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Exploration of pyridine containing heteroaryl analogs of biaryl ureas as DGAT1 inhibitors

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ABSTRACT

The diacylglycerol acyltransferase enzyme, DGAT1, presents itself as a potential target for obesity as this enzyme is dedicated to the final committed step in triglyceride biosynthesis. Biphenyl ureas, exemplified by compound **4**, have been reported to be potent hDGAT1 inhibitors. We have synthesized and evaluated 2-pyridyl and 3-pyridyl containing biaryl ureas as hDGAT1 inhibitors. Our aim was to incorporate a heteroaryl scaffold within these molecules thereby improving the *c* Log *P* profile and making these compounds more drug-like. Compounds within this series exhibited potent hDGAT1 inhibition when evaluated using an in vitro enzymatic assay. Selected compounds were also subjected to an oral fat tolerance test in mice where the percent triglyceride reduction versus a vehicle control was evaluated. Of the studied heteroaryl analogs compound **44** exhibited an in vitro IC_{50} of 17 nM and a plasma triglyceride reduction of 79% along with a 12-fold improvement in solubility over the biphenyl urea compound **4**.

The World Health Organization (WHO) defines obesity as a condition characterized by abnormal or excessive fat accumulation that presents health risks to an individual. Obesity is associated with an array of additional health problems, including increased risk of insulin resistance, type 2 diabetes, fatty liver disease, atherosclerosis, hypertension, and cardiovascular disease.[1] The WHO estimates that more than a billion adults worldwide are overweight, of whom 300 million are clinically obese.[2] Clinical obesity is one where an individual possesses a body mass index (BMI) equal to or greater than 30 kg m^{-2}.[3] It results from an energy imbalance arising when the energy input exceeds energy output. In humans, the excess calories consumed are converted into triglycerides that are principal components of fat and in turn act as a major repository for stored metabolic energy. Obese individuals have significantly greater triglyceride levels. Because this ability to make triglycerides is essential for the accumulation of adipose tissue, inhibition of triglyceride synthesis may act as a strategy to either prevent or reverse obesity and its related medical consequences.

One of the strategies for development of anti-obesity drugs includes a reduction in energy intake through either central or peripheral mechanisms. Orlistat, the only marketed anti-obesity drug prevents absorption of fat from human diet thereby reducing the intake of calories.[4] However, this drug suffers from severe adverse effects such as steatorrhea, fecal incontinence, and frequent or urgent bowel movements.[5] Hence there is a pressing need for developing novel anti-obesity drugs possessing fewer side-effects and targeting newer mechanisms of action. The Acyl CoA:diacylglycerol acyltransferase 1 (DGAT1) is one of two known DGAT enzymes (DGAT1 and DGAT2) that catalyze the final committed step of triglyceride biosynthesis.[6] Of late, several potent inhibitors of the hDGAT1 enzyme have been reported and some of these compounds have also been studied clinically (Fig. 1).[7–9] This particular interest in hDGAT1 inhibition arises from a published phenotype of DGAT1 deficient mice.[10] These animals are viable, resistant to weight gain when fed a high-fat diet, and exhibit increased insulin and leptin sensitivity. Contrary to DGAT1, DGAT2 deficient mice die soon after birth apparently from profound reductions in substrates required for energy metabolism coupled with an impaired permeability barrier function in the skin. Hence inhibition of DGAT1 is an attractive target for developing anti-obesity agents.

A potent series of biphenyl keto acid derivatives, exemplified by structure **3**, for developing anti-obesity agents have been reported by Bayer Pharmaceuticals.[9] The reported IC_{50} value of compound **3**

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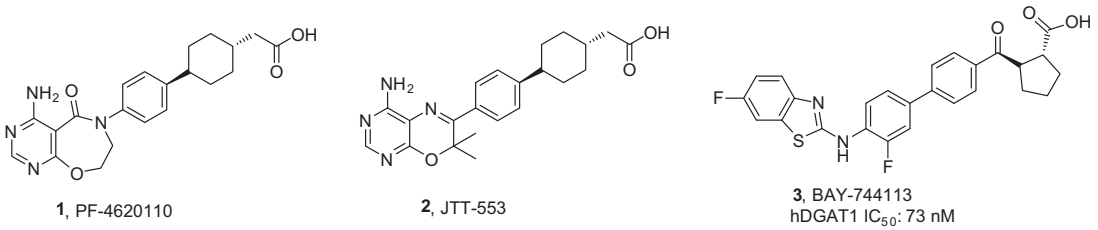


Figure 1. Few hDGAT1 inhibitors studied clinically.

is 73 nM when studied using an hDGAT1 enzymatic assay.[11] Abbott Laboratories discovered compound **4** by replacing the benzothiazole moiety of compound **3** with a phenyl urea resulting in improved potency (IC_{50} = 7 nM).[11] A series of similar compounds have also been patented by Abbott Laboratories.[12,13] Compound **4** was synthesized in-house using a reported procedure[11] and in our hands it exhibited an IC_{50} value of 20 nM.

We have investigated the effects of replacing phenyl ring 'A' within the biphenyl scaffold of compound **4** with a pyridine ring (Fig. 2). This modification would result in reduced Log *P* of these molecules. The pyridine ring can exist in either the 3-pyridyl orientation, exemplified by structure **5**, or the 2-pyridyl orientation, exemplified by structure **6**, as shown in Figure 2 and hence both these orientations have been studied. Inspired by an earlier patent filed by Bayer Pharmaceuticals[14] we have also explored several amino acid head groups for this heteroaryl scaffold. Uncharged polar amino acid head groups such as serine and nonpolar amino acid head groups such as glycine, alanine, valine, and leucine have been studied. The selections of these amino acids were based on their hydrophobicities, contributions towards Log *P*, and molecular weights of the resulting analogs. Our goal was to develop a heteroaryl scaffold that allows for developing drug-like candidates while retaining hDGAT1 inhibition.

To synthesize phenyl urea analogs of the 3-pyridyl heteroaryl scaffold possessing different amino acid head groups (Scheme 1), 4-bromo benzoic acid (**7**) was subjected to esterification using sulfuric acid and methanol to yield compound **8**. This was further converted to its corresponding boronic acid pinacol ester, compound **9**, using a palladium catalyzed reaction incorporating bispinacol diboron. Compound **9** was then subjected to Suzuki coupling with 2-amino-5-bromopyridine yielding compound **10**. Compound **10** was subsequently coupled with a substituted isocyanate to yield the corresponding substituted urea derivative **11,** followed by an ester hydrolysis yielding **12**. Compound **12** was coupled with a variety of amino acid esters to yield compounds **13–21**. Finally compounds **13–21** were hydrolyzed to their corresponding acids **22–30** using lithium hydroxide. A total of six different amino acids were used for synthesis. These included glycine (**22**), L-alanine (**23**), L-serine (**24**), L-valine (**25**), D-valine (**26**), and L-leucine (**27**). Since the biological results indicated L-valine, incorporated as the acid head group, to be optimum for hDGAT1 inhibition this modification was retained while synthesizing analogs displaying the 2-pyridyl and 3-pyridyl orientations required in the study. The synthesis of 3-pyridyl heteroaryl analogs retaining L-valine as the acid head group were conducted similar to that of compound **25** using either appropriately substituted phenyl isocyanates to obtain compounds **28** and **29** or a benzyl isocyanate to yield compound **30**.

To synthesize the 2-pyridyl analogs (Scheme 2), compound **9** was subjected to Suzuki coupling with 2-bromo-5-nitropyridine yielding compound **31**. This was subsequently hydrolyzed using lithium hydroxide to yield compound **32**. Compound **32** was coupled with the methyl ester of L-valine to yield compound **33**. Metallic reduction of the nitro group in compound **33** using Fe/NH_4Cl gave its corresponding amine **34**. Compound **34** was then coupled with various substituted phenyl isocyanates to yield compounds **35–41** that on subsequent hydrolysis with lithium hydroxide yielded the corresponding acids **42–48**. These synthesized compounds were studied for their hDGAT1 inhibitory activity using an in vitro enzymatic assay that measures the output of triolein from diolein and radiolabeled oleoyl-CoA.[15] Results obtained from these studies are discussed ahead.

The in vitro activities for different acid head groups (Table 1) varied from 28% for L-serine (compound **24**) to 88% for L-valine (compound **25**) at a 10 μM concentration. All of the acid head groups with the exception of L-valine exhibited hDGAT1 inhibition less than 75% and were hence not subjected to IC_{50} evaluations. The L-valine head group, yielding the *S* enantiomer (compound **25**), displayed an IC_{50} of 120 nM for hDGAT1 enzyme. To confirm the optimal stereochemistry of this valine head group its corresponding *R* enantiomer (compound **26**) was also synthesized using D-valine and studied for its hDGAT1 inhibition. The *R* enantiomer, exhibiting an hDGAT1 inhibition of 51% was however found to be significantly less potent than the corresponding *S* enantiomer thereby indicating a preference for L-valine over D-valine. As a result of this preference we have retained L-valine as the acid head in our further studies.

Analogs incorporating the 2-pyridyl and 3-pyridyl heteroaryl scaffolds have also been synthesized and evaluated in an effort to understand the effect of pyridine on hDGAT1 inhibition. This modification leads to a decrease in *c* Log *P* of the molecules. Four analogs possessing the 3-pyridyl heteroaryl scaffold and seven

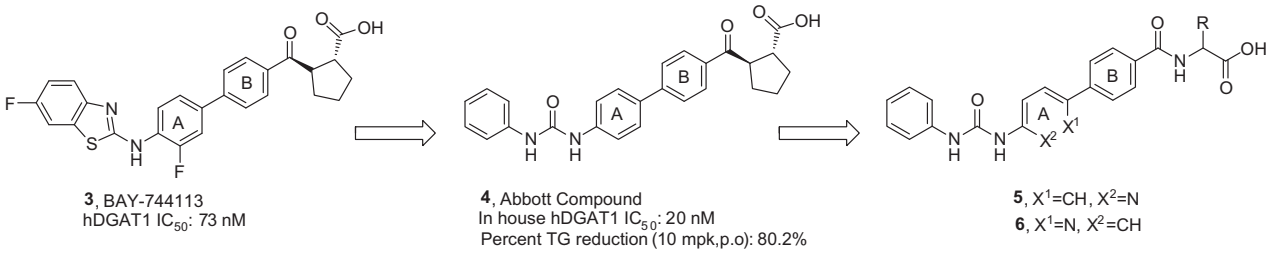
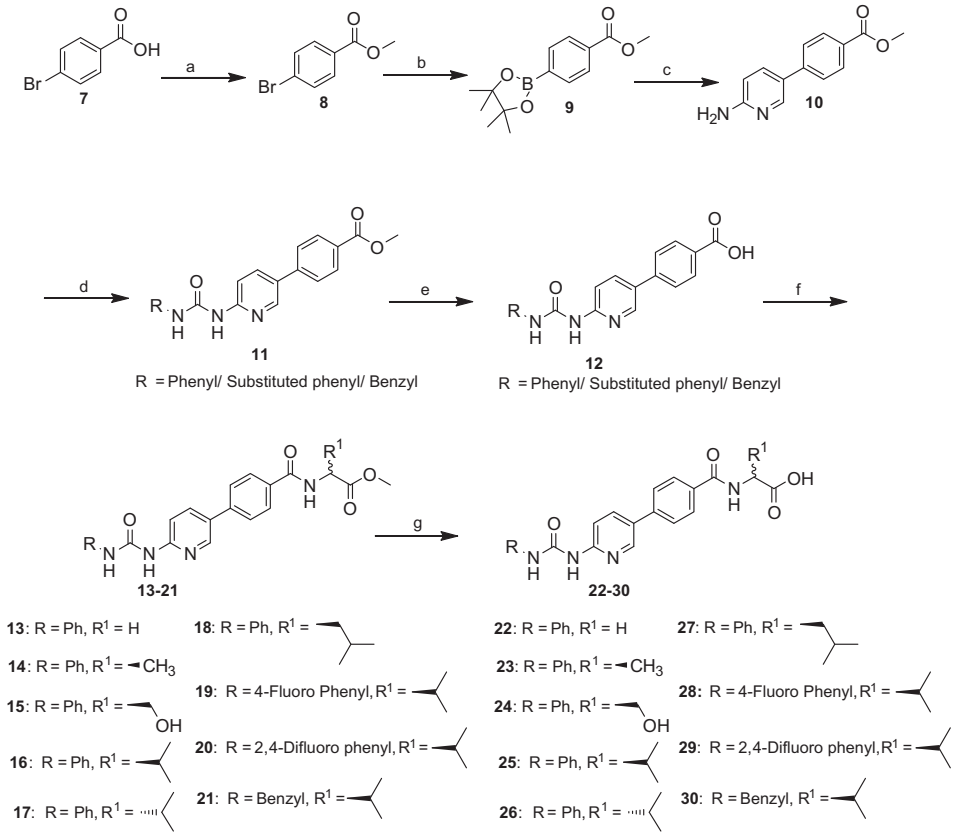
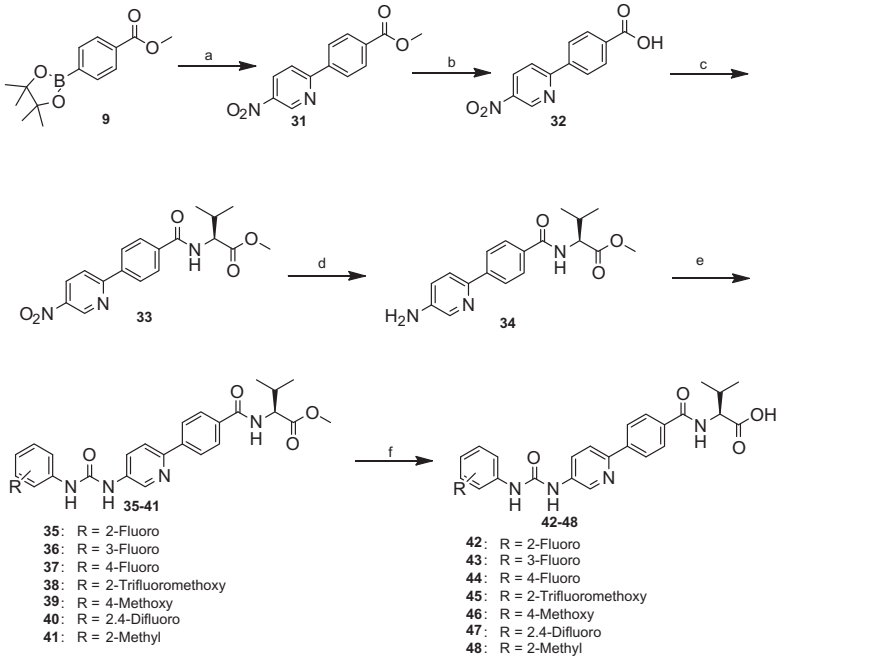


Figure 2. Development of pyridine containing heteroaryl scaffold.



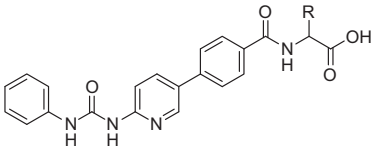
Scheme 1. Reagents and conditions: (a) H_2SO_4/MeOH reflux, 16 h (yield 77%); (b) Bis pinacol diboron/Potassium acetate/$Pd(dppf)_2$: DCM in DMSO 80 °C, 3 h (yield 75%); (c) 2-amino 5-bromopyridine/2 M K_2CO_3/tetrakis in toluene/EtOH/H_2O 80 °C, 5 h (yield 60%); (d) R-NCO in THF rt, 2–5 h (yield 80%); (e) LiOH·H_2O in THF/MeOH rt, 16 h (yield 80%); (f) Amino acid/EDCI·HCl/HOBt/DIPEA in DMF rt, 16 h (yield 60–85%); (g) LiOH·H_2O in THF/MeOH rt, 16 h (yield 70–85%).



Scheme 2. Reagents and conditions: (a) 2-bromo 5-nitropyridine/2 M K_2CO_3/tetrakis in toluene/EtOH/H_2O at 80 °C, 5 h (yield 69%); (b) LiOH·H_2O in THF/MeOH at rt, 5 h (yield 87%); (c) L-Valine/EDCI·HCl/HOBt/DIPEA in DMF at rt, 16 h (yield 47%); (d) Fe/NH_4Cl in EtOH/H_2O at 75 °C, 4 h (yield 81%); (e) R-NCO in THF at rt, 2–5 h (yield 50–85%); (f) LiOH·H_2O in THF/MeOH at rt, 16 h (yield 80–90%).

analogs possessing the 2-pyridyl heteroaryl scaffold have been synthesized of which three compounds (**25**, **28**, and **29**) belonging to the 3-pyridyl series and all seven compounds (**42–48**) belonging to the 2-pyridyl series exhibited hDGAT1 inhibition greater than 75% in the primary screen conducted at 10 μM. Compound **30** (hDGAT1 inhibition = 56%) belonging to the 3-pyridyl heteroaryl

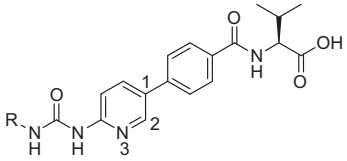
Table 1
In vitro evaluation of different amino acid head groups



Compound number	Parent amino acid	R	hDGATI inhibition IC_{50} (nM)	*c* Log *P*[a]
22	Glycine	–H	30	1.98
23	L-Alanine		72	2.47
24	L-Serine		28	1.61
25	L-Valine		88	3.35
26	D-Valine		51	3.35
27	L-Leucine		32	3.70

[a] *c* Log *P* values were calculated using the Chemdraw version 12.0 ND.

Table 2
In vitro evaluation of 3-pyridyl heteroaryl analogs

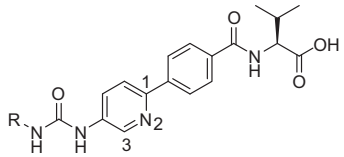


Compound number	R	hDGATI inhibition (% inhibition 10 μM)	hDGATI Inhibition IC50 (nM)	*c* Log *P*[a]
25		88	120	3.35
28		87	99	3.51
29		90	57	3.67
30		56	ND	3.42

ND, Not Determined.
[a] *c* Log *P* values were calculated using the Chemdraw version 12.0.

scaffold exhibited inhibition less than 75% in the primary screen possibly because it possessed a benzyl urea as opposed to a phenyl urea. Following the primary screening, IC_{50} values of these 2-pyridyl heteroaryl analogs (Table 3) and 3-pyridyl heteroaryl analogs (Table 2) were determined. In both the scaffolds, smaller hydrophobic substituents appear to be well tolerated at all positions of the terminal phenyl ring, as various methoxy, trifluoromethoxy, and halogen substituents exhibited good enzymatic potency. Several compounds within the 2-pyridyl heteroaryl scaffold were almost equipotent when compared with compound **4** (In house hDGAT1 IC_{50}: 20 nM). Specifically compounds **44** (IC_{50} = 17 nM) and **47** (IC_{50} = 14 nM) belonging to the 2-pyridyl series exhibited better enzymatic activity as compared to their corresponding analogs **28** (IC_{50} = 99 nM) and **29** (IC_{50} = 57 nM) within the 3-pyridyl series. Additionally three other 2-pyridyl analogs, compounds **42** (IC_{50} = 14 nM), **43** (IC_{50} = 18 nM), and **46** (IC_{50} = 25 nM) also exhibited greater enzymatic inhibition as compared to the studied 3-pyridyl analogs. These results thereby highlight the preference of hDGAT1 towards the 2-pyridyl heteroaryl scaffold over the 3-pyridyl heteroaryl scaffold.

Table 3
In vitro evaluation of 2-pyridyl heteroaryl analogs



Compound number	R	hDGATI inhibition (% inhibition 10 μM)	hDGATI Inhibition IC_{50} (nM)	*c* Log *P*[a]
42		92	14	3.22
43		81	18	3.22
44		94	17	3.22
45		79	64	4.59
46		93	25	2.93
47		82	14	3.38
48		78	ND	3.55

ND—Not Determined.
[a] *c* Log *P* values were calculated using the Chemdraw version 12.0.

Table 4
Plasma triglyceride reduction following an in vivo fat tolerance test and solubility data

Compound number	hDGATI inhibition IC_{50} (nM)	FTT %TG reduction	Solubility[a] (mg/mL)
4	20	80	0.045
28	99	53	0.12
44	17	79	0.56

[a] Solubility determined at pH 7.4.

Compounds incorporating the pyridine scaffold also exhibit an improvement in *c* Log *P* as compared to compound **4**. Compound **44** (*c* Log *P*: 3.22), a potent 2-pyridyl analog exhibited a 3.5 log order improvement over the Bayer compound **3** (*c* Log *P*: 6.72) and a 1 log order improvement over the Abbott compound **4** (*c* Log *P*: 4.22). However, the hDGAT1 enzymatic potency of compound **44** is retained when compared to the potent Abbott compound (**4**). Compounds **42**, **43**, **46**, and **47** exemplify other molecules within the 2-pyridyl series displaying a trend similar to that of compound **44**.

A confirmation of the in vitro results using an acute in vivo fat tolerance test (FTT) was also undertaken. 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 (10 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

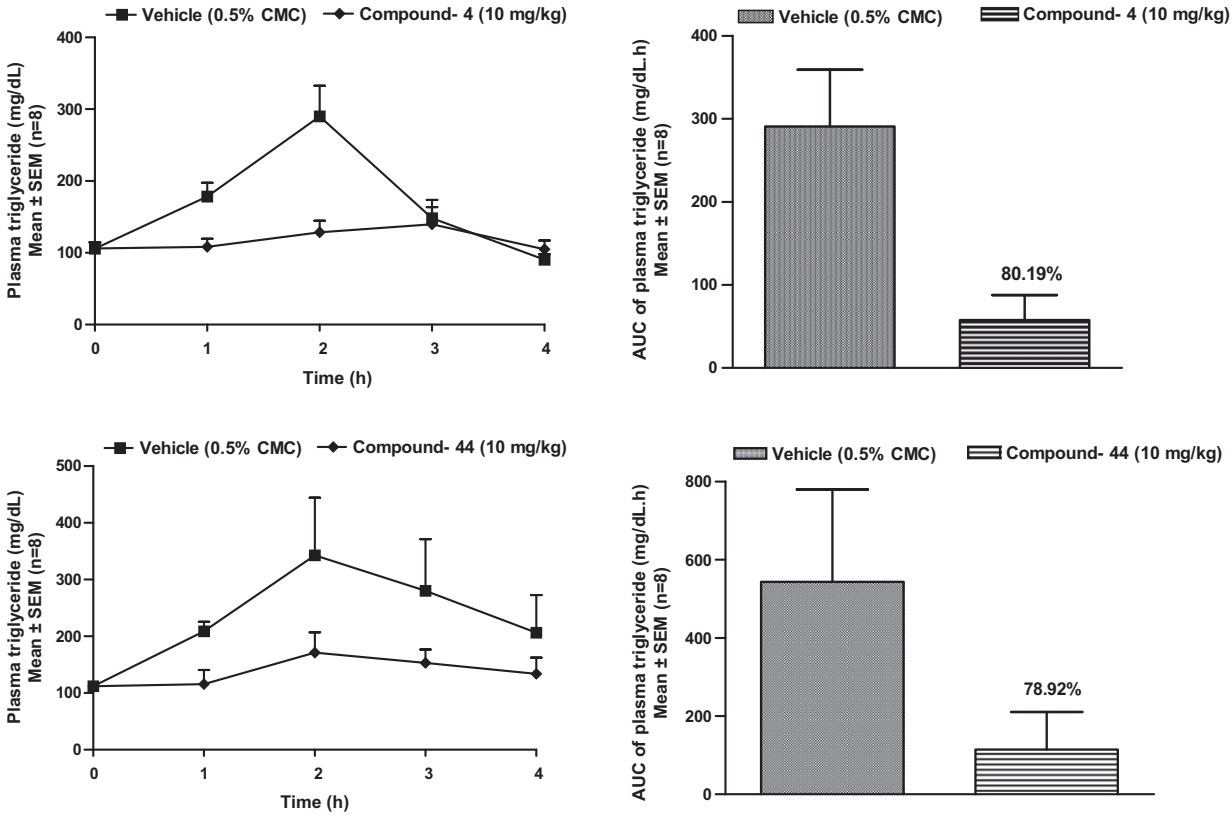


Figure 3. In vivo fat tolerance test (FTT) to measure the percent reduction in triglyceride levels following the administration of a test compound (10 mg/kg) by oral gavage. Data analyses were performed using $AUC_{0-4\ h}$ for the test compound and comparing it with $AUC_{0-4\ h}$ of the vehicle (0.5% CMC) that is considered to be 100%.

(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 the area under curve ($AUC_{0-4\ h}$) of the test compound and comparing it along with the $AUC_{0-4\ h}$ of the vehicle group that is considered to be 100%.

DGAT1 has been reported to be expressed highly in the small intestine of experimental animals and human subjects.[16] DGAT1 shows significant expression within the enterocytes of the small intestine. Enterocytes absorb fatty acids and monoacylglycerol and thereby re-synthesize them into triglycerides. These triglycerides are critical for assembly and secretion of chylomicrons that transport triacylglycerol into the blood stream. So any inhibition of the enzyme within the enterocytes should lead to a reduction in triglyceride synthesis in these cells following an oral lipid load. This in turn results in a decrease in the triglyceride levels of the blood.

The 3-pyridyl analog **28** exhibited a plasma triglyceride reduction of 53% while its corresponding 2-pyridyl analog **44** displayed a plasma triglyceride reduction of 79% (Table 4 and Fig. 3) when dosed at 10 mg/kg. At a similar dose compound **4**, discovered by Abbott, displayed a plasma triglyceride reduction of 80% (Table 4 and Fig. 3). Thus, compound **44** is equi-efficacious at a 10 mg/kg dose as compared to compound **4**.

We also studied solubilities of the three compounds evaluated in vivo in an attempt to understand if introducing a pyridine led to an improvement in the drug-likeness of these molecules (Table 4). Although modest, compound **44** (solubility: 0.56 mg/mL) and compound **28** (solubility: 0.12 mg/mL) possessing the pyridine containing heteroaryl scaffold exhibited a 12-fold and 2.4-fold improvement in their respective solubilities over the biphenyl compound **4** (solubility 0.045 mg/mL).

In this study, we have developed a pyridine containing heteroaryl scaffold possessing improved drug-like properties. We have studied a few amino acid head groups using the 3-pyridyl heteroaryl scaffold and identified L-valine as the preferred acid head group amongst those studied. A comparison between the 2-pyridyl and 3-pyridyl scaffolds highlighted a preference of the 2-pyridyl analogs as hDGAT1 inhibitors. Compounds possessing the 2-pyridyl scaffold were found to be equi-efficacious and compound **44** also exhibited a 12-fold improvement in solubility as compared to the Abbott compound (**4**). This indicated an improvement in the drug-like characteristics of the molecule along with retention of activity. The substantial in vitro hDGAT1 enzymatic inhibition coupled along with in vivo plasma triglyceride reduction of some of these 2-pyridyl heteroaryl analogs, exemplified by compound **44** (IC_{50} = 17 nM and 79% triglyceride reduction), marks them as potential new leads for developing better hDGAT1 inhibitors for therapeutic application.

Acknowledgments

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