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Discovery of a novel series of benzimidazole derivatives as diacylglycerol acyltransferase inhibitors

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ABSTRACT

A novel series of benzimidazole derivatives was prepared and evaluated for their diacylglycerol acyltransferase (DGAT) inhibitory activity using microsome from rat liver. Among the newly synthesized compounds, furfurylamine containing benzimidazole carboxamide **10j** showed the most potent DGAT inhibitory effect (IC_{50} = 4.4 μM) and inhibited triglyceride formation in HepG2 cells. Furthermore, compound **10j** reduced body weight gain of Institute of Cancer Research mice on a high-fat diet and decreased levels of total triglyceride, total cholesterol, and LDL-cholesterol in the blood accompanied with a significant increase in HDL-cholesterol level.

Triglyceride (TG) synthesis in mammals is important in many biochemical processes, including lactation, energy storage in adipose tissue, muscle, and assembly of lipoprotein particles in the liver and small intestine. Triglycerides (TG) are essential for normal physiology. However, excess accumulation of TG in certain organs and tissues can cause a variety of disorders, such as dyslipidemia, obesity, insulin resistance, and hepatic steatosis. Based on these aspects, inhibition of TG synthesis is considered as an efficient strategy for treatment of obesity and type II diabetes.[1,2]

TG is produced by two pathways: the major glycerol phosphate pathway and the minor monoacylglycerol pathway. Diacylglycerol acyltransferase (DGAT), which catalyzes acyl residue transfer from acyl-CoA to diacylglycerol (DAG), is the exclusive key enzyme for the final step common to both pathways.[3–5] Accordingly, DGAT has emerged as an attractive target for the control of obesity and other related disorders.[6] So far, several small molecules including natural products and synthetic compounds (**1–4**) have been reported to possess DGAT inhibitory activity (Fig. 1).[7–11] Recently, detailed pharmacological effect of T863 (**4**) has been reported, in which T863 caused weight loss and reduction in serum and liver TG.[12] In addition, T863 improved insulin sensitivity and reduced level of serum cholesterol. These results suggested pharmacological relevance of DGAT1 inhibitors for treatment of metabolic disorders.[12]

In our search for novel DGAT inhibitors, we have screened our in-house chemical library using rat microsome based DGAT assay, which resulted in identification of a benzimidazole-type compound. Herein, we describe synthesis and biological evaluation of novel benzimidazole derivatives as potential DGAT inhibitors.

A series of benzimidazole carboxamides (**10a–o** and **16a–c**) was prepared as depicted in Schemes 1 and 2. Reaction of commercially available adamantyl phenol **5** with ethyl chloroacetate followed by alkaline hydrolysis furnished compound **7** in good yield. A single-step ring closure of adamantylphenoxy acetic acid 7[13] with methyl-3,4-diaminobezoate in the presence of cyclodehydrating agent PPSE at 140 °C afforded the benzimidazole ester **8** in 90% yield. Initial attempts to hydrolyze the ester under basic hydrolysis conditions using LiOH, KOH, NaOH or LiI resulted in poor yields; however, successful hydrolysis reaction was achieved in good yield by employing acetic acid and hydrochloric acid under reflux condition to provide the corresponding benzimidazole carboxylic acid **9** in considerable yield. Further reaction of **9** with the appropriate amines using coupling agent HATU in the presence of DIPEA yielded the amide derivatives **10a–o** (50–86%).[14]

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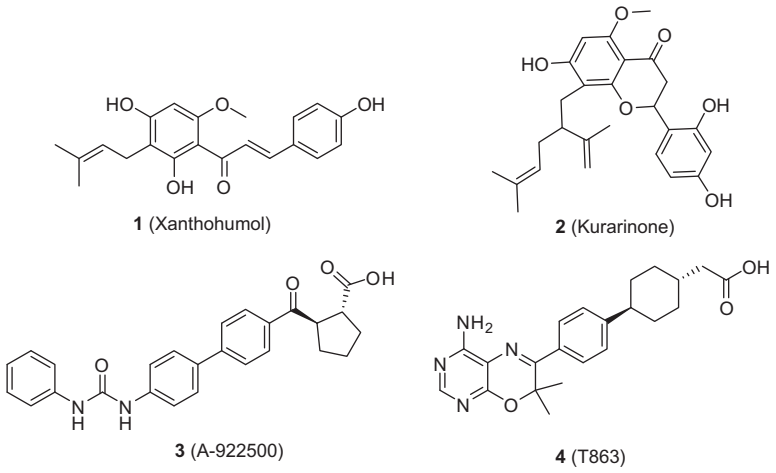


Figure 1. Structures of DGAT inhibitors.

As shown in Scheme 2, compound **16a–c**, in which the adamantyl group was replaced with hydrogen, *tert*-butyl, and phenyl, were prepared starting from the corresponding phenols **11a–c**. Thus, reaction of the appropriate phenols **11a–c** with ethyl chloroacetate followed by subsequent hydrolysis under basic conditions afforded the required aryloxy acetic acids **13a–c**. PPSE-mediated cyclization of the acids **13a–c** with methyl-3,4-diaminobezoate furnished related benzimidazole esters **14a–c** in good yield. The resulting esters **14a–c** were hydrolyzed to give the acids **15a–c**, which were then coupled with 2-furfurylamine to produce the amides **16a–c**, respectively.

The newly synthesized compounds in this study were evaluated for their inhibitory activities against DGAT (Table 1) as IC_{50} values according to the reported method.[15] The known DGAT inhibitors **2**[8] and **4**[10] were used as positive controls for comparison. Compound **4** displayed significant DGAT inhibition with an IC_{50} value of 3.1 μM, whereas compound **2** was shown to be moderately active (IC_{50} = 10.9 μM). Compound **8** identified from the initial screening exhibited moderate DGAT inhibitory activity with an IC_{50} value of 23 μM.

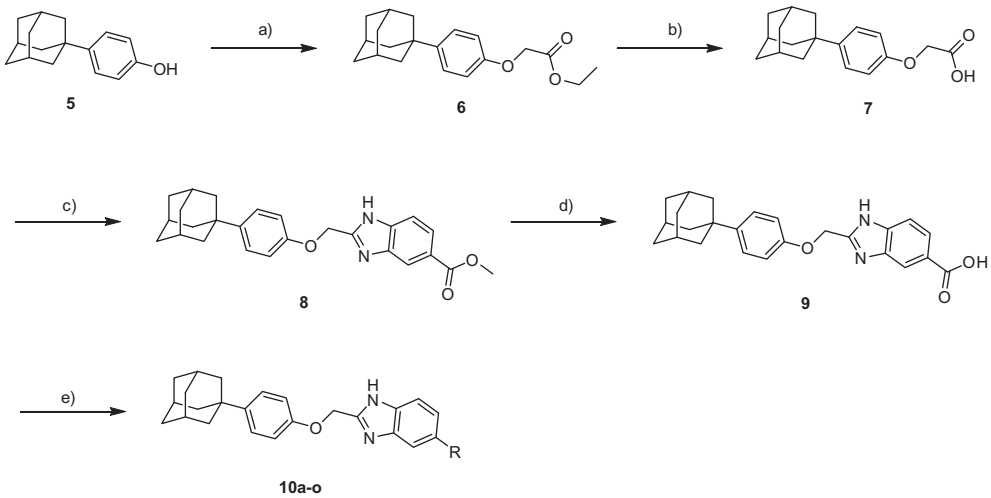
Firstly, in order to assess the effect of ester functional group at 5-position of the benzimidazole ring, we compared the inhibitory activity of the ester **8** with the corresponding acid **9** and its amide analogues, including free amide **10a**, and mono- and di-substituted amides **10b–e**. Of these, pyrrolidine-containing amide **10d** exhibited improved inhibitory activity (IC_{50} = 11.3 μM) than the screening hit **8** and the activity was comparable to that of the reference compound **2**. The isopropyl amide derivative **10c** exhibited moderate inhibitory activity (IC_{50} = 20 μM) with two-fold less potency than the reference **2**, whereas all of the other derivatives displayed poor inhibitory activity. Based on these results, we further evaluated the amide analogue series by preparing compounds **10f–j**. This derivatization resulted in potent DGAT inhibitors, as represented by compound **10j** that demonstrated appreciably high inhibitory activity (IC_{50} = 4.4 μM), which was as potent as the reference compound **4** and more potent than the reference **2**. On the other hand, the pyridinyl methane substituted amide derivative **10i** displayed moderate inhibition with an IC_{50} value of 20 μM and rest of the compounds **10f–h** were found to be poor inhibitors (IC_{50} > 50 μM). In view of potent inhibitory activity of the furfurylamine derivative **10j**, we were interested in exploring the effect of furanyl moiety for DGAT inhibition. Thus, substitution of furanyl moiety with 3-methylfuran, 5-methylfuran, tetrahydrofuran, and thiophene was investigated by synthesizing compounds **10k–o**. Among these, compounds **10l**, **10m**, and **10n** having 5-methylfuranyl and tetrahydrofuranyl linkage demonstrated good inhibitory activities comparable to that of the reference **1** (IC_{50} = 11.4 μM, 8.7 μM, and 9.5 μM, respectively). Compound **10k** with 3-methyl furan exhibited 7-fold less potency than the furan analogue **10j**. This suggests that the methyl group on furan ring is making an unfavorable interaction with the enzyme. Moreover, replacement of oxygen in furan ring by sulfur afforded drastically reduced inhibitory activity (**10o**, IC_{50} > 50 μM), suggesting that oxygen atom in the furan ring is optimal for DGAT inhibition.

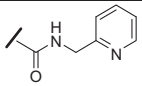
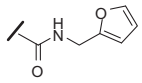
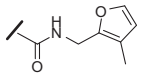
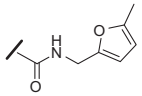
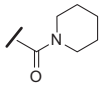
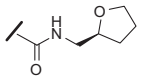
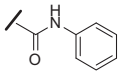
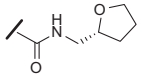
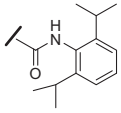
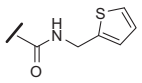
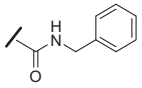
We then explored the significance of the alkyl group of the phenoxymethyl moiety at 2-position of the benzimidazole ring by substituting with *tert*-butyl, phenyl, and hydrogen (**16a–c**) while retaining the furfurylamine moiety in **10j**. However, only *tert*-butyl derivative showed good inhibitory activity with an IC_{50} value of 9.0 μM, indicating that hydrophobic groups such as adamantanyl and *t*-butyl may be more suitable for the inhibition of DGAT than aromatic (phenyl) and H.

To examine whether DGAT inhibitory effect in enzyme level translates into inhibitory effect on TG synthesis in cellular level, the most active analogue **10j** in the series was chosen for further evaluation. Accordingly, **10j** was evaluated for its potential to inhibit cellular formation of TG in HepG2 cells. As shown in Table 2, the HepG2 cells treated with 10 μM of **10j** produced far smaller amounts of triglycerides than the control (without any inhibitor). **10j** was measured to inhibit the biosynthesis of TG by 47% for radio-labeled acetate and 55% for radio-labeled glycerol, respectively. These results revealed that **10j** entered the cells and reduced the cellular formation of TG by inhibiting DGAT activity.

Encouraged by the enzymatic and cellular level activity of **10j**, we then examined its in vivo efficacy in a diet-induced obesity (DIO) mouse model, containing normal diet group, high-fat diet (HFD) group with diet-induced obesity, and HFD group with a drug as summarized in table 3. On the basis of the control, the HFD group administered with compound **10j** at a dose of 10 mg/kg was observed to inhibit body weight gain by 73%. The inhibitory effect of Xenical[17] serving as a positive control (orlistat, a lipase inhibitor) against body weight gain was 88%. A 5-week administration of **10j** greatly decreased body weight without affecting food intake.

It was reported that altered lipid absorption by inhibition of DGAT1 might associate with the improvement in serum lipid



Compd	R	Compd	R
10a		10i	
10b		10j	
10c		10k	
10d		10l	
10e		10m	
10f		10n	
10g		10o	
10h			

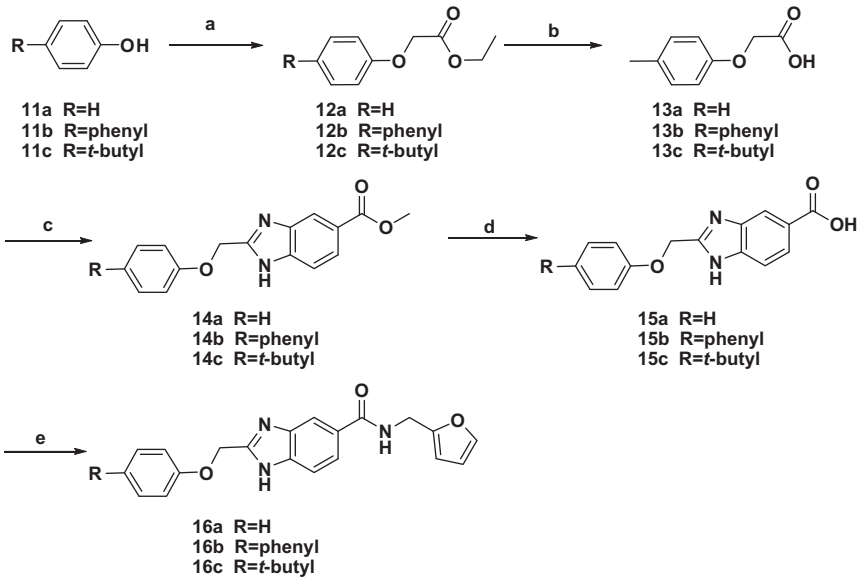
Scheme 1. Synthesis of compounds **10a**–**o**. Reagents: (a) ethyl chloroacetate, K_2CO_3, DMF, 12 h, r.t., 96%; (b) LiOH·H_2O, THF/H_2O, 8 h, r.t., 94%; (c) methyl-3,4-diaminobezoate, PPSE, 140 °C, 5 h, 90%; (d) AcOH/HCl, reflux, 4 h, 84%; (e) HATU, DIPEA, DMF, R-NH_2, 1–12 h, r.t., 50–86%.

profile.[12,19] We then measured the levels of total triglyceride, total cholesterol, low density lipoprotein (LDL)-cholesterol, and high density lipoprotein (HDL)-cholesterol in DIO mice. As shown in Table 4, the administration of the compound **10j** in DIO mice, resulted in decreased levels of total TG, total cholesterol, and LDL-cholesterol in the blood, accompanied by a significant increase in HDL-cholesterol. This improvement in serum lipid profile by **10j** was consistent with the result of A-922500 (**3**)[12] and T863 (**4**).[19]

After completion of administration, the animals were measured for their weights of total fat, abdominal fat, epididymal fat, and retroperitoneal fat (Table 5). The animal group with HFD and **10j** showed a significant decrease in all of total fat, abdominal fat, epididymal fat, and retroperitoneal fat in comparison with HFD model. The data of **10j** was comparable with that of Xenical. Excessive accumulation of body weight was due to HFD-caused obesity. These results suggest that the decrease in body weight is mainly attributed to a reduction of total fat.

In summary, a novel series of benzimidazole analogues was identified to possess DGAT inhibitory activities. Among the series, **10j** has emerged as the most promising DGAT inhibitor, which also demonstrated considerable inhibition of cellular TG formation in HepG2 cells as well as in vivo efficacy.

Taken together, these results merit further investigation toward the development of preclinical candidates for the treatment of obesity and metabolic disorders.



Scheme 2. Synthesis of compounds **16a–c**. Reagents: (a) ethyl chloroacetate, K_2CO_3, DMF, 12 h, r.t., 93–99%; (b) $LiOH{\cdot}H_2O$, THF/H_2O, 8 h, r.t., 89–91%; (c) methyl-3,4-diaminobezoate, PPSE, 140 °C, 5 h, 76–81%; (d) AcOH/HCl, reflux, 4 h, 97–99%; (e) PyBop, DMAP, DMF, 2-furfurylamine, 16 h, r.t., 86–92%.

Table 1
DGAT inhibitory activity of the synthesized compounds

Compound	IC_{50} (μM)[a]	Compound	IC_{50} (μM) [a]
8	23	**10j**	4.4
9	>50	**10k**	25.4
10a	>50	**10l**	11.4
10b	>50	**10m**	8.7
10c	20	**10n**	9.5
10d	11.3	**10o**	>50
10e	27.5	**16a**	>50
10f	>50	**16b**	>50
10g	>50	**16c**	9.0
10h	>50	**2**	10.9
10i	20	**4**	3.1

[a] The inhibitory effects of compounds on DGAT activity were studied with microsomes prepared from rat liver by modification of the method reported by Coleman.[15]

Table 2
Inhibition of formation of TG by compound **10j** in HepG2 cells[16]

Substrate	[^{14}C]-TG synthesis, % of control[a]			Toxicity[b] (μM)
	Control (DMSO)	**10j** (μM)		
		3	10	
[^{14}C]acetate	100 ± 6.2	80.9 ± 2.4	53.0 ± 7.8	>50
[^{14}C]glycerol	100 ± 6.2	69.5 ± 5.3	45.0 ± 4.2	>50

[a] The cell-based assay for confirmation of DGAT inhibition was performed in human hepatocyte HepG2 cells.
[b] The cytotoxic effects on HepG2 cells were determined by MTT assay.

Table 3
Inhibition of body weight gain by **10j** in DIO mice[18]

Groups/diet	Body weight (g) (Mean ± SD)					
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
Normal diet	26.3 ± 1.08	32.2 ± 1.77	36.3 ± 3.83	36.6 ± 3.41	39.1 ± 3.17	39.7 ± 1.20
HFD	25.3 ± 1.09	35.2 ± 1.57	37.8 ± 3.55	45.9 ± 2.29	49.9 ± 1.24	52.9 ± 1.20
HFD + **10j**	25.1 ± 0.99	35.1 ± 1.99	38.9 ± 2.33	40.8 ± 1.75	42.3 ± 1.57	43.2 ± 1.41
HFD + Xenical	26.3 ± 1.45	33.6 ± 1.28	37.4 ± 2.02	39.1 ± 0.90	40.0 ± 1.26	41.2 ± 1.60

Table 4
Levels of total TG, total cholesterol, LDL-cholesterol, and HDL-cholesterol in DIO mice treated with **10j** and Xenical

Groups/diet	Total TG (mg/dl)	Total cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)
Normal diet	46.0 ± 9.89	64.7 ± 10.9	30.1 ± 8.06	26.4 ± 2.69
HFD	120 ± 11.2	160 ± 13.9	119 ± 14.3	17.4 ± 2.15
HFD + **10j**	41.8 ± 9.26	73.2 ± 10.9	43.6 ± 10.1	21.2 ± 2.19
HFD + Xenical	40.8 ± 6.06	53.8 ± 12.4	20.5 ± 8.99	30.3 ± 1.52

Table 5
Weights of total fat, abdominal fat, epididymal fat, and retroperitoneal fat in DIO mice treated with **10j** and Xenical

Groups/diet	(Tissue weight/body weight) × 100		
	Total fat	Abdominal fat + epididymal fat	Retroperitoneal fat
Normal diet	2.77 ± 1.52	2.11 ± 1.09	0.66 ± 0.44
HFD	6.75 ± 1.18	5.34 ± 1.09	1.41 ± 0.29
HFD + **10j**	4.1 ± 1.03	3.16 ± 0.84	0.94 ± 0.24
HFD + Xenical	3.20 ± 1.52	2.45 ± 1.03	0.75 ± 0.50

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.10.046.

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14. Procedure for the synthesis of 2-((4-adamantylphenoxy)methyl)-*N*-(furan-2-ylmethyl)-1H-benzo[*d*]imidazole-5-carboxamide (**10j**): to a solution of benzimidazole carboxylic acid 9 (0.37 mmol), furfurylamine (54.3 mg, 0.55 mmol), and HATU (0.55 mmol) in DMF was added DIPEA (0.55 mmol) at room temperature. The mixture was stirred for 16 h at room temperature and then poured into water. The resulting solid was extracted with EtOAc, washed with brine, aqueous sodium bicarbonate, and water. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The resulted crude product was purified by column chromatography (hexanes/EtOAc, 2:1) to afford **10j** as a white solid (112 mg, 86% yield).
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