ACS Medicinal Chemistry Letters

Letter

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Potent DGAT1 Inhibitors in the Benzimidazole Class with a Pyridyl-oxy-cyclohexanecarboxylic Acid Moiety

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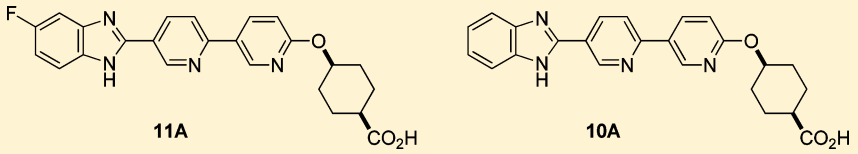
Supporting Information



ABSTRACT: We report the design and synthesis of a series of novel DGAT1 inhibitors in the benzimidazole class with a pyridyl-oxy-cyclohexanecarboxylic acid moiety. In particular, compound **11A** is a potent DGAT1 inhibitor with excellent selectivity against ACAT1. Compound **11A** significantly reduces triglyceride excursion in lipid tolerance tests (LTT) in both mice and dogs at low plasma exposure. An in vivo study in mice with des-fluoro analogue **10A** indicates that this series of compounds appears to distribute in intestine preferentially over plasma. The propensity to target intestine over plasma could be advantageous in reducing potential side effects since lower circulating levels of drug are required for efficacy. However, in the preclinical species, compound **11A** undergoes cis/trans epimerization in vivo, which could complicate further development due to the presence of an active metabolite.

KEYWORDS: *DGAT1, inhibitor, benzimidazole, ACAT1, cyclohexanecarboxylic acid, lipid tolerance test, epimerization, metabolite*

Type II diabetes mellitus (T2DM) and obesity, two interlinked disease conditions, have emerged as the major threats to human health globally.[1,2] In 2008, World Health Organization estimated the number of overweight and obese adults worldwide to be 1 billion and 500 million, respectively. In 2009−2010, more than one-third of the American population was classified as obese.[3] Meanwhile, a recent estimate indicates that there are about 371 million people worldwide living with diabetes.[4] Current drugs available to treat diabetes and obesity have limitations in terms of long-term efficacy and/or side effects.[5−7] The unmet need prompts significant research efforts in this area.

Inhibition of DGAT1 (diglyceride acyltransferase 1) has emerged as a potential mechanism for the treatment of T2DM and obesity.[8,9] The DGAT family (diglyce diglyceride acyltransferase 1 and 2) catalyzes the formation of triglyceride (TG) from diacylglycerol and acyl-CoA, the terminal and committed step in TG synthesis.[10] DGAT1 shares only limited sequence homology with DGAT2, the other known isoform.[11] In contrast, DGAT1 has more sequence homology to acyl CoA:cholesterol acyltransferase (ACAT1 and ACAT2). ACAT plays an important role in cholesterol homeostasis.[12] The strong interest in DGAT1 started after the reports on DGAT1 knockout mouse phenotyping studies. DGAT1 knockout mice were shown to be viable and resistant to diet-induced obesity.[13] Furthermore, these mice appeared to have increased sensitivity to insulin and leptin.[14] This compelling data set has inspired major efforts in identifying small molecule DGAT1 inhibitors for potential treatment of diabetes and obesity (Figure 1).[15−20]

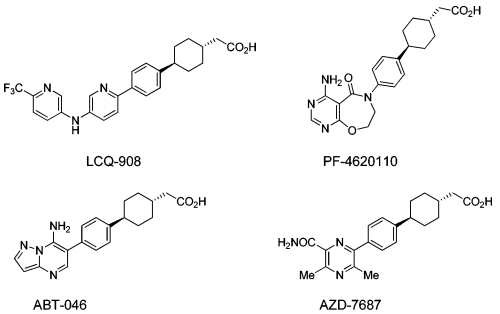


Figure 1. Structures of selected DGAT1 inhibitors.

Received: May 8, 2013
Accepted: June 6, 2013

   dx.doi.org/10.1021/ml400168h | *ACS Med. Chem. Lett.* XXXX, XXX, XXX−XXX

A number of drug candidates have been advanced into clinical trials. Recently, we reported that small molecule DGAT1 inhibitors markedly alter incretin peptide release following oral lipid challenge.[21] Additionally, combination of DGAT1 inhibition with dipeptidyl-peptidase-4 (DPP-4) inhibition led to further enhancements in active GLP-1 in mice and dogs, suggesting potential combinability of DGAT1 inhibitors and DPP-4 inhibitors for treatment of metabolic diseases.[22]

The DGAT1 inhibitor program at Merck was primarily built upon our initial discovery of a novel benzimidazole class of compounds bearing an acid moiety at the terminus of the structure (Figure 2).[23] Compounds **1** and **2** demonstrated

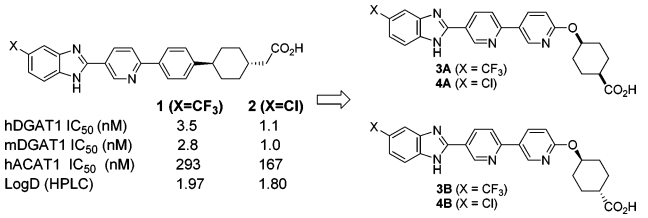
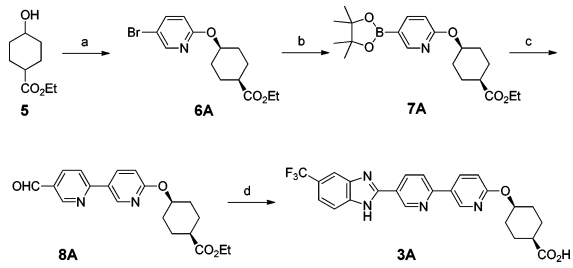


Figure 2. Design of benzimidazole acid class with a pyridyl−pyridyl−ether moiety.

potent inhibition against both human and mouse isoforms of DGAT1.[24] However, both compounds had limited selectivity against ACAT1.[25] We decided to explore analogues incorporating a pyridyl−pyridyl−ether moiety. This modification was expected to render the structure more flexible due to the ether linkage. Furthermore, the addition of nitrogen and oxygen atoms could help reduce log *D*. We began the work focusing on the preparation of the compounds **3A**, **4A**, **3B**, and **4B** since Cl and CF_3 substitutions were shown to be favorable in the series of **1** and **2**. Cyclohexanecarboxylic acid was selected as the terminal moiety since the corresponding starting materials were readily available.[26]

The initial method for the synthesis of this series of compounds was exemplified by the preparation of compound **3A** (Scheme 1). Bromide **6A** (*cis*) was prepared by Mitsunobu reaction of commercially available ethyl 4-hydroxycyclohexanecarboxylate (a *cis* and *trans* mixture) and 5-bromo-2-hydroxypyridine followed by SFC to separate *cis* and *trans* isomers. Next, bromide **6A** was converted into pinacol boronate **7A**, which underwent a Suzuki coupling reaction with 6-bromonicotinaldehyde to furnish aldehyde **8A**. Oxidative

Scheme 1. Synthesis of Compound 3A[a]



[a]Reagents and conditions: (a) 1. PPh_3, 5-bromo-2-hydroxypyridine, DIAD, THF, 55 °C; 2. SFC (ChiralPak AD-H), first peak; (b) bis(pinacolato)diboron, KOAc, $PdCl_2$(dppf), dioxane, 80 °C; (c) 6-bromonicotinaldehyde, Na_2CO_3, $PdCl_2$(dppf), DMF−water, 80 °C; (d) 1. potassium peroxymonosulfate, 4-(trifluoromethyl)benzene-1,2-diamine, DMF−water; 2. LiOH, THF−water.

condensation of **8A** with 4-(trifluoromethyl)benzene-1,2-diamine formed a benzimidazole ring.[27] Finally, the ester group was hydrolyzed to afford acid compound **3A**. Accordingly, other compounds (**3B**, **4A**, and **4B**) were synthesized using the corresponding *cis* or *trans* isomer and the substituted benzene-1,2-diamine.

The profiles of compounds **3A**/**B** and **4A**/**B** are summarized in Table 1. All four compounds exhibit potent inhibition on both human and mouse DGAT1, with potencies comparable to compounds **1** and **2**. These analogues also have reduced log *D* (HPLC) values relative to compounds **1** and **2**. In addition to the excellent in vitro potency on DGAT1, all four compounds reduce triglyceride excursion in mouse LTT (lipid tolerance test).[28] In particular, compound **4A** demonstrates extraordinary efficacy in mouse LTT. It also gives the best selectivity (×1680) against ACAT1, judging by the ratio IC_{50}s of ACAT1 against DGAT1 in human. Compound **4A** was further evaluated for PK in rat (Table 2). It was shown to have low plasma clearance, reasonable half-life, and good plasma exposure after oral dosing.

Given the encouraging profiles for **3A**/**B** and **4A**/**B**, we continued with a SAR study to assess the effect of the substitution pattern on the benzimidazole ring in both the cis and trans series in a library fashion. Scheme 2 details the chemistry for the cis series. We decided to prepare a common intermediate, which would be converted to the final analogues with minimal manipulations. For this purpose, intermediate **9A** was chosen as the common intermediate instead of **8A**. After surveying a few reaction conditions, we identified a good condition for hydrolyzing ester **8A** to **9A** without destroying the aldehyde group.[29] Under the standard oxidative condensation condition, **9A** reacted with a variety of diamines to give the required final compounds. With this protocol, hydrolysis of the ester group was carried out once during the preparation of common intermediate **9A** rather than every time for each compound. The compounds in the trans series were prepared in a similar manner.

Table 3 compares the profiles for the analogues prepared from this library approach. In general, DGAT1 potency correlates well across human and mouse. Small substitutions (e.g., H and F) afford the best potency (compounds **10**−**12**). As an exception, CN on benzimidazole maintains excellent potency on human DGAT1 but loses potency on mouse DGAT1 (compounds **13A** and **13B**). OCF_3 (**14A**) gives good DGAT1 potency but suffers from poor selectivity against ACAT1.[30] The sulfone, a polar group, is not tolerated for DGAT1 potency (**15A** and **15B**). Incorporation of an azabenzimidazole core eliminates DGAT1 potency (**16A** and **16B**). Introduction of the OMe group helps to regain reasonable DGAT1 potency but with deterioration of ACAT1 selectivity (**17A** and **17B**).

Overall, compound **11A** gave the best profile among the analogues. As a significant advantage over **4A**, compound **11A** exhibited excellent selectivity against ACAT1. Furthermore, in the mouse LTT assay, compound **11A** inhibited triglyceride excursion by 72% after 3 mg/kg oral dosing (with plasma trough level < 10 nM at 20 h). In a separate study, the plasma drug level of compound **11A** was determined to be also low (<10 nM) at 4 h time point after oral dosing at 3 mg/kg in mice. Compound **11B**, the trans isomer of **11A**, also showed comparable efficacy, but compound **11A** had more balanced in vitro DGAT1 IC_{50} numbers across human and mouse. The difluoro analogue, compound **12A**, also gave a similar profile,

dx.doi.org/10.1021/ml400168h | ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Table 1. Profiles of Compounds 3A, 3B, 4A, and 4B[a]

compd	human DGAT1 IC_{50} (nM)	mouse DGAT1 IC_{50} (nM)	log *D* HPLC	human ACAT1 IC_{50} (nM)	ratio of IC_{50} human ACAT1 vs DGAT1	mouse LTT triglyceride reduction @ 18 h
3A	1.7	2.2	1.62	1093	643	−84%
3B	3.0	4.4	1.72	1232	410	−84%
4A	2.1	3.7	1.42	3528	1680	−144%
4B	2.0	4.2	1.43	1344	672	−83%

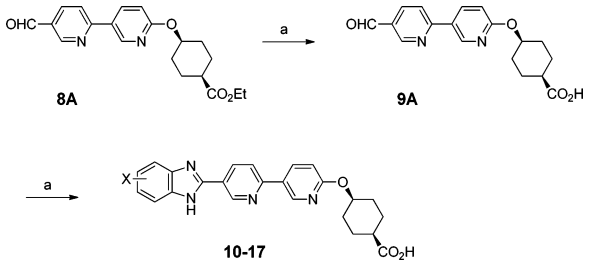
[a]Compounds were dosed in 0.5% methylcellulose at 10 mg/kg p.o. as a suspension.

Table 2. Pharmacokinetic Data of 4A in Rat[a]

PK parameters	rat
F (%)	19
Cl (mL min^{-1} kg^{-1})	3.0
Vdss (L kg^{-1})	0.34
$t_{1/2}$ (h)	3.7
AUCn (μM·h/(mg/kg))	2.4

[a]Compound dosed in Sprague−Dawley rats as a solution in EtOH/PEG400/water (10:50:40) at 1 mg/kg, iv, and 2 mg/kg, p.o.

Scheme 2. Exploration of Substitutions on Benzimidazole Ring[a]

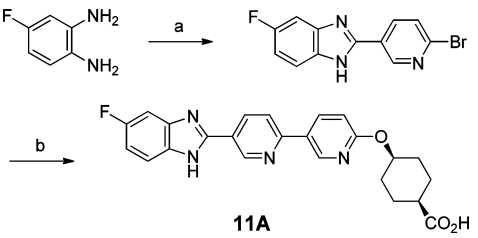


[a]Reagents and conditions: (a) K_2CO_3, MeOH−water, 80 °C, 1.5 h; (b) potassium peroxymonosulfate, substituted diamine, 3% HOAc in DMF, 100 °C.

but it has slightly higher log *D* (1.28) than **11A** (1.02). Therefore, compound **11A** was chosen for further evaluation.

To scale up compound **11A** for additional profiling, we modified the chemistry (Scheme 3). The modified synthesis was more convergent and provided the material needed to support further studies. In addition, compound **10A** was also scaled up according to a procedure similar to Scheme 2.

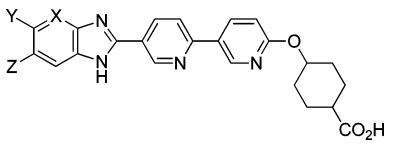
Scheme 3. Chemistry for the Scale-up of 11A[a]



[a]Reagents and conditions: (a) 6-bromonicotinaldehyde, potassium peroxymonosulfate, DMF−water; (b) 1. pinacol boronate **7A**, Na_2CO_3, $PdCl_2$(dppf), DMF−water, 80 °C; 2. LiOH, THF−water.

In addition to the observed mouse LTT efficacy, compound **11A** performed extremely well in the dog LTT assay (Figure 3).[31] One hour after oral dosing of compound **11A** at 3 mg/kg in 0.5% methyl cellulose, the dogs were challenged with lipid. In comparison to the vehicle group, **11A** was shown to abolish lipid excursion at all time points. However, the corresponding plasma exposure at all time-points (0 to 24 h) was low (<10 nM).

Table 3. Profiles of Compounds 10−17



compd	cis/trans	X	Y	Z	human DGAT1 IC_{50} (nM)	mouse DGAT1 IC_{50} (nM)	human ACAT1 IC_{50} (nM)
10A	cis	CH	H	H	4.0	8.1	8080
10B	trans	CH	H	H	5.3	12	6065
11A	cis	CH	F	H	2.0	3.4	>10000
11B	trans	CH	F	H	2.5	8.1	>10000
12A	cis	CH	F	F	1.4	3.4	>10000
12B	trans	CH	F	F	1.3	4.3	8440
13A	cis	CH	−CN	H	3.2	36	>10000
13B	trans	CH	−CN	H	6.0	96	>10000
14A	cis	CH	$-OCF_3$	H	3.9	4.6	772
15A	cis	CH	$-SO_2Me$	H	101	358	>10000
15B	trans	CH	$-SO_2Me$	H	137	727	>10000
16A	cis	N	Me	H	32	62	>10000
16B	trans	N	Me	H	54	161	8782
17A	cis	N	−OMe	H	11	13	2475
17B	trans	N	−OMe	H	14	16	4903

dx.doi.org/10.1021/ml400168h | *ACS Med. Chem. Lett.* XXXX, XXX, XXX−XXX

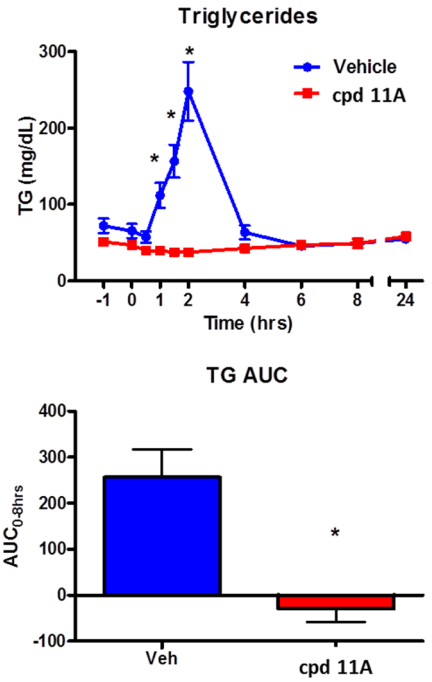


Figure 3. Compound **11A** demonstrates excellent efficacy in dog LTT.

The apparent disconnect between the efficacy and plasma exposure of **11A** could be due to the preferential tissue distribution of compound **11A** in intestine, where the nutrient absorption and reassembly of triglycerides take place. This finding presented a good opportunity to develop a gut-targeting DGAT1 inhibitor, which could minimize potential side effects due to systematic plasma exposure.[32]

To test this hypothesis, we repeated the mouse LTT using compound **10A**, the des-fluoro surrogate, since initial mouse LTT data showed **10A** at 3 mg/kg dosing reduces lipid excursion by 95%, similar to compound **11A**. In this study, after 3 mg/kg oral dosing, the drug levels were measured in different segments of the intestine (duodenum, jejunum, and ileum) as well as in blood at three time points (2, 5, and 25 h) (Table 4).

Table 4. Concentration of 10A in Segments of Intestine and Blood after Oral Dosing at 3 mg/kg in Mouse

	drug level (μM) of **10A**			
time point	blood	duodenum	jejunum	ileum
2 h	0.114	8.32	5.51	3.89
5 h	0.051	8.76	6.01	1.72
25 h	0.01	1.76	3.06	0.62

In agreement with our hypothesis, compound **10A** showed high concentration in the different segments of the intestine, much higher (>30×) than the concentration in blood at all time points.

While the profiling of **11A** continued, a major issue emerged when we carefully analyzed the plasma samples from the in vivo studies. In the preclinical species (rat, dog, and rhesus), after oral dosing of **11A**, we detected a significant conversion of **11A** to **11B** by the epimerization at carbon atom attached to the carboxylic acid group. After oral dosing of **11A** at 10 mg/kg in rat, the plasma exposure of metabolite **11B** was about ten times the exposure of the parent **11A** (Table 5). Moreover, in dog and rhesus, the plasma exposure of metabolite **11B** was also

Table 5. Pharmacokinetic Data for Compound 11A[a,b,c]

PK parameters	rat	dog	rhesus
F (%)	2	2	3
Cl (mL min^{-1} kg^{-1})	31.8	32.3	12.4
Vdss (L kg^{-1})	2.18	0.63	0.37
$t_{1/2}$ (h)	1.69	0.82	2.0
oral dose (mg/kg)	10	2	2
C_{max} (μM)	0.06	0.06	0.01
T_{max} (h)	2.00	0.25	8.00
AUC (μM·h) **11A**	0.25	0.05	0.20
AUC (μM·h) metabolite **11B**	2.47	0.06	0.56

[a]Compound dosed in Sprague–Dawley rats as a solution in EtOH/PEG400/water (10:50:40) at 1 mg/kg, iv, and 10 mg/kg, p.o., as solid dispersion formulation. [b]Compound dosed in beagles as a solution in EtOH/PEG400/water (10:50:40) at 0.55 mg/kg, iv, and as solution in 0.5% methylcellulose at 2 mg/kg, p.o. [c]Compound dosed in rhesus monkeys as a solution in EtOH/PEG400/water (10:50:40) at 1 mg/kg, iv, and as a solution in 0.5% methylcellulose at 2 mg/kg, p.o.

comparable or higher than parent **11A**. The epimerization of carboxylic acid is known and was previously studied in detail in the case of ibuprofen.[33,34] It has been generally accepted that the epimerization of ibuprofen occurs enzymatically through an acyl-CoA intermediate. A similar mechanism may also operate for compound **11A**. Although compound **11A** has many desirable attributes, the fact that the systematic exposure of active metabolite **11B** is comparable or even higher than parent **11A** would likely complicate the development of **11A** according to a recent FDA guidance.[35] Therefore, we decided to halt the further progress of **11A**.

As a follow-up, a pharmacokinetic study of **11B** in rat indicates that **11B** is also partially converted to **11A** but to a lesser extent (Table 6). After oral dosing of **11B** at 2 mg/kg,

Table 6. Pharmacokinetic Data for Compound 11B[a]

PK parameters	rat
F (%)	24
Cl (mL min^{-1} kg^{-1})	4.2
Vdss (L kg^{-1})	0.46
$t_{1/2}$ (h)	0.86
oral dose (mg/kg)	2
AUC (μM·h) **11B**	4.47
AUC (μM·h) metabolite **11A**	0.48

[a]Compound dosed in Sprague–Dawley rats as a solution in EtOH/PEG400/water (10:50:40) at 1 mg/kg, iv, and 2 mg/kg, p.o.

the plasma exposure of metabolite **11A** is roughly 10% of the parent **11B**, approaching the 10% threshold recommended in the FDA guidance.[35] Taking together the pharmacokinetic studies of **11A** and **11B**, we decided to halt this series of compounds with the cyclohexanecarboxylic acid as the terminal moiety.[36]

In summary, we have described the design and synthesis of a novel series of DGAT1 inhibitors in the benzimidazole class with a pyridyl-oxy-cyclohexanecarboxylic acid moiety. Compound **11A** shows excellent potency on DGAT1 and excellent selectivity against ACAT1. In addition, **11A** significantly reduced triglyceride excursion in LTT tests both in mice and dogs with low plasma exposures. The excellent in vivo efficacy at low plasma exposure may be due to the preferential distribution of the compound in intestine over plasma. The ability to target the intestine over plasma could be advanta-

dx.doi.org/10.1021/ml400168h | ACS Med. Chem. Lett. XXXX, XXX, XXX–XXX

geous due to possibly lower risk of potential side effects. However, this series of compounds undergo epimerization in vivo, thereby generating active metabolites. Further efforts to address the epimerization issue 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.

■ ACKNOWLEDGMENTS

We thank Dr. Charles W. Ross III and Mr. Thomas J. Novak at Merck Research Laboratories for measuring high-resolution mass.

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