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Identification of 2-aminooxazole amides as acyl-CoA: Diacylglycerol acyltransferase 1 (DGAT1) inhibitors through scaffold hopping strategy

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ABSTRACT

A scaffold hopping strategy was successfully applied in discovering 2-aminooxazole amides as potent DGAT1 inhibitors for the treatment of dyslipidemia. Further optimization in potency and PK properties resulted in a lead series with oral in vivo efficacy in a mouse postprandial triglyceridemia (PPTG) assay.

Triglycerides are one of the neutral lipids that are utilized to store free long chain fatty acids. Excess accumulation of triglycerides results in obesity and is associated with insulin resistance.[1] Triglycerides in the body are obtained by two major routes, one through absorption from food sources and the other through biosynthesis. Therefore, limiting the absorption of dietary triglycerides and the inhibition of their production in the body would be effective methods to address the diseases caused by lipid imbalance, such as obesity and type 2 diabetes. In the biosynthesis of triglycerides, the final step of the process is carried out by the enzyme called diacylglycerol acyltransferases (DGAT) with co-factor acyl-CoA.[2] DGAT is an enzyme widely expressed at the endoplasmic reticulum, and catalyzes the fatty acid esterification reaction on diacylglycerol in the liver and adipose tissue during the biosynthesis of triglycerides. Additionally, DGAT plays an important role during triglyceride absorption in the small intestine where triglyceride resynthesis is the last step of its absorption.[2]

There are two isoforms of DGAT that are involved in triglyceride synthesis. DGAT2 has been found in a wide range of tissues and is present in especially high levels in the liver. Knockout of the DGAT2 gene in mice was lethal due to severe essential fatty acid deficiency.[3] On the other hand, DGAT1 knockout mice were shown to be viable. Compared to the wild-type mice on a chow diet, DGAT1 knockout mice had 50% less adipose mass and smaller adipocytes.[4] Furthermore, on a high fat diet, DGAT1 knockout mice were resistant to diet-induced obesity, despite normal food intake and intestinal triglyceride resynthesis.[5] Although these mice had lower levels of triglycerides, diacylglycerol and fatty acid levels remained similar to the wild type mice.[5] These results strongly suggested that inhibition of DGAT1 would be a beneficial point of intervention for diseases caused by excessive lipids.

DGAT has been a target of great interest, and many medicinal chemistry programs focusing on DGAT1 inhibition have been disclosed.[6–15] Hoffmann-La Roche reported a class of heterocyclic amide compounds such as **1** as potent DGAT1 inhibitors (Fig. 1).[16] There are two features of **1** that caught our attention when we initiated the DGAT1 inhibitor program. One, unlike several other DGAT1 inhibitors reported in the literature such as **2**,[17–20] **1** does not possess the acid functionality, and the

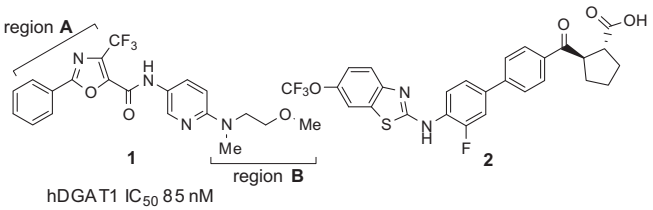

Figure 1. Examples of DGAT1 inhibitors.

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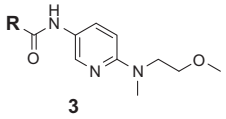
Table 1
Oxazole amide replacements



3

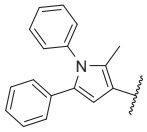
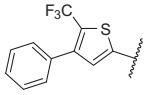
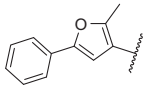
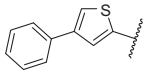
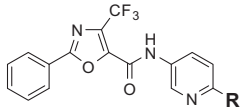
Compd	R	hDGAT-1 IC_{50} (nM)
3a		1712
3b		1616
3c		1560
3d		2837

Table 2
Region **B** SAR study of 2-phenyloxazole amides



4

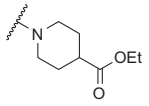
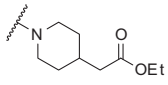
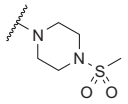
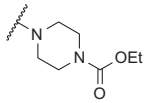
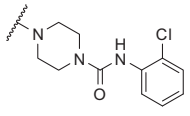
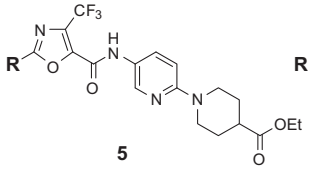
Compd	R	hDGAT-1 IC_{50} (nM)
4a		84
4b		179
4c		474
4d		116
4e		103

Table 3
Saturated heterocycles on the oxazole



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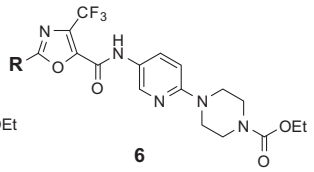
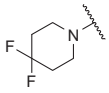
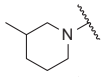
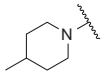
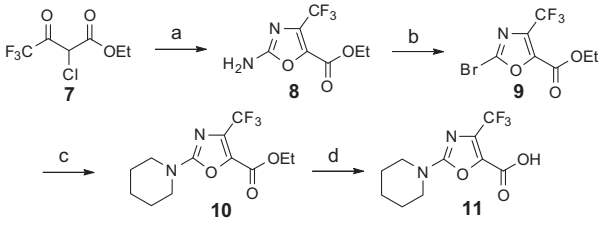
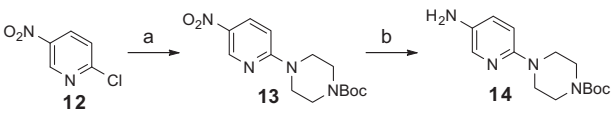


6

Compd	R	hDGAT-1 IC_{50} (nM)
5a		1708
5b		6870
5c		66
5d		93
5e		4749
6a		1508
6b		938
6c		79
6d		228



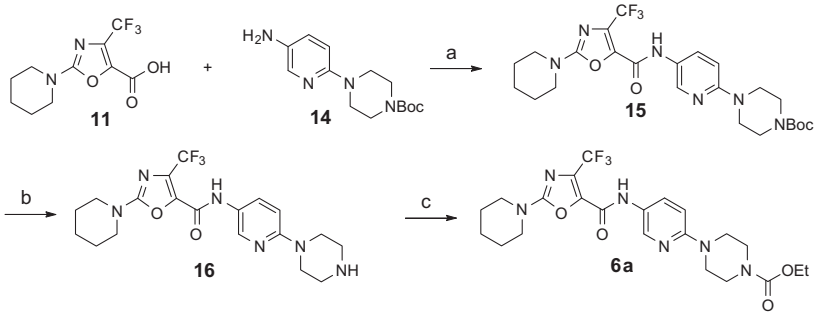
Scheme 1. Reagents and conditions: (a) urea, DMF, 120 °C; (b) *t*-BuONO, $CuBr_2$, CH_3CN; (c) piperidine, α,α,α-trifluorotoluene, microwave, 120 °C; (d) NaOH, THF, H_2O.



Scheme 2. Reagents and conditions: (a) 1-Boc-piperazine, Hunig's base, EtOH, microwave, 140 °C; (b) H_2, Pd/C, EtOH, EtOAc.

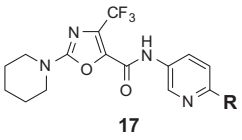
pharmacokinetic properties of **1** were expected to be different from other acid containing inhibitors. Two, its relatively low molecular weight (MW 420) would make it a suitable starting point for further functionalizations in future SAR studies. In our hands, the compound showed human DGAT1 IC_{50} of 85 nM, and mouse DGAT1 IC_{50} of 2849 nM with tractable PK exemplified by Rat PO AUC of 12.7 μM h (6 h).[21] For our initial efforts to identify a novel lead scaffold based on this molecule, two regions of **1** (region **A** and **B**) were explored to obtain suitable potency and pharmacokinetic properties. In addition, the confirmation of in vivo efficacy of the new lead series was deemed to be important prior to the promotion of the program to the lead optimization stage.

Initially, we focused our efforts on replacing oxazole amides with different heterocyclic amides in region **A**. The compounds obtained from this work turned out to be much weaker DGAT1



Scheme 3. Reagents and conditions: (a) HATU, Hunig's base, DMAP, DMF; (b) 4 N HCl/dioxane; (c) ethyl chloroformate, Hunig's base, CH_2Cl_2.

Table 4
Region **B** SAR study of 2-aminooxazole amides



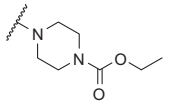
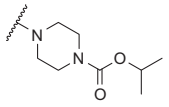
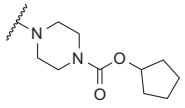
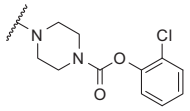
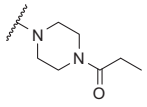
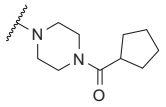
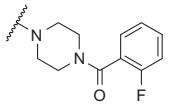
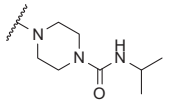
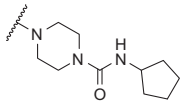
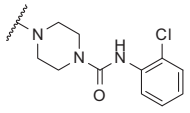
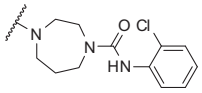
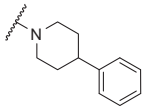
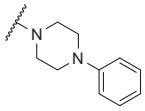
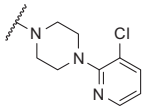
Compd	R	hDGAT1 IC_{50} (nM)	mDGAT1 IC_{50} (nM)
6a		1508	
17a		41	1205
17b		79	
17c		631	1387
17d		5837	
17e		311	
17f		1381	
17g		731	
17h		788	
17i		41	26

Table 4 (*continued*)

Compd	R	hDGAT1 IC_{50} (nM)	mDGAT1 IC_{50} (nM)
17j		46	134
17k		3870	
17l		180	
17m		67	2269

inhibitors (Table 1), suggesting that oxazole amide was the best moiety in region **A** to maintain the inhibitory activity.

Then, we shifted our focus on developing SAR in region **B**. We discovered that 4-substituted piperidine and piperazine produced quite potent compounds (Table 2). Ethyl piperidine-4-carboxylate derivative **4a** possessed similar IC_{50} as **1** while homologated ester **4b** was slightly less potent. Methyl sulfonamide **4c** displayed even less inhibitory activity. However, ethyl carbamate **4d** and urea analog **4e** showed potent inhibition of human DGAT1 enzymatic activities.

With newly gained insights on SAR trends in region **B**, we began to explore possible modifications in region **A** to further improve potency and PK. After extensive efforts to find suitable modifications, we discovered that the C2-phenyl on the oxazole could be replaced with saturated heterocycles such as piperidine without loss of inhibitory activity (Table 3). Replacement of the phenyl group with pyrrolidine (compound **5a**) or morpholine (compound **5b**) showed significant loss of potency (hDGAT1 IC_{50} 1708 nM and 6870 nM, respectively). However, piperidine replacement was more successful, producing potent compounds **5c** and **5d** (hDGAT1 IC_{50} 66 nM and 93 nM, respectively). As noted in the case of **5b** and **5e**, not all six member saturated ring compounds were active and future SAR optimization potentials were observed. For substituted piperidines, 2-, 3-, and 4-methylpiperidine analogs **6b**, **6c**, and **6d** were prepared and compared with unsubstituted piperidine analog **6a**. Parallel to the case of **5c** and **5d**, **6a** and **6b** were similar in potency (hDGAT1 IC_{50} 1508 nM and 938 nM, respectively). Further improvement of the potency was observed for **6c** and **6d** with 3-methylpiperidinyloxazole **6c** being the most potent (hDGAT1 IC_{50} 79 nM).

The synthesis of the representative compound **6a** commenced from the combination of ethyl 4,4,4-trifluoro-2-chloroacetoacetate (**7**) and urea, which resulted in the formation of **8** (Scheme 1).[22] Modified Sandmeyer conditions were utilized to convert **8** to **9**, which was a key intermediate for the syntheses of several 2-aminooxazole compounds. In the particular case of **6a**, **9** was treated with piperidine to provide **10**, which was successfully converted to **11** upon basic hydrolysis.

The preparation of the other key intermediate **14** was initiated by treatment of 2-chloro-5-nitropyridine (**12**) with 1-Boc piperazine to provide **13** (Scheme 2). Alternatively, other amines in place of 1-Boc piperazine were successfully employed for the syntheses of other compounds in this study. The nitro group of **13** was reduced to give aminopyridine **14**.

HATU mediated coupling of acid **11** and aminopyridine **14** in the presence of catalytic amount DMAP provided **15** (Scheme 3). Further functionalization of **15** was carried out by deprotection of the Boc group followed by carbamate formation to yield **6a**. Again, several other reactions including urea formations and sulfonamide formations were also carried out on **16** in the preparation of other compounds described in this communication.

To further probe the SAR of this new scaffold, we decided to have region **A** fixed as piperidinyloxazole as in **6a** and began re-exploring region **B** (Table 4). Among the piperazinyl carbamate compounds (**6a**, **17a**, **17b**, and **17c**), branched alkyl carbamates **17a** and **17b** turned out to be more potent in the series (hDGAT1 IC_{50} 41 nM and 79 nM, respectively). In general, less inhibitory activities were obtained for piperazinyl amide compounds (**17d**, **17e**, and **17f**), although SAR trends similar to the piperazinyl carbamate series were observed. Contrary to the carbamate and amide cases, piperazinyl alkyl ureas did not follow a similar trend in SAR. Thus, branched alkyl ureas **17g** and **17h** were less active, while aryl urea **17i** was one of the most potent in the series (hDGAT1 IC_{50} 41 nM). Homopiperazinyl urea **17j** was also quite active and demonstrated flexibility of the region to accommodate some small changes. In addition, several aryl piperidine and aryl piperazine compounds were prepared (**17k**, **17l**, and **17m**). In general, aryl piperazine compounds exhibited better potencies than aryl piperidine compounds and aryl piperazine **17m** showed the best potency (hDGAT1 IC_{50} 67 nM) among these types. For selected cases, mouse DGAT1 potency was measured as well and **17a** and **17m** showed some selective inhibition favoring human DGAT1 over mouse DGAT1.

As shown for the representative compounds **17a** and **17i** (Table 5), PK properties of this series of compounds were quite acceptable at the hit to lead stage. These compounds have high Rat AUC and good solubility. As for other properties, their *clogP* values were in the acceptable range (*clogP* < 5)[23], even though molecular weights are slightly higher than desired. The Cyp P450 profiles of the series were also acceptable and so were their hERG inhibitions. On the other hand, human hepatocyte clearance for these compounds were high (24.2 μL/min/10^6 cells for **17a** and 16.9 μL/min/10^6 cells for **17i**). Another potential area for improvement was plasma protein binding as both compounds exhibited greater than 99% binding. Overall, the 2-aminooxazole amide series has been shown to possess suitable PK properties as a lead series.

Selected compounds from this series were tested in a mouse postprandial triglyceridemia (PPTG) assay to determine in vivo activity (Table 6).[24,25] The compounds were orally dosed (3 mg/kg or 10 mg/kg) to mice fasted overnight and treated with an oral

Table 5
PK for representative compounds

	17a	17i
Mol Wt	511	578
*c*logP	3.87	3.95
Solubility	100 μM	10 μM
Rat AUC (μM h)	69	25
hHepatocyte Cl (μL/min/10^6 cells)	24.2	16.9
P450 IC_{50} (pre/co μM)	3A4 (>20/>20), 2D6 (>20/>20), 2C9 (>20/>20)	3A4 (5.0/>20), 2D6 (>20/>20), 2C9 (9.7/6.4)
hERG ionwork (% inhib. at 10 μM)	40%	
Plasma protein binding	99.6%	99.9%

Table 6
%Reduction of plasma triglyceride level

	10 mg/kg	3 mg/kg
17a	−12	
17b	−27	
17i	−63	−28

bolus of corn oil 15 min after compound dosing. After 2 h of the corn oil bolus, blood samples were obtained and plasma triglyceride levels were measured. Then the values were compared with those of the control mice. Three compounds showed activity at 10 mg/kg. And the most potent **17i** also showed good activity at 3 mg/kg. Having demonstrated significant reductions in plasma triglyceride levels, the 2-aminooxazole amide series provided a suitable lead series, which warrant further lead optimization studies.

In conclusion, we successfully identified the 2-aminooxazole amide series as the lead for a DGAT1 inhibitor program for the treatment of diabetes and obesity. From the SAR studies, we were able to achieve the desired level of in vitro potency. Several compounds in the series have been shown to possess desirable PK properties. Furthermore, we were able to demonstrate in vivo activity in reducing plasma triglyceride levels in mice challenged with a triglyceride load. Thus, with identification of the 2-aminooxazole amide series as the lead, the DGAT1 inhibitor hit-to-lead program has been successfully concluded and transferred to lead optimization.

References and notes

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25. All animal studies were reviewed and approved by the Merck IACUC. The Guide for the Care and Use of Laboratory Animals was followed in the conduct of the animal studies. Veterinary care was given to any animals requiring medical attention.