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Brief Article

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Imidazopyridine and Pyrazolopiperidine Derivatives as Novel Inhibitors of Serine Palmitoyl Transferase

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ABSTRACT: In order to develop novel treatments for type II diabetes and dyslipidemia we pursued inhibitors of serine palmitoyl transferase (SPT). To this end compounds 1 and 2 were developed as potent SPT inhibitors in vitro. 1 and 2 reduce plasma ceramides in rodents, have a slight trend toward enhanced insulin sensitization in DIO mice and reduce triglycerides and raise HDL in cholesterol/cholic acid fed rats. Unfortunately these molecules cause a gastric enteropathy after chronic dosing in rats.

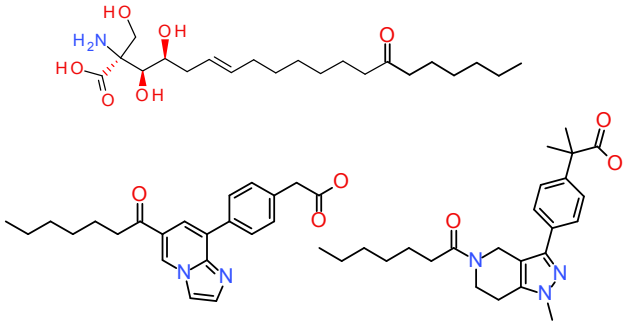
INTRODUCTION

Serine palmitoyl transferase (SPT) is a heteromeric enzyme composed of three sub-units that catalyzes the first step of the sphingolipid biosynthetic pathway leading to various ceramide species that are further metabolized to form complex sphingolipids such as sphingomyelin, glycosphingolipids, etc.[1] The various sphingolipids species are major components of plasma membranes and plasma lipoproteins and excesses have been associated with type 2 diabetes mellitus (T2DM), dyslipidemia and atherosclerosis.[2-8] For example, numerous studies have shown an association between elevated levels of plasma or tissue levels of ceramides (and other downstream ceramide metabolites, i.e. glucosyl ceramides, gangliosides) and various readouts of insulin sensitivity, such as HOMA-IR.[2,4,5]

Further, pro-inflammatory stimuli such as TNFa and IL6 which are associated with insulin resistance are also involved in the generation of ceramides. Lipid infusion and hyperglycemia are two conditions known to elevate plasma TNFa levels and also cause insulin resistance. In addition to activating de-novo synthesis of ceramides, TNFa also activates catabolic production of ceramides from sphingomyelin via induction of sphingomyelinase, which overall results in intracellular accumulation of ceramides in insulin sensitive tissues (liver, muscle, adipose).[9] Finally, plasma TNFa and intracellular ceramides are elevated in T2DM patients and ceramides have been proposed as bioactive species involved directly in mediating insulin resistance.[4]

The role of ceramides in insulin resistance and metabolic syndrome has been somewhat controversial.[5,9] However it has been proposed that the higher levels of plasma ceramides are either direct mediators of or a biomarker of insulin resistance. The exact mechanism by which elevated ceramides cause insulin resistance and dyslipidemia are unknown but ceramide has been shown to activate cellular phosphatases (PP2A) that abrogate insulin signaling via dephosphorylation of AKT.[5,9] There is also evidence that ceramides induces activation of inflammatory pathways via the nuclear factor kB (NFkB) tumor necrosis factor-a (TNFa) axis.[9]

Myriocin



1 **2**

Figure 1. Structures of SPT inhibitor Myriocin and Compounds 1 and 2.

A further complicating factor in the role of ceramides in T2DM and metabolic syndrome are the existence of multiple ceramide species of various chain lengths, and the association of these various plasma ceramides with these diseases.[2-4] Haus et al have observed that plasma ceramides of chain lengths C18:1, C18:0, C20:0, C24:1 and C24:0 are elevated in T2DM patients and these ceramide species may contribute to the severity of insulin resistance, although other evidence exists for

C16:0 being causative.[2-5,9] Insulin sensitivity (mg x kg(-1) x min(-1)) was lower in type 2 diabetic patients (4.90 +/- 0.3) versus control subjects (9.6 +/- 0.4) ($P < 0.0001$). Type 2 diabetic subjects had higher ($P < 0.05$) concentrations of C18:0, C20:0, C24:1, and total ceramide. Insulin sensitivity was inversely correlated with C18:0, C20:0, C24:1, C24:0, and total ceramide (all $P < 0.01$). Plasma TNF-alpha concentration was increased ($P < 0.05$) in type 2 diabetic subjects and correlated with increased C18:1 and C18:0 ceramide subspecies.[4]

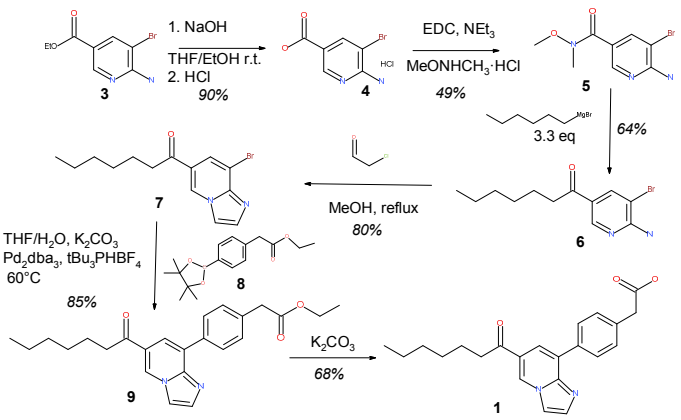
Preclinical models offer additional evidence of the importance of plasma ceramides and SPT in the development of diabetes and dyslipidemia. Although a complete knock-out of either SPT1 or 2 is embryonically lethal, heterozygous SPT-1 or -2 knock out animals are completely healthy, have circulating ceramide levels 40-50% lower than wild type littermates and exhibit resistance to high fat induced insulin resistance and dyslipidemia.[10] Moreover, pharmacological inhibition of SPT with the natural product myriocin has produced similar results in diabetic preclinical models.[5] Thus, preclinical evidence has shown that inhibition of SPT is expected to prevent the accumulation of ceramide in peripheral tissues (chiefly liver and muscle), initiated by elevations in free fatty acids or glucose, and reduce peripheral insulin resistance.[5,9] Furthermore, since ceramide has been linked to apoptosis of b-cells, inhibition of SPT may delay/prevent the progression of b-cell failure in type II diabetes.[5] For the dyslipidemic indication, preclinical mouse models of atherosclerosis have shown that inhibition of SPT was associated with reduction of lipid plaque burden in the aorta and a concomitant reduction in the concentration of multiple lipid markers associated with elevated cardiovascular risk (cholesterol, triglycerides, sphingomyelin).[6-8,10,11]

In summary, although definitive proof of a direct causal role for elevated plasma ceramides and T2DM and dyslipidemia does not exist, there is a considerable body of associative clinical and preclinical evidence which indicate a causal relationship is likely.[2,4,9]

In our efforts to develop novel and efficacious treatments for T2DM and dyslipidemia we have pursued inhibitors of SPT. Via a medium throughput screening effort, we identified novel biaryl acid chemotypes as SPT inhibitors. Several subsequent iterations of design, synthesis and property optimization yielded the imidazopyridine **1** and the pyrazolylpiperidine **2** as novel sub-structurally diverse molecules. Both **1** and **2** are potent and efficacious SPT inhibitors with good in vitro profiles and ADME characteristics.

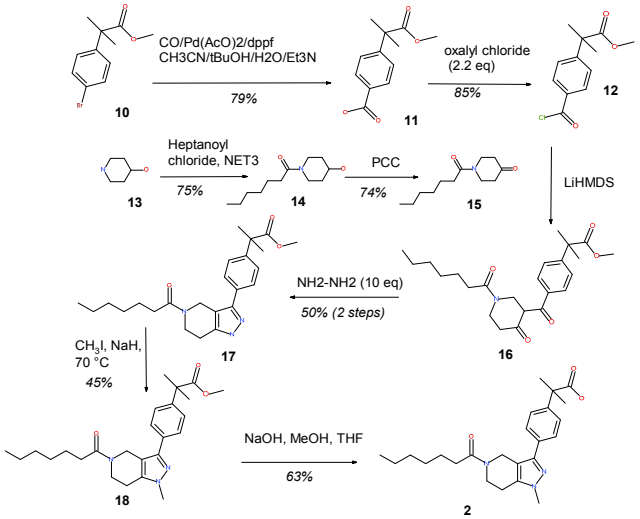
RESULTS AND DISCUSSION

Chemistry. The synthesis of the imidazopyridine compound **1** is outlined in Scheme 1. The commercially available aminopyridine carboxylate **3** was hydrolyzed to provide **4** as an HCl salt upon workup. This material was converted to the Weinreb amide **5** under standard conditions. Reaction of **5** with excess hexylmagnesium bromide provided the key intermediate **6** which when treated with chloroacetaldehyde under reflux provided the desired imidazopyridine system **7**. Coupling of **7** with the boronate ester **8** under standard conditions was followed by ester hydrolysis to yield the desired imidazopyridine SPT inhibitor **1** in six steps.



Scheme 1. Synthesis of compound 1.

Scheme 2 depicts the synthesis of the pyrazolopiperidine SPT inhibitor **2**. The known aryl bromide **10** was carbonylated to give the benzoic acid derivative **11** which was converted to the acid chloride **12** in high yield with oxalyl chloride. This material served as the key partner in the formation of the pyrazolylpiperidine **17.** Thus, treatment of the piperidinone system **15** with LiHMDS followed by **12** gave the diketo intermediate **16**. Reaction of **16** with excess hydrazine yielded the core pyrazole system **17.** Methylation of the pyrazole ring nitrogen provided penultimate intermediate **18** as the ester which upon hydrolysis provided compound **2**. The requisite intermediate piperidinone **15** was prepared via acylation of 4-piperidinol followed by oxidation as shown.



Scheme 2. Synthesis of compound 2.

Biology and Pharmacology. Compounds **1** and **2** were tested for their ability to inhibit SPT in a microsomal preparation from HEK 293 cells. Using an LC/MS detection technique, the inhibition of formation of 3-ketodeoxysphinganine (3-KDS) from serine and palmitoyl coenzyme A was measured. In this system both compounds **1** and **2** were potent inhibitors of SPT (Table 1). The primary activity of the enzyme was tested utilizing a fraction obtained from HEK293 cells as a source of SPT activity. The degree of inhibition of SPT at 10 concentrations of each compound was determined. The imidazopyridine **1** with an IC50 = 5.2 nM was more potent than compound **2**, IC50 = 63.9nM.

Table I. In vitro SPT1 inhibitory activity of 1 and 2.

Compound	hSPT1 Relative IC50 (uM)	C14-serine Ceramide incorporation MCF-7 cells Relative EC50 (uM)
Myriocin	ND	0.0495 (0.0352, n=39)
1	0.00519 (0.00260, n=9)	0.977
2	0.0639 (0.0198, n=23)	4.22 (0.460, n=3)

In addition to the biochemical assay, compounds **1** and **2** were tested in human MCF-7 cells measuring incorporation of 14C-serine into ceramides. Due to the homology between the mouse and human catalytic subunits (96% identity, 98% similarity) an in vitro rodent assay was not employed in compound evaluation. Thus, cells were treated with various concentrations of compound or control media containing DMSO, in the presence of 14C-serine and 25 uM N-(4-hydroxyphenyl)retinamide (4-HPR) that activates SPT and increases cellular ceramide levels. Following incubation for 2h, 14C-serine labeled products were quantified. Both compounds exhibited a dose-dependent inhibition of 14C-serine incorporation into ceramide. Compound **1** and **2** displayed EC50s of 0.98 uM and 4.2 uM respectively in this assay.

Compounds were then tested in vivo in three assays. First, in order to determine target engagement a PD response was measured in normal mice. Compounds with good activity were then tested in models of dyslipidemia and type II diabetes/insulin resistance. Thus, the initial assay utilized for in vivo evaluation of compounds measured the reduction in plasma ceramides in C57bl6 mice in comparison to control animals. In a dose response experiment six doses of each compound were administered to the appropriate group of mice (n=3). Concentration of several main species of ceramides (C16:0, C20:0, C22:0, C24:0, and C24:1) were determined by mass spectrometry, as described in the literature.[12] Reduction in total ceramide (sum of the aforementioned molecular species) was calculated relative to animals treated with vehicle only. At 6 hours post dose both compounds reduced plasma ceramides in a dose responsive fashion. Reflective of the in vitro activity, compound **1** was again more potent with an ED50 = 1.04 mg/kg whereas compound **2** had an ED50 = 8.8 mg/kg (Figure 2).

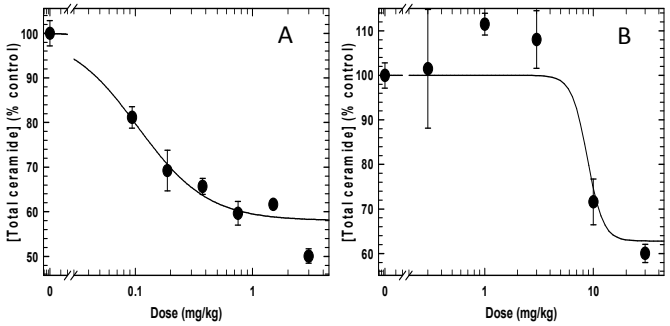


Figure 2. Dose-dependent effect of compounds 1 and 2 on plasma ceramides 6 hours after a single oral dose. Panel A compound 1, Panel B compound 2. The values of ED_{50} were 1.04 and 8.8 mg/kg, respectively. Filled circles represent mean value, error bars represent SEM (n=6).

In light of the positive pharmacodynamic properties, compounds **1** and **2** were evaluated for their effects on plasma lipids in a model of dyslipidemia. The in vivo impact of SPT inhibition on lipoprotein metabolism was performed in Sprague Dawley rats that had been fed a cholic acid/cholesterol diet for 14 days. This assay measures reduction in plasma ceramides, increase in HDL as well as effects on triglycerides, cholesterol, vLDL and apolipoproteins in comparison to vehicle control animals. Five doses of each compound were administered to the appropriate group of rats (n=6) BID for 7 days. Similar to what was observed in mice, both compounds robustly lowered total plasma ceramides albeit at higher doses (Figure 3).

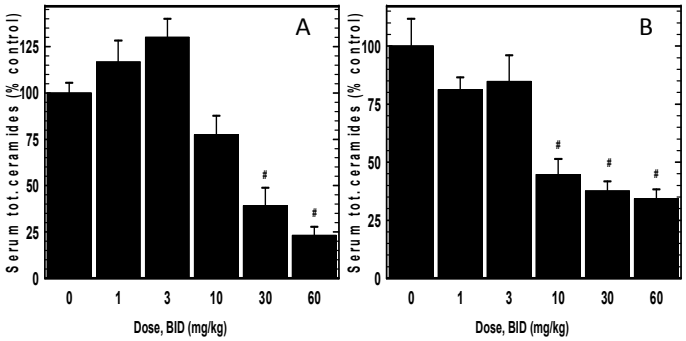


Figure 3. Dose dependent reduction of serum ceramides in rats on cholic acid/cholesterol diet, following one week of oral administration of of compounds 1 and 2. Panel A compound 1, Panel B compound 2. Filled bars represent mean value, error bars represent SEM (n=6). Hash mark indicates statistically significant ($p<0.05$) difference from control (dose 0), assessed with Dunnett's test of the means.

In addition, both compounds caused an elevation in HDL cholesterol (Figure 4). The HDL increases were dose-dependent and statistically different from the vehicle treated group for the groups treated with the two highest doses. The level of vLDL cholesterol was reduced in the group treated with the highest dose of compound **1** but compound **2** did not significantly reduce vLDL at any dose.

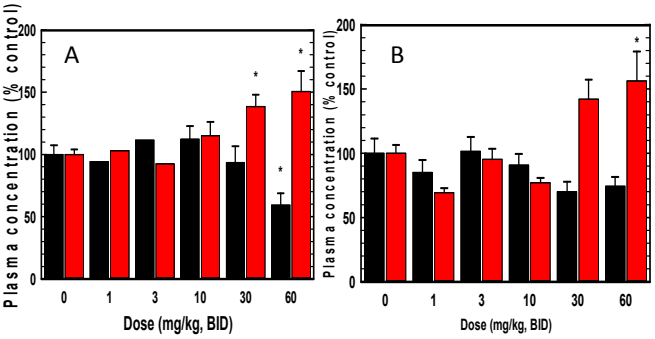


Figure 4. Dose dependent changes in serum VLDL (black) and HDL (red) cholesterol in rats on cholic acid/cholesterol diet, following one week of oral administration of 1 and 2. Panel A compound 1, Panel B compound 2. Filled bars represent mean value, error bars represent SEM (n=6). Asterisk mark indicates statistically significant ($p<0.05$) difference from control (dose 0), assessed with Dunnett's test of the means.

Both compounds were also tested for their effect on diabetes endpoints. The DIO mouse is an animal model of human insulin resistance that displays elevations in plasma ceramides, the magnitude of which are similar to those observed in humans. Additionally, the ceramide chain lengths that show significant elevations in this model (C16:0 and C18:0) are the same ceramide species which we have observed are elevated in humans with type two diabetes. Thus, inhibition of SPT in this model would be expected to show modulations of ceramide levels that would mimic changes in humans. Further, this model although not diabetic per se, displays a mild form of insulin resistance somewhat similar to that seen in humans. Therefore, testing of compounds in this model allowed us to assess effects of SPT inhibition on diabetes efficacy parameters (insulin AUC during OGTT) in a model with a phenotype similar to that of humans.

Thus, the ability of compounds **1** and **2** to lower plasma ceramides in conjunction with positive modulation of insulin AUC was assessed in a 4 week dose response study in DIO mice. Myriocin was used as a positive control and a dose-dependent reduction in plasma ceramides was noted following 28 days of oral administration for all compounds at 2 hours following the last dose (Figure 5).

Both test compounds reduced total plasma ceramides to below the levels seen in lean controls and C18:0 ceramides were reduced to lean levels at higher doses. Relative to one another, compound **2** was slightly more potent than **1** with normalization of total ceramides occurring between 1 and 3 mg/kg whereas compound 1 required a dose between 3 and 10 mg/kg to bring ceramides to normal lean levels. Likewise, myriocin also lowers ceramides at low dose (0.5 mg/kg) however it is difficult to draw direct comparisons of its in vivo effects vs 1 and 2 due to its lack of selectivity and activity at the Edg-1 (S-1-P) receptor.[13,14]

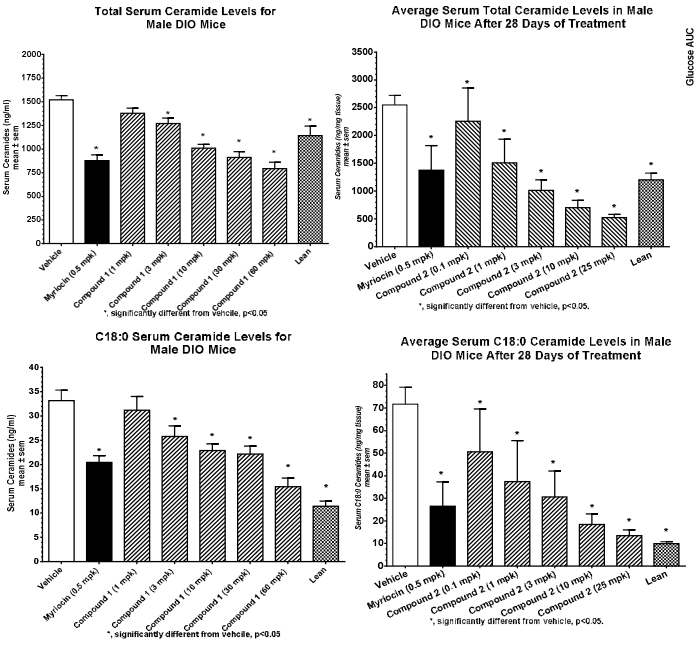


Figure 5. Total and C18:0 plasma ceramide changes for compounds 1 and 2 after 4 weeks of oral administration at 2 hours post the final dose in DIO mice.

At the end of the 4 week dosing period DIO mice were subjected to an oral glucose tolerance test to investigate changes in insulin sensitivity. A characteristic reduction in the area under the curve (AUC) for insulin, but not glucose, as compared to lean mice as shown in Figure 6 was observed. Although compound **1** did not produce a significant reduction in insulin, there was a significant trend towards lowering of the AUC as determined by ANOVA trend analysis. Interestingly compound 2 did significantly reduce insulin in this model and it also had an effect on glucose at 25mg/kg although it is noteworthy that at 25 mg/kg compound **2** caused a significant decrease in food intake thereby confounding the interpretation of the OGTT results at that dose. The 10 mg/kg dose however

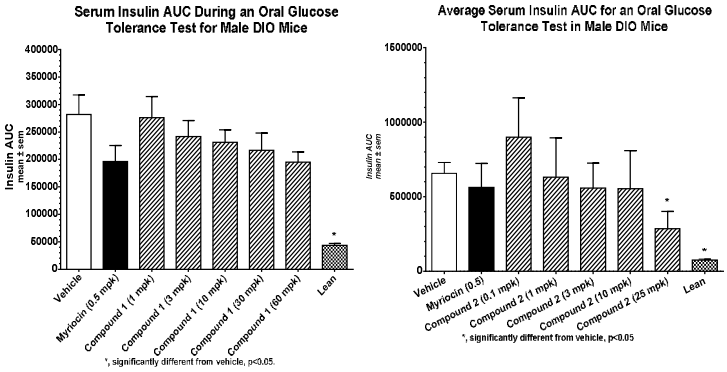


Figure 6. Insulin response during OGTT for compounds 1 and 2 after 4 weeks of oral administration in DIO mice.

did not cause a decrease in food consumption and the glucose AUC was significantly lower (Figure 7). Compound **1** did not affect food intake at any dose.

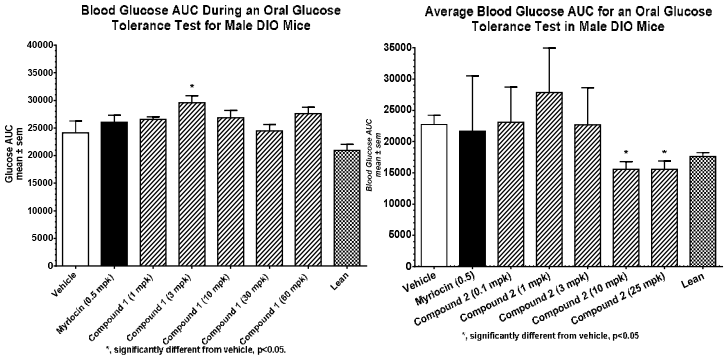


Figure 7. Glucose response during OGTT for Compounds 1 and 2 after 4 weeks of oral administration in DIO mice.

Ultimately, although both compounds reduced plasma ceramides to below lean, neither have appreciable effects on insulin sensitization. Dosing for a longer duration may eventually lead to insulin sensitization via this mechanism but that remains to be tested.

Pharmacokinetics. Pharmacokinetic parameters of compunds **1** and **2** were determined in male Sprague Dawley (SD) rats and results are detailed in Table II. Although compound **1** has higher plasma exposure and absolute bioavailability than compound **2**, the volume of distribution and half- life of **1** are lower than **2**. It was hypothesized that the enhanced Vdss and longer half-life of compound **2** may lead to better target engagement in the liver thereby enhancing the ceramide lowering properties of the molecule in vivo. Thus upon examining the liver from the DIO study above it was found that ceramide

levels in this target organ were lower in the group treated with compound **2** vs compound **1**. Animals were sacrificed 6 hours post last dose, liver and serum were analyzed for compound levels.

Table II: Pharmacokinetic Properties of compounds 1 and 2 following single oral dose in fasted male Sprague Dawley rats.

Parameter	Compound 1		Compound 2	
Route	IV	PO	IV	PO
Dose (mg/kg)	1	3	1	3
$AUC_{0\text{-}24hr}$ (ng*hr/mL)	1270	2920	190	155
Cmax (ng/mL)	2540	1500	756	16.3
T1/2 (hr)	2.13	1.48	5.17	7.65
Vdss (mL/kg)	574	1840	8330	126000
Bioavailability (%)		75 ± 6		33 ± 3

As shown in Table III, it appears that compound **2** is preferentially distributed to the liver, and it clears more slowly from liver than from blood. Additionally, there appears to be a maximal capacity for compound **2** in liver as demonstrated by liver concentrations from the 10 and 25 mg/kg dose groups. The higher volume of distribution (Vdss) of compound **2** could in part explain the increased liver distribution. It is unclear why compound **2** has such a dramatic increase in Vdss however it could be due to the fact that vs compound **1** it has increased lipophilicity (logP 4.66 vs 3.97), is slightly less acidic (pKa 4.19 vs 4.73) and has increased rat fraction unbound (0.02 vs <0.02).

Table III. Mean liver and serum concentrations of compounds 1 and 2 after 4 weeks dosing at 6 hours post last oral dose in male DIO mice.

Compound	Dose (mg/kg)	Serum (ng/mL)	Liver Conc. (ng/g)	Ratio Liver:Serum
1	1	88	463	5
1	3	264	789	3
1	10	341	2846	8
1	30	677	6585	10
1	60	3353	181260	54
2	0.1	5	26459	5292
2	1	141	61230	434
2	3	665	83764	126
2	10	855	130004	152
2	25	1209	119171	99

Conclusion. In summary, a medium throughput screen identified novel biaryl acid chemotypes as SPT inhibitors. Several subsequent iterations of design, synthesis and property optimization yielded the imidazopyridine **1** and the pyrazolylpiperidine **2** as novel sub-structurally diverse chemotypes. Both **1** and **2** are potent and efficacious SPT inhibitors with good in vitro profiles and ADME characteristics. In addition, both compounds lower plasma and liver ceramides in mice and rats, although compound **2** is preferentially distributed to the liver. Compounds **1** and **2** also favorably modulate plasma lipid profiles in rats and have a trend toward enhancing insulin sensitivity in DIO mice. In 14-day rat toxicology studies however these molecules were found to cause a gastric enteropathy that precluded further pre-clinical development. Nevertheless, they remain useful tools for the study of the effects of ceramide modulation on biological processes.

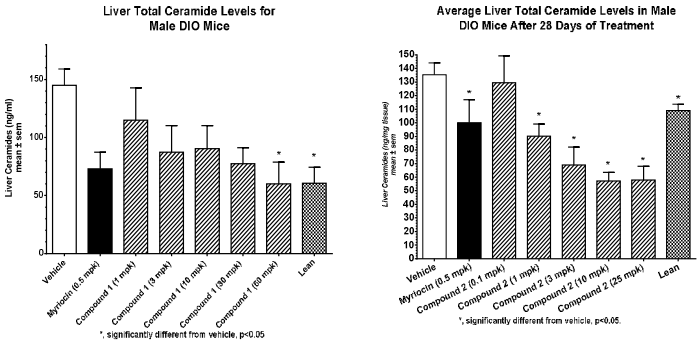


Figure 8. Liver total ceramide levels for compounds 1 and 2 after 4 weeks of oral administration in DIO mice.

Experimental Section.

Chemical Methods. All air or moisture sensitive reactions were performed under positive pressure of nitrogen with dried glassware. Chemical reagents and anhydrous solvents were obtained from commercial sources and used as is. Where necessary compounds were purified using ISCO chromatography systems. 1H NMR spectra were recorded in DMSO-d6 on a Bruker 400 MHz spectrometer. The purities of final compounds were confirmed to be greater than 95% by liquid chromatography and mass spectrometry on an Agilent 6150 series LCMS instrument with a Gemini NX 2x50 C18, 3.5 um column @ 50°C, flow 1.2 ml/min, 5-95% B gradient in 1.5 min, 0.25 min hold at 95% B; A:0.1% TFA in water; B: 0.1%TFA in CAN.

6-Amino-5-bromo-pyridine-3-carboxylic acid (**4**). 5.0 N NaOH (108.2 mmoles, 21.6 mL) is added to a room temperature suspension of methyl 6-amino-5-bromo-pyridine-3-carboxylate (21.6 mmoles, 5.00 g) in methanol (108 mL) and stirred overnight. The methanol is removed under reduced pressure, 5.0 N HCl is added and the resulting white solid is filtered to give **4** (20.5 mmoles, 4.45 g, 95%). ES/MS (m/z) (81Br) 218.9 [M+H]+.

6-Amino-5-bromo-N-methoxy-N-methyl-pyridine-3-carboxamide (**5**). Triethylamine (51.3 mmoles, 7.14 mL) is added to a room temperature suspension of **4** (20.5 mmoles, 4.45 g), N,O-dimethylhydroxylamine hydrochloride (22.6 mmoles, 2.20 g), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (30.8 mmoles, 5.90 g) and 4-N,N-dimethyl-pyridinamine (2.05 mmoles, 251 mg) in DMF (68.3 mL) and stirred overnight. The reaction is poured into saturated $NaHCO_3$ and extracted with ethyl acetate. The extracts are dried over Na_2SO_4, filtered and concentrated. The material is purified by flash chromatography eluting with 0%-70% ethyl acetate in dichloromethane over 60 minutes at 30 mL/ minute to give the title compound (8.34 mmoles, 2.170 g, 41%). ES/MS (m/z) (79Br/81Br) 260.0/ 262.0 [M+H]+.

1-(6-Amino-5-bromo-3-pyridyl)heptan-1-one (**6**). 2.0 M Hexylmagnesium bromide in ether (29.2 mmoles, 14.6 mL) is added to a -10 °C solution of **5** (8.3 mmoles, 2.17 g) in tetrahydrofuran (28 mL). The reaction is allowed to slowly warm to room temperature overnight,

quenched with saturated NH_4Cl and extracted with ethyl acetate. The extracts are dried over magnesium sulfate, filtered and concentrated. The material is purified by flash chromatography eluting with 0%-20% ethyl acetate in dichloromethane over 60 minutes at 30 mL/minute to give the title compound (6.7 mmoles; 1.91 g, 81%). ES/MS (m/z) (79Br/81Br) 285.0/ 287.0 [M+H]+.

1-(8-Bromoimidazo[1,2-a]pyridin-6-yl)heptan-1-one (**7**). Chloroacetaldehyde (50 w%) (8.4 mmoles, 1.08 mL) is added to a solution of **6** (7.0 mmoles, 2.00 g) in methanol (14.0 mL) and refluxed overnight. The reaction is concentrated, 1.0 N NaOH is added and the material is extracted with ethyl acetate. The extracts are concentrated and purified by flash chromatography eluting with 0%-20% ethyl acetate in dichloromethane over 45 minutes at 60 mL/minute gave the title compound (5.6 mmoles, 1.73 g, 80%). ES/MS (m/z) (79Br/81Br) 309.0/ 310.8 [M+H]+.

Ethyl-2-[4-(6-heptanoylimidazo[1,2-a]pyridin-8-yl)phenyl]acetate (**9**). Tris(dibenzylidineacetone)-dipalladium (0) (56.6 µmoles, 51.8 mg) and tri-t-butylphosphonium tetrafluoroborate (113.2 µmoles, 32.8 mg) are added to a degassed solution of **7** (2.3 mmoles, 700 mg), ethyl 2-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]acetate **8** (3.4 mmoles, 985 mg) and K_2CO_3 (4.53 mmoles, 626 mg) in THF (7.0 mL) and water (2.8 mL). The reaction is heated at 60°C overnight, cooled to room temperature, diluted with water and extracted with ethyl acetate. The extracts are dried over $MgSO_4$, filtered and concentrated. The material is purified by flash chromatography eluting with 0%-20% ethyl acetate in dichloromethane over 45 minutes at 30 mL/ minute to give the title compound (1.95 mmoles, 765 mg, 85%). ES/MS (m/z) 393.0 [M+H]+.

2-[4-(6-Heptanoylimidazo[1,2-a]pyridin-8-yl)phenyl]acetic acid (**1**). A solution of **9** (1.94 mmoles, 0.76 g) and K_2CO_3 (5.81 mmoles, 802 mg) in methanol (19.3 mL) and water (3.9 mL) are heated at 60°C. After 4 hours the methanol is removed under reduced pressure, saturated NH_4Cl is added and extracted with ethyl acetate. The extracts are dried over $MgSO_4$, filtered and concentrated. The material is purified by flash chromatography eluting with 0% to 10% methanol in dichloromethane over 45 minutes at 30 mL/ minute to give the title compound (1.31 mmoles, 478 mg, 68%). ES/MS (m/z) 365.0 [M+H]+ LCMS Retention time 0.945 minutes area 100.0%. H1 NMR (399.80 MHz, dmso-d6): 12.01-12.00 (m, 1H), 8.87 (d, J= 1.7 Hz, 1H), 7.56 (d, J= 1.3 Hz, 1H), 7.50-7.47 (d, J= 8.3 Hz, 2H), 7.22 (d, J= 1.7 Hz, 1H), 7.14 (d, J= 1.3 Hz, 1H), 6.81 (d, J= 8.3 Hz, 2H), 3.06 (s, 2H), 2.49-2.46 (m, 2H), 1.11-1.06 (m, 2H), 0.80-0.77 (m, 6H), 0.30-0.27 (m, 3H).

4-(2-Methoxy-1,1-dimethyl-2-oxo-ethyl)benzoic acid (**11**). Add palladium II acetate (1.072 g, 4.77 mmol), 1,1'-bis(diphenylphosphino)ferrocene (3.175 g, 5.73 mmol), anhydrous acetonitrile (270 mL), **10** (8.18 g), tert-butyl alcohol (178 mL), water (1.6 mL) and triethylamine (11.1 mL, 79.6 mmol) to a 1 L Parr autoclave with mechanical stir. Seal and pressurize to 100 psig with CO. Heat to 100 °C and stir the reaction overnight. Cool to room temperature and evaporate to near dryness. Dilute with 0.1 N NaOH and diethyl ether. Filter the mixture and collect the filtrate. Extract the aqueous phase with diethyl ether three times discarding organic phase. Adjust aqueous phase pH to 1-2 using 5.0 N HCl. Extract the mixture with ethyl acetate three times. Combine the organic phases and wash with brine. Dry the material over $MgSO_4$, filter, and concentrate to dryness to provide the title compound (25.15 mmol, 5.59 g, 79.06%). ESI (m/z) 221.0 (M-H).

Methyl-2-(4-chlorocarbonylphenyl)-2-methyl-propanoate (**12**). Mix **11** (6.75 mmol, 1.50 g), toluene (22.50 mL), and oxalyl chloride (13.50 mmol, 6.75 mL) and stir for 1 hour. Add DMF (387.98 µmol, 30.00 µL) and stir for 4 hours. Concentrate the reaction, add diethyl ether, and filter. Collect the filtrate and concentrate to dryness to provide the title compound (5.69 mmol, 1.37 g, 84.33%).

1-(4-Hydroxy-1-piperidyl)heptan-1-one (**14**). Add piperidin-4-ol (3 g, 29.66 mmol) and DMF (1.28 mol, 98.87 mL) together. Triethylamine (103.81 mmol 14.47 mL) is added at -10° C followed by the addition of heptanoyl chloride (29.66 mmol, 4.41 g) slowly over 5 min. Stir the reaction mixture at ambient temperature for 1 hour. Dilute the reaction mixture with water and ethyl acetate. Wash the organic phase with 1.0 N HCl, saturate $NaHCO_3$, water, and brine. Dry the material over $MgSO_4$, filter, and concentrate to dryness to give the title compound for use without further purification (4.77 g; 22.36 mmol, 75.39%). ESI (m/z) 214.0 (M+H).

1-Heptanoylpiperidin-4-one (**15**). Add **14** (4.77 g; 22.36 mmoles), dichloromethane (74.54 mL), and pyridinium chlorochromate (44.72 mmoles; 9.64 g) to a round bottom flask. Stir reaction mixture 7 hours. Filter reaction mixture then concentrate filtrate. Purify the residue by flash chromatography, eluting with ethyl acetate/hexane (0-100% gradient) to provide **15** (3.5 g; 16.56 mmoles; 74.08% yield) (m/z): 212.0 (M+H).

Methyl-2-[4-(5-heptanoyl-1,4,6,7-tetrahydro-pyrazolo[4,3-c]pyridin-3-yl)phenyl]-2-methyl-propanoate (**17**). Mix **15** (1.56 g, 7.40 mmol) and toluene (28.46 mL) together. Add lithium bis(trimethylsilyl)amide (7.68 mmol, 7.68 mL) at 0°C under nitrogen and stir for 1 minute. Add **12** (1.37 g, 5.69 mmol) in toluene (2.00 mL). Remove ice bath and stir for 1 minute. Add acetic acid (28.46 mmol, 1.63 mL), ethanol (56.92 mmol, 3.31 mL) and THF (85.38 mmol 6.95 mL). Add hydrazine (56.92 mmol, 1.82 g) and stir 30 minutes. Add NaOH (1.0 N) to pH 10 and extract with ethyl acetate. Combine the extracts and wash with brine, dry over $MgSO_4$, filter, and concentrate to dryness. Purify the residue by silica gel chromatography, eluting with ethyl acetate:hexane (0-100%) to provide the title compound (2.87 mmol, 1.18 g, 50.37%). ESI (m/z) 412.0 (M+H).

Methyl-2-[4-(5-heptanoyl-1-methyl-6,7-dihydro-4H-pyrazolo[4,3-c]pyridin-3-yl)phenyl]-2-methyl-propanoate (**18**). Mix **17** (2.84 mmol, 1.17 g) and THF (116.45 mmol, 9.48 mL) together under nitrogen. Add NaH (2.84 mmol, 113.71 mg) at 0 °C and stir 30 minutes. Add methyl iodide (8.53 mmol, 531.19 µL) and heat the reaction to 70 °C for 18 hours. Cool to room temperature and quench with saturated NH_4Cl and dilute with ethyl

acetate. Wash the organic phase with brine, dry over MgSO4, filter, and concentrate to dryness. Purify the residue by silica gel chromatography, eluting with 10:40:50 acetonitrile/hexanes/dichloromethane to provide the title compound (1.29 mmol, 550.00 mg, 45.46%). ESI (m/z) 426.2 (M+H).

2-(4-(5-Heptanoyl-1-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-3-yl)phenyl)-2-methylpropanoic acid (**2**). Add **18** (1.29 mmoles; 547.00 mg), THF (31.59 mmoles; 2.57 mL), methanol (63.52 mmoles; 2.57 mL), and NaOH (12.85 mmoles; 2.57 mL) to a round bottom flask sequentially. Stir 8 hours, adjust the pH to 4-5 with 5.0N HCl and extract with ethyl acetate. Combine organic phases, wash with brine and dry over $MgSO_4$. Filter, and concentrate to dryness to provide the title compound (814.01 µmoles; 335.00 mg; 63.33% yield). ESI (m/z) 412 (M+1), LCMS Retention time 1.164min area 100.0%, 1H NMR (400 MHz, DMSO-d6) δ 0.80-0.87 (m, 3 H), 1.17-1.27 (m, 6 H), 1.44-1.53 (m, 8 H), 2.36-2.43 (m, 2 H), 2.65-2.79 (m, 2 H), 3.72-3.79 (m, 5 H), 4.65 (s, 2 H), 7.41 (d, J = 8.4 Hz, 2 H), 7.55-7.61 (m, 2 H), 12.34 (br s, 1 H).

ASSOCIATED CONTENT

Supporting Information. Experimental general procedures, in vitro and in vivo pharmacology procedures and general methods. 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.

ABBREVIATIONS

SPT; serine palmitoyl transferase, T2DM; type 2 diabetes, DIO; diet induced obese, OGTT; oral glucose tolerance test, LDL; low density liproprotein, HDL; high density liproprotein, THF; tetrahydrofuran, DMF; dimethylformamide, TFA; trifluoroacetic acid

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