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Discovery of a Potent and Selective DGAT1 Inhibitor with a Piperidinyl-oxy-cyclohexanecarboxylic Acid Moiety

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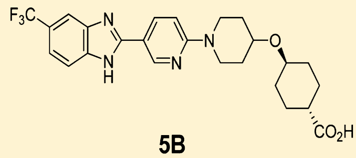
Supporting Information

ABSTRACT: We report the discovery of a novel series of DGAT1 inhibitors in the benzimidazole class with a piperdinyl-oxy-cyclohexanecarboxylic acid moiety. This novel series possesses significantly improved selectivity against the A_{2A} receptor, no ACAT1 off-target activity at 10 μM, and higher aqueous solubility and free fraction in plasma as compared to the previously reported pyridyl-oxy-cyclohexanecarboxylic acid series. In particular, **5B** was shown to possess an excellent selectivity profile by screening it against a panel of more than 100 biological targets. Compound **5B** significantly reduces lipid excursion in LTT in mouse and rat, demonstrates DGAT1 mediated reduction of food intake and body weight in mice, is negative in a 3-strain Ames test, and appears to distribute preferentially in the liver and the intestine in mice. We believe this lead series possesses significant potential to identify optimized compounds for clinical development.



5B

$hDGAT_1$	IC_{50}	3.9 nM
$mDGAT_1$	IC_{50}	23 nM
$ACAT_1$	IC_{50}	> 10 μM
A_{2A}	Ki	4.6 μM

KEYWORDS: *DGAT1, inhibitor, benzimidazole, ACAT1, A_{2A} receptor, cyclohexanecarboxylic acid, lipid tolerance test, epimerization, metabolite, Ames test, skin*

DGAT1 inhibitors have emerged as potential therapeutic agents against diabetes and obesity.[1] DGAT (acyl CoA:diacylglycerol acyltransferase) catalyzes the final and committed step in the synthesis of triglyceride: the formation of triacylglycerol from diacylglycerol and acyl-CoA. DGAT1 is one of the isoforms and shares only limited homology with DGAT2 in the terms of amino acid sequence.[2−4] DGAT1 has more sequence homology to acyl CoA:cholesterol acyltransferase (ACAT1 and ACAT2), which play a crucial role in cholesterol homeostasis.[5] DGAT1 has attracted much attention since the disclosure of the phenotype of DGAT1 knockout mice, which were shown to be viable and resistant to diet-induced obesity.[6] DGAT1 knockout mice were also reported to have enhanced insulin sensitivity compared to wild-type mice.[7] The data, taken together, has prompted significant research effort in identifying small molecule DGAT1 inhibitors as a potential treatment for obesity and diabetes (Figure 1)[8−16] In a phase II clinical trial, 3-week dosing of LCQ-908 (pradigastat) at 20 mg daily resulted in a 40% reduction in fasting triglyceride levels in patients with familial chylomicronemia syndrome, thus demonstrating the clinical proof of concept that inhibition of DGAT1 in humans leads to reductions of plasma triglycerides.[17] Recent reports on the clinical results for AZD-7687 demonstrated the ability of DGAT1 inhibitors to attenuate postprandial triacylglyceride excursion. However, the gastrointestinal side effects could hinder further development of

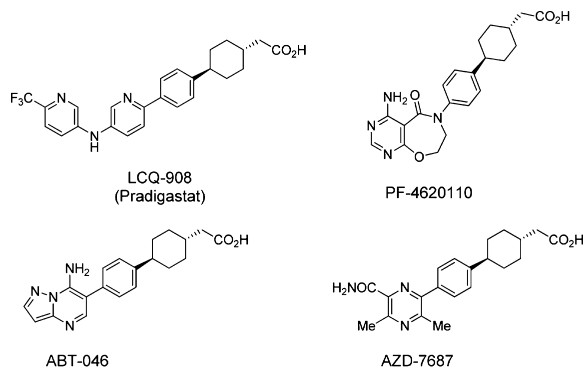


Figure 1. Structures of selected DGAT1 inhibitors.

DGAT1 inhibitors as a novel treatment for diabetes and obesity.[18,19]

Recently, we disclosed a series of novel DGAT1 inhibitors in the benzimidazole class bearing a pyridyl-oxy-cyclohexanecarboxylic acid moiety.[20] A representative of this series, **1A**, is a potent DGAT1 inhibitor with excellent selectivity against

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ACAT1 (Figure 2). Furthermore, **1A** significantly reduces triglyceride excursion in lipid tolerance (LTT) in both mice and dogs. However, **1A** undergoes cis/trans isomerization in vivo, which could complicate the further development.

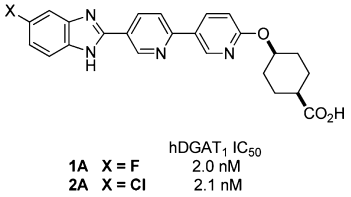


Figure 2. Representative DGAT1 inhibitors of the pyridyl-oxy-cyclohexanecarboxylic acid series.

During the profiling of **1A** as the lead compound, other potential issues for this structural series were uncovered. First, **1A** has low aqueous solubility. In the high throughput solubility test, all the salt forms prepared, free form, formate, and ammonium salt, have solubility less than 10 μM in PBS buffers at pH 2 and 7, respectively. Second, **1A** appears to be tightly bound to plasma proteins. The unbound free fraction of **1A** in human plasma is only 0.36%. In mouse plasma, the unbound free fraction is slightly higher (1.7%). Finally, this series of compounds were shown to have an off-target interaction with the A_{2A} receptor.[21] Compound **2A**, an earlier lead compound with a chloro substitution on the benzimidazole ring, was screened against a panel of known biological targets (Figure 2). In this test, **2A** had an IC_{50} of 247 nM in a radioligand based competition binding assay with recombinant human A_{2A} receptor. The activity was later confirmed in a similar in-house human A_{2A} receptor binding assay. In this assay, **1A** had IC_{50} of 334 nM, which is only ~160-fold selectivity against DGAT1. Consistent with this result, in an A_{2A} cAMP functional assay, compound **1A** displayed antagonist activity (IC_{50} = 269 nM with 101% inhibition at 30 μM). Given the cis/trans isomerization issue and the potential issues discussed above, further profiling of **1A** was discontinued.

While profiling **1A** and related compounds, we continued to explore other structural variations to present both the benzimidazole and the carboxylic acid pharmacophores in the molecule. We became interested in a new design, which incorporates a piperidinyl linker instead of a pyridyl linker (e.g., structure **3A** in Figure 3). We believed the piperidiny linker would impart more flexibility to the structure, as well as a more basic piperidine nitrogen, which could improve aqueous solubility with molecules of essentially the same molecular weight. As an advantage to alternative designs, the symmetrical nature of this piperidinyl linker would not impose additional stereochemical complications to the synthesis. Furthermore, given that many known A_{2A} receptor modulators have multiple aromatic rings in their structures, we expected that the saturation of the pyridyl ring system could decrease the number of aromatic rings and potentially reduce the affinity with the A_{2A} receptor.[22]

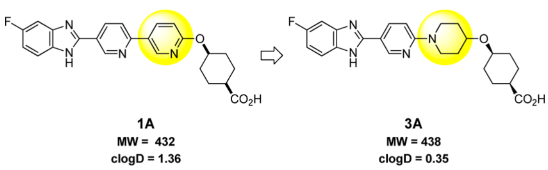
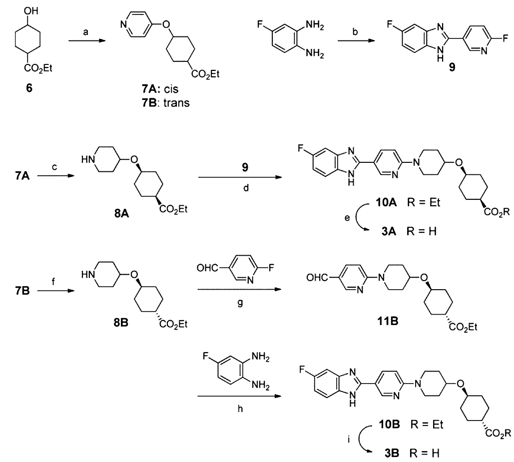


Figure 3. Design of the piperidinyl-oxy-cyclohexanecarboxylic acid series.

In addition, the compounds with a piperidinyl linker series (e.g., **3A**) might have a lower risk to cause adverse effects in the skin. DGAT1 is known to be expressed in the skin of mice and human.[23] DGAT1 KO mice were shown to display deficiency in skin and fur, including sebaceous gland atrophy and hair loss. These findings raised concerns that pharmacological inhibition of DGAT1 in skin could lead to undesirable adverse effects in humans. The calculated logD for **3A** is about one unit lower than that of **1A** (0.35 vs 1.36, Figure 3). Given that the skin tissue is largely lipophilic, we believed that an inhibitor with lower logD is expected to distribute less in the skin and therefore has a lower tendency to elicit undesirable effect.[24]

The synthesis for this series of compounds focused on the preparation of the piperidine substructures **8A** and its trans isomer **8B** (Scheme 1). The initial synthetic route involved saturating a pyridyl ring to furnish the required piperidinyl intermediates. The Mitsunobu reaction of commercially available ethyl 4-hydroxycyclohexanecarboxylate **6** (a cis and trans mixture) and 4-hydroxypyridine followed by SFC separation gave the cis and trans isomers (**7A** and **7B**). Saturation of the pyridyl ring to a piperidinyl ring turned out to be challenging, which we rationalized to be due to the electron donating effect of the alkoxy substituent on 4-position of the pyridyl ring. Eventually, we were able to hydrogenate **7A** to piperidine **8A** with frustratingly long reaction time at 45 psi in a Parr shaker at room temperature. The trans isomer **7B** behaved similarly in the hydrogenation step to give **8B**. Coupling of **8A** with 2-(6-fluoropyridin-3-yl)-5-fluoro-1*H*-benzimidazole **9** gave ethyl ester **10A**. The coupling reaction condition was identified from a comprehensive screening of a variety of solvents and bases. Eventually, we identified that the coupling worked best with a weak inorganic base such as $NaHCO_3$ in polar aprotic solvents (such as DMSO and NMP).[25] The coupling chemistry thus developed proved to be pivotal for the rapid progression of this structural series. Under the same conditions, **8B** was coupled

Scheme 1. Synthesis of 3A and 3B[a]



[a]Reagents and conditions: (a) 1. PPh_3, 4-hydroxypyridine, DIAD, THF, 55 °C, 2 days; 2. SFC (ChiralPak AD-H), **7A** (14% for two steps), **7B** (23% for two steps); (b) 6-fluoronicotinaldehyde, potassium peroxymonosulfate, DMF−water, 69%; (c) PtO_2, TsOH, H_2 (45 psi), EtOH, RT, 5 days; (d) $NaHCO_3$, DMSO, 110 °C, 43% for two steps; (e) LiOH, THF−water, 83%; (f) PtO_2, TsOH, H_2 (45 psi), EtOH, 5 days; (g) $NaHCO_3$, DMSO, 110 °C, 27% for two steps; (h) potassium peroxymonosulfate, 4-fluorobenzene-1,2-diamine, DMF−water, 18%; (i) LiOH, THF−water, 70%.

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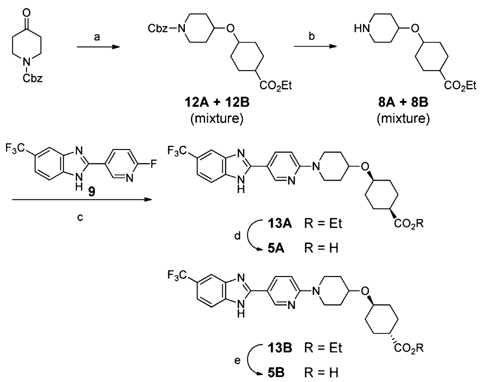
with 6-fluoronicotinaldehyde to give aldehyde intermediate **11B**. Oxidative condensation of **11B** with 4-fluorobenzene-1,2-diamine afforded ester **10B**. Finally, hydrolysis of ethyl esters **10A** and **10B** provided compounds **3A** and **3B**, respectively.

The difficulty in preparing the piperidine pieces (**8A** and **8B**) by the route shown above significantly hampered the synthesis and profiling of this series of compounds. To overcome this synthetic problem, we developed a new route starting with benzyl 4-oxopiperidine-1-carboxylate (Scheme 2). Reductive etherification of this starting material with ethyl 4-hydroxycyclohexanecarboxylate (cis and trans) gave a mixture of **12A** and **12B**.[26] Removal of Cbz-protecting group using standard conditions furnished a mixture of **8A** and **8B**. Following the chemistry described above, **5A** and **5B** were prepared. During the reductive etherification reaction, trans/cis configuration in ethyl 4-hydroxycyclohexanecarboxylate was transferred to the product with complete retention. Therefore, scale-up of this series of compounds could begin with pure *cis*- or *trans*-ethyl 4-hydroxycyclohexanecarboxylate to provide the desired intermediate in cis or trans form, respectively.[27]

Scheme 2. Reductive Etherification Route[a]



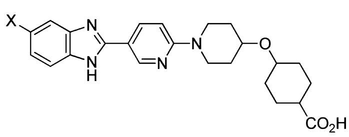
[a]Reagents and conditions: (a) 1. ethyl 4-hydroxycyclohexanecarboxylate (cis/trans mixture), triethylamine, TMSCl, 0 °C; 2. benzyl 4-oxopiperidine-1-carboxylate, Et_3SiH, TMSOTf, −78 to 0 °C, 91%; (b) 1. H_2, Pd/C; 2. $NaHCO_3$, NMP, 110 °C; 3. SFC (ChiralPak AD-H), **13A** (37% for three steps), **13B** (33% for three steps); (d) LiOH, THF−water, RT, 73%; (e) LiOH, THF−water, RT, 81%.

Applying the chemistry described above, we prepared the compounds with several different substitutions on the benzimidazole ring in both the cis and trans series. The compounds and their profiles are listed in Table 1. Most of the compounds in piperidinyl series maintain potent inhibition on both human and mouse DGAT1 except for **3B**, the least potent analogue in this series.[28] The compounds are slightly less potent on mouse DGAT1 than on human DGAT1. The potency loss appears to be more severe for the compounds in the trans series. In contrast, in the pyridyl linked series reported earlier (e.g., **1A**), there is less differentiation of potencies (human vs mouse and cis vs trans).[16] However, the piperidinyl series shows much improved off-target selectivity. All the compounds in the piperdinyl series are now highly selective against ACAT1.[29] In contrast, in the pyridyl linker series, a small substituent on benzimidazole (e.g., F or H) is required to offer good ACAT selectivity, while maintaining potent DGAT1 inhibition. Furthermore, all the piperidinyl linked compounds possess improved selectivity against A_{2A} as compared to **1A** and **2A** of the pyridyl linker series. In particular, **3A**, **4B**, **5A**, and **5B** achieve >1000-fold selectivity (the ratios of hDGAT1 IC_{50} and hA_{2A} K_i). Finally, the piperidinyl series displays improved aqueous solubility and increased free fractions in plasma. For example, the TFA salt of **5B** has solubility of 130 and 151 μM in PBS buffer at pH 2 and 7, respectively. The solubility of the free form is 167 μM and 153 μM at pH 2 and 7, respectively. The free fraction of **5B** is 9.0% and 7.8% in human and mouse plasma, respectively.[30]

The compounds were subsequently tested in rodent lipid tolerance test (LTT), an in vivo lipid excursion assay, to evaluate their ability to inhibit plasma triglyceride accumulation. In mouse LTT screening at 10 mpk oral dose, **3A** and **4A** show modest efficacy reducing lipid excursion (∼50% at 20 h time point).[31] Because of its poor potency on mouse DGAT1, **3B** was excluded from mouse LTT screening. However, **5A** and **5B** with a CF_3 substituent have the best efficacy. Similarly, **4B** with a chloro substituent on benzimidazole ring gave excellent efficacy in mouse LTT screening. However, it suffers from slightly increased off-target activity on hERG ion channel in a competitive binding assay with radiolabeled MK-499 (K_i = 4.0 μM).[32] Because of its excellent efficacy observed in mouse LTT screening, **5B** was also tested in rat LTT model (Figure 4).[33] At 1 mpk and 3 mpk, **5B** reduces lipid excursion in rat by ∼80%. The corresponding plasma concentrations of **5B** were 20 and 58 nM.[34] In this assay, reduction of lipid excursion by the positive control, Cpd A (a known DGAT1 inhibitor from the literature), at 10 mpk was normalized to 100%.[35]

To establish the target engagement on DGAT1, **5B** was evaluated in wild type (WT) vs DGAT1 knockout (KO) mice for the effect on body weight and food intake (Figure 5).[36] In a six day study with daily dosing at 10 mpk, **5B** reduced cumulative

Table 1. Profiles of Compounds 3−5



compd	cis/trans	X	human DGAT1 IC_{50} (nM)	mouse DGAT1 IC_{50} (nM)	human ACAT1 IC_{50} (% inh. at 10 μM)	hA_{2A} binding K_i (μM)	mouse LTT (10 mpk) triglyceride reduction @18 h	hERG binding (K_i, μM)
3A	cis	F	5.8	26	>10 μM (−2%)	>10	55%	11
3B	trans	F	15	110	>10 μM (5%)	9.7	ND	>60
4A	cis	Cl	2.2	11	>10 μM (13%)	ND	52%	15
4B	trans	Cl	2.9	29	>10 μM (4%)	3.5	75%	4.0
5A	cis	CF_3	2.5	8.0	>10 μM (22%)	8.0	75%	7.0
5B	trans	CF_3	3.9	23	>10 μM (23%)	4.6	89%	10

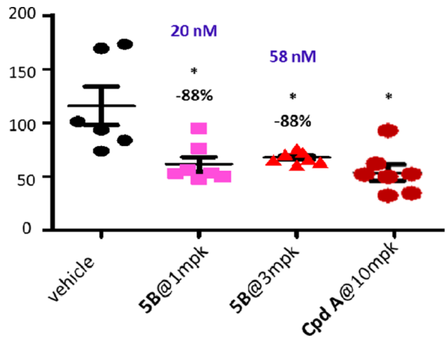


Figure 4. Compound **5B** reduces lipid excursion in rat LTT.

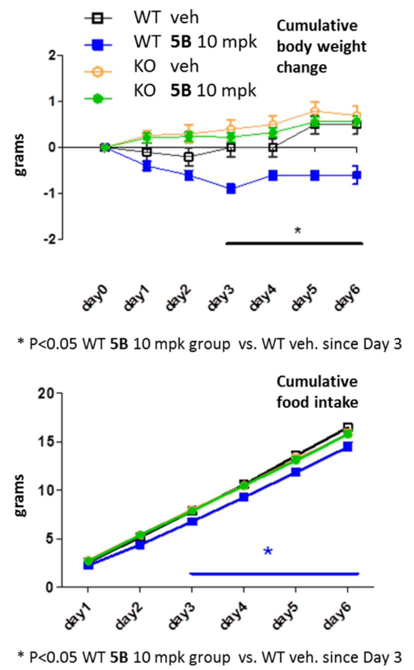


Figure 5. Effect on body weight and food intake of **5B** in DGAT1 KO vs WT mice.

body weight gain and food intake significantly in WT mice as compared to vehicle treated groups starting from day 3, while the body weight and food intake of **5B** treated KO mice were indistinguishable from the vehicle treated group. This result strongly suggests that the reduction of body weight and food intake was due to the inhibition of DGAT1.

During the profiling of **5B**, there was some concern around the possible mutagenicity due to the presence of a benzimidazole moiety in the structure.[37] Initial in silico prediction by MultiCASE and DEREK indicated that the probability of mutagenicity was low.[38] Indeed, **5B** was tested and shown to be negative in a 3-strain microbial mutagenesis assay. Over a concentration range of 30 to 5000 μg/plate, **5B** did not produce any 2-fold or greater increases in revertants relative to the solvent control in any of the three strains tested (TA1535, TA98, and TA100), either with or without metabolic activation. This result offered us more confidence on the overall potential for this structure class.

With the increasing interest in **5B** as a potential development candidate, **5B** was screened against a panel of more than 100 biological targets (receptors and enzymes). In this set of assays, 5B showed an excellent selectivity profile with IC_{50} >10 μM against all targets except A_{2A}.

As **5B** was extensively profiled as a potential development candidate, **1A** in the pyridyl linked series was shown to isomerize to its trans isomer in vivo. Immediately, **5B** was evaluated for this possibility. In a hepatocyte incubation study, **5B** does isomerize but at different levels across the species (Table 2). In dog and monkey, the isomerization was minimal, while in mouse and human there was more isomerization. The isomerization was most pronounced in rat hepatocytes. As a comparison, the isomerization from **5A** (the cis isomer) to *trans*-**5B** occurs more extensively in rat and human.

Table 2. Conversion of 5B to 5A and Vice Versa in Hepatocyte Incubation[a]

conversion of **5B** (parent) to **5A** (metabolite)	
species	**5A** peak area as % of **5B**
mouse	3.3%
rat	34.6%
dog	0%
monkey	0%
human	5.7%
conversion of **5A** (parent) to **5B** (metabolite)	
species	**5B** peak area as % of **5A**
rat	67.0%
human	26.1%

[a]Compound was added with final concentration of 10 μM into hepatocytes diluted to 1×10^6 cell/mL in a buffer. The incubation was performed at 37 °C for 120 min. The plates were centrifuged, and the supernatant was analyzed by LC–MS/MS.

In addition to the in vitro hepatocyte incubation study, **5B** was also tested for its in vivo pharmacokinetics in rat and dog (Table 3). Consistent with the in vitro data, about half of *trans*-**5B** was converted to the cis isomer **5A** in rat in vivo as indicated by the AUCs in plasma. Less isomerization occurred in dog (~5%).

Table 3. Pharmacokinetic Data for 5B in Rat and Dog[a,b]

PK parameters	rat	dog
F (%)	8	126
Cl (mL min^{-1} kg^{-1})	5.82	88.9
Vdss (L kg^{-1})	0.19	4.54
$t_{1/2}$ (h)	0.91	1.16
oral dose (mg kg^{-1})	2	4
AUC (μM·h) **5B**	1.00	1.94
AUC (μM·h) metabolite **5A**	0.97	0.11

[a]Compound **5B** was dosed in Sprague–Dawley rats as a solution in EtOH/PEG400/water (10:50:40) at 1 mg/kg, iv, and 2 mg/kg, po. [b]Compound **5B** was dosed in beagle dogs as a solution in 30% captisol (pH 8) at 1 mg/kg, iv, and in 0.5 methylcellulose at 4 mg/kg, po.

Finally, **5B** was dosed orally in mice to determine the distribution in various tissues of interest (Table 4). Considering the isomerization of **5B** to **5A**, the drug levels of **5A** were also measured. Compound **5B** showed much higher drug levels in liver and small intestine compared with the concentrations in plasma at both 3 and 24 h time points. The level of **5B** in skin is much lower compared to liver and intestine, but still high compared with the IC_{50} on DGAT1 (17- and 7-fold over mouse DGAT1 IC_{50} at 3 and 24 h, respectively). The tissue distribution of **5A** (the metabolite of **5B** from isomerization) tracks that of **5B** albeit at lower concentrations. On the basis of this study, **5B** demonstrated favorable distributions in the target organs (liver

Table 4. Mouse Tissue Distribution after Oral Dosing of 5B[a]

tissue	concentration of **5B** (μM)		concentration of **5A** (μM)	
	3 h	24 h	3 h	24 h
plasma	0.324	<0.01	0.054	<0.01
liver	21.6	4.02	6.91	0.672
intestine (small)	36.6	0.711	5.026	0.076
fat (adipose)	0.237	<0.01	0.021	<0.010
skin	0.404	0.167	0.046	0.018

[a]Compound **5B** was dosed in C57BL/6 mice as a solution in EtOH/PEG400/water (10:50:40) at 10 mg/kg, po.

and small intestine). However, **5B** is present in the skin at a significant concentration. Furthermore, the isomerization of **5B** to an active metabolite (**5A**) could complicate the development process.[39] Therefore, although there were many desirable attributes associated with **5B**, the profiling of **5B** as a potential development candidate was discontinued.

In summary, we have described the discovery of a novel series of DGAT1 inhibitors in the benzimidazole class with a piperdinyl-oxy-cyclohexanecarboxylic acid moiety. This series of compounds abolish the ACAT1 off-target activity associated with the earlier pyridyl-oxy-cyclohexanecarboxylic acid series. Meanwhile, the liability of A_{2A} binding affinity is significantly reduced by the introduction of the piperidinyl linker. The series shows improved aqueous solubility and higher free fraction in plasma. The most interesting compound in this series to date, **5B**, was shown to possess an excellent selectivity profile by screening it against a panel of more than 100 biological targets. In addition to its favorable in vitro profile, **5B** significantly reduced lipid excursion in LTT assays in mouse and rat, respectively. In a WT vs DGAT1 KO mouse study, **5B** demonstrated DGAT1 mediated reduction in food intake and body weight. Upon oral dosing in mice, **5B** seemed to distribute in liver and small intestine preferentially. Furthermore, the risk of mutagenicity of **5B** was low based on a 3-strain Ames test. All the exciting data collected for **5B** helped us to make a critical decision to deprioritize the previous pyridyl linked series and instead focus on this new series. Additional efforts focusing on addressing cis/trans isomerization issue and further reducing the distribution of the DGAT1 inhibitor in skin while maintaining other desirable attributes will be disclosed in the future.

ASSOCIATED CONTENT

Supporting Information

Syntheses and characterization data for the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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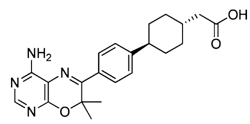

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