Bioorganic & Medicinal Chemistry Letters 26 (2016) 1245–1248

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of diamide compounds as diacylglycerol acyltransferase 1 (DGAT1) inhibitors

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ARTICLE INFO

Article history:
Received 24 November 2015
Revised 8 January 2016
Accepted 9 January 2016
Available online 11 January 2016

Keywords:
DGAT1 inhibitors
Diamide compounds
1,2,4-Triazole
Oral bioavailability
Triglyceride

ABSTRACT

Diamide compounds were identified as potent DGAT1 inhibitors in vitro, but their poor molecular properties resulted in low oral bioavailability, both systemically and to DGAT1 in the enterocytes of the small intestine, resulting in a lack of efficacy in vivo. Replacing an *N*-alkyl group on the diamide with an *N*-aryl group was found to be an effective strategy to confer oral bioavailability and oral efficacy in this lipophilic diamide class of inhibitors.

While triglyceride is an important energy source, its excess accumulation in the body contributes to the pathogenesis of cardiovascular disease[1] and pancreatitis.[2] Inhibition of triglyceride synthesis can offer a potential therapeutic opportunity towards treatment of these diseases.

Triglyceride synthesis involves two biochemical pathways: one starts from monoacylglycerol, a metabolite of hydrolysis of dietary triglyceride in the gut. The other starts from glycerol-3-phosphate. Both routes ultimately provide a common intermediate diacylglycerol, which is then coupled with fatty acyl-CoA to generate triglyceride. This final step is catalyzed by two enzymes known as diacylglycerol acyltransferases, DGAT1[3] and DGAT2.[4] While DGAT2 knockout mice die shortly after birth,[5] DGAT1 knockout mice are healthy, resistant to diet-induced obesity and have improved insulin sensitivity relative to wild-type.[6] This phenotype of DGAT1 knockout mice has led a number of groups to develop DGAT1 inhibitors.[7,8]

DGAT1 is a membrane-bound protein localized to the endoplasmic reticulum. It is ubiquitously expressed, but is most abundant in the enterocytes of the small intestine where it plays an important role in dietary triglyceride uptake.[3,9] In order to suppress absorption of dietary triglyceride, DGAT1 inhibitors must be absorbed into enterocytes, but systemic exposure is not required.[10]

Our high throughput screening using recombinant human DGAT1 enzyme identified diamide compound **1** as a DGAT1 inhibitor (Table 1). Despite excellent potency (DGAT1 IC_{50} = 0.007 μM),[11] **1** was neither bioavailable nor efficacious when dosed orally to rats at 10 mg/kg,[12] suggesting that it does not reach the target in enterocytes. Gut permeability and stability of **1** against CYP enzymes while it resides in the enterocytes are unlikely the problem based on Caco-2 and rat liver microsomal (RLM) clearance[13] data, respectively. Therefore poor stability and/or solubility of **1** in gastrointestinal fluid likely hindered **1** from reaching the target in the enterocytes. A medicinal chemistry program to optimize **1** into an orally efficacious DGAT1 inhibitor was then initiated. Although systemic exposure for analogs of **1** is not required for efficacy, we nevertheless measured it to confirm that molecules were being absorbed into enterocytes.

Our initial approach was to simply replace the ester groups of **1** with groups that were predicted to be more stable to hydrolytic degradation. This provided potent DGAT1 inhibitors **2** and **3** (Table 1). In particular, **2** was stable in vitro in simulated gastric fluid in the presence of pepsin (83 % remained after 1 h of incubation). Nevertheless, after oral suspension administration to rats, these analogs still showed poor systemic exposure

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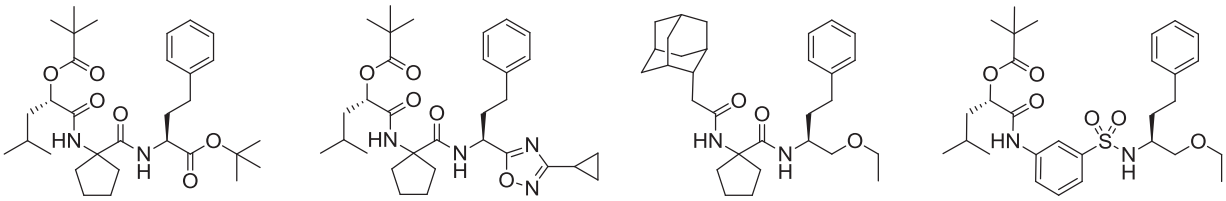
http://dx.doi.org/10.1016/j.bmcl.2016.01.025
0960-894X/

Table 1
DGAT1 inhibitors **1**–**4** and their in vitro/rat PK data

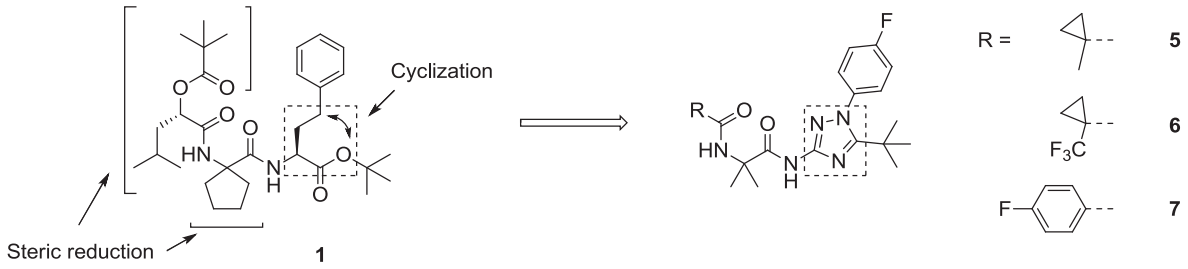


1 **2** **3** **4**

Compounds	**1**	**2**	**3**	**4**
DGAT1 IC_{50} (μM)[11]	0.007	0.011	0.027	>10
Caco-2 A–B ($\times 10^{-6}$ cm/s)	0.6	0.4	1.0	1.1
Caco-2 B–A ($\times 10^{-6}$ cm/s)	0.2	0.1	0.2	0.2
RLM CL_h (mL/min/kg)[13]	33	31	42	53
Equilibrium solubility in pH 6.8 buffer (μM)[13]	<5	<5	<5	<5
Rat PK				
Dose (mg/kg)—suspension[a]	10	10	10	5
Systemic exposure $AUC_{0-24\ h}$ (nM * h/mg/kg)	0	1	5	536
Dose (mg/kg)—solution[b]	—	5	—	—
Systemic exposure $AUC_{0-8\ h}$ (nM * h/mg/kg)	—	4	—	—

[a] $n = 3$, vehicle: 0.5% methylcellulose/0.1% Tween 80.
[b] $n = 3$, vehicle: 10% ethanol, 10% Cremophore, 30% PEG400, 50% vitamin E TPGS (10%).

Table 2
Design of DGAT1 inhibitors **5**–**7** and their in vitro data



Compounds	**5**	**6**	**7**
DGAT1 IC_{50} (μM)[11]	>10	4.8	3.5
Equilibrium solubility in pH 6.8 buffer (μM)[13]	710	60	<5

($AUC_{0-24\ h}$ = <5 nM * h/mg/kg). This poor exposure could not be improved by oral solution administration for **2** ($AUC_{0-8\ h}$ = 4 nM * h/mg/kg). As **2** and **3** are not efflux substrates (Caco-2) and moderately stable in the gut (RLM CL_h), their poor systemic exposure might be attributed to their continued poor solubility (**1**–**3**:<5 μM, in vitro assay).[13]

Interestingly, however, without improvement of in vitro solubility, **4** (DGAT1 IC_{50} = >10 μM) was well absorbed after oral suspension administration to rats (systemic exposure $AUC_{0-24\ h}$ = 536 nM * h/mg/kg) (Table 1). We hypothesized that facile ionization of the more acidic NH proton of the *N*-aryl amide or sulfonamide (**4**) compared to the *N*-alkyl amides (**1**–**3**) improved the solubility in vivo and hence systemic exposure for **4**. As the two adjacent amides were an important pharmacophore for good DGAT potency (**1**–**3**),[14] a heteroaryl group such as 1,2,4-triazole was then introduced into the Eastern amide (**5**–**7**) (Table 2). With concomitant replacement of the cyclopentyl and the Western acyl groups with less lipophilic groups, we were delighted to observe that **6** and **7** showed inhibition of DGAT1 (IC_{50} = 4.8 and 3.5 μM, respectively) with an improvement of solubility for **6** (60 μM).

Subsequent investigation of other triazole substituents on **6** led to identification of more potent analogs **15**–**17**, whose syntheses are shown in Scheme 1. Methyl thiourea **8** was diacylated to give **9**, which was condensed with alkyl hydrazine,[15] providing amidotriazole **10**. Acid-catalyzed deacylation gave aminotriazole **11**. This was coupled with oxazolone **13**, which was in turn prepared from N-Cbz protected aminobutyric acid **12**, furnishing **14**. The N-Cbz group of **14** was removed, and the resulting free amine was acylated to give **15**–**17**. Table 3 shows DGAT1 IC_{50} and solubility data. Substitution of a methyl group of the *t*-butyl group (**6**) with a 4-fluorophenyl group (**15**) improved potency by 2–3 fold (IC_{50} = 1.4 μM). Replacement of the whole *t*-butyl group (**6**) with a 4-fluorophenyl group (**16**) was more effective, slightly improving potency (IC_{50} = 0.76 μM) with three less carbon atoms from **15**. Potency was further improved when a methyl group was added to one of the two 4-fluorophenyl groups (**17**) (IC_{50} = 0.062 μM).[16] All of these analogs improved DGAT1 potency from **6** while they maintained similar solubility (8–15 μM) to **6** (60 μM).

As **17** is not only a potent DGAT1 inhibitor but also has good gut permeability (Caco-2: A–B = 20 × 10^{-6} cm/s; B–A = 20 × 10^{-6} cm/s)