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Letter

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Successful strategies for mitigation of a preclinical signal for phototoxicity in a DGAT1 inhibitor

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ABSTRACT: Diacylglycerol O-acyltransferase 1 (DGAT1) inhibitor Pradigastat (**1**) was shown to be effective at decreasing postprandial triglyceride levels in a patient population with familial chylomicronemia syndrome (FCS). Although pradigastat does not cause photosensitization in humans at the high clinical dose of 40 mg, a positive signal was observed in preclinical models of phototoxicity. Herein we describe a preclinical phototoxicity mitigation strategy for diarylamine containing molecules utilizing the introduction of an amide or suitable heterocyclic function. This strategy led to the development of two second-generation compounds with low risk of phototoxicity, disparate exposure profiles and comparable efficacy to **1** in a rodent lipid bolus model for post prandial plasma triglycerides.

KEYWORDS: *DGAT1, diacylglycerol acyltransferase inhibitor, phototoxicity, pradigastat, diaryl amines, triglycerides*

The accumulation of abnormal levels of triglyceride (TG) in circulation and in tissues is associated with common diseases like obesity and cardiovascular disease as well as rare diseases such as familial chylomicronemia syndrome (FCS).[1,2,3] DGAT1 plays a critical role in postprandial absorption of dietary fat catalyzing the terminal and committed step in TG synthesis in enterocyctes of the gut wall.[4,5,6] Mice lacking DGAT1 are long-lived[7] and protected from diet-induced obesity[8], diabetes[9] and atherosclerosis[10] which is largely believed to be a consequence of delayed lipid absorption in the intestine[11]. Pradigastat (**1**) was developed as a novel DGAT1 inhibitor for the treatment of cardiometabolic diseases and FCS and has been shown in this patient population to decrease fasting and postprandial TG levels by reducing the rate of chylomicron-TG secretion.[12]

Drug induced phototoxicity is an acute, usually cutaneous, adverse reaction which potentially limits therapeutic use.[13,14] This does not only apply to topically applied chemicals absorbing ultraviolet (UV) and/or visible (vis) light, but also to those that reach light-exposed tissues such as skin (or in some cases, eye) following systemic exposure. Compounds that absorb sufficiently within sunlight range (290-700 nm) have the potential to induce a phototoxic response[15,16] and often require further evaluation (usually, if the molar absorptivity or molar extinction coefficient, MEC, exceeds 1000 $Lmol^{-1}cm^{-1}$).[17] For regulatory purposes, the in vitro 3T3-Neutral Red Uptake (NRU) Phototoxicity Test is typically used.[18] Compounds that demonstrate phototoxicity in this in vitro assay (Photo-Irritation Factor, PIF, above 5)[18] may be further studied in vivo using the murine photo-Local Lymph Node Assay (photo-LLNA).[19] If necessary, photosensitivity risk may be further assessed in clinic.

Pradigastat is highly exposed in plasma and has a long $t_{1/2}$ in humans (125 h)[20,21]. As a result of the UV/vis absorption (maxima at 310 nm and 330 nm; MEC, for both: ~30,000 $Lmol^{-1}cm^{-1}$), pradigastat was profiled in both in vitro and in vivo preclinical models and demonstrated the potential to induce phototoxicity. Pradigastat did not, however, induce any clinically relevant photosensitization at the high clinical dose of 40 mg per day in a dedicated clinical photosensitivity study and it can be used without the need for sun-protective measures.[22]

Due to the potential concern over the observed preclinical signal for phototoxicity, follow-on efforts were initiated prior to the read-out of the clinical photosensitization study with pradigastat. The proposed compound would be free from an in vitro signal for phototoxicity in the 3T3-NRU assay (PIF below 5), would maintain an efficacy profile comparable to

pradigastat and would offer diversification to guard against unforeseen issues in late stage development.

The strong UV/vis absorption of **1** was hypothesized to be derived from an efficient chromophore defined by the extended conjugation of the A, B, C-aryl ring system. Initial efforts focused on breaking up that conjugation (Figure 1). Saturation of the central B-ring pyridine successfully abrogated the phototoxic signal in the 3T3-NRU assay, but also completely eliminated activity for DGAT1 inhibition.

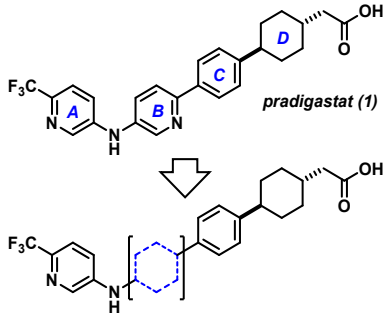


Figure 1. Initial strategy for abolishing phototoxic potential from pradigastat scaffold

As a subsequent strategy, a new hypothesis was developed which centered around blocking the known photodegredation/photocyclization of diarylamines[23,24], which has been utilized in the photochemical synthesis of complex carbazoles.[25] To the extent that pradigastat could undergo a photo-decomposition via a similar pathway (Figure 2), we hypothesized that masking the available ortho-CH units in a suitable heterocycle may be an effective means to block the putative photocyclization event. We proposed that an oxazole or oxadiazole could work in that regard.

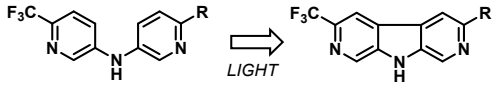


Figure 2. Proposed photodecomposition of predigastat via diaryl amine photocyclization

Pradigastat analogue **2** was available from our internal archive and the benzoxazole ring provided an initial test of the hypothesis. We were excited to see that although this modification to the scaffold did not abolish the in vitro phototox signal, it did provide an improvement in observed phototoxic potential in the 3T3-NRU assay relative to pradigastat (Figure 3).

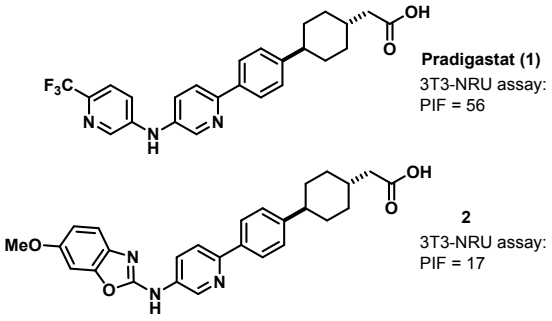
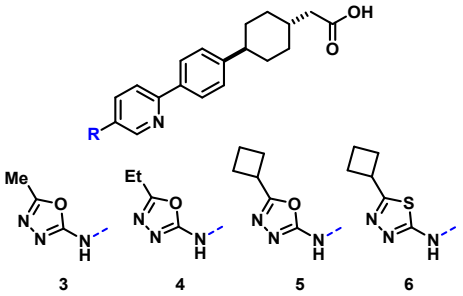


Figure 3. Benzoxazole **2** improves phototoxic potential in 3T3-NRU assay.

Although the benzoxazole offered a moderate improvement to the signal for phototoxicity and lent support to our hypothesis, a fused bicyclic aromatic system also introduces additional conjugation to the molecule and could carry an inherent phototoxic risk. Existing SAR suggested that the extended aromaticity at the A-ring was not necessary and the benzoxazole ring was subsequently deconstructed.

Substituted oxadiazoles were investigated as potential benzoxazole replacements where it was found that 1,3,4-oxadiazoles offered superior metabolic stability over 1,2,4-oxadiazoles in liver microsomes. A series of alkyl-substituted 1,3,4-oxadiazoles were prepared in order to probe the impact on phototoxicity in the 3T3-NRU assay (Table 1).

Table 1. In vitro data for compounds 1 and 3-6



Compound	DGAT1 IC_{50} (nM)[a]	Cell EC_{50} (nM)[b]	PIF[c]
1	**55±50 (n=377)**	**71 ±55 (n=17)**	**56**
3	**540 (n=1)**	**206 (n=1)**	**NT**
4	**150±79 (n=7)**	**27 ±15 (n=3)**	**NT**
5	**41 ±20 (n=6)**	**12 ±6 (n=3)**	**1**
6	**9±7 (n=2)**	**8 (n=1)**	**22**

a) DGAT1 membrane preparation assessing conversion of diolein to triolein by LC/MS/MS; b) DGAT1 inhibitor effect on cellular triglyceride production with intact C2C12 cells (see supporting info); c) PIF from 3T3-NRU assay = $IC_{50\ -irradiation}/IC_{50\ +irradiation}$; chlorpromazine-HCl used as positive control in 3T3-NRU assay (with PIF>6); NT=not tested; n=number of experiments

Alkyl substitution on the oxadiazole ring appears to be well tolerated whereby it was found that alkyl groups larger than methyl are generally required for optimal potency. Larger substituents such as cyclobutyl or tertiary-butyl (*not shown*) offered good potency and did not show signs of phototoxicity in the 3T3-NRU assay (PIF=1), further supporting the original hypothesis. Thiadiazaole analogue, **6**, was also prepared and while it maintained good DGAT1 potency in vitro, a risk of phototoxicity was observed in the 3T3-NRU assay (PIF=22).

Our previously reported benzimidazole series of DGAT1 inhibitors[26,27] demonstrated an amide function as a suitable linker between the A and B rings and we hypothesized that an amide linker could also be sufficient to prevent the putative photocyclization event.

Introduction of an oxazole amide A-ring in **7** (Figure 4) proved effective at maintaining DGAT1 potency while removing the phototoxicity signal in the 3T3-NRU assay.

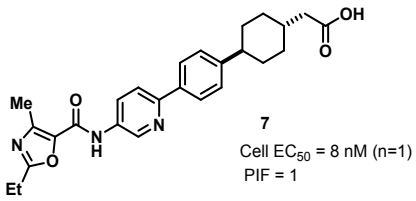
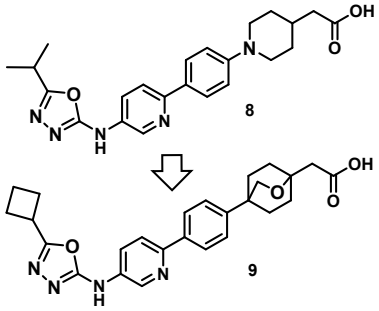


Figure 4. Amide function of Compound **7** is tolerated and non-phototoxic in vitro

With the identification of A-ring modifications that could lead to elimination of the in vitro phototoxicity signal, as in **5** and **7**, we wanted to confirm that this strategy was consistent across alternative C- and D-ring combinations in order to guard against unexpected issues that could emerge in late stage development. Internal SAR suggested that the C ring was less amenable to modification and in the interest of diversifying away from the pradigastat C,D-ring system, we instead focused efforts on heteroatom insertion into the D-ring. Saturated nitrogen heterocycles at the D-ring position, as in piperidine analogue, **8**, resulted in substantial loss of activity in most cases. Oxygen-containing heterocycles were considered as suitable alternatives and an oxabicyclooctane ring system (**9**, Table 2) was eventually identified.[28]

Table 2. In vitro data comparison for compounds 1, 5, 8, 9



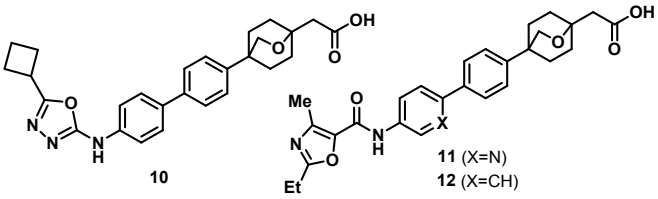
Compound	DGAT1[a] IC_{50} (nM)	Cell[b] EC_{50} (nM)	PSA (Å^2)	$logD_{7.4}$	PAMPA $logP_E$ pH6.8	pKa
1	55 ±50 (n=377)	71 ±55 (n=17)	75	3.6	-5	4.1
5	41 ±20 (n=6)	12 ±6 (n=3)	101	2.8	-5.8	NT
8	12600±5900 (n=2)	2300 (n=1)	104	0.7	< -6.7	3.8
9	120 ±28 (n=2)	413 ±120 (n=2)	110	1.5	-7.1	3.9

a) DGAT1 membrane preparation assessing conversion of diolein to triolein by LC/MS/MS; b) DGAT1 inhibitor effect on cellular triglyceride production with intact C2C12 cells (see supporting info); NT=not tested; n=number of experiments

Compound **9** showed minimal loss of potency in the biochemical assay (IC_{50} = 120 nM), but potency in the cell based assay was more significantly impacted (>30-fold less potent than **5**). The decrease in cell-based potency was attributed to reduced permeability and although changes to pKa were modest, it coincided with a reduction in $logD_{7.4}$ by 2 log units and an increase in polar surface area (PSA). Upon closer inspection of our diarylamine SAR, we realized that potent DGAT1 inhibitors in this series tended toward PSA values less than ~100 Å^2. To that end, we targeted the pyridine B-ring as an accessible means to test the hypothesis of reducing the PSA of **9** to below this perceived threshold. Removal of the pyridine nitrogen to generate biphenyl analogue **10** (Table 3) resulted in PSA = 97 Å^2, $logD_{7.4}$ = 2.4 and restored cell-based activity (EC_{50} = 12 nM).

In line with this discovery, we were pleased to find that the oxabicyclooctane ring system was tolerated with the amide linker as well, resulting in **11** (Table 3). The corresponding biphenyl modification afforded **12** with minimal change to DGAT1 potency. Importantly, both **10** and **12** remained free of phototoxic potential in the 3T3-NRU assay (PIF=1).

Table 3. In vitro data for oxabicyclooctanes 10-12

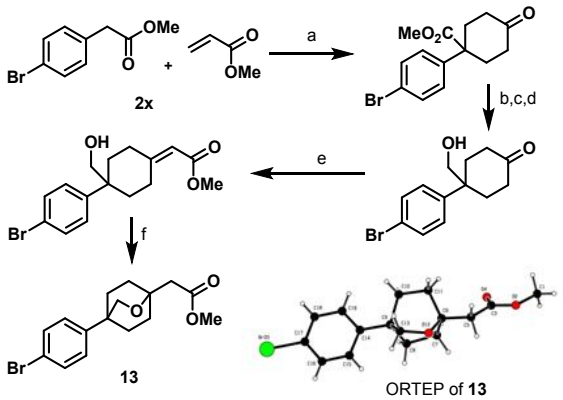


Compound	DGAT1 IC_{50} (nM)[a]	Cell EC_{50} (nM)[b]	PSA (Å^2)	PIF[c]
10	83 ±41 (n=5)	12 ±14 (n=5)	97	1
11	390 ±230 (n=3)	98 ±17 (n=2)	115	NT
12	540 ±170 (n=10)	56 ±49 (n=2)	102	1

a) DGAT1 membrane preparation assessing conversion of diolein to triolein by LC/MS/MS; b) DGAT1 inhibitor effect on cellular triglyceride production with intact C2C12 cells (see supporting info); c) PIF from 3T3-NRU assay = $IC_{50\ -irradiation}/IC_{50\ +irradiation}$; chlorpromazine-HCl used as positive control in 3T3-NRU assay (with PIF>6); NT=not tested; n=number of experiments

In order to access oxabicyclooctane containing analogues such as **10** and **12**, the synthetic strategy utilized key intermediate **13** which was built up from methyl 2-(4-bromophenyl) acetate. A double Michael-Dieckmann cyclization-decarboxylation sequence (Scheme 1) was used to construct the corresponding cyclohexanone.[29] Overall reduction of the resulting methyl ester to the neopentyl alcohol followed by Horner-Wadsworth-Emmons olefination afforded the desired substrate for the intramolecular oxa-Michael addition, which proceeded smoothly with base to give key bromide intermediate **13**.[28] The structure of the oxabicyclooctane ring system was confirmed in the solid state by X-ray crystallographic analysis (ORTEP of **13** shown in Scheme 1).[30]

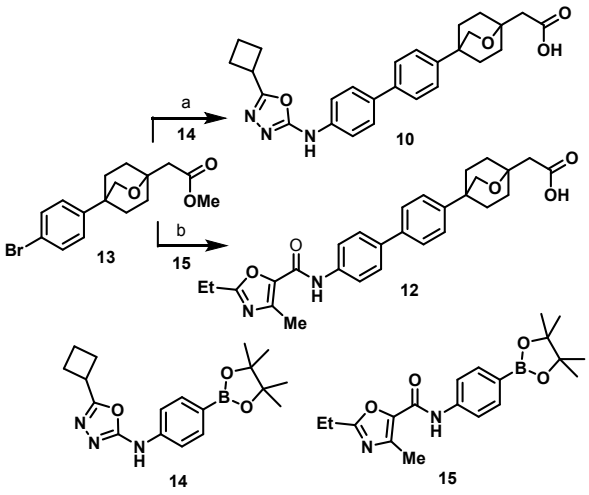
Scheme 1. Assembly of oxabicyclooctane intermediate 13



Reagents and conditions: (a) i) KO*t*Bu, THF ii) H_2O, 85 °C, 21 h, 59%; (b) toluene, ethylene glycol, toluenesulfonic acid, 80 °C, 2 h; (c) DIBAl-H, dichloromethane, -78 °C, 30 min; (d) acetone, H_2O, toluenesulfonic acid, 75 °C, 1 h; (e) trimethyl phosphonoacetate, NaH, MeOH,rt, 18 h 86% over 4 steps; (f) NaH, 1,4-dioxane, 100 °C, 30 min, 78 %.

Intermediate **13** was coupled to either arylaminophenyl boronic ester **14** or to amidophenylboronic ester **15** to afford final compounds **10** or **12** after methyl ester saponification, respectively (Scheme 2).

Scheme 2. Elaboration of oxabicyclooctane intermediate 13 into 10 and 12



Reagents and conditions: (a) i) **14**, Pd(amphos)Cl_2, CsF, 1,4-dioxane-H_2O, 90 °C, 15 h, 88%; ii) LiOH, THF-methanol-H_2O, 40 °C, 5 h, 73% (b) **15**, Pd(amphos)Cl_2, CsF, 1,4-dioxane-H_2O, 90 °C, 18 h, 58%; ii) LiOH, THF-methanol-H_2O, rt, 18 h, 87%.

In general, both the oxadiazole and amide head groups offered solutions to the issue of in vitro phototoxicity. The amide head group resulted in high permeability, low in vivo clearance in rat and high exposures, consistent with the pradigastat profile. The oxadiazole **10**, by comparison, afforded lower exposures in a rat pharmacokinetic (PK) study (Table 4).

Table 4. Pharmacokinetic data for 1, 10 and 12 in rat

Compound	1	10	12
Solubility $pH_{6.8}$ (μM)	**<5**	**<5**	**13**
Caco-2: A-B (x 10^{-6} cm/s)	**8.1**	**5.9**	**18.3**
Caco-2: B-A (x 10^{-6} cm/s)	**2.3**	**11.8**	**7.7**
B-A/A-B ratio	**0.28**	**2**	**0.4**
Plasma protein binding (Rat, %)	**99.7**	**>99**	**>99**
IV dose, n = 2 (mg/kg)	**1[a]**	**0.5[b]**	**0.5[c]**
$AUC_{0\text{-}24h}$ (nM*h)	**46000**	**5160**	**51400**
CL (mL/min/kg)	**1**	**3.5**	**0.2**
V_d (L/kg)	**0.1**	**0.7**	**0.3**
$T_{1/2}$ (h)	**5.1**	**3.2**	**20**
PO dose, n = 3 (mg/kg)	**5[a]**	**1.5[b]**	**1.5[c]**
$AUC_{0\text{-}24h}$ (nM*h)	**190000**	**25800**	**180000**
C_{max} (nM)	**42000**	**6096**	**28038**
T_{max} (h)	**0.7**	**1**	**3**
F (%)	**83**	**>100**	**>100**

[a]Vehicle (**1**): 5% NMP, 5%Captisol, 50mM Tris buffer; [b]Vehicle (**10**): 4% 1N NaOH, 20% PEG300, 50% of 20%Cremaphore EL, 50 mM Tris buffer pH 7.4; [c]Vehicle (**12**): 2% 1N NaOH, 20% PEG300, 50% of 20%Cremaphore EL, 50 mM Tris buffer pH 7.4 and 2N HCl for pH adjustment

In order to understand the efficacy profile of **10** and **12** in vivo, the compounds were studied in rats subjected to an acute oral lipid challenge to observe effects on postprandial triglycerides. When administered at 5 mg/kg, 4 hours prior to lipid challenge, both compounds (**10** and **12**) provided significant blunting of the corresponding plasma triglyceride excursion (Figure 5), demonstrating efficacy comparable to pradigastat from a related study (while not compared head-to-head with compounds **10** and **12**, in separate rat studies pradigastat similarly reduces TG excursions after a lipid challenge by ~50-70%, consistent with what is observed in human[20]).

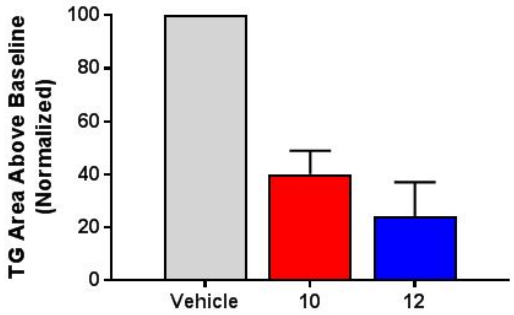


Figure 5: Rat lipid bolus data for **10** and **12**; 5 mg/kg PO administration (suspension, oral gavage) 4 h prior to lipid challenge

Consistent with rodent PK studies, exposure of **12** over the course of the study was high in comparison to the moderate exposure observed for **10** (Figure 6). Despite the differences in exposure, comparable blunting of the triglyceride excursion was observed in the rodent efficacy model, which is consistent with efficacy driven by gut exposure. Adipose tissue specific deletion of DGAT1 decreases fat mass and increases energy expenditure suggesting that high systemic exposure of DGAT1 inhibitors like amide **12** may be beneficial.[31, 32] However, in these same studies, loss of DGAT1 in adipose tissue was reported to lead to lipotoxic stress suggesting that lower systemic exposure as observed with oxadiazole **10** may be desired to minimize lipotoxicity.

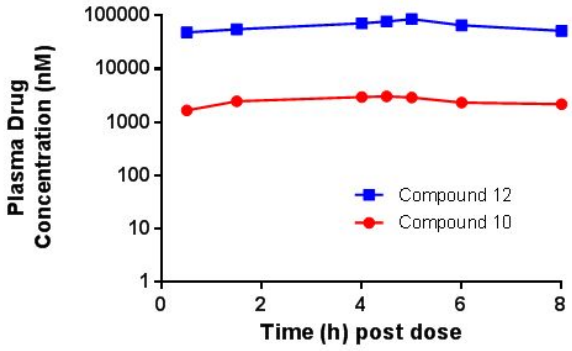


Figure 6: Compound **10** and **12** plasma exposures in rat lipid bolus study

Neither **10** nor **12** showed a worsened GI tolerability profile in preclinical models when compared to pradigastat.[33] DGAT1 inhibitors as a class, however, have struggled with GI tolerability and all DGAT1 inhibitors tested in clinic to date show GI tolerability limitations.[34] This is believed to be an on-target effect and is consistent with human genetics.[35] Until a mitigation strategy for the GI side effects is known, DGAT1 inhibitors will possess an uncertain future for the treatment of cardiometabolic disease.

In summary, two second-generation compounds have been identified from the pradigastat scaffold. Candidate molecules **10** and **12** have no observable preclinical signal for phototoxicity, have a comparable GI tolerability profile to pradigastat and retain full efficacy in a rodent lipid bolus model for postprandial plasma triglycerides.[36] In addition, the two compounds identified offer disparate PK profiles with respect to exposure and half-life as well as some structural diversification relative to pradigastat. Importantly, it was found that masking of the A-ring ortho-hydrogens of the diaryl amine with a suitable heterocycle or amide function successfully removed the phototox liability and provides potential for a general solution to phototoxicity arising from the diarylamine substructure.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Synthetic Procedures, analytical data and assay protocols (PDF)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

CsF, caesium fluoride; DGAT1, Diacylglycerol O-acyltransferase 1; DIBAl-H, diisobutyl aluminum hydride; FCS, familial chylomicronemia syndrome; GI, gastrointestinal; h, hours; HCl, hydrochloric acid; KO*t*Bu, potassium *tert*-butoxide; LiOH, lithium hydroxide; LLNA, local lymph node assay; MEC, molar extinction coefficient; NaH, sodium hydride; NaOH, sodium hydroxide; NRU, neutral red uptake; NT, not tested; PAMPA, parallel artificial membrane permeability assay; PEG, polyethylene glycol; PK, pharmacokinetics; PIF, photo-irritation factor; PSA, polar surface area; SAR, structure activity relationship; TG, triglyceride; UV, ultraviolet; vis, visible.

REFERENCES

(1) Brunzell J. Deeb S. Familial lipoprotein lipase deficiency and hepatic lipase deficiency. In: Scriver CR. Beaudet AL. Sly WS. Valle D. editors. The metabolic and molecular basis of inherited disease. 8th ed. New York: McGraw-Hill Medical Publishing Division; 2001. pp 2789-816.

(2) Santamarina-Fojo, S. The familial chyolomicronemia syndrome. *Endocrinol. Metab. Clin. North Am.* **1998**, *27*, 551-567.

(3) Brahm, A.J.; Hegele, R.A. Chylomicronemia – current diagnosis and future therapies. *Nat. Rev. Endocrinol.* **2015**, *11*, 352-362.

(4) Cases, S.; Smith, S.J.; Zheng, Y.-W.; Myers, H.M.; Lear, S.R.; Sande, E.; Novak, S.; Collins, C.; Welch, C.B.; Lusis, A.J.; Erickson, S.K.; Farese, R.V., Jr. Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 13018-13023.

(5) Yen, C-L.E.; Stone, S.J.; Koliwad, S.; Harris, C.; Farese, R.V. DGAT enzymes and triacylglycerol biosynthesis. *J. Lipid Res.* **2008**, *49*, 2283-2301.

(6) Zammit, V.A.; Buckett, L.K.; Turnbull, A.V.; Wure, H.; Proven, A. Diacylglycerol acyltransferases: Potential roles as pharmacological targets. *Pharmacol. Ther.* **2008**, *118*, 295-302.

(7) Streeper, R.S.; Grueter, C.A.; Salomonis, N.; Cases, S.; Levin, M.C.; Koliwad, S.K.; Zhou, P.; Hirschey, M.D.; Verdin, E.; Farese Jr., R.V. Deficiency of the lipid synthesis enzyme, DGAT1, extends longevity in mice *Aging* **2012**, *4*, 13-27.

(8) Smith, S.J.; Cases, S.; Jensen, D.R.; Chen, H.C.; Sande, E.; Tow, B.; Sanan, D.A.; Raber, J.; Eckel, R.H.; Farese Jr., R.V. Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. *Nat. Genet.* **2000**, *25*, 87-90.

(9) Chen, H.C.; Smith, S.J.; Ladha, Z.; Jensen, D.R.; Ferreira, L.D.; Pulawa, L.K.; McGuire, J.G.; Pitas, R.E.; Eckel, R.H.; Farese Jr., R.V. Increased insulin and leptin sensitivity in mice lacking acyl CoA:diacylglycerol acyltransferase 1. *J. Clin. Investig.* **2002**, *109*, 1049-1055.

(10) Chandak, P.G.; Obrowsky, S.; Radovic, B.; Doddapattar, P.; Aflaki, E.; Kratzer, A.; Doshi, L.S.; Povoden, S.; Ahammer, H.; Hoefler, G.; Levak-Frank, S.; Kratky, D. Lack of acyl-CoA:diacylglycerol acyltransferase 1 reduces intestinal cholesterol absorption and attenuates atherosclerosis in apolipoprotein E knockout mice. *Biochim. Biophys. Acta* **2011**, *1811*, 1011-1020.

(11) Lee, B.; Fast, A.M.; Zhu, J.; Cheng, J.-X.; Buhman, K.K. Intestine-specific expression of acyl CoA:diacylglycerol acyltransferase 1 reverses resistance to diet-induced hepatic steatosis and obesity in Dgat1-/- mice. *J. Lipid Res.* **2010**, *51*, 1770-1780.

(12) Meyers, C.D.; Tremblay, K.; Amer, A.; Chen, J.; Jiang, L.; Gaudet, D. Effect of DGAT1 inhibitor pradigastat on triglyceride and apoB48 levels in patients with familial chylomicronemia syndrome. *Lipids Health Dis.* **2015**, *14* (8).

(13) Moore, D.E. Drug-induced cutaneous photosensitivity. *Drug Saf.* **2002**, *25*, 345-372.

(14) Drucker, A.M.; Rosen, C.F. Drug-Induced Photosensitivity. *Drug Saf.* **2011**, *34*, 821-837.

(15) Bauer, D.; Averett, L.A; De Smedt, A.; Kleinman, M.H.; Muster, W.; Pettersen, B.A.; Robles, C. Standardized UV-vis spectra as the foundation for a threshold-based, integrated photosafety evaluation. *Regul. Toxicol. Pharmacol.* **2014**, *68*, 70-75.

(16) Peukert, S.; Nunez, J.; He, F.; Dai, M.; Yuseff, N.; DiPesa, A.; Miller-Moslin, K.; Karki, R.; Lagu, B.; Harwell, C.; Zhang, Y.; Bauer, D.; Kelleher, J.F.; Egan, W. A method for estimating the risk of drug-induced phototoxicity and its application to smoothened inhibitors. *Med. Chem. Commun.* **2011**, *2*, 973-976.

(17) ICH Harmonized Tripartite Guideline S10 "Photosafety evaluation of pharmaceuticals" **2013**, http://www.ich.org/products/guidelines/safety/article/safety-guidelines.html

(18) OECD (2004), Test No. 432: In vitro 3T3 NRU Phototoxicity Test, OECD Guidelines for the testing of chemicals, Section 4, OECD publishing.

(19) Schümann, J.; Boudon, S.; Ulrich, P.; Loll, N.; Garcia, D.; Schaffner, R.; Streich, J.; Kittel, B.; Bauer, D. Integrated preclinical

photosafety testing strategy for systemically applied pharmaceuticals. *Toxicol. Sci.* **2014**, *139*, 245-256.

(20) Meyers, C.D.; Amer, A.; Majumdar, T.; Chen, J. Pharmacokinetics, pharmacodynamics, safety and tolerability of pradigastat, a novel diacylglycerol acyltransferase 1 inhibitor in overweight or obese, but otherwise healthy human subjects. *J. Clin. Pharmacol.* **2015**, *55*, 1031-1041.

(21) Chen, J.; Meyers, D.; Keefe, D.; Yu, J.; Sunkara, G. Clinical Pharmacokinetics of pradigastat, a novel diacylglycerol acyltransferase 1 inhibitor *J. Drug Des. Res.* **2017**, *4*, 1044.

(22) Bauer, D.; Soon, R.L.; Kulmatycki, K.; Chen, Y.; Noe, A.; Chen, J.; Dosik, J.S.; Meyers, D. The DGAT1 inhibitor pradigastat does not induce photosensitivity in healthy human subjects: a randomized controlled trial using three defined sunlight exposure conditions. *Photochem. Photobiol. Sci.* **2016**, *15*, 1155-1162.

(23) Encinas, S.; Boscá, F.; Miranda, M.A. Photochemistry of 2,6-dichlorodiphenylamine and 1-chlorocarbazole, the photoactive chromophores of dichlofenac, meclofenamic acid and their major photoproducts. *Photochem. Photobiol.* **1998**, *68*, 640-645.

(24) Görner, H. Photoinduced oxygen uptake of diphenylamines in solution and their ring closure revisited. *J. Phys. Chem. A* **2008**, *112*, 1245-1250.

(25) Hernandex-Perez, A.C.; Caron, A.; Collins, S.K. Photochemical synthesis of complex carbazoles: evaluation of electronic effects in both UV- and visible-light methods in continuous flow. *Chem. Eur. J.* **2015**, *21*, 16673-16678.

(26) Serrano-Wu, M.H.; Coppola, G.M.; Gong, Y.; Neubert, A.D.; Chatelain, R.; Clairmont, K.B.; Commerford, R.; Cosker, T.; Daniels, T.; Hou, Y.; Jain, M.; Juedes, M.; Li, L.; Mullarkey, T.; Rocheford, E.; Sung, M.J.; Tyler, A.; Yang, Q.; Yoon, T.; Hubbard, B.K. Intestinally targeted diacylglycerol acyltransferase 1 (DGAT1) inhibitors robustly suppress postprandial triglyceride. *ACS Med. Chem. Lett.* **2012**, *3*, 411-415.

(27) Nakajima, K.; Chatelain, R.; Clairmont, K. B.; Commerford, R.; Coppola, G. M.; Daniels, T.; Forster, C. J.; Gilmore, T. A.; Gong, Y.; Jain, M.; Kanter, A.; Kwak, Y.; Li, J.; Meyers, C. D.; Neubert, A. D.; Szklennik, P.; Tedesco, V.; Thompson, J.; Truong, D.; Yang, Q.; Hubbard, B. K.; Serrano-Wu, M. H. Discovery of an orally bioavailable benzimidazole diacylglycerol acyltransferase 1 (DGAT1) inhibitor that suppresses body weight gain in diet-induced obese dogs and postprandial triglycerides in humans. *J. Med. Chem.* **2017**, *60*, 4657-4664.

(28) For synthesis of compounds: Chen, X.; Ding, Y.; Duvadie, R.; Gai, Y.; Harrison, T.; Liu, Q.; Larrow, J.; Mao, J.Y.C.; Patel, S.; Ye.J. Preparation of cyclic bridgehead ethers as DGAT1 inhibitors. WO 2013160873, 2013.

(29) DeGraffenreid, M. R; Bennett, S; Caille, S; Gonzalez-Lopez de Turiso, F; Hungate, R. W.; Julian, L. D.; Kaizerman, J. A.; McMinn, D. L.; Rew, Y.; Sun, D.; Yan, X.; Powers, J.P. An efficient and scalable one-pot double Michael addition-Dieckmann condensation for the synthesis of 4,4-disubstituted cyclohexane β-keto esters. *J. Org. Chem.* **2007**, *72*, 7455–7458.

(30) See supporting information for solid-state characterization of oxabicyclooctane intermediate **13**. CCDC 1896964 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures

(31) Chitraju, C.; Mejhert, N.; Hass, J.T.; Diaz-Ramirez, L.G.; Grueter, C.A.; Imbriglio, J.E.; Pinto, S.; Koliwad, S.K.; Walther, T.C.; Farese Jr., R.V. Triglyceride synthesis by DGAT1 protects adipocytes from lipid-induced ER stress during lipolysis. *Cell Metab.* **2017**, *26*, 407-418.

(32) Chitraju, C.; Walther, T.C; Farese Jr., R.V. The triglyceride synthesis enzymes DGAT1 and DGAT2 have distinct and overlapping functions in adipocytes. *J. Lipid Res.* **2019**, advanced online publication, doi: 10.1194/jlr.M093112.

(33) Description of preclinical models for GI tolerability will be published in due course.

(34) DeVita, R.J.; Pinto, S. Current status of the research and development of diacylglycerol O-acyltransferase 1 (DGAT1) inhibitors. *J. Med. Chem.* **2013**, *56*, 9820-9825.

(35) Haas, J.T.; Winter, H.S.; Lim, E.; Kirby, A.; Blumenstiel, B.; DeFelice, M.; Gabriel, S.; Jalas, C.; Branski, D.; Grueter, C.A.; Toporovski, M.S.; Walther, T.C.; Daly, M.J.; Farese Jr., R.V. DGAT1 mutation is linked to a congenital diarrheal disorder. *J. Clin. Investig.* **2012**, *122*, 4680-4684.

(36) Compounds **10** and **12** are currently parked prior to IND-enabling studies.

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