European Journal of Medicinal Chemistry 79 (2014) 203–215

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and evaluation of cyclohexane carboxylic acid head group containing isoxazole and thiazole analogs as DGAT1 inhibitors

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ARTICLE INFO

Article history:
Received 6 February 2014
Received in revised form
26 March 2014
Accepted 27 March 2014
Available online 28 March 2014

Keywords:
Diacylglycerol acyltransferase (DGAT)
Obesity
Plasma triglycerides
Fat tolerance test (FTT)

ABSTRACT

Diacylglycerol acyltransferase 1 (DGAT1) is known to play an important catalytic role in the final step of triglyceride biosynthesis. High fat diet fed DGAT1 knockout mice were resistant to weight gain and exhibited increased insulin and leptin sensitivity thereby indicating a plausible role for DGAT1 inhibitors in the treatment of obesity. 4-Phenylpiperidine-1-carbonyl cyclohexanecarboxylic acid (compound **6**, DGAT1 IC_{50} = 57 nM) has been lately reported as a potent DGAT1 inhibitor. In our search for newer scaffolds possessing potent DGAT1 activity we undertook a systematic diversification of compound **6** to identify a 4-(5-phenylthiazole-2-carboxamido)cyclohexanecarboxylic acid scaffold. Further linker optimization of this scaffold identified compound **9e** (DGAT1 IC_{50} = 14.8 nM) as a potent DGAT1 inhibitor. Coupled with its *in vitro* potency, compound **9e** also exhibited 112 percent plasma triglyceride reduction at a 3 mpk dose in an oral fat tolerance test (FTT) when studied in Swiss mice.

1. Introduction

Diacylglycerol acyltransferase 1 (DGAT1) is abundantly expressed in the small intestine, liver, and adipose tissues. It is known to catalyze the final committed step of triglyceride biosynthesis [1]. DGAT1 knockout mice phenotypes are viable and exhibit reduction in the postprandial rise of plasma triglycerides, resistance to diet induced obesity (DIO), and increased sensitivity for both insulin and leptin [2, 3]. In contrast, DGAT2 deficient mice were found to be smaller in size than the wild type and died soon following their birth [4]. It was observed that the DGAT2 deficient mice lacked essential fatty acids resulting in skin lipid abnormalities and impaired epidermal barrier function. As a result specific inhibitors of the DGAT1 enzyme are being explored for metabolic disorders such as weight gain, dyslipidemia, type 2 diabetes, and endothelial dysfunction. Of late an increased interest towards this attractive target has resulted in the identification of several potent and selective DGAT1 inhibitors (Fig. 1) [5–10].

LCQ-908 (**1**), a DGAT1 inhibitor being developed by Novartis is under evaluation for the treatment of familial chylomicronemia syndrome (FCS), a condition characterized by elevated triglyceride levels [5]. Other potent DGAT1 inhibitors include a series of biphenyl cyclopentane carboxylic acids represented by BAY-744113 (**2**, IC_{50} = 73 nM) [6]. Replacing the benzothiazole moiety of **2** with a phenyl urea resulted in an orally potent carboxylic acid represented by compound **3** (DGAT1 IC_{50} = 7 nM) [7]. Other scaffolds also identified as DGAT1 inhibitors include a bicyclic heterocycle core (**4**, DGAT1 IC_{50} = 15 nM) disclosed by Japan Tobacco/Tularik [8] and PF-04620110 (**5**, DGAT1 IC_{50} = 19 nM) reported by Pfizer [9]. Hoffmann-La Roche has recently reported a 4-phenylpiperidine-1-carbonyl cyclohexanecarboxylic acid scaffold represented by compound **6**, (DGAT1 IC_{50} = 57 nM) as an active DGAT1 inhibitor [10]. Despite several advances in pre-clinical drug discovery and application of stringent selection criteria for advancing clinical candidates, a high attrition rate is observed in case of compounds that enter clinical development. According to a published study [11] the success rate for compounds that entered clinical trials in between 1993 and 2004 was 32% for large molecules and a meager 13% for small molecules. One of the possible scientific approaches for overcoming such unforeseen developmental hurdles is to invest in efforts towards the identification of diverse chemical scaffolds for a

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http://dx.doi.org/10.1016/j.ejmech.2014.03.077
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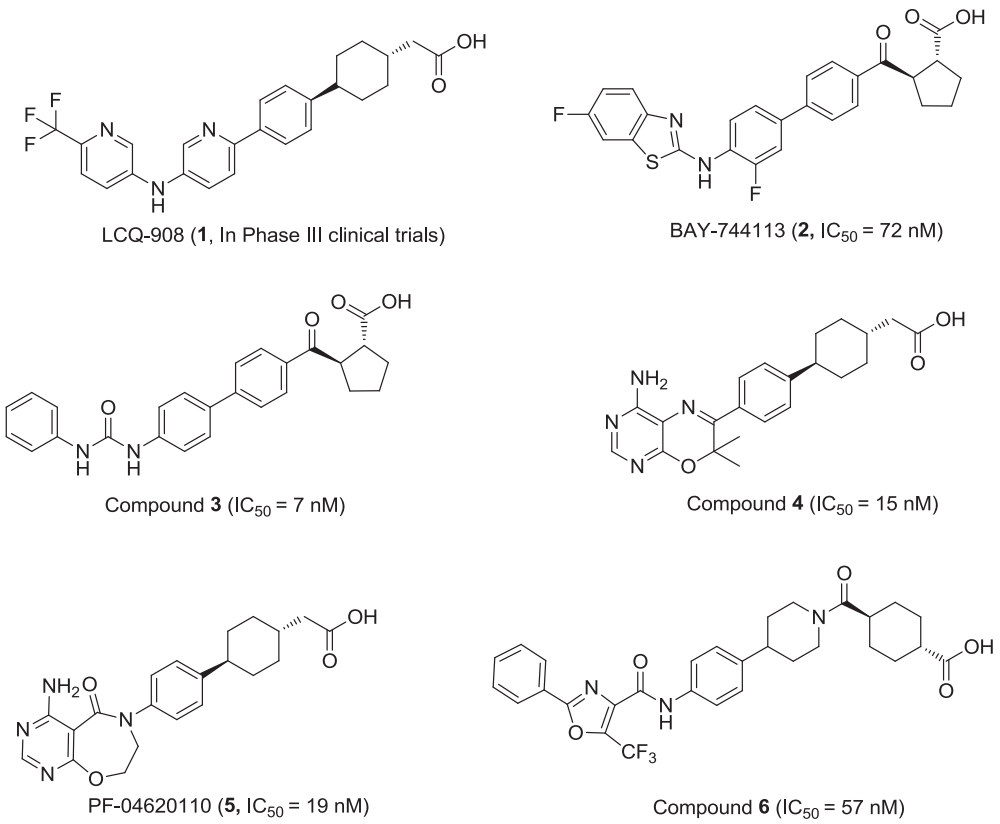


Fig. 1. Selected DGAT1 inhibitors from literature.

validated protein target like DGAT1. The present study highlights our systematic efforts towards the identification of a 4-(5-phenylthiazole-2-carboxamido)cyclohexanecarboxylic acid scaffold.

In our attempts to develop a novel and selective DGAT1 chemotype, we undertook the modification of compound **6** (in-house DGAT1 IC_{50} = 114 nM). In our hands, compound **6** also demonstrated 89% triglyceride reduction in an acute fat tolerance test conducted in mice. Compound **6** appears to be comprised of four domains (aryl, linker, central core, and acid head) as shown in Fig. 2. In the present study we have explored replacing the piperidine ring in the central core with either isoxazole and thiazole structural motifs thereby resulting in either a 3-phenylisoxazole core (represented by compound **7**), a 4-phenylthiazole core (represented by compound **8**), or a 5-phenylthiazole core (represented by compound **9**). Having replaced a six-membered heteroaliphatic piperidine ring with five-membered heteroaromatic rings, we compensated the loss of this singular ring atom by introducing a nitrogen atom in the acid head portion of the molecule. This modification resulted in an amide linker preceding the cyclohexyl acid head. Taken together, these modifications resulted in either a 4-(3-phenylisoxazole-5-carboxamido)cyclohexanecarboxylic acid scaffold (represented by compound **7**, DGAT1 inhibition [1 μM] = 73%), 4-(4-phenylthiazole-2-carboxamido)cyclohexanecarboxylic acid scaffold (represented by compound **8**, DGAT1 inhibition [1 μM] = 7%), or 4-(5-phenylthiazole-2-carboxamido)cyclohexanecarboxylic acid scaffold (represented by compound **9**, DGAT1 inhibition [1 μM] = 94%). Amongst these three heterocyclic scaffolds, the 4-(5-phenylthiazole-2-carboxamido) cyclohexanecarboxylic acid scaffold thus appeared to exhibit better DGAT1 potency than the other two heterocyclic scaffolds. We evaluated the DGAT1 IC_{50} of compound **9** and found it to be 73 nM. Although this initial modification led to an improvement in DGAT1 potency, it was also observed that this modification led to a molecular weight increase from 569.5 Da for compound **6** to 584.5 Da for compound **9**. With an aim to reduce the molecular weight of our resulting molecules to the more acceptable 500 Da range we chose to replace the trifluoromethyl oxazole amide linker in compound **6** with either amide (compound **9a**, Molecular weight = 485.5 Da), sulfonamide (compound **9b**, Molecular weight = 521.5 Da), or urea (compound **9c**, Molecular weight = 500.5 Da) linkers as shown in Fig. 3. Our efforts involving a systematic diversification of compound **6** were thus focused on developing and optimizing a novel heteroaryl scaffold that retains DGAT1 inhibitory activity.

2. Chemistry

Compound **11** a key intermediate for the synthesis of compound **6** was prepared following the selective hydrolysis [12] of commercially available dimethyl *trans*-1,4-cyclohexane dicarboxylate (**10**) to *trans*-1,4-cyclohexanedicarboxylic acid monomethyl ester (**11**) as shown in Scheme 1. Compound **6** was prepared (Scheme 2) following the nitration of commercially available 4-phenylpiperidine (**12**) using nitric acid [13] to yield 4-(4-nitrophenyl)piperidine (**13**). Compound **13** was coupled with **11** using HATU to give methyl 4-(4-(4-nitrophenyl)piperidine-1-carbonyl)cyclohexanecarboxylate (**14**). Reduction of compound **14** using iron-ammonium chloride resulted in the corresponding amine (**15**) that on coupling with commercially available 2-phenyl-5-trifluoromethyloxazole-4-carboxylic acid using HATU followed by alkaline hydrolysis resulted in the synthesis of compound **6**.

The 4-(3-phenylisoxazole-5-carboxamido)cyclohexanecarboxylic acid scaffold represented by compound **7** was synthesized as depicted in Scheme 3. Refluxing 4-Nitro

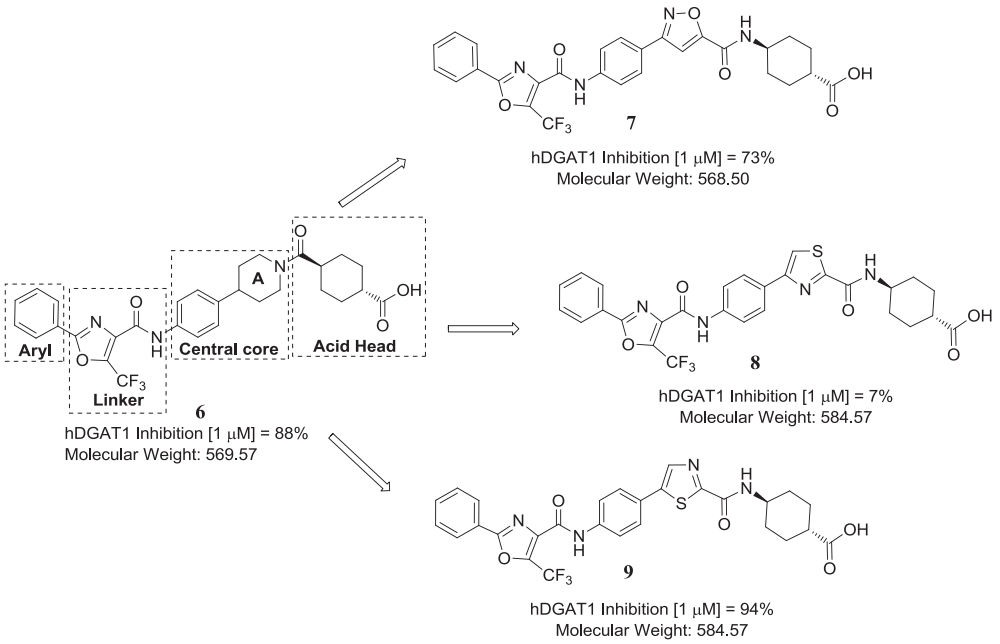


Fig. 2. Replacement of aliphatic heterocycle by five membered aromatic heterocycles.

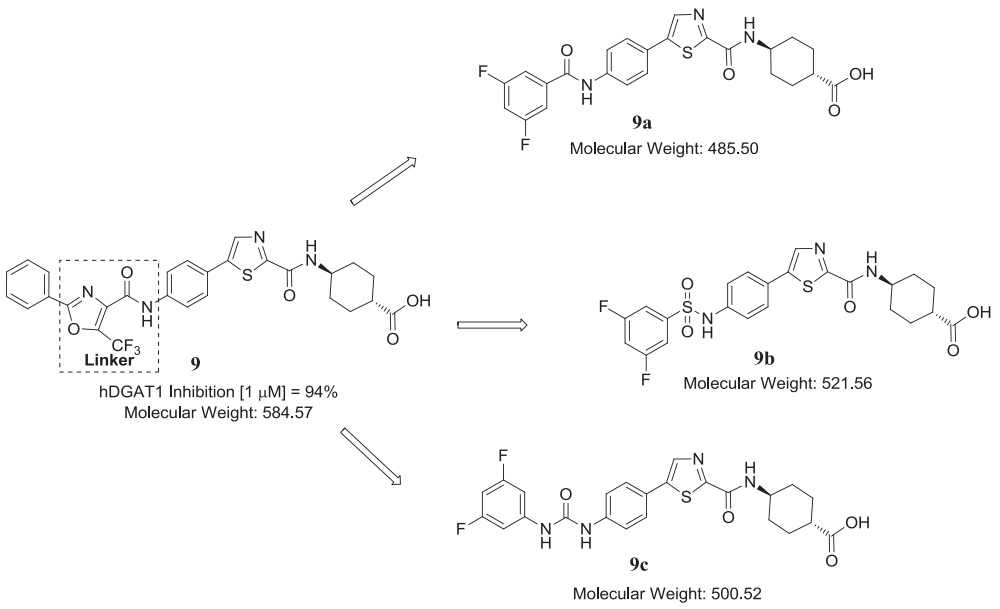
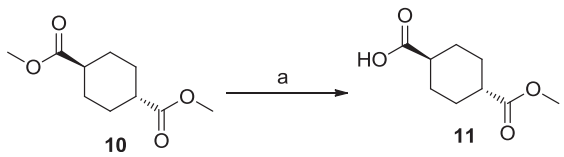
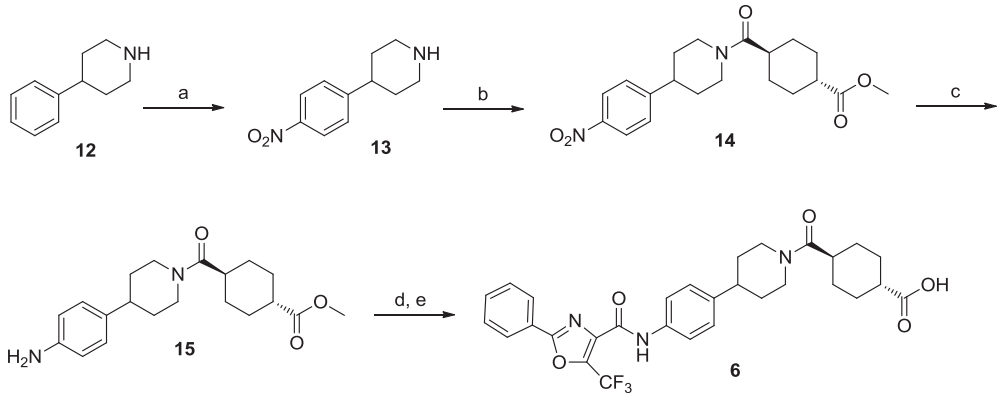


Fig. 3. Linker modifications aimed at reducing molecular weight.

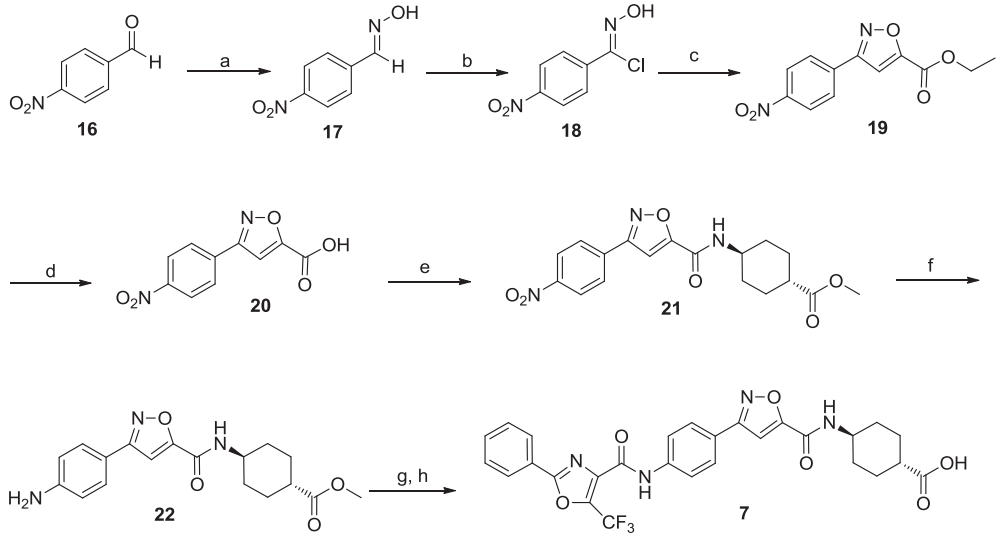


Scheme 1. Synthesis of intermediate **11**. *Reaction conditions*: (a) KOH, methanol reflux, 5 h.

benzaldehyde (**16**) in methanol with hydroxylamine hydrochloride yielded the corresponding oxime (**17**) that was subsequently treated with *N*-chlorosuccinimide in DMF to yield *N*-hydroxy-4-nitrobenzimidoyl chloride (**18**). Compound **18** was further cyclized to ethyl 3-(4-nitrophenyl)isoxazole-5-carboxylate (**19**) following treatment with ethyl propiolate [14]. Compound **19** was hydrolyzed to its corresponding acid (**20**) using sodium hydroxide that was subsequently coupled with methyl *trans*-4-aminocyclohexanecarboxylate to obtain methyl 4-(3-(4-nitrophenyl)isoxazole-5-carboxamido)cyclohexanecarboxylate (**21**). Compound **21** was reduced to its corresponding amino derivative (**22**). Compound **22** was further coupled with commercially available 2-phenyl-5-trifluoromethyloxazole-4-carboxylic acid using HATU as the peptide coupling reagent followed by alkaline hydrolysis to yield compound **7**.



Scheme 2. Synthesis of compound **6**. *Reaction conditions*: (a) HNO_3, H_2SO_4; (b) Compound **11**, HATU, TEA, DMF, 16 h; (c) Fe, NH_4Cl, THF, EtOH, water, 75 °C, 3 h; (d) 2-phenyl-5-trifluoromethyloxazole-4-carboxylic acid, HATU, TEA, DMF, 16 h; (e) NaOH, MeOH, 8 h.



Scheme 3. Synthesis of compound **7**. *Reaction conditions*: (a) $NH_2OH.HCl$, MeOH, reflux, 5 h; (b) NCS, DMF, 2 h; (c) Ethyl propiolate, TEA, Toluene, 80 °C, 8 h; (d) NaOH, THF, H_2O, 1 h; (e) Methyl *trans*-4-aminocyclohexanecarboxylate hydrochloride, N-Methyl morpholin, IBCF, TEA, THF, -20 to 25 °C, 16 h; (f) Fe, NH_4Cl, THF, EtOH, water, 75 °C, 3 h; (g) 2-phenyl-5-trifluoromethyloxazole-4-carboxylic acid, HATU, TEA, DMF, 16 h; (h) NaOH, MeOH, THF, 8 h.

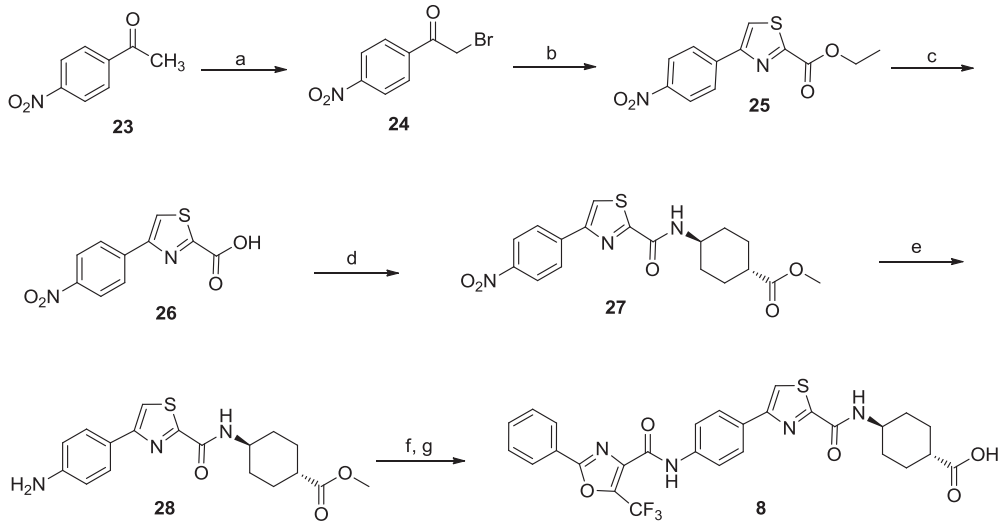
Compound **8** representing the 4-(4-phenylthiazole-2-carboxamido)cyclohexanecarboxylic acid scaffold was synthesized as shown in Scheme 4 using commercially available 4-nitroacetophenone (**23**). Bromination of compound **23** using bromine solution yielded 2-bromo-1-(4-nitrophenyl)ethanone (**24**) that was cyclized using ethyl thioxoacetate to yield ethyl 4-(4-nitrophenyl)thiazole-2-carboxylate (**25**). Subsequent hydrolysis of **25** to its corresponding acid [15] compound (**26**) followed by coupling with methyl *trans*-4-aminocyclohexanecarboxylate yielded methyl 4-(4-(4-nitrophenyl)thiazole-2-carboxamido)cyclohexanecarboxylate (**27**). Reduction of **27** to its corresponding amine (**28**) was achieved using iron-ammonium chloride. Coupling of compound **28** with 2-phenyl-5-trifluoromethyloxazole-4-carboxylic acid followed by hydrolysis resulted in the synthesis of compound **8**.

The synthesis of compounds **9** and **9a**–**9i** possessing the 4-(5-phenylthiazole-2-carboxamido)cyclohexanecarboxylic acid scaffold (Scheme 4) was initiated with the treatment of 2-bromo-1-(4-nitrophenyl)ethanone (**24**) with hexamine and hydrochloric acid to yield 2-amino-1-(4-nitrophenyl)ethanone hydrochloride (**29**). Compound **29** when refluxed with ethyl chloroxoacetate in ethyl acetate yielded compound **30** that was further cyclized using Lawesson's reagent to obtain ethyl 5-(4-nitrophenyl)thiazole-2-carboxylate (**31**). Subsequent hydrolysis of compound **31** yielded its corresponding acid [15] compound (**32**) that on coupling with methyl *trans*-4-aminocyclohexanecarboxylate yielded methyl 4-(5-(4-nitrophenyl)thiazole-2-carboxamido)cyclohexanecarboxylate (**33**). Reduction of compound **33** with iron-ammonium chloride yielded the key amine intermediate methyl 4-(5-(4-aminophenyl) thiazole-2-carboxamido)cyclohexanecarboxylate **(34)**. The amine intermediate (**34**) was coupled with 2-phenyl-5-trifluoromethyloxazole-4-carboxylic acid followed by hydrolysis to yield compound **9**. Compound **34** was also diversified using 3,5-difluorobenzoyl chloride to yield compound **9a**, 3,5-difluorobenzenesulfonyl chloride to yield compound **9b**, and appropriate phenyl isocyanates to yield compounds **9c**–**9i**.Scheme 5

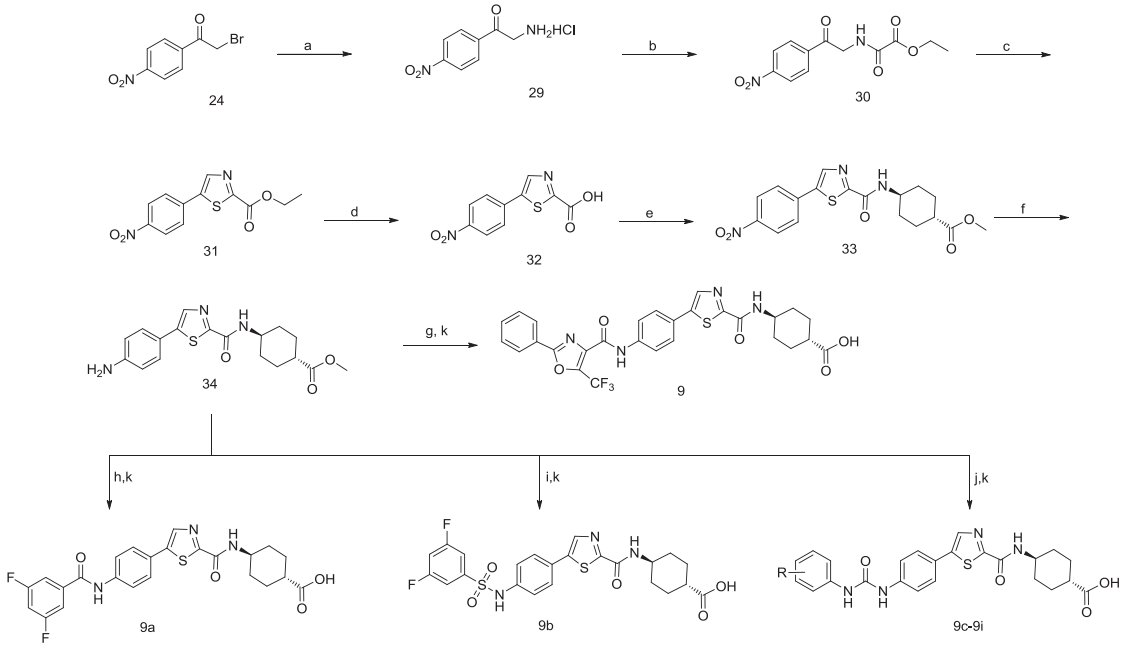
3. Results and discussion

3.1. In vitro pharmacology

The compounds thus synthesized were assayed using an *in vitro* enzymatic assay that measured a triolein output from diolein and radiolabeled oleoyl-CoA [16]. The DGAT1 assay was performed using 2.5 μg of the protein from a post nuclear supernatant pre-incubated with 100 μl of the assay buffer [100 mM Tris–HCl (pH



Scheme 4. Synthesis of compound **8**. Reaction condition: (a) Br_2, $AlCl_3$, DEE, 1 h; (b) ethyl 2-amino-2-thioxoacetate MeOH, reflux, 3 h; (c) NaOH, THF, H_2O, 1 h; (d) Methyl *trans*-4-aminocyclohexanecarboxylate hydrochloride, HATU, TEA, DMF, 16 h; (e) Fe, NH_4Cl, THF, EtOH, water, 75 °C, 3 h; (f) 2-phenyl-5-trifluoromethyloxazole-4-carboxylic acid, HATU, TEA, DMF, 16 h; (g) NaOH, MeOH, THF, 8 h.



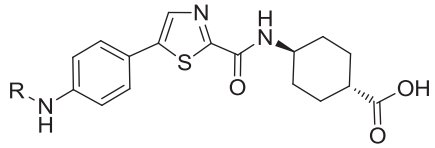
Scheme 5. Synthesis of compounds **9** and **9a**–**9i**. *Reaction condition*: (a) Hexamine, Conc. HCl, EtOH, 24 h; (b) Ethyl chloroxoacetate, TEA, EtOAc, reflux, 3 h; (c) Lawesson's reagent, dioxane, reflux 1.5 h; (d) 1 N NaOH, THF, 0.5 h; (e) Methyl *trans* 4-aminocyclohexanecarboxylate hydrochloride, IBCF, N-methyl morpholine, TEA, THF, -20 to 25 °C, 16 h; (f) Fe, NH_4Cl, THF, EtOH, water, 75 °C, 3 h; (g) 2-phenyl-5-trifluoromethyloxazole-4-carboxylic acid, HATU, TEA, DMF, 16 h; (h) 3,5-Difluorobenzoyl chloride; pyridine, DCM, 55 °C, 16 h; (i) 3,5-Difluorobenzenesulfonyl chloride; pyridine, DCM, 55 °C, 16 h; (j) Substituted phenyl isocyanate, THF, 55 °C, 16 h; (k) 1 N NaOH, MeOH, THF, 8 h.

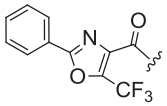
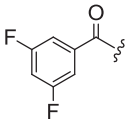
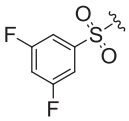
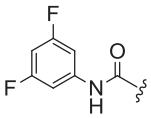
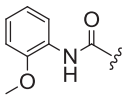
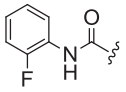
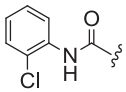
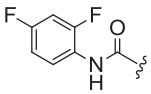
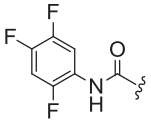
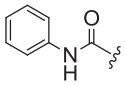
7.5), 250 mM sucrose, and 1.25 mg/ml fatty acid free BSA] containing known concentration of the inhibitor and supplemented using 2047.5 μM of 1,2-dioleoylglycerol. The hDGAT1 reaction was initiated following an addition of 16.8 nCi of [^{14}C]-oleoyl CoA and after 10 min of incubation at 37 °C, the reaction 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 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.

Compound **9a** (DGAT1 inhibition [1 μM] = 86%, IC_{50} = 76 nM) possessing the amide linker and compound **9c** (DGAT1 inhibition [1 μM] = 91%, IC_{50} = 34 nM) possessing a urea linker exhibited retention of DGAT1 potency. On the contrary, compound **9b** (DGAT1 inhibition [1 μM] = 17%) possessing a sulfonamide linker resulted in greatly diminished DGAT1 potency. Each of these three

compounds possesses 2,4-difluorophenyl as the aryl substituent. In comparison with compound **6**, compound **9a** possessing the amide linker appeared 1.5-fold more active whereas compound **9c** possessing the urea linker appeared 3.3-fold more active, indicating a preference for the urea linker over the amide linker. Hence we explored a few additional urea linked analogs (**9d**–**9i**) belonging to this unique 4-(5-phenylthiazole-2-carboxamido)cyclohexanecarboxylic acid scaffold (Table 1). Compound **9i** (DGAT1 inhibition [1 μM] = 89%, IC_{50} = 55 nM) possessing a phenyl urea substituent appeared well tolerated at the DGAT1 enzyme. The presence of electron withdrawing halo-substituents on the phenyl urea as seen in compounds **9e** (DGAT1 inhibition [1 μM] = 93%, IC_{50} = 15 nM), **9f** (DGAT1 inhibition [1 μM] = 91%, IC_{50} = 20 nM), **9g** (DGAT1 inhibition [1 μM] = 81%, IC_{50} = 39 nM), and **9h** (DGAT1 inhibition [1 μM] = 80%, IC_{50} = 26 nM) led to an improvement in DGAT1 potency. The presence of an electron donating 2-methoxy substituent on the phenyl urea as in case of compound **9d** (DGAT1 inhibition [1 μM] = 93%, IC_{50} = 150 nM) led to a 3-fold loss in DGAT1 potency. This result indicates that while electronegative substituents on the phenyl urea led to an increase in DGAT1 potency the presence of electron donating substituent led to a decrease in the same. Compound **9e** was further subjected to an *in vivo* acute fat tolerance test and compared alongside compound **6** for its ability to reduce the triglyceride levels following a 10 ml/kg bolus dose of olive oil.

Table 1
In vitro evaluation of 4-(5-phenylthiazole-2-carboxamido)cyclohexanecarboxylic acid analogs.



Compound number	R	*In vitro* hDGAT1 inhibition [1 μM]	IC_{50} (nM)
9		93.7%	72.85
9a		86.4%	75.59
9b		16.5%	ND
9c		91.9%	34.15
9d		92.7%	149.9
9e		93.3%	14.8
9f		90.9%	20.4
9g		61.0%	38.88
9h		80.2%	26.2
9i		89.3%	54.85
6	–	88.0%	114

ND: not determined.

3.2. 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 (**6** and **9e**) 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_{0-4h}) of the test compounds and comparing it along with an AUC_{0-4h} of the vehicle group that is considered to be 100 percent (Fig. 4). In this study, compound **6** exhibited an 89% triglyceride reduction. Compound **9e**, 4-(5-(4-(3-(2-fluorophenyl)ureido) phenyl)thiazole-2-carboxamido)cyclohexanecarboxylic acid, exhibited 112% triglyceride reduction. Thus both the compounds appear equi-efficacious when evaluated *in vivo*.

4. Conclusions

In this study, we developed a novel 4-(5-phenylthiazole-2-carboxamido)cyclohexanecarboxylic acid scaffold exhibiting improved DGAT1 potency. A preliminary comparison in between the 3-phenylisoxazole, 4-phenylthiazole, and 5-phenylthiazole central cores highlighted a preference for the 5-phenylthiazole scaffold at the DGAT1 enzyme. A subsequent study undertaken to evaluate the urea, amide, and sulfonamide linkers of this 5-phenylthiazole scaffold identified the urea and amide linkers to be well tolerated at the DGAT1 enzyme when analyzed *in vitro*. These linker modifications also resulted in bringing down the molecular weight of this scaffold to the more acceptable 500 Da range. The *in vitro* studies identified compound **9e** (DGAT1 inhibition [1 μM] = 93%, IC_{50} = 15 nM) to be the most potent amongst the series of compounds studied. This compound was subsequently evaluated *in vivo* for its ability to reduce triglycerides in HFD-fed Swiss mice when dosed at 3 mg/kg. Compound **9e** (Triglyceride reduction = 112%) appeared equipotent to compound **6** (Triglyceride reduction = 88%) at the same dose. These attributes identify compound **9e** as a potential new lead for developing DGAT1 inhibitors for bringing about therapeutic intervention in conditions involving high triglyceride levels.

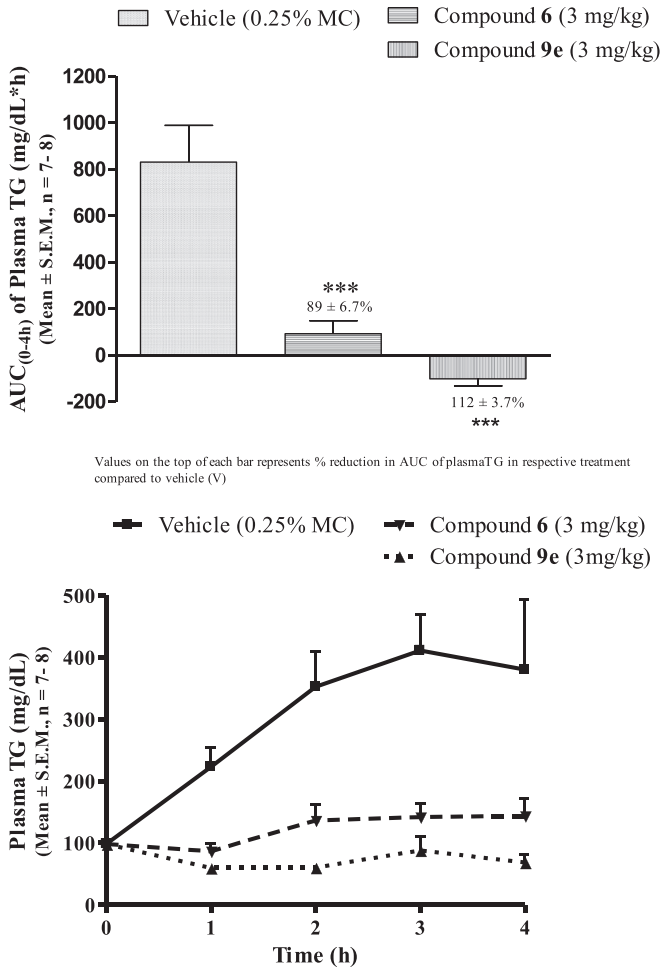


Fig. 4. Effect of compound **6** and compound **9e** on plasma triglyceride in an acute fat tolerance test in Swiss mice.

5. Experimental

5.1. Analytical methods

^{1}H NMR and ^{13}C NMR spectras were recorded on a Bruker spectrometer (300 MHz or 500 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.

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 an Ascentis TM Express (50 × 4.6 mm I.D.), 2.7 μm operated at 1 ml/min, detection at 288 nm.

5.2. Chemistry

Unless mentioned otherwise all reactions were performed under atmosphere. Unless otherwise specified all reagents were obtained from Aldrich and solvents were obtained from Spectrochem and used without further purification.

5.2.1. Trans 1,4-cyclohexanecarboxylic acid monomethyl ester (**11**)

To a solution of *trans* dimethyl 1,4-cyclohexanedicarboxylate (9 g, 45 mmol, 1.0 equiv) in methanol (90 ml) was added a solution of methanolic KOH (3 g, 45 mmol, 1.0 equiv) and the reaction mixture was refluxed for 5 h. Following reaction completion methanol was evaporated under reduced pressure. The obtained residue was dissolved in 50 ml water and extracted using diethyl ether to remove any unreacted *trans* dimethyl 1,4-cyclohexanedicarboxylate. Aqueous layer was acidified to pH 6 using 6.0 N HCl and the precipitated solid was filtered. Solid cake was washed with 100 ml cold water and dried under high vacuum to obtain 6.1 g (72%) white solid compound.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.07 (bs, 1H), 3.58 (s, 3H), 2.25–2.30 (m, 1H), 2.16 (s, 1H), 1.90 (d, $J = 7.2$ Hz, 4H), 1.25–1.41 (m, 4H); MS (ESI-) *m/z* 185.2 $[M - H]^-$.

5.2.2. 4-(4-Nitrophenyl)piperidine (**13**)

4-Phenylpiperidine (5 g, 31.0 mmol, 1.0 equiv) was slowly added to conc. H_2SO_4 (10.74 ml, 202 mmol, 6.5 equiv) at rt and the reaction mass was stirred for 10 min. The mixture was then cooled to 0 °C, fuming nitric acid (1.540 ml, 31.0 mmol, 1.0 equiv) was added to it over 10 min, and the reaction mixture was stirred at rt for 24 h. Following reaction completion the reaction mass was slowly dumped in a mixture of water and ice. The reaction mixture was basified to pH 8–9 using aqueous 1.0 M NaOH. 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 4.3 g (67%) yellow colored solid that was used as such without further purification.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.15 (d, $J = 8.7$ Hz, 2H), 7.51 (d, $J = 8.4$ Hz, 2H), 3.02 (d, $J = 11.7$ Hz, 3H), 2.70–2.78 (m, 1H), 2.57 (t, $J = 11.1$ & 12 Hz, 2H), 1.69 (d, $J = 11.7$ Hz, 2H), 1.48–1.44 (m, 2H); MS (ESI+) *m/z* 207.1 $[M + H]^+$.

5.2.3. Methyl 4-(4-(4-nitrophenyl)piperidine-1-carbonyl) cyclohexanecarboxylate (**14**)

To a solution of compound **11** (1.8 g, 9.68 mmol, 1.0 equiv) in DMF (5 ml), HATU (5.50 g, 14.52 mmol, 1.5 equiv), compound **13** (2 g, 9.68 mmol, 1.0 equiv) and triethylamine (2.7 ml, 19.36 mmol, 2.0 equiv) were added sequentially and the reaction mass was stirred at rt for 2 h. Following reaction completion, water was added and extracted with EtOAc, the organic layer was washed with water, brine, dried over sodium sulfate and concentrated under reduced pressure to obtain pale yellow liquid. The oil was further purified by column chromatography using 2:8 EtOAc:Pet ether to get 1.6 g (44%) pale yellow solid compound.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.20 (d, $J = 8.7$ Hz, 2H), 7.38 (d, $J = 8.7$ Hz, 2H), 4.85 (d, $J = 12.9$ Hz, 1H), 4.06 (d, $J = 13.2$ Hz, 1H), 3.69 (s, 3H), 3.18 (t, $J = 12.6$ Hz, 1H), 2.90 (t, $J = 12$ & 12.3 Hz, 1H), 2.50–2.68 (m, 2H), 2.32–2.41 (m, 1H), 2.05 (d, $J = 12.3$ Hz, 2H), 1.84–2.00 (m, 4H), 1.51–1.69 (m, 6H); MS (ESI+) *m/z* 375.2 $[M + H]^+$.

5.2.4. Methyl 4-(4-(4-aminophenyl)piperidine-1-carbonyl) cyclohexanecarboxylate (**15**)

To a solution of compound **14** (1.6 g, 4.27 mmol, 1.0 equiv) in ethyl acetate (35 ml) was added 10% Pd-C (160 mg, 10% w/w) and the mixture was subjected to hydrogenation at 40 psi for 1 h. The

reaction mixture was filtered through celite and the filtrate was concentrated to give a yellow residue that was purified by flash column chromatography (3:7 Ethyl acetate/Chloroform) to obtain 450 mg (30%) off white solid compound.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 6.87 (d, J = 8.1 Hz, 2H), 6.49 (d, J = 8.1 Hz, 2H), 4.84 (s, 2H), 4.51 (d, J = 12.3 Hz, 1H), 4.03 (d, J = 10.5 Hz, 1H), 3.58 (s, 3H), 3.04 (t, J = 12.3 Hz, 1H), 2.50–2.55 (m, 3H), 2.29 (s, 1H), 1.89 (s, 2H), 1.71 (s, 4H), 1.28–1.42 (m, 6H); MS (ESI+) m/z 345.2 $(M + H)^+$; HPLC Retention time – 3.23 min, Purity – 97.45%.

5.2.5. 4-(4-(4-(2-Phenyl-5-(trifluoromethyl)oxazole-4-carboxamido)phenyl)piperidine-1-carbonyl)cyclohexanecarboxylic acid (**6**)

To a solution of 2-phenyl-5-(trifluoromethyl)oxazole-4-carboxylic acid **15** (74.7 mg, 0.290 mmol, 1.0 equiv) in DMF (2 ml), HATU (165 mg, 0.435 mmol, 1.5 equiv), methyl 4-(4-(4-aminophenyl)piperidine-1-carbonyl)cyclohexanecarboxylate (100 mg, 0.290 mmol, 1.0 equiv) and triethylamine (0.060 ml, 0.435 mmol, 1.5 equiv) were sequentially added and the reaction mixture was stirred at rt for 16 h. Following reaction completion, the 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 obtain an oily residue. The residue was subjected to column chromatography using 1:9 EtOAc:Chloroform to get a white solid. This solid was dissolved in THF (0.9 ml) and to it was added 1.0 N NaOH (1.16 ml, 1.16 mmol, 4.0 equiv) and the resulting mixture was stirred at rt for 16 h. Organic solvent was removed and 2 ml water was added. The reaction mixture was acidified to pH 2 using 1.0 M HCl, precipitated solid was filtered, washed with acetone, and dried to obtain 93 mg (56%) white solid compound.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.02 (s, 1H), 10.52 (s, 1H), 8.15 (d, J = 6.9 Hz, 2H), 7.72 (d, J = 8.1 Hz, 2H), 7.64–7.70 (m, 3H), 7.26 (d, J = 8.1 Hz, 2H), 4.57 (d, J = 15.3 Hz, 1H), 4.06 (d, J = 13.8 Hz, 1H), 3.10 (t, J = 11.3 & 12.7 Hz, 1H), 2.80 (t, J = 9.6 Hz, 1H), 2.60 (d, J = 12 Hz, 2H), 2.18 (s, 1H), 1.72–1.90 (m, 6H), 1.40 (bs, 6H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 177.04, 173.29, 161.26, 157.01, 142.42, 139.00, 137.55, 136.34, 133.00, 129.89 (2C), 127.62 (2C), 127.43 (2C), 125.25, 121.19 (2C), 120.05, 45.74, 42.51 (2C), 42.06, 41.88, 34.30 (2C), 28.82 (2C), 28.66 (2C); MS (ESI+) m/z 569.2 $(M + H)^+$; HRMS (ESI+) calcd for $C_{30}H_{31}F_3N_3O_5$ $[M + H]^+$ 570.2210, found 570.2185, (mean error 4.42 ppm); melting point 142–144 °C; HPLC Retention time – 4.93 min, Purity – 98.42%.

5.2.6. 4-Nitrobenzaldoxime (**17**)

To a solution of 4-nitronenzaldehyde (50 g, 331 mmol, 1.0 equiv) in methanol (500 ml) was added hydroxylamine hydrochloride (34.5 g, 497 mmol, 1.5 equiv) and refluxed for 4 h. Following reaction completion, the methanol was evaporated under reduced pressure. The obtained material was dissolved in ethyl acetate, washed with water, brine, dried over sodium sulfate, and the solvent was removed under reduced pressure. The solid thus obtained was then recrystallized from ethyl acetate and petroleum ether to afford the title compound (50 g, 91%) as an off white solid compound.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.67 (d, J = 9.0 Hz, 2H), 8.62 (s, 1H), 8.17 (d, J = 9.0 Hz, 2H); MS (ESI-) m/z 165.0 $[M - H]^-$.

5.2.7. N-hydroxy-4-nitrobenzimidoyl chloride (**18**)

To a solution of compound **17** (50.0 g, 301 mmol, 1.0 equiv) in DMF (250 ml) was added N-chlorosuccinimide (52.3 g, 391 mmol, 1.3 equiv) and stirred at rt for 5 h. Following reaction completion DMF was removed under reduced pressure and the obtained material was dissolved in ethyl acetate, washed with water, brine, dried over sodium sulfate, and the solvent was removed under reduced pressure to obtain a crude solid that was purified by column using 1:9 Ethyl acetate:Pet ether to obtain 51 g (84%) white solid compound.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.45 (bs, 1H), 8.26 (d, J = 7.2 Hz, 2H), 8.04 (d, J = 7.2 Hz, 2H); MS (ESI-) m/z 166.0 $[M - Cl]^-$.

5.2.8. Ethyl 3-(4-nitrophenyl)isoxazole-5-carboxylate (**19**)

To a solution of compound **18** (20.0 g, 10 mmol, 1.0 equiv) and ethyl propiolate (20.2 ml, 200 mmol, 2.0 equiv) in toluene (250 ml) was added Et_3N (14.6 ml, 105 mmol, 1.05 equiv) over 10 min. The resulting reaction mixture was heated at 80 °C for 2.5 h. Following completion the reaction mixture was cooled to rt and then further diluted with EtOAc (500 ml). The organic layer was washed with 0.1 M HCl, water, and brine. The organic layer was dried over anhydrous sodium sulfate, the solvent was evaporated, and the residue was crystallized from chloroform and pet ether to afford the title compound (23 g, 88%) as off white solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.35 (d, J = 9.0 Hz, 2H), 8.03 (d, J = 8.7 Hz, 2H), 7.32 (s, 1H), 4.48 (q, J = 7.2 Hz, 2H), 1.45 (t, J = 7.2 Hz, 3H); MS (ESI+) m/z 263.1 $[M + H]^+$; HPLC Retention time – 5.17 min, Purity – 99.53%.

5.2.9. 3-(4-Nitrophenyl)isoxazole-5-carboxylic acid (**20**)

To a solution of compound **19** (20 g, 76 mmol, 1.0 equiv) in THF (200 ml) was added 1.0 M aqueous solution of NaOH (92 ml, 92 mmol, 1.2 equiv) and stirred at rt for 1 h. The reaction mass was acidified with 1.0 M HCl in water, the precipitated slid was filtered, washed with water and dried to obtain 10.2 g (57%) white solid compound.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.38 (d, J = 7.8 Hz, 2H), 8.26 (d, J = 7.8 Hz, 2H), 7.97 (s, 1H); MS (ESI+) m/z 235.1 $[M + H]^+$; HPLC Retention time – 1.59 min, Purity – 99.99%.

5.2.10. Methyl 4-(3-(4-nitrophenyl)isoxazole-5-carboxamido) cyclohexanecarboxylate (**21**)

To a solution of compound **20** (4 g, 17.08 mmol, 1.0 equiv) in THF (20 ml) was added N-methyl morpholine (1.878 ml, 17.08 mmol, 1.0 equiv) and the reaction mixture was cooled to −20 °C. To this was added isobutyl chloroformate (2.243 ml, 17.08 mmol, 1.0 equiv) and the reaction mixture was stirred for 30 min. To this mixture were added methyl *trans*-4-aminocyclohexanecarboxylate hydrochloride (3.31 g, 17.08 mmol, 1.0 equiv) and triethylamine (7.14 ml, 51.2 mmol, 3.0 equiv), stirred reaction mixture at rt overnight. Following reaction completion solvent was removed under reduced pressure and 20 ml water was added, the product was separated from the aqueous mixture using EtOAc. The organic layer was washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure to get a solid residue. The residue was subjected to column chromatography using 3:7 EtOAc:Pet ether to get 2 g (32%) an off white solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.78 (d, J = 7.8 Hz, 1H), 8.32 (d, J = 8.1 Hz, 2H), 8.10 (d, J = 8.1 Hz, 2H), 7.88 (s, 1H), 3.70 (s, 1H), 3.54 (s, 3H), 2.29 (s, 1H), 1.95 (d, J = 15.9 Hz, 4H), 1.42–1.96 (m, 4H); Mass (ESI+) m/z 374.1 $[M + H]^+$.

5.2.11. Methyl 4-(3-(4-aminophenyl)isoxazole-5-carboxamido) cyclohexanecarboxylate (**22**)

To a solution of compound **21** (2 g, 5.36 mmol, 1.0 equiv) in ethanol (20 ml), THF (10.0 ml) and Water (10.0 ml) were added iron (0.89 g, 16.08 mmol, 3.0 equiv) and ammonium chloride (0.86 g, 16.08 mmol, 3.0 equiv) and reaction mixture was stirred at 75 °C for 3 h. The reaction mixture was filtered through celite and the filtrate was concentrated to give a yellow residue. To this $NaHCO_3$ solution was added and, the product was separated from the aqueous

mixture using EtOAc, the organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure to get solid residue. The residue was subjected to column chromatography using 1:9 EtOAc:Chloroform to obtain 1.4 g (76%) light brown solid compound.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.76 (d, J = 8.1 Hz, 1H), 7.56 (d, J = 8.4 Hz, 2H), 7.39 (s, 1H), 6.63 (d, J = 8.4 Hz, 2H), 5.62 (s, 2H), 3.71 (s, 1H), 3.60 (s, 3H), 2.27 (s, 1H), 1.91 (d, J = 16.8 Hz, 4H), 1.38–1.45 (m, 4H); MS (ESI+) m/z 344.1 $(M + H)^+$; HPLC Retention time – 3.16 min, Purity – 99.22%.

5.2.12. 4-(3-(4-(2-Phenyl-5-(trifluoromethyl)oxazole-4-carboxamido)phenyl)isoxazole-5-carboxamido) cyclohexanecarboxylic acid (**7**)

To a solution of 2-phenyl-5-(trifluoromethyl)oxazole-4-carboxylic acid (112 mg, 0.437 mmol, 1.0 equiv) in DMF (2 ml), HATU (183 mg, 0.481 mmol, 1.1 equiv), compound **22** (150 mg, 0.437 mmol, 1.0 equiv) and triethylamine (0.183 ml, 1.311 mmol, 3.0 equiv) were added sequentially and the reaction mixture was stirred overnight at rt. Following this reaction the solvent was removed under reduced pressure to obtain a dark brown residue that was taken up in water and extracted using ethyl acetate. The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate, and concentrated to obtain a dark brown residue that was purified using flash column chromatography (3:7 Ethyl acetate/Pet ether) to afford a white solid. This solid was dissolved in a mixture of THF (2 ml) and MeOH (2 ml), 1.0 N NaOH (1.75 ml, 1.75 mmol, 4.0 equiv) was added, and the mixture was stirred overnight at rt. Following reaction completion, the organic solvent was removed and 2 ml of water was added. The reaction mixture was acidified to pH 2.0 using 1.0 M HCl, the precipitated solid was filtered, washed with acetone, and dried to obtain 64 mg (56%) of white solid compound.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.10 (s, 1H), 10.81 (s, 1H), 8.87 (d, J = 8.1 Hz, 1H), 8.17 (d, J = 7.2 Hz, 2H), 8.02 (d, J = 8.7 Hz, 2H), 7.92 (d, J = 8.4 Hz, 2H), 7.62–7.70 (m, 3H), 7.56 (s, 1H), 3.72 (s, 1H), 2.16 (s, 1H), 1.91 (d, J = 19.8 Hz, 4H), 1.38–1.44 (m, 4H); ^{13}H NMR (DMSO-d_6, 300 MHz) δ 176.93, 162.06, 161.00, 158.37, 157.24, 155.58, 138.29, 137.10, 133.04, 130.60, 128.70, 129.20 (2C), 127.54 (2C), 127.75 (2C), 123.83, 119.73 (2C), 117.23, 110.53, 48.54, 43.07, 31.38 (2C), 28.43 (2C); MS (ESI+) m/z 569.2 $(M + H)^+$; HRMS (ESI+) calcd for $C_{28}H_{24}F_3N_4O_6$ $[M + H]^+$ 569.1642, found 569.1626, (mean error 3.03 ppm); melting point >250 °C; HPLC Retention time – 4.89 min, Purity – 93.82%.

5.2.13. 2-Bromo-1-(4-nitrophenyl)ethanone (**24**)

To a solution of 4-nitroacetophenone (50 g, 302 mmol, 1.0 equiv) in diethyl ether (500 ml) was added catalytic amount of aluminum chloride (2 g, 15 mmol, 0.05 equiv) and the reaction mixture was stirred for 10 min. To this bromine (15.4 ml, 302 mmol) was added using an addition funnel over 30 min at rt and the resulting mixture was stirred for 1 h. Following reaction completion the reaction mixture was quenched using aqueous sodium bicarbonate. The ether layer was separated, dried over sodium sulfate, and evaporated under reduced pressure. The solid thus obtained was recrystallized using ethyl acetate and petroleum ether to afford the title compound (43 g, 62%).

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

5.2.14. Ethyl 4-(4-nitrophenyl)thiazole-2-carboxylate (**25**)

To a solution of compound **24** (3 g, 12.2 mmol, 1.0 equiv) in methanol (60 ml) was added ethyl 2-amino-2-thioxoacetate (1.64 g, 12.2 mmol, 1.0 equiv) and reaction mixture was refluxed for 2 h. Following reaction completion, the reaction mixture was cooled to rt and the precipitated solid was filtered, washed with cold methanol, and dried to yield 2.9 g (85%) of the desired compound as a white solid.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.33 (d, J = 8 Hz, 2H), 8.16 (d, J = 8 Hz, 2H), 7.96 (s, 1H), 4.55 (q, J = 7 Hz, 2H), 1.50 (t, J = 7 Hz, 3H); Mass (ESI+) m/z 278.6 $[M + H]^+$.

5.2.15. 4-(4-Nitrophenyl)thiazole-2-carboxylic acid (**26**)

Compound **25** (2.78 g, 10 mmol, 1.0 equiv) was taken in THF (60 ml) and 1.0 M LiOH (40 ml, 40 mmol, 4.0 equiv) solution in water was added to it. The reaction mixture was stirred at rt for 4 h. After removing the solvent the obtained residue was diluted with water and acidified to pH 2 using 2.0 M HCl. The solid material thus obtained was filtered, washed with water and dried to obtain 2.4 g (96%) of the desired compound as a white solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.01 (s, 1H), 8.78 (s, 1H), 8.28–8.32 (m, 4H); Mass (ESI+) m/z 250.6 $[M + H]^+$.

5.2.16. Methyl 4-(4-(4-nitrophenyl)thiazole-2-carboxamido) cyclohexanecarboxylate (**27**)

To a solution of compound **26** (800 mg, 3.20 mmol, 1.0 equiv) in DMF (8 ml), HATU (1337 mg, 3.52 mmol, 1.1 equiv), methyl 4-aminocyclohexanecarboxylate hydrochloride (619 mg, 3.20 mmol, 1.0 equiv) and triethylamine (0.891 ml, 6.39 mmol, 2.0 equiv) were added sequentially and the reaction mixture was stirred at rt for 16 h. Following reaction completion, the reaction mixture was diluted with water and extracted using ethyl acetate. The organic layer was separated, washed with water, dried over anhydrous sodium sulfate, and filtered. The solvent was removed under reduced pressure to obtain a solid that was stirred in methanol at rt and filtered to obtain the title compound as an off-white solid (750 mg, 58%).

^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.78 (d, J = 8.4 Hz, 1H), 8.73 (s, 1H), 8.36 (bs, 4H), 3.80 (s, 1H), 3.61 (s, 3H), 2.29 (s, 1H), 1.88–2.00 (m, 4H), 1.42–1.60 (m, 4H); Mass (ESI+) m/z 390.1 $[M + H]^+$; HPLC Retention time – 5.13 min, Purity – 96.28%.

5.2.17. Methyl 4-(4-(4-aminophenyl)thiazole-2-carboxamido) cyclohexanecarboxylate (**28**)

Compound **27** (710 mg, 1.82 mmol, 1.0 equiv) was dissolved in a mixture of ethanol (14 ml), THF (7 ml) and water (7 ml). To this solution ammonium chloride (294 mg, 5.47 mmol, 3.0 equiv) and iron powder (306 mg, 5.47 mmol, 3.0 equiv) was added and reaction mixture was stirred at 75 °C for 3 h. Following completion the reaction mixture was cooled, filtered through celite, and the solvent was removed under reduced pressure to obtain a dark yellow residue. This residue was taken up in sodium bicarbonate solution and extracted using ethyl acetate. The organic layer was separated, dried over anhydrous sodium sulfate, and concentrated to give a dark brown residue that was purified using flash column chromatography (1:9 ethyl acetate/chloroform) to afford a yellow solid that was crystallized in DCM:Pet ether to yield the title compound (533 mg, 81%) as a pale yellow solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.52 (d, J = 8.4 Hz, 1H), 7.96 (s, 1H), 7.75 (d, J = 8.4 Hz, 2H), 6.62 (d, J = 8.4 Hz, 2H), 5.35 (s, 2H), 3.78 (s, 1H), 3.61 (s, 3H), 2.80 (s, 1H), 1.87–1.99 (m, 4H), 1.38–1.60 (m, 4H); Mass (ESI+) m/z 360.1 $[M + H]^+$; HPLC Retention time – 3.80 min, Purity – 99.50%.

5.2.18. 4-(4-(4-(2-Phenyl-5-(trifluoromethyl)oxazole-4-carboxamido)phenyl)thiazole-2-carboxamido) cyclohexanecarboxylic acid (**8**)

To a solution of 2-phenyl-5-(trifluoromethyl)oxazole-4-carboxylic acid (107 mg, 0.417 mmol, 1.0 equiv) in DMF (5 ml), HATU (175 mg, 0.459 mmol, 1.1 equiv), compound **28** (150 mg,

0.417 mmol, 1.0 equiv) and triethylamine (0.116 ml, 0.835 mmol, 2.0 equiv) were added sequentially and reaction mixture was stirred overnight at rt. The reaction was then quenched with water and extracted using ethyl acetate. The organic layer was separated, dried over sodium sulfate, and evaporated under reduced pressure to obtain a crude compound that was purified using flash column chromatography (3:7 Ethyl acetate/Petroleum ether) to afford a white solid. This solid was taken in THF (5 ml) and added 1.0 M aqueous solution of NaOH (1.67 ml, 1.67 mmol, 4.0 equiv) and reaction mixture was stirred at rt overnight. The solvent was removed and the obtained residue was diluted with water and acidified to pH 2 using 2.0 M HCl. The solid material thus obtained was filtered, washed with water and acetone to obtain 87 mg (36%) of the title compound as an off white solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.12 (s, 1H), 10.69 (s, 1H), 8.66 (d, J = 8.1 Hz, 1H), 8.36 (s, 1H), 8.17 (d, J = 6.6 Hz, 2H), 8.13 (d, J = 8.1 Hz, 2H), 7.97 (d, J = 7.8 Hz, 2H), 7.66–7.68 (m, 3H), 3.77 (s, 1H), 2.18 (s, 1H), 1.88–1.99 (m, 4H), 1.35–1.56 (m, 4H); ^{13}H NMR (DMSO-d_6, 300 MHz) δ 176.83 (2C), 164.18, 161.28, 158.67, 157.16, 155.28, 138.51, 137.40, 133.04, 130.22, 129.91 (2C), 127.64 (2C), 127.25 (2C), 125.23, 121.03 (2C), 120.83, 119.13, 48.44, 42.17, 31.48 (2C), 28.23 (2C); Mass (ESI+) m/z 585.1 $[M + H]^+$; HRMS (ESI+) calcd for $C_{28}H_{24}F_3N_4O_5S$ $[M + H]^+$ 585.1414, found 585.1377, (mean error 5.91 ppm); melting point: 270–272 °C; HPLC Retention time − 4.64 min, Purity − 98.51%.

*5.2.19. 2-amino-1-(4-nitrophenyl)ethanone hydrochloride (**29**)*

To a stirred solution of compound **24** (40 g, 164 mmol, 1.0 equiv) in DCM (600 ml) was added hexamine (27.6 g, 196 mmol, 1.2 equiv) and reaction mixture was stirred at rt for 8 h. Following completion the reaction mass was filtered, washed with 200 ml DCM, and dried on a rotavap to give an off-white solid that was dissolved in ethanol (600 ml) and conc. HCl (35 ml, 410 mmol, 2.5 equiv). The reaction mixture was stirred at rt for 16 h. The off white solid thus obtained was filtered, washed with water, and dried to give an off white solid that was crystallized in water to yield 23 g (65%) of the title compound as a light brown solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.53 (bs, 2H), 8.40 (d, J = 9 Hz, 2H), 8.27 (d, J = 9 Hz, 2H), 4.68 (s, 2H); Mass (ESI+) m/z 181.1 $[M + H]^+$.

*5.2.20. Ethyl 2-((2-(4-nitrophenyl)-2-oxoethyl)amino)-2-oxoacetate (**30**)*

To a solution of 2-amino-1-(4-nitrophenyl)ethanone hydrochloride **29** (20 g, 92 mmol, 1.0 equiv) in ethyl acetate (400 ml) was added triethylamine (15.5 ml, 111 mmol, 1.2 equiv) and reaction mixture was cooled to 5 °C. To this mixture ethyloxalylchloride (12.4 ml, 111 mmol, 1.2 equiv) was added dropwise and the reaction mixture was refluxed for 3 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 obtain 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, 34%).

^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.19 (t, J = 5.4 Hz, 1H), 8.35 (d, J = 8.7 Hz, 2H), 8.20 (d, J = 8.7 Hz, 2H), 4.75 (d, J = 5.4 Hz, 2H), 4.26 (q, J = 7.2 Hz, 2H), 1.27 (t, J = 7.2 Hz, 3H); Mass (ESI+) m/z 303.1 $[M + Na]^+$; HPLC Retention time − 2.83 min, Purity − 91.74%.

*5.2.21. Ethyl 5-(4-nitrophenyl)thiazole-2-carboxylate (**31**)*

To a solution of ethyl 2-((2-(4-nitrophenyl)-2-oxoethyl)amino)-2-oxoacetate **28** (5 g, 17.84 mmol, 1.0 equiv) in 1,4-Dioxane (100 ml) was added Lawesson's Reagent (7.9 g, 19.64 mmol, 1.1 equiv) and the reaction 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 subjected to column chromatography using 2:8 EtOAc:Pet ether to yield a dark brown colored solid that was stirred in methanol and filtered to obtain the title compound as a light brown solid (3.4 g, 69%).

^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.34 (d, J = 8.4 Hz, 2H), 8.30 (s, 1H), 7.82 (d, J = 8.4 Hz, 2H), 4.54 (q, J = 7.2 Hz, 2H), 1.49 (t, J = 7.2 Hz, 3H); Mass (ESI+) m/z 279.0 $[M + H]^+$; HPLC Retention time − 7.02 min, Purity − 96.90%.

*5.2.22. 5-(4-Nitrophenyl)thiazole-2-carboxylic acid (**32**)*

To a solution of compound **31** (3 g, 10.78 mmol) in THF (250 ml) was added 1.0 M aqueous NaOH (21.5 ml, 21.56 mmol, 2.0 equiv) and the reaction mixture was stirred for 30 min. 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 brown colored solid. Recrystallization with EtOAc/Pet ether yielded the desired product as a light brown solid (2.2 g, 82%).

^{1}H NMR (DMSO-d_6, 300 MHz) δ 14.14 (bs, 1H), 8.68 (s, 1H), 8.32 (d, J = 8.7 Hz, 2H)), 8.08 (d, J = 8.7 Hz, 2H); Mass (ESI+) m/z 251.0 $[M + H]^+$.

*5.2.23. Methyl 4-(5-(4-nitrophenyl)thiazole-2-carboxamido) cyclohexanecarboxylate (**33**)*

To a solution of 5-(4-nitrophenyl)thiazole-2-carboxylic acid **32** (1.6 g, 6.39 mmol, 1.0 equiv) in THF (10 ml) was added N-methyl morpholine (0.703 ml, 6.39 mmol, 1.0 equiv) at rt and the reaction mixture was cooled to −20 °C. To this isobutyl chloroformate (0.840 ml, 6.39 mmol, 1.0 equiv) was added and the reaction mixture was stirred for 20 min at −20 °C. To this reaction mass was added a solution of methyl *trans* 4-aminocyclohexanecarboxylate hydrochloride (1.238 g, 6.39 mmol, 1.0 equiv) in THF (20 ml) after neutralizing with triethylamine (1.33 ml, 9.58 mmol, 1.5 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. Following reaction completion, the organic solvent was removed under reduced pressure and the crude material was subjected to column chromatography using 1:9 EtOAc:$CHCl_3$ to get the desired compound as an off white solid (1.65 g, 66%).

^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.84 (d, J = 8.7 Hz, 1H), 8.65 (s, 1H), 8.31 (d, J = 8.7 Hz, 2H), 8.08 (d, J = 8.7 Hz, 2H), 3.76 (m, 1H), 3.60 (s, 3H), 2.55 (s, 1H), 1.95 (d, J = 10.5 Hz, 2H), 1.86 (d, J = 10.5 Hz, 2H), 1.40–1.53 (m, 4H); Mass (ESI+) m/z 390.1 $[M + H]^+$.

*5.2.24. Methyl 4-(5-(4-aminophenyl)thiazole-2-carboxamido) cyclohexanecarboxylate (**34**)*

To a solution of compound **33** (1.6 g, 4.11 mmol, 1.0 equiv) in EtOH (25 ml), THF (12.5 ml) and water (12.5 ml) were added iron (0.918 g, 16.43 mmol, 4.0 equiv) and ammonium chloride (0.879 g, 16.43 mmol, 4.0 equiv). The resulting reaction mixture was stirred at 75 °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 aqueous sodium bicarbonate solution and extracted using ethyl acetate. The organic layer was dried using sodium sulfate and concentrated to obtain a brown residue that was purified by column chromatography using 2:8 EtOAc:$CHCl_3$ to afford the title compound as a yellow solid (1.06 g, 72%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.83 (s, 1H), 7.41 (d, J = 8.4 Hz, 2H), 7.06 (d, J = 8.4 Hz, 1H), 6.72 (d, J = 8.4 Hz, 2H), 3.94–3.96 (m, 1H),

3.91 (s, 2H), 3.70 (s, 3H), 2.27–2.35 (m, 1H), 2.18 (d, J = 9.6 Hz, 2H), 2.10 (d, J = 15.6 Hz, 2H), 1.57–1.68 (m, 2H), 1.27–1.40 (m, 2H); Mass (ESI+) m/z 360.1 $[M + H]^+$; HPLC Retention time – 3.75 min, Purity – 99.83%.

5.2.25. 4-(5-(4-(2-Phenyl-5-(trifluoromethyl)oxazole-4-carboxamido)phenyl)thiazole-2-carboxamido) cyclohexanecarboxylic acid (**9**)

To a solution of 2-phenyl-5-(trifluoromethyl) oxazole-4-carboxylic acid (64.4 mg, 0.250 mmol, 1.0 equiv) in DMF (3 ml), HATU (105 mg, 0.275 mmol, 1.1 equiv), methyl 4-(5-(4-aminophenyl)thiazole-2-carboxamido)cyclohexanecarboxylate **34** (90 mg, 0.250 mmol, 1.0 equiv) and triethylamine (0.070 ml, 0.501 mmol, 2.0 equiv) were added sequentially and the reaction mixture was stirred at rt for 16 h. Following completion the reaction mixture was quenched with water and extracted using ethyl acetate. The organic layer was separated, dried over sodium sulfate, and evaporated under reduced pressure to obtain a crude compound that was purified using flash column chromatography (3:7 Ethyl acetate/Pet ether) to afford a white solid. This solid was taken in THF (5 ml), 1.0 M aqueous solution of NaOH (1.0 ml, 1.0 mmol, 4.0 equiv) was added, and the resulting mixture was stirred overnight 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 solid material thus obtained was filtered, washed with water and acetone to obtain the title compound (52 mg, 35%) as an off white solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.09 (bs, 1H), 10.77 (s, 1H), 8.68 (d, J = 8.7 Hz, 1H), 8.38 (s, 1H), 8.17 (d, J = 6.3 Hz, 2H), 7.96 (d, J = 8.7 Hz, 2H), 7.82 (d, J = 8.4 Hz, 2H), 7.62–7.70 (m, 3H), 3.75 (s, 1H), 2.13 (bs, 1H), 1.95 (d, J = 12 Hz, 2H), 1.85 (d, J = 12.6 Hz, 2H), 1.38–1.55 (m, 4H); ^{13}H NMR (DMSO-d_6, 300 MHz) δ 175.59, 162.63, 161.29, 158.69, 157.30, 143.79, 139.82 (2C), 139.09, 137.26, 133.06, 129.92 (2C), 127.76 (2C), 127.65 (2C), 126.79, 125.22, 121.47 (2C), 117.22, 51.85, 48.28, 41.92, 31.26, 29.47, 28.10; Mass (ESI+) m/z 585.2 $[M + H]^+$; HRMS (ESI+) calcd for $C_{28}H_{24}F_3N_4O_5S$ $[M + H]^+$ 585.1414, found 585.1380, (mean error 4.78 ppm); melting point >280 °C; HPLC Retention time – 5.21 min, Purity – 99.18%.

5.2.26. 4-(5-(4-(3,5-Difluorobenzamido)phenyl)thiazole-2-carboxamido)cyclohexanecarboxylic acid (**9a**)

To a solution of compound **34** (90 mg, 0.250 mmol, 1.0 equiv) in DCM (2 ml) were added pyridine (0.06 ml, 0.750 mmol, 3.0 equiv) and 3,5-difluorobenzoyl chloride (0.045 ml, 376 mmol, 1.5 equiv) and the resulting mass was stirred at 55 °C for 16 h. Following completion the reaction mass was diluted with DCM, washed with water followed by brine, and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the crude solid so obtained was purified by flash column chromatography (3:7 EtOAc/Pet ether) to provide the methyl ester as a white colored solid. This solid was taken in THF (5 ml) and added to 1.0 M aqueous NaOH solution (1.0 ml, 1.0 mmol, 4.0 equiv) and the reaction mixture was stirred at rt overnight. The solvent was removed, the residue was diluted with water, and acidified to pH 2 using 2.0 M HCl. The resulting solid was filtered, washed with water and acetone to obtain (66.6 mg, 55%) of the title compound as an off white solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.09 (s, 1H), 10.54 (s, 1H), 8.67 (d, J = 8.4 Hz, 1H), 8.36 (s, 1H), 7.89 (d, J = 8.7 Hz, 2H), 7.80 (d, J = 8.7 Hz, 2H), 7.69–7.72 (m, 2H), 7.52–7.58 (m, 1H), 3.75 (s, 1H), 2.13 (s, 1H), 1.95 (d, J = 12.3 Hz, 2H), 1.84 (d, 2H), 1.37–1.52 (m, 4H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 176.87, 164.38, 164.23, 163.44, 162.53, 161.10, 160.93, 158.67, 143.88, 139.92, 139.62, 127.72 (2C), 126.35, 121.25 (2C), 111.83, 111.61, 48.40, 42.10, 31.39 (2C), 28.18 (2C).

Mass (ESI+) m/z 486.1 $[M + H]^+$; HRMS (ESI+) calcd for $C_{24}H_{22}F_2N_3O_4S$ $[M + H]^+$ 486.1294, found 486.1277, (mean error 3.25 ppm); melting point >250 °C; HPLC Retention time – 3.90 min, Purity – 98.24%.

5.2.27. 4-(5-(4-(3,5-difluorophenylsulfonamido)phenyl)thiazole-2-carboxamido)cyclohexanecarboxylic acid (**9b**)

To a solution of compound **34** (90 mg, 0.250 mmol, 1.0 equiv) in DCM (2 ml) were added pyridine (0.06 ml, 0.750 mmol, 3.0 equiv) and 3,5-difluorobenzenesulfonyl chloride (80 mg, 0.376 mmol, 1.5 equiv) and the resulting mixture was stirred at 55 °C for 16 h. Following completion the reaction mass was diluted with DCM, washed with water, followed by brine and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the crude solid so obtained was purified by flash column chromatography (3:7 EtOAc/Petroleum ether) to provide the methyl ester as a white solid. This solid was taken in THF (5 ml) and to it 1.0 M aqueous solution of LiOH (1 ml, 1.0 mmol, 4.0 equiv) was added and the reaction mixture was stirred overnight at rt. The solvent was removed and the resulting residue was diluted with water and acidified to pH 2 using 2.0 M HCl. The solid material thus obtained was filtered, washed with water and acetone to obtain 70 mg (54%) as off white solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.08 (s, 1H), 10.78 (s, 1H), 8.66 (d, J = 8.4 Hz, 1H), 8.29 (s, 1H), 7.69 (d, J = 8.4 Hz, 2H), 7.62–7.65 (m, 1H), 7.49–7.51 (m, 2H), 7.21 (d, J = 8.4 Hz, 2H), 3.73 (s, 1H), 2.12 (s, 1H), 1.94 (d, J = 11.4 Hz, 2H), 1.80 (d, J = 17.1 Hz, 2H), 1.36–1.50 (m, 4H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 176.82, 164.20, 162.82, 160.90, 158.60, 143.32, 142.93, 139.88, 138.12, 128.38 (2C), 126.97, 121.12 (2C), 111.18, 110.81, 109.61, 48.38, 42.08, 31.36 (2C), 28.16 (2C); Mass (ESI+) m/z 522.1 $[M + H]^+$; HRMS (ESI+) calcd for $C_{23}H_{22}F_2N_3O_5S_2$ $[M + H]^+$ 522.0963, found, 522.0932 (mean error 6.00 ppm); melting point >250 °C; HPLC Retention time – 3.58 min, Purity – 98.98%.

5.2.28. General procedure for the synthesis of urea derivatives of phenylthiazole series

To a solution of compound **34** (90 mg, 0.250 mmol, 1.0 equiv) in THF (5 ml) was added appropriate phenyl isocyanate (1.1 equiv) and reaction mixture was stirred at 55 °C for 16 h. Following reaction completion the precipitated solid was filtered and washed with diethyl ether to obtain a white solid as ester compound. To a solution of ester compound (1.0 equiv) in THF (3 ml) and methanol (3 ml) was added 1 N aqueous NaOH (4 equiv) solution and stirred at rt for 16 h. 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 filtered and washed with acetone to obtain the desired product.

*5.2.28.1. 4-(5-(4-(3-(3,5-Difluorophenyl)ureido)phenyl)thiazole-2-carboxamido)cyclohexanecarboxylic acid (**9c**).* Prepared as described above in the general procedure using 3,5-difluorophenyl isocyanate (43 mg, 0.275 mmol, 1.1 equiv) as the substituted isocyanate to afford the title compounds (81.5 mg, 65%) as off white solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.09 (s, 1H), 9.16 (s, 1H), 9.11 (s, 1H), 8.65 (d, J = 8.7 Hz, 1H), 8.30 (s, 1H), 7.71 (d, J = 8.4 Hz, 2H), 7.57 (d, J = 8.4 Hz, 2H), 7.23 (s, 1H), 7.19 (s, 1H), 6.78–6.84 (m, 1H), 3.74 (s, 1H), 2.13 (s, 1H), 1.95 (m, J = 12.6 Hz, 2H), 1.83 (d, 2H), 1.37–1.51 (m, 4H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 176.86, 164.76, 162.07, 166.55, 158.71, 152.50, 144.17, 142.71, 140.64, 139.14, 127.93 (2C), 124.55, 119.28 (2C), 101.73, 101.34, 97.40, 48.36, 42.10, 31.39 (2C), 28.18 (2C); Mass (ESI+) m/z 501.1 $[M + H]^+$; HRMS (ESI+) calcd for $C_{24}H_{23}F_2N_4O_4S$ $[M + H]^+$ 501.1403, found 501.1380, (mean error

4.62 ppm); melting point >250 °C; HPLC Retention time – 3.98 min, Purity – 99.23%.

*5.2.28.2. 4-(5-(4-(3-(2-Methoxyphenyl)ureido)phenyl)thiazole-2-carboxamido)cyclohexanecarboxylic acid (**9d**).* Prepared as described above in the general procedure using 2-methoxyphenyl isocyanate (41 mg, 0.275 mmol, 1.1 equiv) as the substituted isocyanate to afford the title compounds (74.8 mg, 60%) as off white solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.09 (s, 1H), 9.56 (s, 1H), 8.64 (d, J = 8.4 Hz, 1H), 8.29 (s, 2H), 8.13 (d, J = 7.5 Hz, 1H), 7.69 (d, J = 8.7 Hz, 2H), 7.56 (d, J = 8.7 Hz, 2H), 7.03 (d, J = 8.1 Hz, 1H), 6.88–6.99 (m, 2H), 3.89 (s, 3H), 3.74 (s, 1H), 2.13 (s, 1H), 1.94 (d, J = 11.1 Hz, 2H), 1.85 (d, J = 10.8 Hz, 2H), 1.37–1.51 (m, 4H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 176.84, 161.90, 158.74, 152.62, 148.16, 144.32, 141.29, 138.96, 128.86, 127.99 (2C), 124.00, 122.49, 121.01, 118.82, 118.65, (2C), 111.21, 56.24, 48.37, 42.10, 31.40 (2C), 28.18 (2C); Mass (ESI+) m/z 495.1 [M + H]$^+$; HRMS (ESI+) calcd for $C_{25}H_{27}N_4O_5S$ [M + H]$^+$ 495.1697, found 495.1649, (mean error 9.53 ppm); melting point >250 °C; HPLC Retention time – 3.71 min, Purity – 98.64%.

*5.2.28.3. 4-(5-(4-(3-(2-Fluorophenyl)ureido)phenyl)thiazole-2-carboxamido)cyclohexanecarboxylic acid (**9e**).* Prepared as described above in the general procedure using 2-fluorophenyl isocyanate (38 mg, 0.275 mmol, 1.1 equiv) as the substituted isocyanate to afford the title compounds (75.7 mg, 63%) as off white solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.09 (s, 1H), 9.31 (s, 1H), 8.64 (d, J = 9.0 Hz, 2H), 8.30 (s, 1H), 8.15 (t, J = 7.8 & 8.4 Hz, 1H), 8.71 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.7 Hz, 2H), 7.22–7.28 (m, 1H), 7.16 (t, J = 7.5 & 7.8 Hz 1H), 7.02–7.05 (m, 1H), 3.75 (s, 1H), 2.13 (s, 1H), 1.94 (m, J = 11.4 Hz, 2H), 1.83 (d, 2H), 1.37–1.51 (m, 4H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 176.85, 162.01, 158.72, 152.45, 144.21, 140.86, 139.08, 128.01 (2C), 127.83, 127.69, 124.98, 124.33, 123.23, 121.15, 118.86 (2C), 115.60, 48.36, 42.10, 31.39 (2C), 28.18 (2C); Mass (ESI+) m/z 483.1 [M + H]$^+$; HRMS (ESI+) calcd for $C_{24}H_{24}F_1N_4O_4S$ [M + H]$^+$ 483.1497, found 483.1469, (mean error 6.72 ppm); melting point >250 °C; HPLC Retention time – 3.63 min, Purity – 98.33%.

*5.2.28.4. 4-(5-(4-(3-(2-Chlorophenyl)ureido)phenyl)thiazole-2-carboxamido)cyclohexanecarboxylic acid (**9f**).* Prepared as described above in the general procedure using 2-chlorophenyl isocyanate (42 mg, 0.275 mmol, 1.1 equiv) as the substituted isocyanate to afford the title compounds (78.5 mg, 63%) as off white solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.09 (s, 1H), 9.65 (s, 1H), 8.65 (d, J = 8.4 Hz, 1H), 8.37 (s, 1H), 8.30 (s, 1H), 8.17 (d, J = 8.1 Hz, 1H), 7.72 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 8.7 Hz, 2H), 7.48 (d, J = 7.8 Hz, 1H), 7.32 (t, J = 7.5 Hz & 7.8 Hz, 1H), 7.05 (t, J = 7.8 Hz, 1H), 3.75 (s, 1H), 2.13 (s, 1H), 1.94 (d, J = 10.8 Hz, 2H), 1.85 (d, J = 11.1 Hz, 2H), 1.33–1.55 (m, 4H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 176.84, 162.02, 158.72, 152.41, 144.21, 140.87, 139.10, 136.20, 129.70, 128.02 (3C), 124.39, 123.99, 122.55, 121.88, 118.95 (2C), 48.36, 42.10, 31.39 (2C), 28.18 (2C); Mass (ESI+) m/z 499.1 [M + H]$^+$; HRMS (ESI+) calcd for $C_{24}H_{24}Cl_1N_4O_4S$ [M + H]$^+$ 499.1201, found 499.1199, (mean error 1.10 ppm); melting Point >250 °C; HPLC Retention time – 3.98 min, Purity – 99.89%.

*5.2.28.5. 4-(5-(4-(3-(2,4-Difluorophenyl)ureido)phenyl)thiazole-2-carboxamido)cyclohexanecarboxylic acid (**9g**).* Prepared as described above in the general procedure using 2,4-difluorophenyl isocyanate (43 mg, 0.275 mmol, 1.1 equiv) as the substituted isocyanate to afford the title compounds (61.8 mg, 49%) as off white solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.10 (s, 1H), 9.27 (s, 1H), 8.64 (d, J = 8.4 Hz, 1H), 8.58 (s, 1H), 8.29 (s, 1H), 8.03–8.11 (m, 1H), 7.71 (d, J = 8.1 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.29–7.35 (m, 1H), 7.00–7.06 (m, 1H), 3.74 (s, 1H), 2.13 (s, 1H), 1.95 (d, J = 11.1 Hz, 2H), 1.83 (d, 2H), 1.37–1.51 (m, 4H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 176.88, 162.00, 158.72, 152.59, 144.21, 140.86, 139.07, 127.99 (2C), 124.33, 122.70, 122.61, 118.88 (2C), 111.68, 111.37, 104.61, 103.98, 48.37, 42.12, 31.40 (2C), 28.18 (2C); Mass (ESI+) m/z 501.1 [M + H]$^+$; HRMS (ESI+) calcd for $C_{24}H_{23}F_2N_4O_4S$ [M + H]$^+$ 501.1403, found 501.1380, (mean error 4.54 ppm); melting point ≥ 250 °C; HPLC Retention time – 3.71 min, Purity – 99.16%.

*5.2.28.6. 4-(5-(4-(3-(2,4,5-Trifluorophenyl)ureido)phenyl)thiazole-2-carboxamido)cyclohexanecarboxylic acid (**9h**).* Prepared as described above in the general procedure using 2,4,5-trifluorophenyl isocyanate (48 mg, 0.275 mmol, 1.1 equiv) as the substituted isocyanate to afford the title compounds (90.7 mg, 70%) as off white solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.09 (s, 1H), 9.32 (s, 1H), 8.78 (s, 1H), 8.65 (d, J = 8.4 Hz, 1H), 8.30 (s, 1H), 8.14–8.24 (m, 1H), 7.72 (d, J = 8.4 Hz, 2H), 7.60–7.66 (m, 1H), 7.56 (d, J = 8.4 Hz, 2H), 3.74 (s, 1H), 2.13 (s, 1H), 1.95 (d, J = 12.3 Hz, 2H), 1.83 (d, 2H), 1.37–1.55 (m, 4H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 176.85, 162.09, 158.70, 152.29, 144.12, 140.48, 139.15, 128.01 (2C), 124.61, 124.60, 118.99 (2C), 109.09, 108.81, 106.34, 105.98, 105.71, 48.36, 42.10, 31.39 (2C), 28.18 (2C); Mass (ESI+) m/z 519.1 [M + H]$^+$; HRMS (ESI+) calcd for $C_{24}H_{22}F_3N_4O_4S$ [M + H]$^+$ 519.1308, found 519.1283, (mean error 4.80 ppm); melting point ≥ 250 °C; HPLC Retention time – 4.08 min, Purity – 98.29%.

*5.2.28.7. 4-(5-(4-(3-Phenylureido)phenyl)thiazole-2-carboxamido) cyclohexanecarboxylic acid (**9i**).* Prepared as described above in the general procedure using phenyl isocyanate (33 mg, 0.275 mmol, 1.1 equiv) as the substituted isocyanate to afford the title compounds (74 mg, 64%) as off white solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.10 (s, 1H), 8.93 (s, 1H), 8.74 (s, 1H), 8.65 (d, J = 8.7 Hz, 1H), 8.29 (s, 1H), 7.70 (d, J = 8.4 Hz, 2H), 7.57 (d, J = 8.7 Hz, 2H), 7.46 (d, J = 8.1 Hz, 2H), 7.29 (t, J = 7.5 Hz & 8.1 Hz, 2H), 6.99 (t, J = 6.9 Hz & 7.2 Hz, 1H), 3.75 (s, 1H), 2.13 (s, 1H), 1.94 (d, J = 11.4 Hz, 2H), 1.83 (d, 2H), 1.37–1.51 (m, 4H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 176.84, 161.92, 158.73, 152.78, 144.31, 141.19, 139.91, 138.99, 126.26 (2C), 127.93 (2C), 124.06, 122.49, 118.91 (2C), 118.79 (2C), 48.36, 42.10, 31.40 (2C), 28.18 (2C); Mass (ESI+) m/z 465.1 [M + H]$^+$; HRMS (ESI+) calcd for $C_{24}H_{25}N_4O_4S$ [M + H]$^+$ 465.1591, found 465.1570, (mean error 2.87 ppm); melting point >280 °C; HPLC Retention time – 3.38 min, Purity – 99.98%.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.03.077.

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