Validation of Diacyl Glycerolacyltransferase I as a Novel Target for the Treatment of Obesity and Dyslipidemia Using a Potent and Selective Small Molecule Inhibitor

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Received November 5, 2007

Abstract: A highly potent and selective DGAT-1 inhibitor was identified and used in rodent models of obesity and postprandial chylomicron excursion to validate DGAT-1 inhibition as a novel approach for the treatment of metabolic diseases. Specifically, compound **4a** conferred weight loss and a reduction in liver triglycerides when dosed chronically in DIO mice and depleted serum triglycerides following a lipid challenge in a dose-dependent manner, thus, reproducing major phenotypical characteristics of DGAT-1$^{-/-}$ mice.

Diacyl glycerolacyltransferase 1 (DGAT-1[a]) is one of two known DGAT enzymes that catalyze the final and only committed step in triglyceride synthesis.[1,2] Multiple lines of evidence stemming from DGAT-1 deficient mice implicate this enzyme in the development of both obesity and insulin resistance.[3–5] DGAT-1 deficient mice are resistant to diet-induced obesity (DIO) and have decreased adiposity through a mechanism involving increased energy expenditure. This phenotype is at least partially attributable to increased ambulatory activity on a high-fat diet and an increase in the expression of uncoupling proteins, which play a major role in nonshivering thermogenesis in rodents. Interestingly, these animals are hyperphagic relative to their wild-type counterparts, a possible compensatory mechanism for the increased energy expenditure. The DGAT-1$^{-/-}$ mice are also protected against diet-induced hepatic steatosis and show decreased levels of triglycerides in lipogenic tissues when fed a high-fat diet. Furthermore, genetic ablation of DGAT-1 leads to increased sensitivity to insulin and leptin, and DGAT-1 deficiency protects against insulin resistance and obesity in agouti yellow mice.[6]

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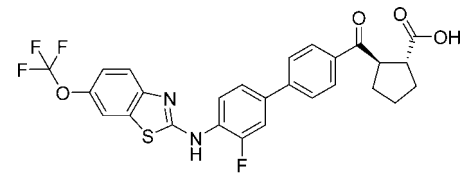
[a]Abbreviations: DGAT-1, diacylglycerol acyltransferase 1; DIO, diet-induced obesity; VLDL, very low-density lipoprotein; hu, human; mse, mouse; DMF, dimethylformamide; EtOH, ethanol; THF, tetrahydrofuran; ACAT, acylcoenzyme A/cholesterol acyltransferase; D-fen, D-fenfluoramine; Veh, vehicle.



1

huDGAT1 IC_{50} 73 nM
mseDGAT1 IC_{50} 207 nM

Figure 1. DGAT-1 inhibitor **1**.

Despite a significant decrease in tissue triglycerides, DGAT-1 mice have normal plasma triglyceride concentrations. This suggests that DGAT-1 is not solely responsible for the secretion of very low-density lipoproteins (VLDL) from the liver, and it is possible that other enzymes are upregulated in the genetically altered animals to compensate for its loss.[7] Intestinal DGAT-1 has been shown to play a major role in postprandial chylomicron secretion, however, as DGAT-1$^{-/-}$ mice show substantially decreased levels of chylomicron-derived plasma triglycerides following a lipid challenge.

These and other data indicate that an orally available small molecule inhibitor could be capable of reducing body weight through decreased triglyceride absorption, among other mechanisms, while improving insulin resistance.[8] To provide further validation for DGAT-1 inhibition as a target for the treatment of obesity and dyslipidemia, we sought to evaluate a potent and selective small molecule DGAT-1 inhibitor in rodent models of metabolic disease. Specifically, we desired a compound capable of delivering mechanism-based weight loss in DIO mice in addition to lowering liver triglycerides, thus reproducing two major components of the DGAT-1$^{-/-}$ phenotype. Additionally, because intestinal DGAT-1 plays a major role in chylomicron secretion,[7] we felt that a compound-mediated decrease in postprandial plasma triglycerides would provide further evidence of mechanism-based efficacy. To this end, we optimized an existing small molecule for potency and evaluated the resulting lead compound in rodent models of DIO and acute postprandial lipemia.

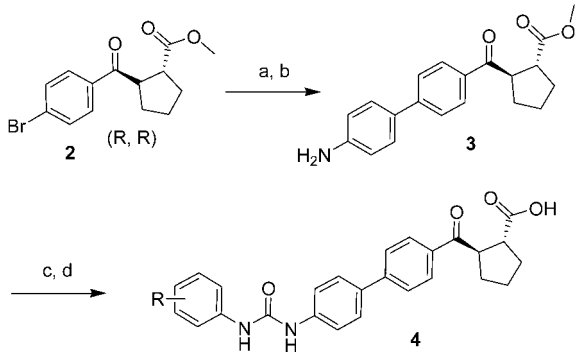
Bayer reported a class of biaryl keto acids, exemplified by structure **1**, that had potency against DGAT-1 in an enzymatic assay measuring the output of triolein from diolein and oleoylCoA (Figure 1).[10] In our hands, **1** had an IC_{50} value of 73 and 207 nM against human and mouse DGAT-1, respectively, while several analogs showed enzymatic activities in the hundreds of nanomolar. Our initial goal was to enhance the potency of analog **1** against both isoforms of DGAT-1. Additionally, the relatively high molecular weight of compound **1** and related analogs prompted us to simplify the heterocyclic terminus of the pharmacophore. We reasoned that the terminal benzthiazole heterocycle could be opened, revealing a more polar urea. To this end, we initiated the synthesis of urea-terminated analogs of **1**.

The synthesis route to the targeted analogs commenced with the production of (1*R*,2*R*)-2-(4-bromo-benzoyl)-cyclopentanecarboxylic acid methyl ester (**2**).[9,10] Subsequent Suzuki coupling to 4-nitrophenyl boronic acid and iron-mediated reduction afforded the primary aniline **3** (Scheme 1). The urea-based analogs (**4**) were then rendered via isocyanate coupling and saponification.

Several of the urea analogs shown in Table 1 demonstrated good to excellent enzymatic inhibition against both mammalian

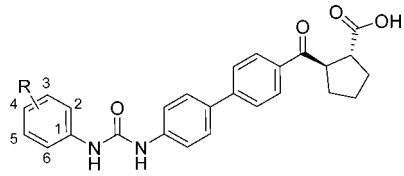
10.1021/jm7013887 CCC: $40.75
Published on Web 01/10/2008

Scheme 1[a]



[a] Reagents and conditions: (a) 4,4,5,5-tetramethyl-2-(4-nitrophenyl)-1,3,2-dioxaborolane, tris(triphenlyphosphine)palladium, potassium fluoride, DMF/toluene/EtOH/H_2O, 90 °C, o/n, 97%; (b) Fe, NH_4Cl, H_2O/EtOH, 65 °C, 98%; (c) isocyanate, THF; (d) LiOH, THF/H_2O.

Table 1. SAR of Analogs of **4**[a]



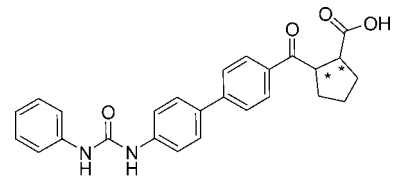
cmpd	R	huDGAT-1 IC_{50}[b] (μM)	mseDGAT-1 IC_{50}[c] (μM)
4a	H	0.007	0.024
4b	2-CF_3	0.009	0.016
4c	3-CF_3	0.010	0.017
4d	3-F	0.011	0.039
4e	3-Cl	0.006	0.019
4f	2-F, 5-CF_3	0.005	0.021
4g	3-C(O)NHMe	0.994	NT
4h	4-MeO	0.009	0.028
4i	4-CN	0.035	0.603
4j	4-CF_3	0.009	0.014
4k	3,4 OCH_2O	0.007	0.063

[a] All compounds were >95% pure by HPLC and characterized by ^1H NMR and MS or EA. See Supporting Information for measurement number (n) and IC_{50} range. [b] Insect cells (e.g., *Sf*9 or High Five) were infected for 24 to 72 h with recombinant human DGAT-1 containing an N-terminal His_6-epitope tag produced in baculovirus expression system. [c] Insect cells (e.g., *Sf*9 or High Five) were infected for 24 to 72 h with recombinant mouse DGAT-1 containing an N-terminal His_6-epitope tag produced in baculovirus expression system.

and mouse DGAT-1 enzymes, although they were generally between 2- and 4-fold less potent against the latter isoform. Remarkably, the first analog synthesized (**4a**, entry 1) showed a 10-fold boost in activity against both isoforms relative to compound **1**. Hydrophobic substituents were tolerated at all positions of the terminal ring, as various trifluoromethyl, halogen, and methoxy-substituted analogs showed good enzymatic potency. Substituents that are both polar and hydrophilic at any position were less-tolerated, as exemplified by the terminal amide-containing compound **4g**. Fortuitously, none of the compounds synthesized showed any measurable activity against the hERG channel in a dofetilide binding assay (data not shown).

Because several of the initial analogs were undifferentiated with respect to potency, we chose the first analog **4a** for further study. To confirm the optimal stereochemistry, the *S*,*S* analog was prepared, along with the racemic *cis*-diastereomer. As demonstrated in Table 2, both isomers were significantly less potent toward the human isoform of DGAT-1, showing 10-fold diminution in both cases. After confirming the optimal stereochemical arrangement, compound **4a** was screened against the

Table 2. SAR of Stereoisomers of **4a**[a]



cmpd	configuration	DGAT-1 IC_{50}[b] (μM)
4a	*R*,*R*	0.007
5	*S*,*S*	0.096
6	*R*,*S*	0.098

[a] See Table 1, footnote a. [b] See Table 1, footnote b.

Table 3. Selected Pharmacokinetic Properties of **4a**[a]

	mse (10 mg/kg)	rat (5 mg/kg)	dog (2.5 mg/kg)
	iv[b]		
$T_{1/2}$ (hr)	NA	3.9	3.3
Vβ (L·kg^{-1})[c]	NA	0.21	1.21
AUC (μg·hr·mL^{-1})[d]	NA	145.4	11.1
Clp (L·hr^{-1}·kg^{-1})[e]	NA	0.04	0.23
	oral[b]		
$T_{1/2}$ (hr)	6.5	2.7	3.6
C_{max} (μg·mL^{-1})	3.51	8.53	2.47
AUC (μg·hr·mL^{-1})[d]	15.2	80.2	5.5
F (%)[f]	NA	55.2	49.2

[a] All values are mean values (n = 3 unless specified otherwise). [b] A total of 1% Tween-80 in water. [c] Volume of distribution. [d] Total exposure. [e] Total plasma clearance. [f] Bioavailability.

acyltransferases DGAT-2[11] and ACAT1/2,[12] as activity at either off-target could affect any potential in vivo results. No activity was seen in either assay up to the measured concentration. Similarly, in a CEREP profile,[13] no inhibition greater than 50% was observed against any of the receptors, ion channels, or enzymes.

The oral pharmacokinetic properties of **4a** were explored in DIO mice, the model chosen for the preliminary in vivo evaluation. Single dose studies with both intravenous and oral routes were also executed in rat and dog. As demonstrated in Table 3, the oral exposure of **4a** was substantial in all species. The rat and dog pharmacokinetic profiles were both characterized by high oral bioavailability and low clearance, while the volume of distribution was significantly higher in the latter species.

Satisfied with the oral exposure of **4a**, we next explored the effects of administration of **4a** in a study measuring body weight, food intake, and liver triglycerides in DIO mice. For a four-week period, DIO mice fed a high-fat diet ad libitum were dosed orally with **4a** at 3 mg/kg bid, D-fenfluoramine (D-fen, 10 mg/kg, qd), or vehicle. Food intake and body weight were measured at days 1, 5, 7, 14, and 27 for each group. The vehicle group showed a nonstatistically significant decrease in body weight through day 14. After this time, the mean group weight rebounded, ultimately resulting in a nonstatistically significant increase by day 28. D-Fen caused a rapid decrease in body weight until day 14, followed by a characteristic rebound. Compound **4a** administered at 3 mg/kg conferred significant weight loss by day 7. By the end of the study, the drug-treated animal group weighed 8.45 ± 0.02% (starting weight, 44.70 ± 0.76 g; ending weight 41.44 ± 0.84 g; body weight change = −3.35 ± 0.46 g, $p < 0.01$) less than the DIO vehicle (starting weight, 44.74 ± 0.56 g; ending weight 44.95 ± 0.37 g; body weight change = +0.21 ± 0.52 g; Figures 1 and 2). No significant changes in cumulative food intake were observed for the drug-treated animals (see Supporting Information),

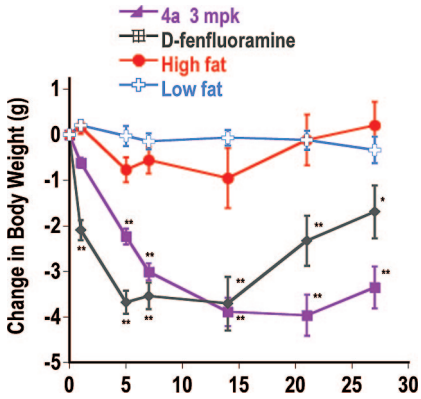


Figure 2. Effect of compound **4a** (dosed at 3 mg/kg, po, bid in 1% Tween-80 in water) and D-fen (10 mg/kg, po, qd) on the body weight of DIO mice. Change is registered as the number of grams of body weight difference for each measurement time point relative to day zero. All values are mean values ± SEM for $n = 9$ (*$p < 0.05$; **$p < 0.01$) for comparisons against vehicle group.

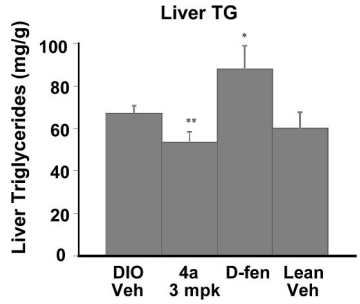


Figure 3. Reduction of liver triglycerides following treatment with compound **4a** (Veh, vehicle; D-fen). All values are mean values ± SEM for $n = 9$ (*$p < 0.05$; **$p < 0.01$), for comparisons against vehicle group.

Table 4. Exposure Levels of **4a** at 1 h (C_{max}) and 17 h ($C_{17\ h}$) Postfinal Dose on Day 28 (3 mg/kg, bid)

time (hours)	plasma[a] (μg/mL)
1[b]	0.91 ± 0.07
17[c]	0.21 ± 0.05

[a] All values are mean values ± SEM. [b] $n = 3$. [c] $n = 9$.

indicating a possible role for increased energy expenditure. Further studies to decipher this result are ongoing.

Consistent with the DGAT-1$^{-/-}$ phenotype, the liver triglycerides were significantly decreased in animals treated chronically with 3 mg/kg compound **4a** (Figure 3). That the D-fen treated animals lost weight yet showed increased liver triglycerides provides evidence for a mechanism-based efficacy profile of **4a**, as body weight loss can occur without concomitant loss of liver triglycerides.[14]

Gratifyingly, the favorable effects observed with chronic dosing of DGAT-1 inhibitor **4a** occurred at low therapeutic levels, with peak (1 h post final dose) drug concentrations being less than 1 μg/mL (Table 4). No measurable differences in locomotor activity were observed in the drug-treated mice throughout the study, indicating that treatment did not cause any overt behavioral effects. Additionally, there were no statistically significant changes in ALT and AST levels following the study (data not shown).

To investigate the in vivo effects of specific inhibition of intestinal DGAT-1, **4a** was evaluated in an acute lipid challenge model measuring chylomicron-derived plasma triglycerides following a corn oil bolus.[9] In a five-dose, three-day study in

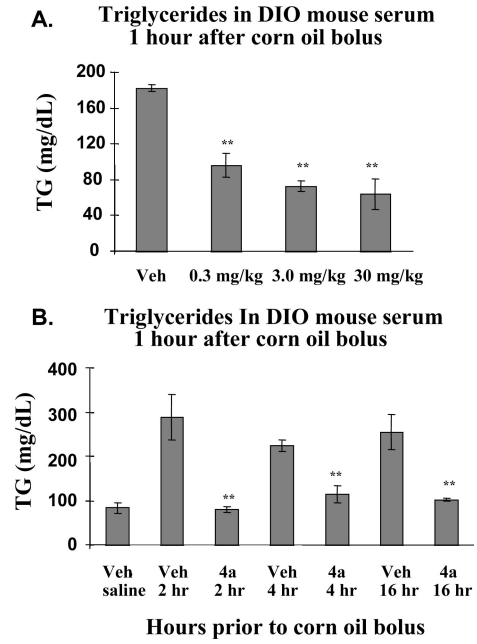


Figure 4. (A) Dose-dependent reduction of chylomicron derived triglycerides following drug treatment. (B) Reduction of plasma triglycerides following drug treatment at 2, 4, and 16 h prior to corn oil challenge. Plasma measurements were taken 1 h after the corn oil bolus. All values are mean values ± SEM for $n = 9$ (**$p < 0.01$).

DIO mice (bid dosing for days 1–2, fasting overnight, and qd dosing on day 3), **4a** was assessed at 0.3, 3, and 30 mg/kg. One hour after the final dose, a corn oil bolus was administered via gavage, and the plasma triglycerides were then measured one hour later. As demonstrated in Figure 4A, compound **4a** shows a dose-dependent reduction of plasma triglycerides starting at 0.3 mg/kg and continuing through the higher doses. The percent reductions in plasma triglycerides corresponding with the three doses are 73, 93, and 100%.[15] Additionally, the 3 mg/kg dose was shown in a subsequent study to retain full inhibition of chylomicron secretion as measured by plasma triglyceride concentration when dosed 2, 4, and 16 h before the corn oil challenge (Figure 4B). These data indicate that inhibition of intestinal DGAT-1 by **4a** has a prolonged action.

Compound **4a** shows potent inhibition of both human and mouse DGAT-1 isoforms, good selectivity over related acyltransferases, hERG, and a panel of antitargets, and good oral pharmacokinetics in multiple species. Chronic drug treatment in DIO mice confers a phenotype that reproduces characteristics of the DGAT-1$^{-/-}$ mice with respect to body fat and liver triglyceride mass. Furthermore, the ability of this compound to substantially deplete serum triglycerides following a corn oil bolus indicates an on-target mechanism of intestinal DGAT-1 inhibition. Together, this combination of data provides compelling validation for the hypothesis that small molecule DGAT-1 inhibition is a viable strategy for the treatment of obesity and various sequelae of metabolic syndrome. Further in vivo characterization of this compound will be disclosed in due course.

Acknowledgment. The authors thank Jan Waters for support with NMR analysis and Peter Dandliker and Michael Wendt for valuable discussion.

Supporting Information Available: Experimental procedures and characterization data for all compounds and experimental procedure for biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Cross-reactivity assays were performed by CEREP; www.cerep.com.

(14) Liver triglycerides were determined on day 28 only.

(15) The 30 mg/kg dose decreases the plasma triglyceride levels to those of the saline-gavaged animals. Thus, the percentage inhibition of the 0.3 and 3.0 mg/kg doses are relative to the 30 mg/kg dose.

JM7013887