ACS Medicinal Chemistry Letters

LETTER

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Discovery of PF-04620110, a Potent, Selective, and Orally Bioavailable Inhibitor of DGAT-1

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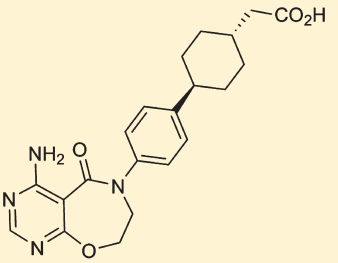
Supporting Information

ABSTRACT: Acyl-CoA:diacylglycerol acyltransferase-1 (DGAT-1) catalyzes the final committed step in the biosynthesis of triglycerides. DGAT-1 knockout mice have been shown to be resistant to diet-induced obesity and have increased insulin sensitivity. Thus, inhibition of DGAT-1 may represent an attractive target for the treatment of obesity or type II diabetes. Herein, we report the discovery and characterization of a potent and selective DGAT-1 inhibitor PF-04620110 (**3**). Compound **3** inhibits DGAT-1 with an IC_{50} of 19 nM and shows high selectivity versus a broad panel of off-target pharmacologic end points. In vivo DGAT-1 inhibition has been demonstrated through reduction of plasma triglyceride levels in rodents at doses of ≥0.1 mg/kg following a lipid challenge. On the basis of this pharmacologic and pharmacokinetic profile, compound **3** has been advanced to human clinical studies.



PF-04620110 (**3**)

KEYWORDS: DGAT-1, diabetes, obesity, triglyceride, phototoxicity, acyl glucuronide

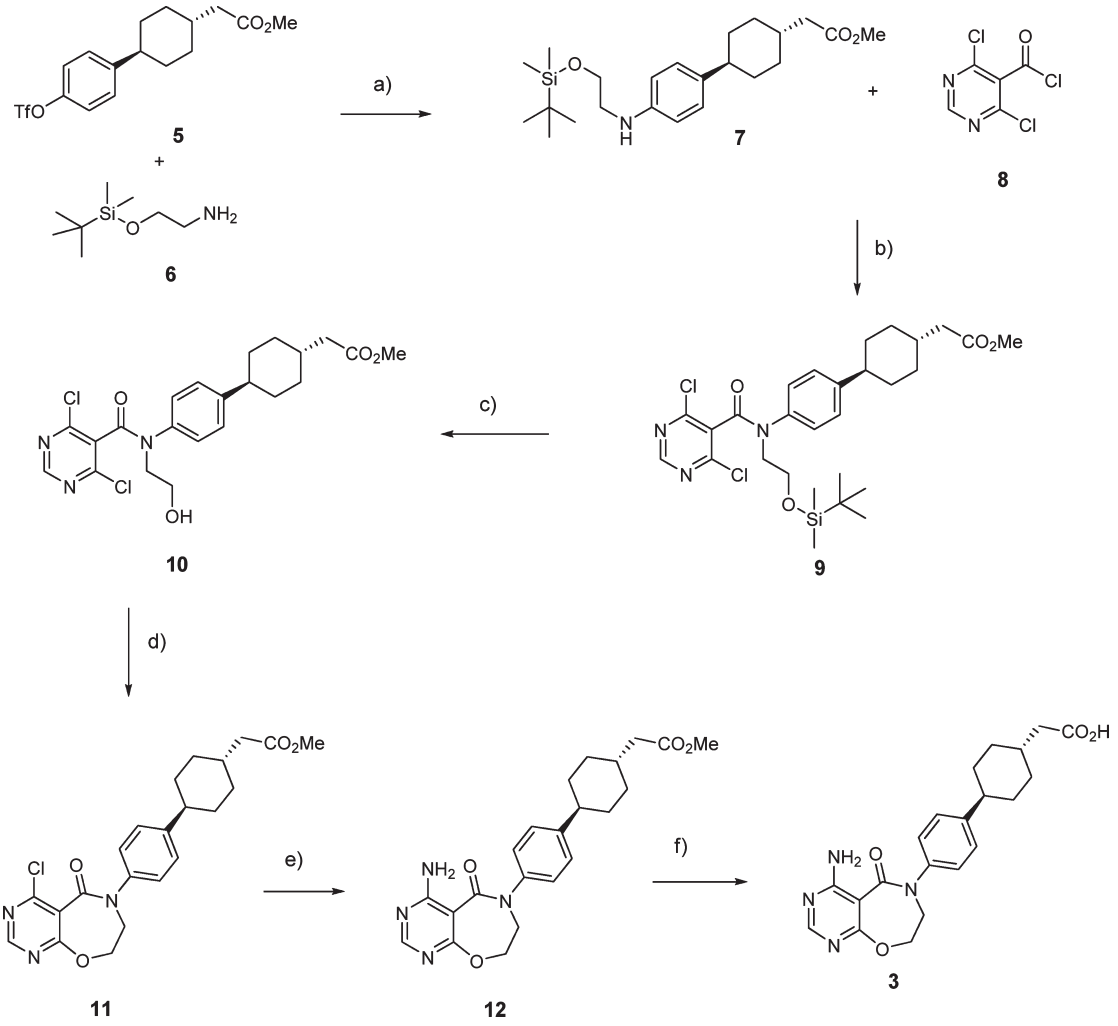
Dysregulation of fatty acid metabolism is recognized as a major determinant in a number of human diseases including obesity, insulin resistance, and hepatic steatosis.[1] Excess disposition of triglycerides in various tissues has been implicated in driving these disease end points. On the basis of this connection, there has been considerable interest in the acyl-CoA:diacylglycerol acyltransferase (DGAT) family of enzymes that catalyze the final committed step in triglyceride synthesis.[2] Two members of this enzyme family have been reported, DGAT-1[3] and DGAT-2.[4] Mice lacking DGAT-1 ($DGAT\text{-}1^{-/-}$) are resistant to diet-induced obesity and have increased insulin sensitivity and energy expenditure.[5,6] Furthermore, transplantation of white adipose tissue from these mice into the wild-type strain confers the enhanced metabolic profile observed in the DGAT-1 knockout mice.[7] These findings have spurred research efforts to determine whether selective, small molecule inhibitors of DGAT-1 can produce the same improved metabolic profile observed in $DGAT\text{-}1^{-/-}$ mice.

Several research groups have disclosed potent and selective DGAT-1 inhibitors from several distinct chemical series.[8,9] Preclinical studies with these compounds have confirmed that small molecule DGAT-1 inhibitors can elicit metabolic outcomes comparable to those observed in $DGAT\text{-}1^{-/-}$ mice.[10,11] While a number of distinct chemotypes have been reported as inhibitors of DGAT-1, a substantial portion of the early chemical matter reported at the time that we began our work in 2006 were high molecular weight and lipophilic in nature.[12] For example, >65% of the compounds disclosed in patent applications up to this point were in property space, which violated at least two of Lipinski's rule of five cutoffs.[13] On the basis of this analysis, we chose to focus our efforts on a pyrimidinooxazine-based series reported by scientists at Japan Tobacco and Tularik.[14] A representative example for this series is pyrimidinooxazine **1**, which is a potent (IC_{50} = 72 nM), selective (DGAT-2 IC_{50} > 10 μM) inhibitor of the human DGAT-1 enzyme and has a $\log D_{7.4}$ of 1.4.[15]

While possessing an encouraging profile, two chemical features of this series raised concerns. Compound **1** and related analogues exhibit substantial absorption of light at wavelengths greater than 290 nm, which can be indicative of phototoxicity potential.[16,17] The molar extinction coefficient for **1** is 7693 at 320 nm, with both solution and solid phase instabilities observed for **1**.[18] On the basis of this data, design strategies based on mimicking the spatial arrangement of the key pharmacophore elements while eliminating the pyrimidinooxazine core were prioritized. The second issue that we chose to evaluate with analogues of this series is the potential to form reactive acyl glucuronide metabolites.[19] During the course of our studies, workers at Astra Zeneca reported that the major route of in vivo clearance of **1** is via the acyl glucuronide, although stability data are not reported.[20] However, it has been shown that aliphatic acids containing no α-branching (e.g., **1**) tend to form the most reactive acyl glucuronides.[21] To address this issue, the Astra Zeneca group utilized increased steric hindrance in the vicinity of the carboxylic acid (**2**) as a means of increasing the stability of the acyl glucuronide metabolite.[20] Part of our program strategy included monitoring both the formation and the stability of the

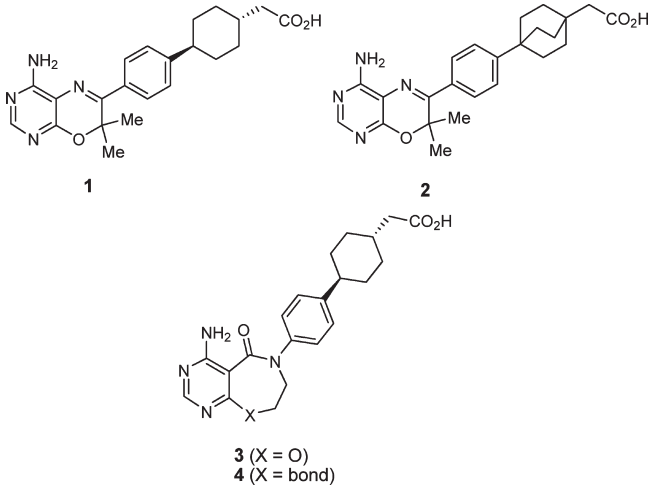
Received: February 17, 2011
Accepted: March 15, 2011
Published: March 18, 2011

Scheme 1. Synthesis of Pyrimidooxazepinone 3[a]



[a] Reagents and conditions: (a) $Pd(OAc)_2$, $CsCO_3$, XPhos, toluene, 120 °C. (b) Et_3N, THF, 0 °C. (c) MeOH, aqueous HCl(c), 25 °C. (d) Et_3N, DMF, 80 °C. (e) NH_3, *p*-dioxane, 25 °C. (f) LiOH, H_2O, *p*-dioxane.

potential acyl glucuronide metabolites of key analogues to minimize the associated safety concerns.

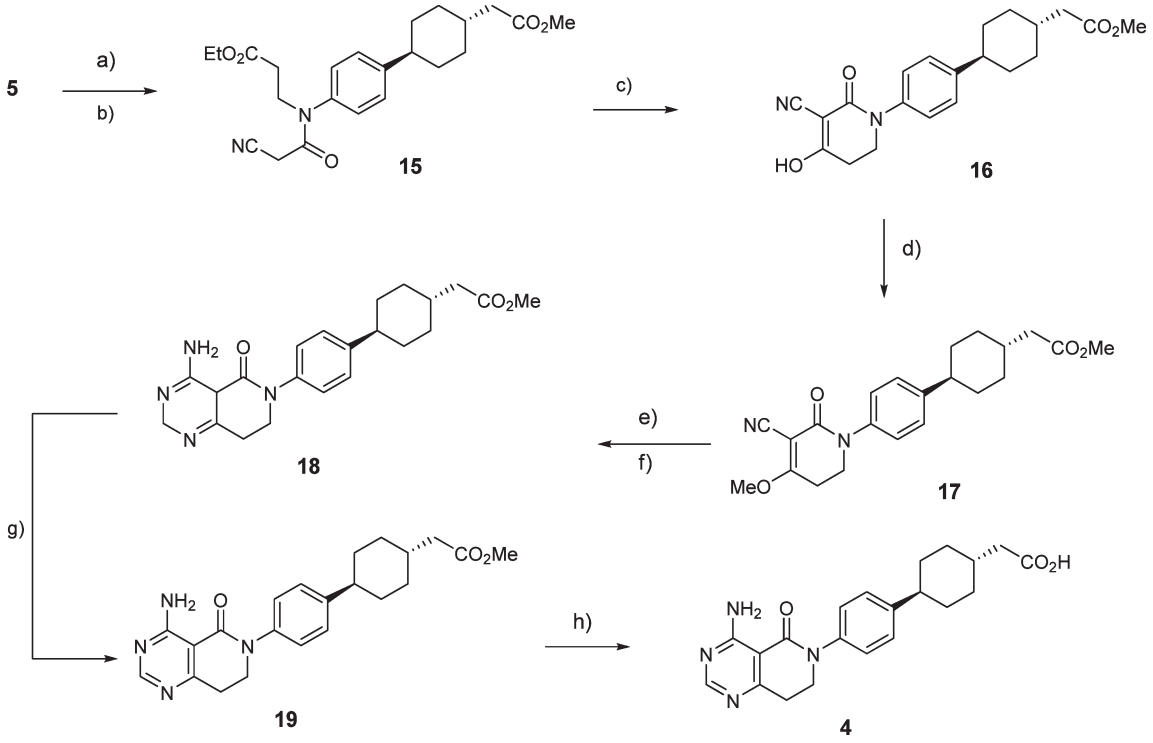


In evaluating alternative designs in which the pyrimidinooxazine core of **1** is modified, there are multiple pharmacophore and spatial features that need to be retained based on previously reported structure−activity relationship studies.[14] The 10-fold loss in potency when the oxazine carbon−nitrogen bond is reduced to the corresponding dihydrooxazine suggests that an in-plane orientation of the bicyclic core to the phenylcyclohexyl side chain is critical. For the aminopyrimidine portion of the bicyclic core, replacement of either one or both of the aminopyrimidine ring nitrogens with carbon produces a 10-/100-fold reduction in potency, respectively. In evaluating alternative designs that would retain these pharmacophore requirements, pyrimidooxazepinone **3** provided a good overlay with the key pharmacophore features with **1**. We also speculated that the lactam-based bicyclic core present in **3** would potentially have a different UV/vis absorbance/stability profile relative to **1**.

In part driven by synthetic limitations, there are no reported analogues of **1** where the oxygen atom of the pyrimidooxazine ring is replaced with carbon have been reported to date.[14,20] Because of the resulting uncertainty surrounding the importance of this group, the pyrimidodihydropyridone-based analogue **4** was also a goal in our efforts. This communication details the syntheses and DGAT-1 inhibitory profiles of these novel aminobicyclic targets. A summary of pharmacokinetic, efficacy, and

dx.doi.org/10.1021/ml200051p |*ACS Med. Chem. Lett.* 2011, 2, 407–412

Scheme 2. Synthesis of Pyrimidodihydropyridinone 4[a]



[a] Reagents and conditions: (a) β-Alanine ethyl ester, Cs_2CO_3, X-Phos, $Pd(OAc)_2$ toluene, reflux. (b) Cyanoacetyl chloride, triethylamine, 0 °C. (c) DBU, MeOH, reflux. (d) Oxalyl chloride, DMF, CH_2Cl_2, 25 °C; then MeOH, reflux. (e) Cyanamide, NaH, *p*-dioxane, 25 °C; then 4 M HCl in *p*-dioxane, 100 °C. (f) 20% $Pd(OH)_2$ on carbon, H_2 (800 psi), 25 °C. (g) 2,3-Dichloro-5,6-dicyanobenzoquinone, CH_3CN, 25 °C. (h) LiOH, H_2O, *p*-dioxane, 45 °C.

safety parameters is also discussed, leading to the advancement of PF-04620110 (**3**) to clinical trials.

Scheme 1 details the synthetic route developed for the preparation of the pyrimidooxazepinone **3**. Amination of triflate **5**[14] with the protected aminoalcohol **6**[22] utilizing Buchwald conditions[23] affords **7** in 70% yield. Acylation with acid chloride **8**[24] followed by deprotection of the silyl protecting group with aqueous hydrochloric acid provides alcohol **10**. Protection of the alcohol was necessary since substantial amounts of O-acylation are observed in its absence. Treatment of **10** with triethylamine at 80 °C forms the pyrimidooxazepinone core, with **11** isolated in 83% overall yield from **7**. Treatment of this chloropyrimidine with ammonia in *p*-dioxane at room temperature affords **12** in a near quantitative yield. Lithium hydroxide-mediated hydrolysis provided the target carboxylic acid **3** in 95% isolated yield as a crystalline solid.

Preparation of pyrimidodihydropyridinone **4** (Scheme 2) is initiated through coupling of ethyl 3-aminopropanoate utilizing the conditions employed for the synthesis of intermediate **7**. Acylation of the resulting amine with cyanoacetyl chloride affords amide **15** in 77% overall yield. Base-catalyzed cyclization generates the dihydropyridinone core, and conversion of the 4-hydroxy group of **16** to the corresponding methyl ether (**17**) is achieved in 64% overall yield for this two-step process. Construction of the aminopyrimidine ring follows the generalized route developed by Schmidt and co-workers.[25] Reaction of **17** with cyanamide, followed by cyclization with hydrogen chloride in *p*-dioxane, creates the fused 2-chloro-4-aminopyrimidine ring. Hydrogenolysis of the 2-chloro substituent requires somewhat forcing conditions [$Pd(OH)_2$/800 psi H_2], leading to a 12:1 mixture of **18**:**19** in 63% combined yield. Tetrahydropyrimidopyridinone **18** can be converted to **19** by oxidation with DDQ, and subsequent hydrolysis provides pyrimidodihydropyridinone **4** in 62% overall yield.

Table 1. In Vitro Pharmacology and Microsomal Stability Data for 1, 3, and 4

	IC_{50} (nM)			
compound	DGAT-1[a]	DGAT-2[b]	TG synthesis[c]	HLM CL_{app} (mL/min/kg)
1	72	>10000	16	<8
3	19	>30000	8	<8
4	60	>30000	71	12

[a] Average of ≥five determinations run in triplicate. [b] Average of two determinations run in triplicate. [c] Inhibition of triglyceride synthesis determined in HT-29 cells. Average of ≥three determinations run in triplicate.

Pyrimidooxazepinone **3** is a potent inhibitor of human DGAT-1 possessing an IC_{50} of 19 nM (Table 1). This result confirmed our design hypothesis that the oxazepinone ring system could function as an effective replacement for the fused oxazine ring of **1**. This result answered two additional pharmacophore questions that were part of our design analysis leading up to the pursuit of **3**. The first of these was whether the lactam carbonyl moiety would affect potency through steric encumbrance in a spatial vector that is not occupied in **1**. This concern was enhanced by the previously mentioned significant reduction in potency observed when the oxazine ring of **1** is reduced, placing a hydrogen in space occupied by the carbonyl oxygen of **3**.

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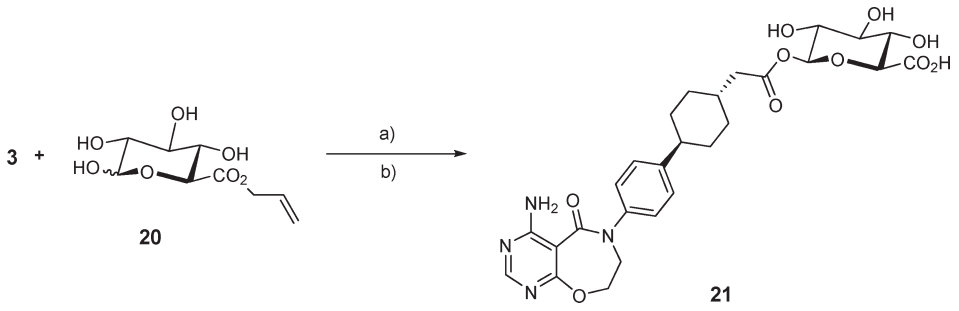
Scheme 3. Synthesis of Acyl Glucuronide 21[a]



[a] Reagents and conditions: (a) HATU, *N*-methylmorpholine, acetonitrile, 25 °C. (b) $Pd(Ph_3)_4$, pyrrolidine, THF, 25 °C.

The second question revolved around the criticality of the *gem*-dimethyl substituents in determining the DGAT-1 inhibitory potency of **1**. On the basis of the potency of **3**, it appears that these methyl groups do not contribute significantly to the potency of **1**, which is consistent with the trends reported for *des*-cyclohexylacetic acid analogues of **1**.[14]

Compound **3** is a highly selective inhibitor of DGAT-1 with >100-fold selectivity against a panel of lipid processing enzymes (human DGAT-2, human acyl-CoA:cholesterol acyltransferase-1, human acyl-CoA:wax alcohol acyltransferase-1/-2, and human acyl-CoA:monacylglycerol acyltransferase-2/-3 and mouse MGAT-1) . The lack of significant activity (IC_{50} > 10 μM) against 200 enzyme, ion channel, and receptor pharmacology end points confirmed high DGAT-1 specificity for **3**. Analysis of inhibition of triglyceride synthesis of **3** in the intestinal-derived HT-29 cell line shows good translation of potency from the isolated enzyme to a whole cell environment (IC_{50} = 8 nM, Table 1). With **3** possessing both a low logD (−0.15) and passive permeability (1×10^{-6}cm/s^{-1}),[26] there was some concern that this profile might limit cellular uptake and thus inhibition of triglyceride synthesis in the HT-29 assay. This is in comparison to **1**, which also shows good translation of DGAT-1 enzymatic inhibitory activity in this assay but has a logD of 1.4 and a high passive permeability of 10×10^{-6}cm/s^{-1}.

Pyrimidodihydropyridinone **4** is also a relatively potent DGAT-1 inhibition with enzymatic and whole-cell IC_{50} values of 60 and 71 nM, respectively. However, when compound **4** was advanced to an in vitro ADME screening panel, it was found to have moderate turnover in human liver microsomes (12 mL/min/kg). This is significantly higher oxidative metabolism than is observed for either **1** or **3**, which are <8 mL/min/kg in this high-throughput assay. Based on the combination of DGAT-1 potency and projected oxidative metabolism, compound **3** was selected for further profiling.

While **1** has substantial absorbance in the near visible light region, pyrimidinooxazepinone **3** has a greatly reduced absorbance profile with an MEC of 1315 L/mol cm^{-1}, which returns to baseline by 340 nM. Compound **3** was advanced to photostability testing where no degradation was observed in solution or solid state.

Because of the potential for idiosyncratic toxicology associated with the acyl glucuronide of **1** and the structural equivalency of the carboxylic acid side chain of **3**, analysis of the metabolite profile was initiated. An authentic sample of the acyl glucuronide of **3** was prepared to serve as a standard for in vitro and in vivo metabolite identification as well for stability measurements of this potential reactive intermediate. Scheme 3 details the

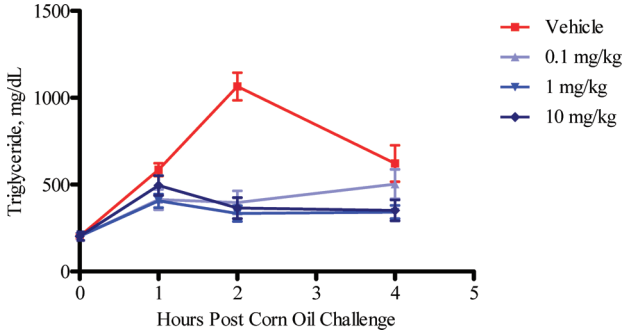


Figure 1. Rat triglyceride tolerance test with **3**.

preparation of **21** via ester coupling of **3** with ally α/β-D-glucopyranuronate[27] followed by removal of the allyl protecting group. Exposure of **3** to both rat and human liver microsomal fractions did not produce detectible quantities of **21**. In rat, 3% of an oral dose is excreted in the bile as a 1:1 mixture of **3** and **21**. While 19% of the dose is excreted in urine of these rats, with no acyl glucuronide or rearrangement products were observed. To further understand the potential risk of any acyl glucuronide formed in vivo, the rates of acyl migration and/or hydrolytic cleavage of **21** were measured by NMR.[28] In pH 7.4 aqueous sodium phosphate at 37 °C, the $T_{1/2}$ for loss of **21** was greater than 9.5 h with only direct hydrolysis to **3**, and no rearrangement products observed. Half lives of greater than 1.5 h under these experimental conditions have been proposed to be associated with a low risk of adverse side effects.[29]

In anticipation of advancing **3** to preclinical in vivo efficacy studies, pharmacokinetic profiling was performed in the rat. Both plasma clearance and volume in the rat are in the moderate range at 6.7 mL/min/kg and 1.8 L/kg, respectively. High oral bioavailability (100%) and a moderate half-life of 6.8 h were observed with an oral dose of 5 mg/kg of crystalline **3** in 0.5% methylcellulose vehicle.[30] To confirm that this pharmacokinetic profile translated into the pharmacodynamic effect via DGAT-1 inhibition in vivo, **3** was evaluated in an acute lipid challenge model measuring plasma triglycerides after a corn oil bolus. Sprague−Dawley rats (n = 7 per dose) were treated with vehicle (5% methyl cellulose), 0.1, 1, or 10 mg/kg of **3** 30 min prior to corn oil dosing. Blood samples for triglyceride and pharmacokinetic analyses were taken at 1, 2, and 4 h postlipid challenge. All three doses produced a statistically significant (p < 0.05) reduction in plasma triglyceride excursion at 2 h to near prelipid load levels (Figure 1). Free C_{avg} plasma concentrations[31] of **3** over the

dx.doi.org/10.1021/ml200051p |*ACS Med. Chem. Lett.* 2011, 2, 407–412

course of the 4 h study were 8, 37, and 398 nM for the 0.1, 1, and 10 mg/kg doses, respectively. While the plasma free C_{avg} for the two lower doses were below the rat DGAT-1 IC_{50} (64 nM), it is likely that in the enterocytes, a key DGAT-1 expressing tissue, concentrations of **3** were considerably higher. On the basis of this excellent potency and efficacy in an acute setting, compound **3** was advanced to a chronic preclinical model of obesity and diabetes, which will be reported separately.

In conclusion, PF-04620110 (**3**) is a potent and selective inhibitor of DGAT-1. Results for this compound demonstrate the low risk of potential adverse effects mediated via the acyl glucuronide metabolite or via photochemical processes. This compound has excellent pharmacokinetic properties that are consistent with once daily oral administration.[32] Compound **3** has been advanced to human clinical trials for the treatment of type II diabetes.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details for the syntheses and pharmacological characterization of **3**, **4**, and **21**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

We thank Chris Wood and Chris Foti for UV−vis spectral/photostability analyses and Kay Ahn for MGAT enzyme inhibition data.

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(12) See the Supporting Information for a property space analysis of DGAT-1 patent literature for 2004−2006.

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(18) A solution of **1** in methanol showed a 46% reduction in UV area (214 nM) after irradiation with near UV (intensity 38 Wh/m^2) and visible (intensity 28000 lx h) light for 24 h. In the solid state, 0.7% degradation of **1** was observed at 18900 KJ/m^2.

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(30) In rats at 5 mg/kg, po in 5% aqueous methylcellulose: C_{max} = 2130 ng/mL, T_{max} = 3.2 h, and $AUC_{0\text{-inf}}$ = 16700 ng h/mL. An intravenous dose of 1 mg/mL had a clearance of 1.8 L/kg.

(31) Free C_{avg} plasma concentrations were calculated from the $AUC_{0-4\,h}$; data are included in Supporting Information. The free fraction of **3** in rat plasma is 0.09.

(32) The human pharmacokinetic and pharmacodynamic predictions of **3** will be the subject of a separate report.