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Lead optimization of a pyridine-carboxamide series as DGAT-1 inhibitors

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ABSTRACT

The structure–activity relationship studies of a novel series of carboxylic acid derivatives of pyridine-carboxamides as DGAT-1 inhibitors is described. The optimization of the initial lead compound **6** based on in vitro and in vivo activity led to the discovery of key compounds **10j** and **17h**.

Obesity is a serious health risk that is characterized by an excess accumulation of triglycerides (TG) and can lead to a number of additional conditions including type 2 diabetes, atherosclerosis, hypertension, and cardiovascular disease.[1] Dietary TGs are broken down in the gut to monoacylglycerol and then absorbed in the small intestines. TGs are then reassembled with the sequential addition of two acyl chains. The final step of TG synthesis is catalyzed by the enzyme acyl CoA: diacylglycerol acyltransferase (DGAT) of which there are two forms.[2] Although both DGAT-1 and DGAT-2 are transmembrane proteins found in white adipose tissue, small intestine, liver, and mammary gland, they are from different gene families with distinctive functions. DGAT-1 knockout mice are viable, exhibit resistance to weight gain when fed a high-fat diet, have increased insulin sensitivity, and have increased leptin sensitivity.[3] In contrast, DGAT-2 has an essential role since these knockout mice are not viable due to lipopenia and skin homeostasis abnormalities.[4] DGAT-1 is a member of the acyl CoA: cholesterol acyltransferase (ACAT) gene family and is more homologous with ACAT-1 and ACAT-2 than with DGAT-2 which is more closely related to the monoacylglycerol acyl transferase (MGAT) enzymes.[2] With the resistance to weight gain and positive physiological effects seen in DGAT-1 null mice, an inhibitor of DGAT-1 may be a useful therapy for obesity.

The discovery of a selective DGAT-1 inhibitor has been the subject of a number of recent reviews and publications.[5–13] First generation DGAT-1 inhibitors from Japan Tobacco, Pfizer, Bayer, and Abbott are shown in Figure 1 and have a terminal carboxylic acid moiety which mimics the fatty acid substrate of DGAT-1.[14–17] Beginning with the non-carboxylic acid, oxazole DGAT-1 inhibitors by Hoffman-LaRoche/Via Pharmaceuticals, our exploratory chemistry group began work on developing novel DGAT-1 inhibitors.[18] Initial investigations led to the identification of the lead compound **6** with reasonable DGAT-1 inhibitory activity.

SAR optimization of compound **6** began with left hand side analogs which were synthesized according to the route depicted in Scheme 1.[19] Addition of *N*-BOC-piperazine to 2-chloro-5-nitropyridine **7** gave pyridyl-piperazine **8**. Removal of the BOC protecting group and reaction with 2-fluorophenyl isocyanate provided nitro intermediate **9**. Reduction of the nitro group by hydrogenation and HATU promoted amide formation produced the target compound **10**.

Representative examples of left hand side modifications are summarized in Table 1. From a large screen of diverse substituents, we found that the 2-piperidinyl-4-trifluoromethyl-oxazole ring could be replaced by a bicyclic indole ring in compound **10a** with increased inhibition of the human DGAT-1 enzyme in our in vitro screening assay.[20] The bicyclic benzofuran ring in compound **10b** and benzothiophene ring in compound **10c** exhibited further improvement of DGAT-1 activity. The 3-trifluoromethyl substituent appeared to be a positive factor since the hydrogen analog **10d** displayed lower activity relative to the methyl **10b**, fluoromethyl **10e**, and difluoromethyl **10f** analogs. Substitution on the phenyl ring of the benzofuran was tolerated in analogs **10g–k**.

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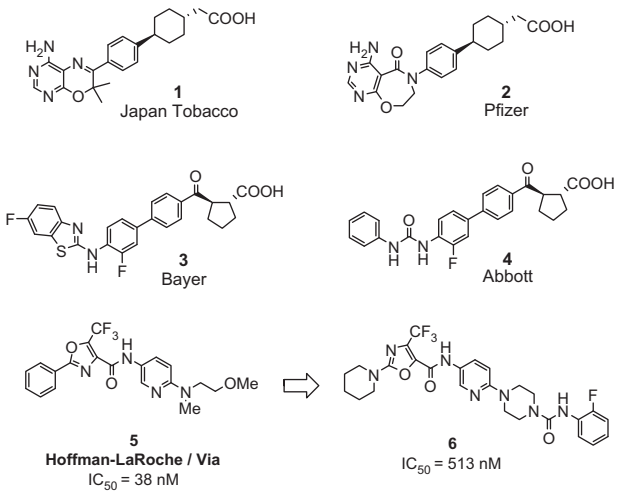
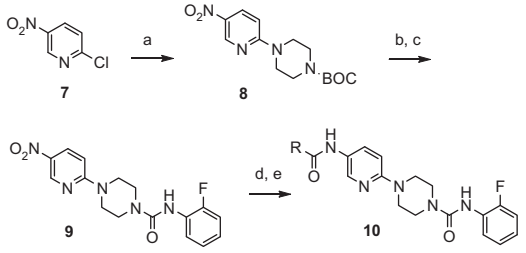


Figure 1. Selected examples of literature inhibitors for DGAT-1.



Scheme 1. Reagents and conditions: (a) *N*-BOC-piperazine, Hunig's base, DMF, 100 °C, 100%; (b) TFA, CH_2Cl_2, 100%; (c) 2-fluorophenyl isocyanate, Et_3N, THF, reflux, 100%; (d) H_2, PtO_2, iPrOH, EtOAc, 92%; (e) RCOOH, HATU, Hunig's base, DMF.

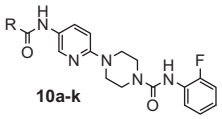
For the SAR optimization of the right hand side, it was decided to explore the inclusion of the key carboxylic acid moiety present in the original DGAT-1 inhibitors from Figure 1. Analogs were constructed by the synthetic routes outlined in Schemes 2 and 3. In Scheme 2, the methylene-oxy linker was introduced by condensation of 2-chloro-5-nitro-pyridine **7** with 4-hydroxymethyl-piperidine. Activation of alcohol **11** as the mesylate and subsequent coupling with methyl 3-hydroxybenzoate provided intermediate **12**. Standard hydrogenation of the nitro group, HATU promoted amide formation, and hydrolysis of the ester produced target **13c** and similar analogs. The reverse oxy-methylene linker was assembled by addition of 4-hydroxy-piperidine to 2-chloro-5-nitro-pyridine **7** (Scheme 3). Subsequent alkylation with methyl 3-(bromomethyl)benzoate yielded intermediate **15** which was converted to target **13e** by the usual steps.

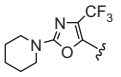
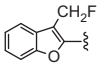
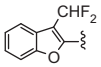
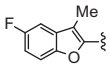
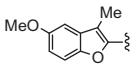
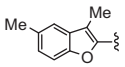
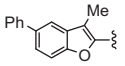
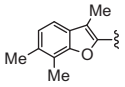
Selected biological data are collated in Table 2. Compounds which were active in the in vitro assay (IC_{50} <100 nM) were subsequently tested in the in vivo mouse postprandial triglyceride (PPTG) assay.[21] Our first discovery was that introduction of the carboxylic acid in the urea analog **13a** exhibited an increase in biological activity and a significant increase in blood levels. Replacement of the urea linker by a methylene ether linker (analogs **13b–d**) indicated a preference for the carboxylic acid at the *meta* position in **13c**. The reverse oxy-methylene linker in **13e** displayed slightly lower biological activity. Substituting the piperidine ring with a pyrrolidine ring in analogs **13f–g** or inserting a methylene linker to the carboxylic acid in **13h** did not improve inhibition of the DGAT-1 enzyme.

The central pyridine ring appeared to be important for biological activity (Table 3). Substitution by a pyrimidine ring **16a** or phenyl ring **16b–c** resulted in a decrease in DGAT-1 inhibition. These analogs were synthesized by the route depicted in Scheme 2 using 2-chloro-5-nitropyrimidine, 1-fluoro-4-nitrobenzene, or 1,2-difluoro-4-nitrobenzene as starting reagents.

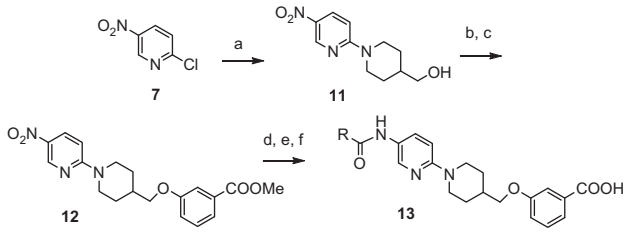
With the identified 2-pyridinyl-4-piperidinyl-methoxybenzoic acid right hand side, reoptimization of the left hand side was surveyed, and key analogs are presented in Table 4. Compound **13c** demonstrated selectivity over hDGAT-2 (IC_{50} = 1.3 μM), hACAT-2 (IC_{50} = 4.7 μM), and hACAT-1 (IC_{50} = 0.8 μM) and revealed no hERG, PXR, or liver enzyme (2D6, 3A4, and 2C9) inhibition issues.

Table 1
Replacement of oxazole left hand side in **10a–k**

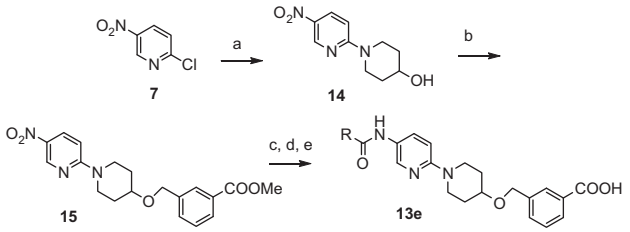


Compd.	R	hDGAT-1[a] IC_{50} (nM)
6		513
10a		190
10b		25
10c		55
10d		145
10e		60
10f		40
10g		39
10h		51
10i		42
10j		103
10k		24

[a] Assay values are the average of at least two independent determinations.

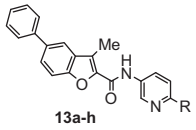


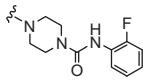
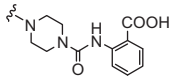
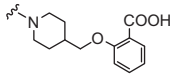
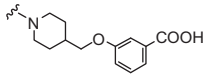
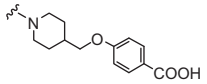
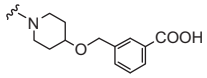
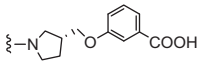
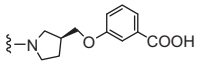
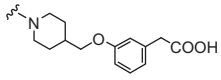
Scheme 2. Reagents and conditions: (a) 4-hydroxymethyl-piperidine, Hunig's base, DMF, 100 °C, 100%; (b) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0 °C to rt, 100%; (c) methyl 3-hydroxybenzoate, NaH, DMF, 90 °C, 78%; (d) H_2, PtO_2, iPrOH, EtOAc, 100%; (e) RCOOH, HATU, Hunig's base, DMF; (f) LiOH, H_2O, MeOH, THF, reflux.



Scheme 3. Reagents and conditions: (a) 4-hydroxy-piperidine HCl, Hunig's base, DMF, 100 °C, 97%; (b) methyl 3-(bromomethyl)benzoate, NaH, nBu_4NI, THF, reflux, 58%; (c) H_2, PtO_2, iPrOH, EtOAc, 100%; (d) RCOOH, HATU, Hunig's base, DMF; (e) LiOH, H_2O, MeOH, THF, reflux.

Table 2
Replacement of phenyl urea piperazine right hand side in **13a–h**[a]

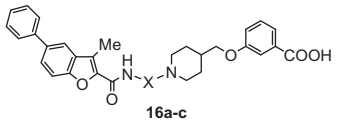


Compd.	R	hDGAT-1[a] IC_{50} (nM)	mPPTG[b] 3 mg/kg	AUC (0–6 h) μM h 10 mg/kg po
10j		103	−32%	0.44
13a		70	−51%	7.5
13b		311	NT	NT
13c		52	−64%	0.23
13d		280	NT	NT
13e		125	−47%	2.3
13f		152	NT	NT
13g		119	NT	NT
13h		120	NT	NT

NT = not tested.
[a] Assay values are the average of at least two independent determinations.
[b] $n = 8$ Mice.

Substitution of the 5-phenyl-benzofuran **13c** with the 5-phenyl-thiophene **17a** showed a slight decrease of in vivo activity. Conversion of the 5-phenyl-benzofuran **13c** to the 5-oxazole-benzofuran **17b** or 5-thiazole-benzofuran **17c** produced a decrease in DGAT-1 activity. Compound **17d** which incorporated our original piperidinyl-oxazole left hand side was not superior to the 5-phenyl-benzofuran moiety. Replacement of the 5-phenyl-benzofuran **13c** with the napthalene analog **17e** or quinoline analog **17f** also resulted in a decrease in biological activity. While the biphenyl ether compounds **17g–h** both exhibited good in vitro DGAT-1 inhibition and similar rat pharmacokinetic profiles, the *meta* substituted analog **17h** showed better in vivo activity in the mouse

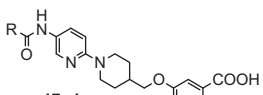
Table 3
Replacement of pyridine ring in **16a–c**

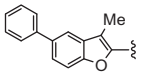
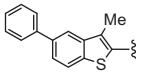
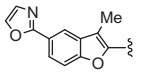
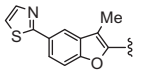
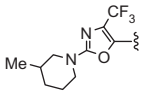
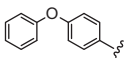
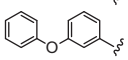
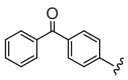
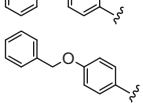
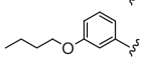


Compd.	X	hDGAT-1[a] IC_{50} (nM)
13c		52
16a		240
16b		774
16c		482

[a] Assay values are the average of at least two independent determinations.

Table 4
Replacement of benzofuran left hand side in **17a–k**[a]



Compd.	R	hDGAT-1[a] IC_{50} (nM)	mPPTG[b] 3 mg/kg	AUC (0–6 h) μM h 10 mg/kg po
13c		52	−64%	0.23
17a		33	−32%	NT
17b		344	NT	NT
17c		194	NT	NT
17d		79	−36%	4.1
17e		116	−29%	15.0
17f		460	NT	NT
17g		54	−10%	7.0
17h		29	−63%	11.2
17i		392	NT	NT
17j		149	NT	NT
17k		41	−35%	6.7

NT = not tested.
[a] Assay values are the average of at least two independent determinations.
[b] $n = 8$ Mice.

PPTG assay. Target compound **17h** was selective over hDGAT-2 (IC_{50} >50 μM), hACAT-2 (IC_{50} >40 μM), and hACAT-1 (IC_{50} = 3 μM), presented no hERG or PXR issues, but did slightly inhibit liver

enzymes 3A4 and 2C9 (IC_{50} = 5 μM). In comparison to the ether compounds **17g–h**, the keto analog **17i** and extended ether analog **17j** demonstrated reduced activity. The alkylphenyl ether **17k** displayed lower in vivo activity while retaining reasonable blood levels.

In conclusion, novel DGAT-1 inhibitors based on a pyridine-carboxamide core have been discovered. Systematic SAR investigation has optimized the original lead compound **6** into compounds **13c** and **17h**. Both compounds **13c** and **17h** exhibit selectivity for hDGAT-1, lower triglyceride levels at a 3 mg/kg dose in our in vivo mouse PPTG assay, and exhibit minimal off-target issues. Additional SAR results will be the subject of future publications.

References and notes

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20. The in vitro assay uses human DGAT1 enzyme expressed in Sf9 insect cells prepared as microsomes. The reaction was initiated by the addition of the combined substrates 1,2-dioleoyl-sn-glycerol and [14C]-palmitoyl-Co A to test compounds and microsomal membranes for 2 h of incubation at room temperature. The assay was stopped by adding 0.5 mg wheat germ agglutinin beads in assay buffer with 1% Brij-35 and 1% 3-cholamidopropyl-dimethyl-ammonio-1-propane sulfonate. Plates were sealed with TopSeal and incubated for 18 h to allow the radioactive triglyceride product to come into proximity with the bead. Plates were read on a TopCount instrument.
21. The mouse postprandial triglyceride (PPTG) in vivo assay uses C57BL/6J male mice (7 weeks old, $n = 8$) which were fasted overnight (~16 h). The mice were dosed with 20% hydroxypropyl-β-cyclodextrin vehicle or test compound in vehicle 15 min before time 0. Mice were challenged with 0.25 mL corn oil bolus at time 0 then euthanized and terminal blood was collected by heart puncture at time of 120 min. Plasma triglyceride levels were determined by colorimetric method using Wako L-Type TG M Enzyme Color assay reagents A and B (Wako Chemicals USA, Inc., 1600 Bellwood Road, Richmond, VA 23237-1326). Absorbance values were determined in an automated microplate reader at a wavelength of 600 nM.