coupling compound **27** with substituted acid chlorides followed by a hydrolysis using 1.0 M lithium hydroxide.

Compound **40**, the dimethyl cyano analog, and compound **41**, the diethyl cyano analog, were synthesized (Scheme 5) starting from methyl 4-(cyanomethyl)benzoate (**37**). Incorporation of the dimethyl group was undertaken by reacting compound **37** with methyl iodide in the presence of potassium *tert*-butoxide to yield the dimethyl intermediate, compound **38**. Similarly replacing methyl iodide with ethyl iodide resulted in the synthesis of the diethyl intermediate, compound **39**. Compounds **38** and **39** were coupled with compound **27** in the presence of trimethyl aluminum and subsequently deprotected using 1.0 N LiOH to yield the desired compounds **40** and **41** respectively.

3. Results and discussion

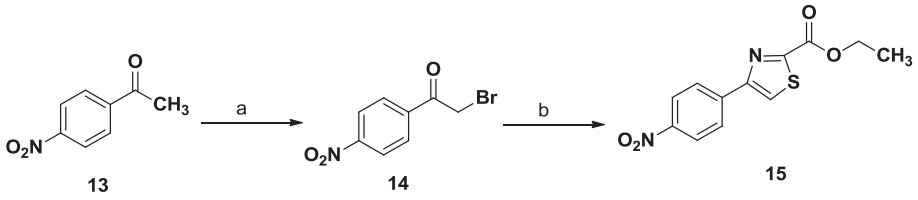
3.1. In vitro pharmacology

The synthesized compounds thus synthesized were assayed using an in vitro enzymatic assay that measured a triolein output from diolein and radiolabeled oleoyl-CoA [20]. The DGAT1 assay was performed using 2.5 μg of the protein from a post nuclear supernatant preincubated with 100 μl of the assay buffer [100 mM Tris-HCl (pH 7.5), 250 mM sucrose, and 1.25 mg/ml fatty acid free BSA] containing known concentration of the inhibitor and supplemented using 2047.5 μM of 1,2-dioleoylglycerol. The hDGAT1 reaction was initiated following an addition of 16.8 nCi of [^{14}C]-oleoyl CoA and after 10 min of incubation at 37 °C, the reaction was terminated by adding 300 μl of alkaline ethanol stop solution mix (AESSM) [12.5% of 100% non-denatured ethanol, 10% deionized water, 2.5% NaOH, and 75% stop solution (78.4% isopropanol, 19.6% n-heptane, 2% deionized water)]. The reaction mixture was properly mixed and the ^{14}C triglyceride formed was extracted using 600 μl of heptane. 250 μl of this extracted heptane was added to the scintillation fluid and subjected to radioactivity measurement. The



10 11 12

Scheme 1. (a) Lawesson's reagent, dioxane, 80 °C; (b) ethyl bromopyruvate, EtOH 80 °C.



Scheme 2. (a) Bromine, $AlCl_3$, diethyl ether; (b) ethyl 2-amino-2-thioxoacetate, methanol, reflux.

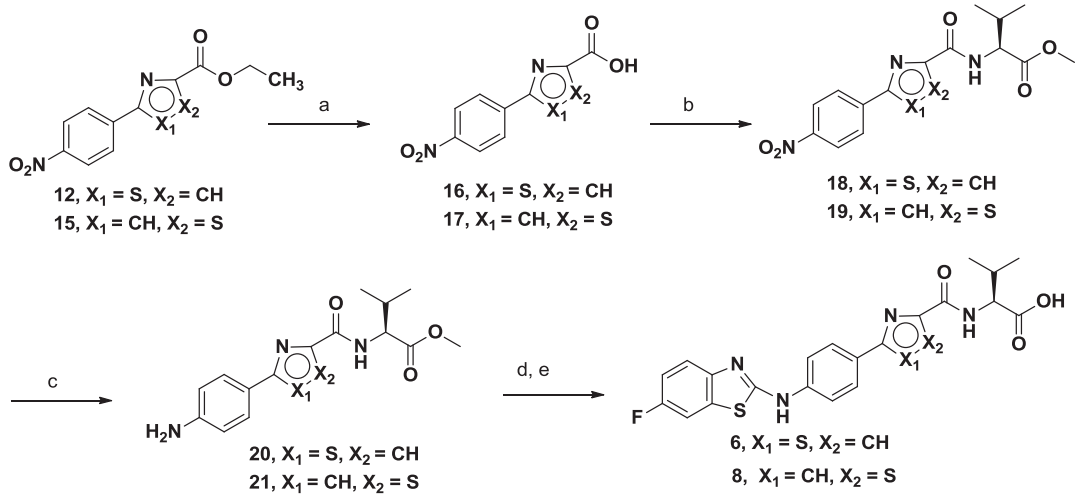
primary screening of hDGAT1 inhibitors was carried out at 1.0 μM concentration. Subsequent IC_{50} determinations were undertaken for compounds exhibiting inhibition greater that 75% in this primary screening. The IC_{50} values were determined by evaluating compounds at nine concentrations ranging from 0.1 nM to 1.0 μM and are presented in Table 1.

A preliminary study involving compounds **6** (Percent Inhibition = 31%), **7** (Percent Inhibition = 81%, IC_{50} = 10 nM), and **8** (Percent Inhibition = 25%) identified the 5-phenylthiazole scaffold represented by compound **7** as an inhibitor of DGAT1. The potent 5-phenylthiazole scaffold was investigated further by replacing the amino fluoro benzothiazole subunit of compound **7** with either a substituted phenyl urea (**28**–**32**) or a substituted phenyl amide (**33**–**38**). Following a primary screening at 1.0 μM concentration, these twelve compounds (**28**–**38**) were evaluated for their IC_{50} values. In case of the phenyl urea analogs, compounds possessing electron donating substituents on the phenyl such as 3-trifluoromethyl (**28**, IC_{50} = 22 nM), 3,4-dimethyl (**30**, IC_{50} = 20 nM), and 2-phenoxy (**31**, IC_{50} = 23 nM) have been evaluated alongside an electron withdrawing 2-chloro (**29**, IC_{50} = 16 nM) substituent. This study highlights an equal preference for both electron donating and electron withdrawing substituents on the phenyl ring. A 2-phenoxy, 4-chloro substituted phenyl urea analog (**32**, IC_{50} = 58 nM) also exhibits DGAT1 activity. In case of the phenyl amide analogs a *para*-*tert*-butyl (**33**, IC_{50} = 23 nM), a *para*-isobutyronitrile (**40**, IC_{50} = 97 nM), and a *para*-2-ethylbutanenitrile (**41**, IC_{50} = 101 nM) substituents on the phenyl ring have been evaluated. The *para*-tert-butyl substituent (**33**) appears to be a potent DGAT1 inhibitor as compared to compounds **40** and **41**. Compounds **34** (IC_{50} = 37 nM) and **35** (IC_{50} = 82 nM) possessing aliphatic substituents in place of an aromatic phenyl substituent and compound **36** (IC_{50} = 49 nM) possessing a heteroaromatic ring in place of an aromatic phenyl have also been evaluated. Each of these substitutions in case of the phenyl amide analogs appear to be tolerated at the DGAT1 enzyme. In case of the 5-phenylthiazole scaffold we have thus observed both the urea and amide linkers to be well tolerated for DGAT1 inhibition. This result is contrary to our earlier observations, involving a 3-phenylisoxazole scaffold, wherein the urea linker appeared to be well tolerated at the DGAT1 enzyme as compared to the amide linker [18]. This study has helped identify several DGAT1 inhibitors possessing potent in vitro activity that belong to the 5-phenylthiazole scaffold.

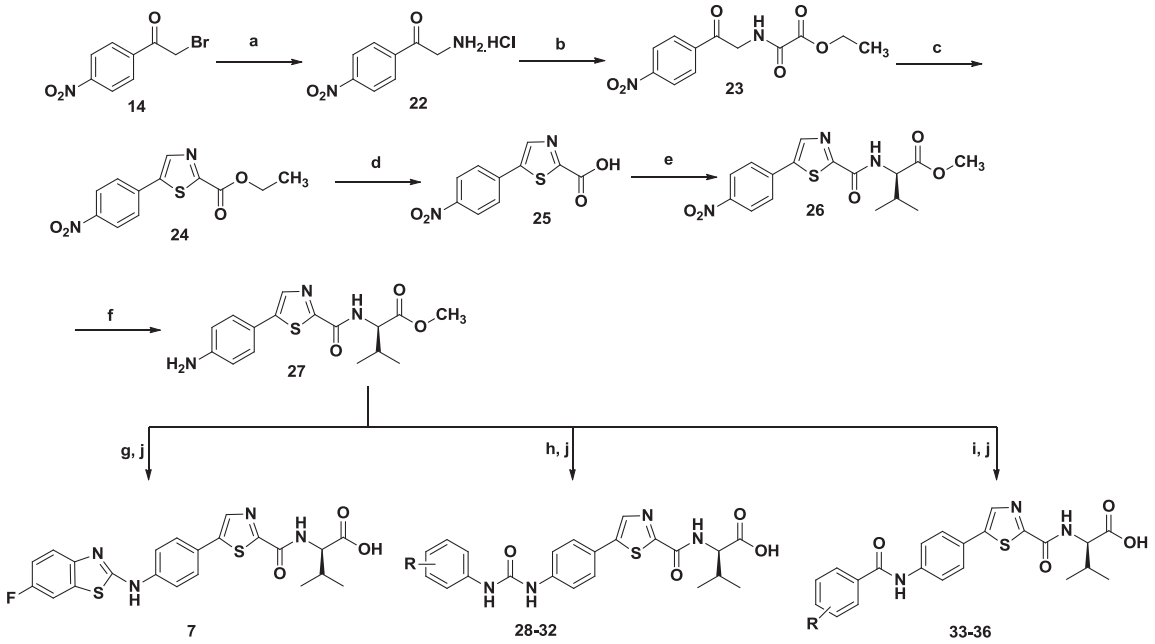
Following in vitro evaluation, a few selected 5-phenylthiazole analogs were assessed for their oral pharmacokinetic parameters and subsequently evaluated in vivo using an acute fat tolerance test (FTT). For these evaluations, the most potent phenyl urea analog, compound **29**, and the most potent phenyl amide analog, compound **33**, were studied and compared with compound **5**.

3.2. Pharmacokinetic properties evaluation

Oral pharmacokinetic parameters of the test compounds (**5**, **29**, **33**) were assessed following the preparation of a suspension [1 mg/ml] in 0.5% CMC and Tween 80. Swiss mice were weighed and the compounds were administered orally (10 mg/kg, n = 4). Blood samples were withdrawn at 0.08, 0.17, 0.25, 0.5, 0.75, 1.0, 2.0, 6.0, 8.0 and 24 h after dosing. Plasma samples were maintained on ice before being centrifuged (4 °C for 5 min at 1411 g), and aliquots were stored at −80 °C pending the assay. Concentrations of the compounds were determined using an ESI-LC/MS/MS method developed at Piramal Enterprises Limited. Pharmacokinetic parameters were determined by non-compartmental analysis using WinNonlin Professional (Version 4.1). C_{max} and T_{max} were taken directly from the plasma concentration-time profile. The area under the curve from time 0 to the last blood sampling time (AUC_{0-t}) was calculated using the linear trapezoidal rule. The area under the



Scheme 3. (a) 1.0 N LiOH, THF, rt; (b) NMM, IBCF, L-valine methyl ester hydrochloride, TEA, THF, −20 °C to rt; (c) Fe, NH_4Cl, EtOH, THF, H_2O, 80 °C; (d) phenyl isocyanate, THF, rt; (e) 1 N LiOH, THF, rt.



Scheme 4. (a) Hexamethylenetetramine, dichloromethane, HCl/ethanol; (b) ethyl 2-chloro-2-oxoacetate, triethylamine, ethyl acetate, reflux; (c) Lawesson's reagent, dioxane, 80 °C; (d) aq. NaOH, THF; (e) NMM, IBCF, L-valine methyl ester hydrochloride, TEA, THF, −20 °C to rt; (f) Fe, NH_4Cl, EtOH, H_2O; (g) 6-fluoro-2-chlorobenzothiazole, 4.0 M HCl in dioxane, EtOH, 80 °C, 20 h; (h) substituted phenyl isocyanates, THF, rt, 16 h; (i) substituted acid chloride, pyridine, dichloromethane, rt; (j) 1 N LiOH, THF, rt.

curve extrapolated to infinity (AUC_{inf}) was calculated by using the plasma concentration at time t divided by slope λz, where λz is estimated by linear regression of the terminal log-linear phase of the plasma concentration–time curve. Terminal plasma elimination half life ($T_{1/2}$) was calculated as $0.693/\lambda z$.

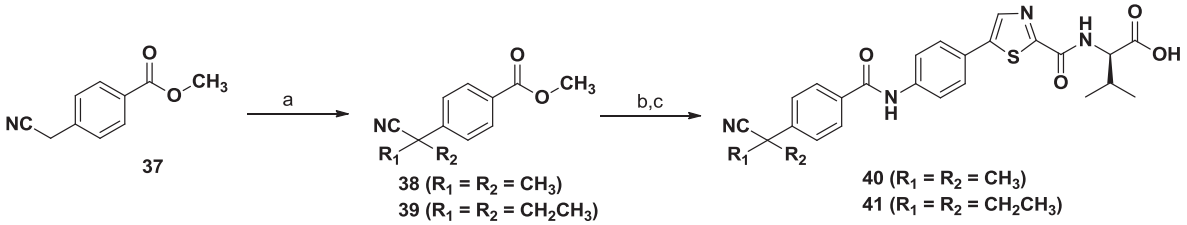
The assessed pharmacokinetic parameters of compounds **5**, **29**, and **33** following oral administration are presented in Table 2. The systemic exposure (AUC_{inf}) of compound **33** was approximately 10-fold higher than that of compound **29** and 1-fold higher than that of compound **5** following oral administration. The elimination half life ($T_{1/2}$) of each of the three compounds was observed to be less than 1 h. However amongst these three compounds, compound **33** ($T_{1/2} = 0.83$ h) possessed the longest elimination half life as compared to that of compound **5** ($T_{1/2} = 0.62$ h) and compound **29** ($T_{1/2} = 0.49$ h). Thus with respect to oral pharmacokinetic parameters the phenyl amide analog **33** appears slightly better than the Bayer compound **5** and significantly better than the phenyl urea derivative **29**.

3.3. Acute fat tolerance test (FTT)

In an FTT fasted Swiss mice, belonging to the age range of 4–5 weeks and body weight range of 25–30 g, were administered with either a vehicle (0.5% CMC) or the test compound [3 mg/kg] by oral gavage. The test compounds were formulated as a suspension in 0.5% CMC containing Tween 80 (25 μL). An hour later, a bolus dose of olive oil (10 ml/kg) was given to the animals. Blood samples were subsequently collected at 1, 2, 3, and 4 h, the plasma was separated, and triglyceride levels were monitored using a commercially available kit (Diasys, Germany). Percent reduction in triglyceride levels were calculated using an area under curve ($AUC_{0-4\ h}$) of the test compound and comparing it along with an $AUC_{0-4\ h}$ of the vehicle group that is considered to be 100 percent. In this study, compound **5** exhibited a 90% triglyceride reduction. Compound **29**, the 5-phenylthiazole analog possessing a urea linker exhibited 82% triglyceride reduction. Compound **33** possessing an amide linker exhibited 87% triglyceride reduction. Thus all three compounds appear equi-efficacious to each other when evaluated in vivo.

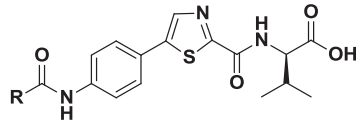
4. Conclusions

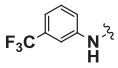
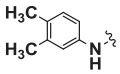
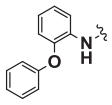
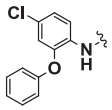
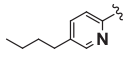
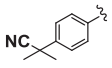
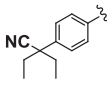
In this study, we have developed a 5-phenylthiazole containing heteroaryl scaffold possessing improved oral pharmacokinetic properties. A preliminary comparison in between the 2-phenyl thiazole, 4-phenylthiazole, and 5-phenylthiazole scaffolds highlighted a preference for the 5-phenylthiazole scaffold at the DGAT1 enzyme. A subsequent study undertaken to evaluate phenyl urea and phenyl amide analogs of the 5-phenylthiazole scaffold identified both these modifications to be well tolerated at the DGAT1 enzyme when analyzed in vitro. Compounds **29** and **33** were



Scheme 5. (a) Methyl iodide (for **38**)/ethyl iodide (for **39**), potassium *tert*-butoxide, THF, −30 °C, 2 h; (b) compound **27**, 2.0 M trimethyl aluminum in toluene, 80 °C, 4 h; (c) 1 N LiOH, THF, rt.

Table 1
In vitro evaluation of 5-phenylthiazole analogs.



Compound number	R	hDGAT1 inhibition (% inhibition 1 μM)	hDGAT1 inhibition IC_{50} (nM)
28		81	22
29		82	16
30		78	20
31		80	23
32		81	58
33		84	23
34		86	37
35		83	82
36		86	49
40		88	97
41		87	101

evaluated for their in vivo ability to reduce triglycerides in fasted Swiss mice when dosed at 3 mg/kg. Compound **29** (Triglyceride reduction = 87%) and compound **33** (Triglyceride reduction = 82%) appear equivalent to compound **5** (Triglyceride reduction = 90%) at the same dose. However when studied for their oral pharmacokinetic properties the phenyl amide analog, compound **33** (AUC_{inf} = 7058 ng*h/ml, $T_{1/2}$ = 0.83 h) appeared better than both compound **29** (AUC_{inf} = 623 ng*h/ml, $T_{1/2}$ = 0.49 h) and compound **5** (AUC_{inf} = 5344 ng*h/ml, $T_{1/2}$ = 0.62 h). Thus with the phenyl amide analog, compound **33**, we were able to improve the pharmacokinetic properties while retaining its in vitro DGAT1 enzymatic activity and its in vivo ability to reduce plasma triglyceride levels. These attributes earmark compound **33** as a potential new lead for developing therapeutically applicable DGAT1 inhibitors.

Table 2
Plasma triglyceride reduction following an in vivo fat tolerance test [3 mg/kg] and pharmacokinetic parameters following oral administration [10 mg/kg] to Swiss mice.

Parameter	Compound **5**	Compound **29**	Compound **33**
Percent TG reduction (FTT)	90	82	87
C_{max} (ng/ml)	2452	933	5449
C_{max} (μM)	5.3	1.9	11.3
T_{max} (h)	0.25	0.16	0.17
$T_{1/2}$ (h)	0.62	0.49	0.83
AUC_{0-t} (ng*h/ml)	5344	623	7058
AUC_{inf} (ng*h/ml)	5350	672	7072

5. Experimental

5.1. Chemistry

Unless mentioned otherwise all reactions were performed under atmosphere. Unless otherwise specified all reagents were obtained from Aldrich and solvents were obtained from Thomas Baker and used without further purification. ^{1}H NMR spectra were recorded on a Bruker spectrometer (300 MHz) using either $CDCl_3$ or DMSO-d_6 as the solvent. Chemical shifts, δ, are reported in ppm relative to the solvent peak. Multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Coupling constants, *J*, are reported in Hertz. Mass spectral (MS) data were obtained on a Bruker Daltonics spectrometer using an electrospray ionization-quadrapole-time of flight (ESI-QTOF) analyzer. All melting points have been determined on a manually operated Veego (VMP-1) melting point apparatus and are reported uncorrected. HPLC purities have been determined for the final compounds using a Waters Alliances 2695 system implementing the following method for chromatographic separation.

5.2. HPLC method

Elution with 20–80% linear gradient of acetonitrile in 6 min followed by 20–80% linear gradient of 0.01 M NH_4OAc + 0.5% TEA, pH 5.0 with AcOH in 1 min that is continued using an isocratic elution with 80% 0.01 M NH_4OAc + 0.5% TEA, pH 5.0 with AcOH for 3 min using a Ascentis TM Express (50 × 4.6 mm I.D.), 2.7 μm operated at 1 ml/min, detection at 288 nm.

5.2.1. 4-Nitrobenzothioamide (**11**)

To a solution of 4-Nitrobenzamide (4 g, 24.08 mmol, 1.0 equiv) in Dioxane (100 ml) was added Lawesson's reagent (7.79 g, 19.26 mmol, 0.8 equiv) and the mixture was heated to 80 °C for 2 h. The reaction mixture was subsequently cooled, added to water and neutralized with a saturated solution of sodium carbonate. The product was separated from the aqueous mixture using EtOAc. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to get a dark brown residue. The residue was subjected to column chromatography using 2:8 EtOAc:Pet ether to get a dark yellow colored solid that was crystallized in DCM/Pet ether to yield the title compound as a pale yellow colored solid (3.52 g, 80%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 10.23 (s, 1H), 9.82 (s, 1H), 8.26 (d, *J* = 8.4 Hz, 2H), 8.03 (d, *J* = 8.4 Hz, 2H); MS (ESI+): *m*/*z* 183 $[M + H]^+$.

5.2.2. Ethyl 2-(4-nitrophenyl)thiazole-4-carboxylate (**12**)

To a suspension of compound **11** (3.4 g, 18.66 mmol, 1.0 equiv) in Ethanol (50 ml) was added Ethyl bromopyruvate (2.348 ml, 18.66 mmol, 1.0 equiv) and the reaction mixture was heated to 80 °C for 4 h. This reaction mass was cooled and TEA (2.60 ml, 18.66 mmol, 1.0 equiv) was added. The solid was filtered, washed

with water, and dried to obtain the title compound as a white solid (4.1 g, 79%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, J = 8.4 Hz, 2H), 8.29 (s, 1H), 8.22 (d, J = 8.4 Hz, 2H), 4.48 (q, J = 6.9 Hz, 2H), 1.46 (t, J = 7.2 Hz, 3H); MS (ESI+): m/z 279.3 $[M + H]^+$.

*5.2.3. 2-Bromo-1-(4-nitrophenyl)ethanone (**14**)*

To a solution of 4-Nitroacetophenone (25.0 g, 151 mmol, 1.0 equiv) in ether (250 ml) bromine (7.77 ml, 151 mmol, 1.0 equiv) was added over 10 min. The reaction mixture was stirred for 30 min in the presence of catalytic amount of aluminum chloride (1.0 g, 7.5 mmol, 0.05 equiv). Following reaction completion the reaction mixture was quenched with aqueous sodium bicarbonate. The ether layer was separated and dried over sodium sulfate and evaporated under reduced pressure. The off white residue thus obtained was crystallized in EtOAc:Pet ether to afford the title compound (25.5 g, 69%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (d, J = 8.1 Hz, 2H), 8.18 (d, J = 8.1 Hz, 2H), 4.48 (s, 2H); MS (ESI+) m/z 245.0 $[M + H]^+$.

*5.2.4. Ethyl 4-(4-nitrophenyl)thiazole-2-carboxylate (**15**)*

To a stirred solution of 2-bromo-1-(4-nitrophenyl)ethanone (9.2 g, 37.7 mmol, 1.0 equiv) in methanol (200 ml) was added ethyl 2-amino-2-thioxoacetate (5.0 g, 37.7 mmol, 1.0 equiv) and reaction mixture was refluxed for 2 h. Following reaction completion the reaction mass was cooled to rt and the precipitated solid was filtered, washed with cold methanol, and dried to yield (7.1 g, 67%) of the title compound as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.33 (d, J = 8.0 Hz, 2H), 8.16 (d, J = 8.0 Hz, 2H), 7.96 (s, 1H), 4.55 (q, J = 7.0 Hz, 2H), 1.50 (t, J = 7.0 Hz, 3H); MS (ESI+) m/z 276.6 $[M + H]^+$.

*5.2.5. 2-(4-Nitrophenyl)thiazole-4-carboxylic acid (**16**)*

To a solution of ethyl 2-(4-nitrophenyl)thiazole-4-carboxylate (4 g, 14.37 mmol, 1.0 equiv) in THF (80 ml) was added 1.0 M aq. Lithium hydroxide (57.5 ml, 57.5 mmol, 4.0 equiv) and the reaction mixture was stirred at rt for 2 h. The reaction mixture was acidified to pH = 2 using 1.0 M HCl. The resulting mixture was extracted with EtOAc, the organic layer was washed with brine, dried over sodium sulfate and concentrated under reduced pressure to obtain a pale yellow colored solid. Recrystallization with EtOAc/Pet ether yielded the desired product as an off white solid (3.12 g, 87%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 13.30 (bs, 1H), 8.66 (s, 1H), 8.37 (d, J = 8.4 Hz, 2H), 8.26 (d, J = 8.4 Hz, 2H); MS (ESI+): m/z 251 $[M + H]^+$.

*5.2.6. 4-(4-Nitrophenyl)thiazole-2-carboxylic acid (**17**)*

To a solution of ethyl 4-(4-nitrophenyl) thiazole-2-carboxylate (7.0 g, 25.0 mmol, 1.0 equiv) in THF (70 ml) was added 1.0 N lithium hydroxide (1.05 g, 25.0 mmol, 1.0 equiv) solution and the reaction mixture was stirred at rt for 4 h. After reaction completion THF was removed under reduced pressure and dil. HCl was added to the mixture. The precipitated solid was filtered, washed with water, and dried to yield (6.2 g, 98%) of the title compound as an off white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.78 (s, 1H), 8.32–8.28 (m, 4H); MS (ESI+) m/z 251.6 $[M + H]^+$.

*5.2.7. Methyl 3-methyl-2-(2-(4-nitrophenyl)thiazole-4-carboxamido) butanoate (**18**)*

To a solution of 2-(4-nitrophenyl)thiazole-4-carboxylic acid (3.0 g, 11.99 mmol, 1.0 equiv) in DMF (60 ml) was sequentially added HATU (6.84 g, 17.98 mmol, 1.5 equiv), methyl 2-amino-3-methylbutanoate hydrochloride (2.412 g, 14.39 mmol, 1.2 equiv) and TEA (2.507 ml, 17.98 mmol, 1.5 equiv) and stirred for 24 h. Reaction mixture was quenched with water and the product was separated from the aqueous mixture using EtOAc. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to get a dark brown residue. The residue was subjected to column chromatography using 3:7 EtOAc:Pet ether to get a pale brown colored solid that was crystallized in $CHCl_3$/Pet ether to yield the product as an off white colored solid (2.9 g, 66%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.53 (s, 1H), 8.51–8.48 (d, J = 8.4 Hz 1H), 8.37 (bs, 4H), 4.39 (t, J = 7.5 Hz, 1H), 3.69 (s, 3H), 2.30–2.23 (m, 1H), 0.96 (t, J = 6.6 Hz, 6H); MS (ESI+): m/z 364 $[M + H]^+$.

*5.2.8. Methyl 3-methyl-2-(4-(4-nitrophenyl)thiazole-2-carboxamido) butanoate (**19**)*

To a solution of 4-(4-nitrophenyl)thiazole-2-carboxylic acid (4.5 g, 18 mmol, 1.0 equiv) in THF (75 ml) was added *N*-methyl morpholine (1.8 g, 18 mmol, 1.0 equiv) and cooled the reaction mass to −20 °C. To this was added isopropyl chloroformate (2.4 g, 18 mmol, 1.0 equiv) and the reaction mixture was stirred for 20 min. In another flask containing a solution of D-valine hydrochloride (7.2 g, 43 mmol, 2.4 equiv) in THF (20 ml) was neutralized using triethylamine (4.3 g, 43 mmol, 2.4 equiv) by stirring the mixture for 5 min. This neutralized valine ester solution was added into the reaction mass and stirred at rt for 3 h. Following reaction completion solvent was removed under reduced pressure. The desired compound was isolated by flash column chromatography using 20:80 EtOAc:Pet ether (4.4 g, 67%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.34 (d, J = 8.1 Hz, 2H), 8.13 (d, J = 8.1 Hz, 2H), 7.94 (s, 1H), 7.79 (d, J = 6.9 Hz, 1H), 4.76 (t, J = 4.8 Hz, 1H), 3.83 (s, 3H), 2.36 (m, 1H), 1.07 (s, 6H); MS (ESI+) m/z 364 $[M + H]^+$.

*5.2.9. Methyl 2-(4-(4-aminophenyl)thiazolidine-2-carboxamido)-3-methylbutanoate (**20**)*

To a solution of Methyl 3-methyl-2-(2-(4-nitrophenyl)thiazole-4-carboxamido)butanoate (1.8 g, 4.95 mmol, 1.0 equiv) in EtOH (36 ml), THF (16 ml), and water (16 ml) were added Iron (0.830 g, 14.86 mmol, 3.0 equiv) and Ammonium chloride (0.795 g, 14.86 mmol, 3.0 equiv) and the mixture was refluxed at 80 °C for 4 h. Following this the resulting mixture was cooled, filtered through celite and solvent was removed under pressure to get a dark brown residue. The residue was taken in water and extracted using ethyl acetate. The organic layer was dried using sodium sulfate and concentrated to obtain a dark brown residue which was purified by column chromatography using 4:6 EtOAc:Pet ether to afford the title compound as a pale yellow solid (1.34 g, 81%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.20–8.17 (d, J = 8.4 Hz, 1H), 8.10 (s, 1H), 7.73 (d, J = 8.4 Hz, 2H), 6.65 (d, J = 8.4 Hz, 2H), 5.78 (bs, 2H), 4.39 (t, J = 7.8 Hz, 1H), 3.68 (s, 3H), 2.28–2.21 (m, 1H), 0.94 (t, J = 3.9 Hz, 6H); MS (ESI+): m/z 334.4 $[M + H]^+$.

*5.2.10. Methyl 2-(4-(4-aminophenyl)thiazole-2-carboxamido)-3-methylbutanoate (**21**)*

To a solution of compound **19** (4.27 g, 11.78 mmol) in ethanol (40 ml) and water (20 ml) were added iron (1.97 g, 35.34 mmol) and ammonium chloride (1.89 g, 35.36 mmol) and the reaction mixture was stirred at 80–85 °C for 3 h. Following reaction completion, the mass was cooled to rt and filtered through Celite. Organic solvent was removed and a saturated sodium bicarbonate solution was added to obtain a pH of 7.0 and the compound was extracted using ethyl acetate. The solvent was evaporated to yield a solid which was further purified by column chromatography using 5:95 EtOAc:chloroform to yield (2.7 g, 68%) the title compound as an off white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.70 (d, J = 7.8 Hz, 1H), 8.03 (s, 1H), 7.76 (d, J = 7.5 Hz, 2H), 6.63 (d, J = 7.8 Hz, 2H), 5.38 (s, 2H), 4.36 (t, J = 7.2 Hz, 1H), 3.96 (s, 3H), 2.30–2.28 (m, 1H), 0.96 (d, J = 5.7 Hz, 6H); MS (ESI+) m/z 334 $[M + H]^+$.

5.2.11. 2-(2-(4-((6-Fluorobenzo[d]thiazol-2-yl)amino)phenyl)thiazole-4-carboxamido)-3-methylbutanoic acid (**6**)

Compound **20** (300 mg, 0.900 mmol, 1.0 equiv) and 2-chloro-6-fluorobenzo[*d*]thiazole (203 mg, 1.080 mmol, 1.2 equiv) were taken in EtOH (10 ml) and heated at 55–60 °C to obtain a clear solution. To this solution was added 4.0 M aq. HCl in 1,4-Dioxane HCl (0.027 ml, 0.900 mmol, 1.0 equiv) and the resulting mixture was refluxed at 80 °C for 20 h. Following this the EtOH was removed completely under pressure and the residue was purified by column chromatography using 3:8 EtOAc:Pet ether to obtain an pale brown solid. The solid was crystallized in EtOAc/Pet ether to yield the corresponding methyl ester. To a solution of this methyl ester (1 equiv) in THF was added 1.0 M solution of LiOH in water (5 equiv) and stirred for 16 h at rt. The solvent was removed and the obtained residue was diluted with water and acidified to pH 2 using 2.0 M HCl. The material thus obtained was extracted using ethyl acetate, washed with water, and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to obtain a solid that was crystallized using ethyl acetate to yield the title compound as off-white solid (171 mg, 40%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 11.03 (s, 1H), 8.21–8.18 (m, 2H), 8.04 (d, J = 8.4 Hz, 2H), 7.99 (d, J = 8.4 Hz, 2H), 7.78–7.52 (m, 1H), 7.66–7.61 (m, 1H), 7.22–7.16 (m, 1H), 4.18 (m, 1H), 2.23 (m, 1H), 0.93 (d, J = 6.3 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 173.23, 167.74, 161.48, 160.30, 156.96, 150.83, 149.01, 143.20, 131.90, 127.88 (2C), 126.35, 123.51, 120.72, 118.31 (2C), 114.14, 108.63; 58.63, 31.27, 19.97, 18.63; HRMS (ESI+) calcd for $C_{22}H_{19}FN_4O_3S_2$ $[M + H]^+$ 471.0955, found 471.0930 (error 5.27 ppm); mp 240–242 °C; HPLC Retention time – 3.95 min, Purity – 97.98%.

5.2.12. 2-(4-(4-((6-Fluorobenzo[d]thiazol-2-yl)amino)phenyl) thiazole-2-carboxamido)-3-methylbutanoic acid (**8**)

To a solution of compound **21** (300 mg, 0.900 mmol, 1.0 equiv) in *n*-butanol (5 ml) was added 2-chloro-6-fluorobenzo[*d*]thiazole (202 mg, 1.1 mmol, 1.1 equiv) and the reaction mixture was stirred at 70 °C followed by the addition of 4.0 M HCl (131 mg). The resulting reaction mixture was stirred at 90 °C for 16 h. Following reaction completion the solvent was removed under reduced pressure and the compound was purified by flash column chromatography using 10:90 EtOAc:Pet ether to yield methyl 2-(4-(4-((6-fluorobenzo[*d*]thiazol-2-yl)amino)phenyl)thiazole-2-carboxamido)-3-methylbutanoate (130 mg, 29%) as a solid compound. To this butanoate ester (105 mg, 0.216 mmol) in THF (2 ml) was added 1.0 N lithium hydroxide solution (45 mg, 1.1 mmol, 1.1 equiv) and the reaction mixture was stirred at rt for 4 h. Following reaction completion THF was removed under reduced pressure and dil. HCl was added to acidify the reaction mixture. The solid that precipitated as a result was filtered, washed with water, and dried to yield (70 mg, 68%) of the title compound as a white solid compound.

^{1}H NMR (300 MHz, DMSO-d_6) δ 10.71 (s, 1H), 8.41 (d, J = 8.1 Hz, 1H), 8.29 (s, 1H), 8.05 (d, J = 7.8 Hz, 2H), 7.88 (d, J = 8.1 Hz, 2H), 7.74 (d, J = 7.5 Hz, 1H), 7.60 (m, 1H), 7.17 (t, J = 6.9 Hz, 1H), 4.24 (m, 1H), 2.27–2.25 (m, 1H), 0.94 (d, J = 6.0 Hz, 6H); ^{13}C NMR (300 MHz, DMSO-d_6): δ 172.86, 163.38, 161.65, 159.19, 157.52, 155.75, 149.18, 141.29, 131.76, 127.69, 127.56 (2C), 120.53, 118.62, 118.26 (2C), 114.01, 108.57, 58.82, 30.84, 19.87, 18.77; HRMS (ESI+) calcd for $C_{22}H_{19}FN_4O_3S_2$ $[M + H]^+$ 471.0955, found 471.0935 (mean error = 4.28); mp 226–228 °C; HPLC Retention time – 3.891 min, Purity – 98.9%.

5.2.13. 2-Amino-1-(4-nitrophenyl)ethanone hydrochloride (**22**)

To a solution of 2-bromo-1-(4-nitrophenyl)ethanone (25.0 g, 102 mmol, 1.0 equiv) was in DCM (250 ml) was added hexamethylenetetramine (20.1 g, 143 mmol, 1.4 equiv) and the reaction mixture was stirred for 1 h. Following completion the reaction was filtered to yield 30.0 g of an off-white solid that was dissolved in ethanol (162 ml) and conc. HCl (40 ml). The mixture was stirred for 3 h and then left standing for two days. The off white solid thus obtained was filtered, washed with water, and dried to yield the title compound (11.8 g, 72%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.57 (bs, 3H), 8.38 (d, J = 9.0 Hz, 2H), 8.25 (d, J = 9.0 Hz, 2H), 4.68 (s, 2H); MS (ESI+) m/z 181 $[M + H]^+$.

5.2.14. Ethyl 2-((2-(4-nitrophenyl)-2-oxoethyl)amino)-2-oxoacetate (**23**)

To a solution of 2-amino-1-(4-nitrophenyl)ethanone hydrochloride (11.5 g, 53.1 mmol, 1.0 equiv) in EtOAc (115 ml), was added triethylamine (8.88 ml, 63.7 mmol, 1.2 equiv). To this mixture ethylchlorooxoacetate (7.11 ml, 63.7 mmol, 1.2 equiv) dissolved in ethyl acetate (35 ml) was added dropwise and refluxed for 2 h. The reaction mass was cooled, quenched with water, and the resulting organic layer was separated, dried over sodium sulfate and evaporated under reduced pressure to get a dark brown oil. The oil was further purified by column chromatography using 3:7 EtOAc:Pet ether to get a yellow solid. The solid was further crystallized in EtOAc/Pet ether to yield the desired compound as a yellow colored solid (8.9 g, 59%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.21 (m, 1H), 8.35 (d, J = 9.0 Hz, 2H), 8.24 (d, J = 9.0 Hz, 2H), 4.78 (d, J = 6.0 Hz, 2H), 4.29 (q, J = 6.0 Hz, 2H), 1.29 (t, J = 6.0 Hz, 3H); MS (ESI–) m/z 278.8 $[M - H]^-$.

5.2.15. Ethyl 5-(4-nitrophenyl)thiazole-2-carboxylate (**24**)

To a solution of ethyl 2-(2-(4-nitrophenyl)-2-oxoethylamino)-2-oxoacetate (5 g, 17.84 mmol, 1.0 equiv) in 1,4-Dioxane (100 ml) was added Lawesson's reagent (7.22 g, 17.84 mmol, 1.0 equiv) and the mixture was refluxed for 2 h. The reaction mixture was subsequently cooled, added to water and neutralized with a saturated solution of sodium carbonate. The product was separated from the aqueous mixture using EtOAc. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to get a dark brown residue. The residue was chromatographed using 0.5:9.5 EtOAc:$CHCl_3$ to get a dark yellow colored solid that was crystallized in $CHCl_3$/Pet ether to yield the product as a pale yellow colored solid (3.65 g, 73%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (d, J = 9.0 Hz, 2H), 8.30 (s, 1H), 7.83 (d, J = 9.0 Hz, 2H), 4.54 (q, J = 6.0 Hz, 2H), 1.49 (t, J = 6.0 Hz, 3H); MS (ESI+): m/z 279 $[M + H]^+$.

5.2.16. 5-(4-Nitrophenyl)thiazole-2-carboxylic acid (**25**)

To a solution of ethyl 5-(4-nitrophenyl)thiazole-2-carboxylate (3.6 g, 12.94 mmol, 1.0 equiv) in THF (90 ml) was added 1.0 M aq. NaOH (52 ml) and the reaction mixture was stirred at rt for 15–20 min. The reaction mixture was acidified to pH 2.0 using 1.0 M HCl. The resulting mixture was extracted with EtOAc, the organic layer was washed with brine, dried over sodium sulfate and concentrated under reduced pressure to obtain a pale yellow colored solid. Recrystallization with EtOAc/Pet ether yielded the desired product as an off white solid (2.48 g, 76%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 14.21 (bs, 1H), 8.70 (s, 1H), 8.31 (d, J = 9.0 Hz, 2H), 8.10 (d, J = 9.0 Hz, 2H); MS (ESI+): m/z 251 $[M + H]^+$.

5.2.17. Methyl 3-methyl-2-(5-(4-nitrophenyl)thiazole-2-carboxamido) butanoate (**26**)

To a solution of 5-(4-nitrophenyl)thiazole-2-carboxylic acid (2.3 g, 9.19 mmol, 1.0 equiv) in THF (72 ml) was added *N*-methyl

morpholine (1.01 ml, 9.19 mmol, 1.0 equiv) and the reaction mixture was stirred for 10 min at rt. The resulting mixture was cooled to −20 °C and to this isobutyl chloroformate (1.19 ml, 9.19 mmol, 1.0 equiv) was added and stirred for 15–20 min at −20 to −30 °C. To this reaction mass was added a solution of L-valine methyl ester hydrochloride (2.15 g, 12.87 mmol, 1.4 equiv) in THF (20 ml) after neutralizing with triethylamine (1.8 ml, 12.87 mmol, 1.4 equiv). This mixture was stirred at −20 to −30 °C for 5 min. Following this the reaction mixture was gradually warmed up to rt over a period of 1 h. The organic solvent was removed under reduced pressure and the crude material was subjected to column chromatography using 15:85 EtOAc:$CHCl_3$ to get a pale yellow colored solid. The title compound (2.25 g, 67%) was obtained as an off white solid on crystallization with EtOAc/Pet ether.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.95 (d, J = 9.0 Hz, 1H), 8.70 (s, 1H), 8.34 (d, J = 9.0 Hz, 2H), 8.09 (d, J = 9.0 Hz, 2H), 4.33 (m, 1H), 3.68 (s, 3H), 2.27 (m, 1H), 0.95 (t, J = 6.0 Hz, 6H); MS (ESI+): m/z 364 $[M + H]^+$.

5.2.18. Methyl 2-(5-(4-aminophenyl)thiazole-2-carboxamido)-3-methylbutanoate (**27**)

To a solution of methyl 3-methyl-2-(5-(4-nitrophenyl)thiazole-2-carboxamido)butanoate (2.15 g, 5.92 mmol, 1.0 equiv) in EtOH (21.5 ml), THF (8.6 ml), and water (8.6 ml) were added ammonium chloride (1.04 g, 17.75 mmol, 3.0 equiv) and iron (777 mg, 13.90 mmol, 2.35 equiv) and the mixture was refluxed at 80 °C for 3 h. Following this the resulting mixture was cooled, filtered through Celite and solvent was removed under pressure to get a dark brown residue. The residue was taken in water and extracted using ethyl acetate. The organic layer was dried using sodium sulfate and concentrated to obtain a dark brown residue which was purified by column chromatography using 2.5:7.5 EtOAc:$CHCl_3$ to afford the title compound as a sticky yellow solid (1.82 g, 91%).

^{1}HNMR (300 MHz, DMSO-d_6) δ 8.59 (d, J = 9.0 Hz, 1H), 8.15 (s, 1H), 7.42 (d, J = 9.0 Hz, 2H), 6.63 (d, J = 9.0 Hz, 2H), 5.76 (bs, 2H), 4.31 (m, 1H), 3.67 (s, 3H), 2.27 (m, 1H), 0.91 (d, J = 6.0 Hz, 6H); MS (ESI+): m/z 334 $[M + H]^+$.

5.2.19. 2-(5-(4-((6-Fluorobenzo[d]thiazol-2-yl)amino)phenyl)thiazole-2-carboxamido)-3-methylbutanoic acid (**7**)

Compound **27** (150 mg, 0.45 mmol, 1.0 equiv) and 2-chloro-6-fluorobenzo[d]thiazole (101 mg, 0.54 mmol, 1.2 equiv) were taken in EtOH (3 ml) and heated at 55–60 °C to obtain a clear solution. To this solution was added 4.0 M aq. HCl in 1,4-Dioxane (0.11 ml, 0.45 mmol, 1.0 equiv) and the resulting mixture was refluxed at 80 °C for 20 h. Following this the EtOH was removed completely under pressure and the residue was purified by column chromatography using 2:8 EtOAc:Pet ether to obtain an yellow solid. The solid was crystallized in EtOAc/Pet ether to yield the corresponding methyl ester. To a solution of this methyl ester (1 equiv) in THF was added 1.0 M solution of LiOH in water (5 equiv) and stirred for 16 h at rt. The solvent was removed and the obtained residue was diluted with water and acidified to pH 2 using 2.0 M HCl. The material thus obtained was extracted using ethyl acetate, washed with water, and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to obtain a solid that was crystallized using ethyl acetate to yield the title compound as a pale yellow solid (87 mg, 41%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 12.99 (bs, 1H), 10.76 (s, 1H), 8.36 (s, 1H), 8.34 (d, 1H), 7.88 (d, J = 9.0 Hz, 2H), 7.81–7.76 (m, 3H), 7.65 (dd, J = 6.0 Hz, 1H), 7.20 (m, 1H), 4.32, (m, 1H), 2.27 (m, 1H), 0.97 (d, J = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.79, 161.49, 160.66, 159.56, 157.60, 149.07, 144.91, 141.85, 139.38, 131.76, 128.24 (2C), 124.08, 120.69, 118.54 (2C), 114.12, 108.63, 58.25, 30.31, 19.62, 18.74; HRMS (ESI+) calcd for $C_{22}H_{19}FN_4O_3S_2$ $[M + H]^+$ 471.0955, found 471.0939 (error 3.02 ppm); mp 244–246 °C; HPLC Retention time – 3.81 min, Purity – 99.72%.

5.2.20. General procedure for the synthesis of urea analogs

To a solution of Compound **27** (0.36 mmol, 1.0 equiv) in THF (3 ml) was added the appropriately substituted phenyl isocyanate (1.1 equiv) and the mixture was stirred for 16 h at rt. The reaction mixture was then concentrated and purified using flash column chromatography (2:8 EtOAc/Pet ether) to afford the desired methyl ester. To a solution of the methyl ester (1 equiv) in THF was added 1.0 M solution of LiOH in water (5 equiv) and stirred for 16 h at rt. The solvent was removed and the obtained residue was diluted with water and acidified to pH 2.0 using 2.0 M HCl. The material thus obtained was extracted with ethyl acetate, washed with water, and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to obtain a solid that was crystallized in ethyl acetate to yield the desired acid.

5.2.20.1. 3-Methyl-2-(5-(4-(3-(3-(trifluoromethyl)phenyl)ureido)phenyl)thiazole-2-carboxamido)butanoic acid (**28**).

Prepared as described above in the general procedure using 3-trifluorophenyl isocyanate (74.12 mg, 0.39 mmol, 1.1 equiv) as the substituted isocyanate to afford the title compound (94 mg, 52%) as an off white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 12.97 (bs, 1H), 9.18 (s, 1H), 9.13 (s, 1H), 8.37 (m, 1H), 8.03 (bs, 1H), 7.71 (d, J = 9.0 Hz, 2H), 7.61 (d, J = 9.0 Hz, 2H), 7.55–7.50 (m, 2H), 7.33 (d, J = 6.0 Hz, 1H), 4.31, (m, 1H), 2.28 (m, 1H), 0.95 (d, J = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.91, 161.71, 159.49, 152.82, 144.85, 141.05, 140.89, 139.37, 130.38, 128.03 (3C), 124.19, 122.43, 119.20 (3C), 118.71, 114.72, 58.33, 30.35, 19.63, 18.74; HRMS (ESI+) calcd for $C_{23}H_{21}F_3N_4O_4S$ $[M + H]^+$ 507.1308, found 507.1286 (error 4.30 ppm); mp 220–222 °C; HPLC Retention time – 3.78 min, Purity – 99.14%.

5.2.20.2. 2-(5-(4-(3-(2-Chlorophenyl)ureido)phenyl)thiazole-2-carboxamido)-3-methylbutanoic acid (**29**).

Prepared as described above in the general procedure using 2-chlorophenyl isocyanate (60.84 mg, 0.396 mmol, 1.1 equiv) as the substituted isocyanate to afford the title compound (108 mg, 63%) as an off white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.67 (s, 1H), 8.38–8.34 (m, 3H), 8.18 (d, J = 9.0 Hz, 1H), 7.74 (d, J = 9.0 Hz, 2H), 7.59 (d, J = 9.0 Hz, 2H), 7.49 (d, J = 9.0 Hz, 1H), 7.32 (m, 1H), 7.06 (m, 1H), 4.33, (m, 1H), 3.68 (s, 3H), 2.26 (m, 1H), 0.95 (d, J = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.83, 160.71, 159.48, 152.43, 144.83, 141.04, 139.37, 136.20, 129.70, 128.12 (3C), 124.20, 124.00, 122.60, 121.93, 118.95 (2C), 58.31, 30.34, 19.64, 18.73; HRMS (ESI+) calcd for $C_{22}H_{21}ClN_4O_4S$ $[M + H]^+$ 473.1045, found 473.1028 (error 3.59 ppm); mp 206–208 °C; HPLC Retention time – 3.49 min, Purity – 99.65%.

5.2.20.3. 2-(5-(4-(3-(3,4-Dimethylphenyl)ureido)phenyl)thiazole-2-carboxamido)-3-methylbutanoic acid (**30**).

Prepared as described above in the general procedure using 3,4-Dimethylphenyl isocyanate (58.21 mg, 0.396 mmol, 1.1 equiv) as the substituted isocyanate to afford the title compound (95 mg, 56%) as an off white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 12.98 (bs, 1H), 8.91 (s, 1H), 8.56 (s, 1H), 8.36 (m, 2H), 8.34 (s, 1H), 7.71 (d, J = 9.0 Hz, 2H), 7.57 (d, J = 9.0 Hz, 2H), 7.24 (bs, 1H), 7.19–7.16 (m, 1H), 7.04 (d, J = 9.0 Hz, 1H), 4.31, (m, 1H), 2.27 (m, 1H), 2.23 (s, 3H), 2.20 (s, 3H), 0.95 (d, J = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.80, 160.50, 159.54, 152.78, 144.99, 141.50, 139.22, 137.56, 136.82, 130.11 (2C), 128.01 (2C), 123.71, 120.11, 118.80 (2C), 116.34, 58.25, 30.28, 20.10, 19.62, 19.14, 18.74; HRMS (ESI+) calcd for $C_{24}H_{26}N_4O^4S$ $[M + H]^+$ 467.1748, found 467.1724 (error 4.91 ppm); mp 188–190 °C; HPLC Retention time – 3.58 min, Purity – 99.63%.

*5.2.20.4. 3-Methyl-2-(5-(4-(3-(2-phenoxyphenyl)ureido)phenyl) thiazole-2-carboxamido)butanoic acid (**31**).* Prepared as described above in the general procedure using 2-phenoxyphenyl isocyanate (83.55 mg, 0.396 mmol, 1.1 equiv) as the substituted isocyanate to afford the title compound (83 mg, 44%) as an off white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 12.98 (bs, 1H), 9.53 (s, 1H), 8.54 (s, 1H), 8.37 (m, 2H), 8.30–8.27 (m, 1H), 7.72 (d, J = 9.0 Hz, 2H), 7.56 (d, J = 9.0 Hz, 2H), 7.43 (dd, J = 9.0 Hz, 2H), 7.19–7.13 (m, 2H), 7.06 (d, J = 9.0 Hz, 2H), 7.00 (m, 1H), 6.86 (m, 1H), 4.31 (m, 1H), 2.27 (m, 1H), 0.94 (d, J = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.78, 160.57, 159.54, 157.17, 152.59, 145.52, 144.91, 141.21, 139.30, 131.54, 130.52 (2C), 128.10 (2C), 124.45, 124.04, 123.97, 122.91, 120.19, 118.81 (3C), 118.75 (2C), 58.24, 30.26, 19.61, 18.74; HRMS (ESI+) calcd for $C_{28}H_{26}N_4O_5S$ [M + H]$^+$ 531.1697, found 531.1665 (error 6.02 ppm); mp 172–174 °C; HPLC Retention time – 4.28 min, Purity – 99.59%.

*5.2.20.5. 2-(5-(4-(3-(4-Chloro-2-phenoxyphenyl)ureido)phenyl)thiazole-2-carboxamido)-3-methylbutanoic acid (**32**).* Prepared as des cribed above in the general procedure using 2-phenoxy, 4-chlorophenyl isocyanate (97.25 mg, 0.396 mmol, 1.1 equiv) as the substituted isocyanate to afford the title compound (85 mg, 40%) as an off white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 12.99 (bs, 1H), 9.61 (s, 1H), 8.73 (s, 1H), 8.39 (d, J = 9.0 Hz, 1H), 8.37–8.34 (m, 2H), 7.73 (d, J = 9.0 Hz, 2H), 7.56 (d, J = 9.0 Hz, 2H), 7.47–7.42 (m, 2H), 7.2 (dd, J = 9.0 Hz, 1H), 7.10 (d, J = 9.0 Hz, 2H), 7.03–6.99 (m, 1H), 6.86 (d, J = 9.0 Hz, 1H), 4.31 (m, 1H), 2.27 (m, 1H), 0.94 (d, J = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.80, 160.72, 159.49, 156.74, 152.44, 144.79, 144.41, 140.85, 139.40, 132.74, 130.64 (2C), 128.12 (2C), 128.06, 124.49, 124.27, 122.19, 119.84, 119.10 (3C), 118.90 (2C), 58.30, 30.32, 19.64, 18.73; HRMS (ESI+) calcd for $C_{28}H_{25}ClN_4O_5S$ [M + H]$^+$ 565.1307, found 565.1303 (error 0.70 ppm); mp 180–182 °C; HPLC Retention time – 4.89 min, Purity – 99.77%.

5.2.21. General procedure for the synthesis of thiazole analogs possessing an amide linker

To a solution of compound 27 (150 mg, 0.45 mmol, 1 equiv) in DCM (3 ml) was added pyridine (0.11 ml, 1.35 mmol, 3.0 equiv) and stirred for 5 min. To this reaction mixture the appropriate acid chloride (1.5 equiv) was added and stirred for 1 h. The reaction mixture was then concentrated and purified using flash column chromatography using 2:8 EtOAc:$CHCl_3$ to yield the corresponding methyl ester. To a solution of the methyl ester (1 equiv) in THF was added 1.0 M solution of LiOH in water (5 equiv) and stirred for 16 h at rt. The solvent was removed and the obtained residue was diluted with water and acidified to pH 2.0 using 2.0 M HCl. The material thus obtained was extracted with ethyl acetate, washed with water, and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to obtain a solid that was crystallized in ethyl acetate to yield the desired acid.

*5.2.21.1. 2-(5-(4-(4-(tert-Butyl)benzamido)phenyl)thiazole-2-carbo xamido)-3-methylbutanoic acid (**33**).* The methyl ester was synthesized by general procedure III using 4-*tert*-butylbenzoyl chloride (0.125 ml, 0.68 mmol, 1.5 equiv) as the substituted acid chloride at the first step and subsequently deprotected by following general procedure II at the second step to afford the title compound (126 mg, 59%) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 13.02 (bs, 1H), 10.38 (s, 1H), 8.40–8.36 (m, 2H), 7.94 (d, J = 9.0 Hz, 2H), 7.91 (d, J = 9.0 Hz, 2H), 7.79 (d, J = 9.0 Hz, 2H), 7.57 (d, J = 9.0 Hz, 2H), 4.32 (m, 1H), 2.26 (m, 1H), 1.33 (s, 9H), 0.95 (d, J = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.78, 166.13, 161.00, 159.53, 155.12, 144.73, 140.81, 139.73, 134.90, 132.46, 128.07 (2C), 127.79 (2C), 125.68 (2C), 121.02 (2C), 58.24, 35.18, 31.39 (3C), 30.29, 19.63, 18.76; HRMS (ESI+) calcd for $C_{26}H_{29}N_3O_4S$ [M + H]$^+$ 480.1952, found 480.1928 (error 3.95 ppm); mp 226–228 °C; HPLC Retention time – 4.28 min, Purity – 99.95%.

*5.2.21.2. 2-(5-(4-(3-Cyclohexylpropanamido)phenyl)thiazole-2-carboxamido)-3-methylbutanoic acid (**34**).* The methyl ester was synthesized by general procedure III using 3-cyclohexylpropanoyl chloride (118 mg, 0.67 mmol, 1.5 equiv) as the substituted acid chloride at the first step and subsequently deprotected by following general procedure II at the second step to afford the title compound (78 mg, 38%) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 13.01 (bs, 1H), 10.08 (s, 1H), 8.36–8.33 (m, 2H), 7.74–7.68 (m, 4H), 4.30 (d, J = 6 Hz, 1H), 2.34 (t, J = 7.5 Hz, 2H), 2.29–2.20 (m, 1H), 1.73–1.54 (m, 4H), 1.50 (q, J = 6.9 Hz, 2H), 1.25–1.09 (m, 4H), 1.94 (d, J = 6.9 Hz, 6H), 0.94–0.91 (m, 3H); ^{13}C NMR (300 MHz, DMSO-d_6): δ 172.77, 172.25, 160.82, 159.51, 144.75, 140.83, 139.53, 172.88 (2C), 124.98, 119.81 (2C), 58.24, 37.24, 34.50, 33.05 (2C), 32.94, 30.25, 26.56, 26.22 (2C), 19.61, 18.74; HRMS (ESI+) calcd for $C_{24}H_{32}N_3O_4S$ [M + H]$^+$ 458.2108, found 458.2092 (error = 4.57); mp 198–200 °C; HPLC Retention time – 4.39 min, Purity – 96.4%.

*5.2.21.3. 2-(5-(4-(4,4-Difluorocyclohexanecarboxamido)phenyl)thiazole-2-carboxamido)-3-methylbutanoic acid (**35**).* The methyl ester was synthesized by general procedure III using 4,4-difluoro cyclohexanecarbonyl chloride (123 mg, 0.67 mmol, 1.5 equiv) as the substituted acid chloride at the first step and subsequently deprotected by following general procedure II at the second step to afford the title compound (116 mg, 55%) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 12.99 (bs, 1H), 10.19 (s, 1H), 8.36 (s, 2H), 7.72 (s, 4H), 4.29 (d, J = 6.3 Hz, 1H), 2.27–2.25 (m, 1H), 2.20–2.00 (m, 2H), 1.95–1.91 (m, 3H), 1.80–1.67 (m, 3H), 0.95 (d, J = 6 Hz, 6H); ^{13}C NMR (300 MHz, DMSO-d_6): δ 173.61, 172.85, 160.97, 159.47, 144.68, 140.71, 139.60, 127.87 (2C), 125.21, 120.04 (2C), 58.32, 42.48, 32.68, 30.35 (2C), 26.03, 25.95 (2C), 19.64, 18.73; HRMS (ESI+) calcd for $C_{22}H_{26}F_2N_3O_4S$ [M + H]$^+$ 466.1607, found 466.1596 (error = 1.40); MS (ESI+): m/z 466 [M + H]$^+$; mp 254–256 °C; HPLC Retention time – 3.15 min, Purity – 99.7%.

*5.2.21.4. 2-(5-(4-(5-Butylpicolinamido)phenyl)thiazole-2-carboxami do)-3-methylbutanoic acid (**36**).* The methyl ester was synthesized by general procedure III using 5-butylpicolinoyl chloride (133 mg, 0.67 mmol, 1.5 equiv) as the substituted acid chloride at the first step and subsequently deprotected by following general procedure II at the second step to afford the title compound (64 mg, 30%) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 12.96 (bs, 1H), 10.81 (s, 1H), 8.59 (s, 1H), 8.40–8.35 (m, 2H), 8.07–7.98 (m, 3H), 7.91 (d, J = 7.8 Hz, 1H), 7.79 (d, J = 7.2 Hz, 2H), 4.30 (m, 1H), 2.73–2.62 (m, 2H), 1.35–1.26 (m, 1H), 1.61 (m, 2H), 1.33–1.32 (m, 2H), 0.94 (s, 9H); ^{13}C NMR (300 MHz, DMSO-d_6): δ 172.81, 163.26, 161.11, 159.49, 148.84, 148.01, 144.65, 142.04, 139.97, 139.83, 138.05, 127.80 (2C), 125.87, 122.71, 121.12 (2C), 58.30, 33.04, 32.21, 30.35, 22.14, 19.64, 18.73, 14.15; HRMS (ESI+) calcd for $C_{25}H_{29}N_4O_4S$ [M + H]$^+$ 481.1904, found 481.1877 (error = 4.56); MS (ESI+): m/z 481.2 [M + H]$^+$; mp 138–140 °C; HPLC Retention time – 4.75 min, Purity – 88.1%.

*5.2.22. Methyl 4-(2-cyanopropan-2-yl)benzoate (**38**)*

To a solution of potassium tert-butoxide (8.0 g, 0.08 mmol, 2.5 equiv) in THF (25 ml) at −30 °C was added a solution of methyl iodide (5.35 ml, 0.09 mmol, 3.0 equiv) and methyl 4-(cyanomethyl) benzoate (5.0 g, 0.03 mmol, 1.0 equiv) in THF (25 ml) under nitrogen atmosphere over 20 min. The cooling bath was removed and the reaction mixture was allowed to warm to rt and stirred for 2 h. Following this the reaction was quenched with water (10 ml) and

ethyl acetate was added and the organic and aqueous layers were separated. The organic layer was washed with water, brine, and dried over sodium sulfate. The organic solvent was removed under vacuum to obtain a violet residue that was purified by column chromatography using 20:80 EtOAc:Pet ether to obtain an off white solid that was crystallized in $CHCl_3$:Pet ether to afford the title compound (4.0 g, 69%) as a white solid.

^{1}HNMR (300 MHz, $CDCl_3$): δ 8.08 (d, J = 9.0 Hz, 2H), 7.57 (d, J = 9.0 Hz, 2H), 3.97 (s, 3H), 1.76 (s, 6H); MS (ESI+): m/z 204.1 $[M + H]^+$.

*5.2.23. Methyl 4-(3-cyanopentan-3-yl)benzoate (**39**)*

Synthesized compound **39** using the same procedure as described for compound **38** by replacing ethyl iodide (4.11 ml, 0.05 mmol, 3.0 equiv) in place of methyl iodide to afford the title compound (2.5 g, 63%) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07 (d, J = 9.0 Hz, 2H), 7.50 (d, J = 9.0 Hz, 2H), 3.94 (s, 3H), 2.0 (m, 2H), 2.00–1.89 (m, 2H), 0.91 (t, J = 6.0 Hz, 6H); MS (ESI+): m/z 232.1 $[M + H]^+$.

*5.2.24. 2-(5-(4-(4-(2-Cyanopropan-2-yl)benzamido)phenyl)thiazole-2-carboxamido)-3-methylbutanoic acid (**40**)*

To a solution of methyl 2-(5-(4-aminophenyl)thiazole-2-carboxamido)-3-methylbutanoate (150 mg, 0.45 mmol, 1.0 equiv) and methyl 4-(2-cyanopropan-2-yl)benzoate (100 mg, 0.495 mmol, 1.1 equiv) in toluene (12 ml) was added a 2.0 M solution of trimethyl aluminum (0.36 ml, 0.72 mmol, 1.6 equiv) in toluene and the reaction mixture was sealed and heated to 80 °C for 4 h. Following this the reaction mass was cooled to rt and neutralized with saturated aqueous solution of ammonium chloride. The reaction mixture was extracted with CH_2Cl_2 and the organic layer was washed successively with 1.0 N HCl followed sequentially with saturated sodium bicarbonate and brine solution. The organic layer was dried over sodium sulfate and the solvent was evaporated under reduced pressure to obtain dark brown residue which was purified by column chromatography in EtOAc:Pet ether to obtain a pale brown colored solid. The solid was crystallized from $CHCl_3$:Pet ether to yield the desired methyl ester. To a solution of the methyl ester (1 equiv) in THF was added 1.0 M solution of LiOH in water (4 equiv) and stirred for 16 h at rt. Following removal of the solvent obtained residue was diluted with water and acidified to pH 2 using 2.0 M HCl. The material thus obtained was extracted with EtOAc, washed with water, and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to obtain a solid that was crystallized in EtOAc to yield the desired acid as white solid (25 mg, 12%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 13.00 (bs, 1H), 10.49 (s, 1H), 8.40–8.37 (m, 2H), 8.03 (d, J = 9.0 Hz, 2H), 7.92 (d, J = 9.0 Hz, 2H), 7.80 (d, J = 9.0 Hz, 2H), 7.70 (d, J = 9.0 Hz, 2H), 4.31 (m, 1H), 2.25 (m, 1H), 1.74 (s, 6H), 0.97 (d, J = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.78, 165.63, 161.07, 159.53, 145.49, 144.66, 140.60, 139.80, 134.66, 128.85 (2C), 127.82 (2C), 125.79 (3C), 124.78, 121.12 (2C), 58.28, 37.31, 30.29 (2C), 28.63, 19.63, 18.76; HRMS (ESI+) calcd for $C_{26}H_{26}N_4O_4S$ $[M + H]^+$ 491.1748, found 491.1730 (error 3.43 ppm); mp 256–258 °C; HPLC Retention time – 3.46 min, Purity – 92.97%.

*5.2.25. 2-(5-(4-(4-(3-Cyanopentan-3-yl)benzamido)phenyl)thiazole-2-carboxamido)-3-methylbutanoic acid (**41**)*

Synthesized following a similar procedure as described for compound **40** by replacing methyl 4-(3-cyanopentan-3-yl)benzoate (114 mg, 0.495 mmol, 1.1 equiv) in place of methyl 4-(2-cyanopropan-2-yl)benzoate to afford the title compound (58 mg, 25%) as an off-white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 13.03 (bs, 1H), 10.52 (s, 1H), 8.40–8.37 (m, 2H), 8.02 (d, J = 9.0 Hz, 2H), 7.92 (d, J = 9.0 Hz, 2H), 7.80 (d, J = 9.0 Hz, 2H), 7.61 (d, J = 9.0 Hz, 2H), 4.29 (m, 1H), 2.25 (m, 1H), 2.12–1.99 (m, 4H), 0.96 (d, J = 6.0 Hz, 6H), 0.81 (t, J = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.77, 165.71, 161.13, 159.47, 144.64, 142.19, 140.63, 139.78, 134.61, 128.86 (2C), 127.81 (2C), 126.64 (2C), 125.78, 122.38, 121.12 (2C), 58.34, 50.05, 33.06 (2C), 30.78, 19.65, 18.73, 10.03 (2C); HRMS (ESI+) calcd for $C_{28}H_{30}N_4O_4S$ $[M + H]^+$ 519.2061, found 519.2042 (error 3.27 ppm); mp 242–244 °C; HPLC Retention time – 3.91 min, Purity – 98.27%.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.05.006.

References

[1] J. Zhou, G. Qin, Adipocyte dysfunction and hypertension, Am. J. Cardiovasc. Dis. 2 (2012) 143–149.
[2] E.V. Kuklina, X. Tong, M.G. George, P. Bansil, Epidemiology and prevention of stroke: a worldwide perspective, Expert Rev. Neurother. 12 (2012) 199–208.
[3] C.J. Roos, P.H. Quax, J.W. Jukema, Cardiovascular metabolic syndrome: mediators involved in the pathophysiology from obesity to coronary heart disease, Biomarkers Med. 6 (2012) 35–52.
[4] F. Wang, B.Y. Wang, L. Qiao, Association between obesity and gallbladder cancer, Front. Biosci. 17 (2012) 2550–2558.
[5] F.P. de Heredia, S. Gómez-Martínez, A. Marcos, Obesity, inflammation and the immune system, Proc. Nutr. Soc. 71 (2012) 332–338.
[6] W.M. Mellana, W.S. Aronow, C. Palaniswamy, S. Khera, Rheumatoid arthritis: cardiovascular manifestations, pathogenesis, and therapy, Curr. Pharm. Des. 18 (2012) 1450–1456.
[7] A. Viardot, L.K. Heilbronn, D. Samocha-Bonet, F. Mackay, L.V. Campbell, K. Samaras, Obesity is associated with activated and insulin resistant immune cells, Diabetes Metab. Res. Rev. 28 (2012) 447–454.
[8] K.S. McClendon, D.M. Riche, G.I. Uwaifo, Orlistat: current status in clinical therapeutics, Expert Opin. Drug Saf. 8 (2009) 727–744.
[9] M.C. Mancini, A. Halpern, Pharmacological treatment of obesity, Arq. Bras. Endocrinol. Metabol. 50 (2006) 377–389.
[10] H.C. Chen, R.V. Farese Jr., DGAT and triglyceride synthesis: a new target for obesity treatment? Trends Cardiovasc. Med. 10 (2000) 188–192.
[11] S.J. Stone, H.M. Myers, S.M. Watkins, B.E. Brown, K.R. Feingold, P.M. Elias, R.V. Farese Jr., Lipopenia and skin barrier abnormalities in DGAT2-deficient mice, J. Biol. Chem. 279 (2004) 11767–11776.
[12] H.C. Chen, R.V. Farese Jr., Inhibition of triglyceride synthesis as a treatment strategy for obesity: lessons from DGAT1-deficient mice, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 482–486.
[13] R.L. Dow, M.J. Munchhoff, Substituted bicyclolactam compounds, WO2009016462, 2009.
[14] B.M. Fox, N. Furukawa, X. Hao, K. Iio, T. Inaba, S.M. Jackson, F. Kayser, M. Labelle, K. Li, T. Matsui, D. McMinn, N. Ogawa, S. Rubenstein, S. Sagawa, K. Sugimoto, M. Suzuki, M. Tanaka, G. Ye, A. Yoshida, J. Zhang, Fused bicyclic nitrogen-containing heterocycles, WO2004047755, 2004.
[15] R. Smith, A.M. Campbell, P. Coish, M. Dai, S. Jenkins, D. Lowe, S.O'Connor, G. Wang, M. Zhang, L.Z. Zhu, Preparation and use of aryl alkyl acid derivatives for the treatment of obesity, US7091228, 2006.
[16] M.H. Serrano-Wu, Y.S. Kwak, W. Liu, New compounds, Patent WO 2007126957, 2007.
[17] R. Smith, S.J. O'Connor, P. Coish, D. Lowe, R.B. Clark, J. Stebbins, A.M. Campbell, C. Akuche, T. Shelekhin, Preparation and use of biphenyl-4-yl-carbonylamino acid derivatives for the treatment of obesity, Patent WO 2006044775, 2005.
[18] R.D. Jadhav, K.S. Kadam, S. Kandre, T. Guha, M.M.K. Reddy, M.K. Brahma, N.J. Deshmukh, A. Dixit, L. Doshi, N. Potdar, A.A. Enose, R.A. Vishwakarma, H. Sivaramakrishnan, S. Srinivasan, K.V.S. Nemmani, A. Gupte, A.K. Gangopadhyay, R. Sharma, Synthesis and biological evaluation of isoxazole, oxazole, and oxadiazole containing heteroaryl analogs of biaryl ureas as DGAT1 inhibitors, Eur. J. Med. Chem. 54 (2012) 324–342.
[19] H. Motiwala, S. Kandre, V. Birar, K.S. Kadam, A. Rodge, R.D. Jadhav, M.M.K. Reddy, M.K. Brahma, N.J. Deshmukh, A. Dixit, L. Doshi, A. Gupte, A.K. Gangopadhyay, R.A. Vishwakarma, S. Srinivasan, M. Sharma, K.V.S. Nemmani, R. Sharma, Exploration of pyridine containing heteroaryl analogs of biaryl ureas as DGAT1 inhibitors, Bioorg. Med. Chem. Lett. 21 (2011) 5812–5817.
[20] R.R. Ramharack, M.A. Spahr, Diacylglycerol acyltransferase (DGAT) assay, US20020127627, 2002.