Novel Acyl Coenzyme A: Diacylglycerol Acyltransferase 1 Inhibitors—Synthesis and Biological Activities of *N*-(Substituted heteroaryl)-4-(substituted phenyl)-4-oxobutanamides

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In a program to discover new small molecule diacylglycerol acyltransferase (DGAT)-1 inhibitors, screening of our in-house chemical library was carried out using recombinant human DGAT-1 enzyme. From this library, the lead compound 1a was identified as a new class of DGAT-1 inhibitor. A series of novel *N*-(substituted heteroaryl)-4-(substituted phenyl)-4-oxobutanamides 2 was designed from 1a, synthesized and evaluated for inhibitory activity against DGAT-1 enzyme. Among these compounds, *N*-(5-benzyl-4-phenyl-1,3-thiazol-2-yl)-4-(4,5-diethoxy-2-methylphenyl)-4-oxobutanamide 9 was found to exhibit potent inhibitory activity and good enzyme selectivities. Following administration in KKA^y mice with 3 mg/kg high fat diet admixture for four weeks, 9 reduced body weight gain and white adipose tissue weight without affecting total food intake. These results suggested that the small molecule DGAT-1 inhibitor might have potential in the treatment of obesity and metabolic syndrome.

Key words acyl coenzyme A: diacylglycerol acyltransferase-1 inhibitor; obesity; KKA^y

Obesity is a major health problem and a risk factor for hypertension, diabetes, and cardiovascular disease. The current treatments of obesity attempt to restore energy balance primarily by decreasing energy input, either by suppressing appetite or interfering with lipid absorption in the small intestine. Due to both of the rapid increase in the prevalence of obesity worldwide and the lack of effective medical therapies, efforts to discover novel pharmacological therapies for obesity have been intensified.[1)] One of potential therapeutic strategies involves inhibiting triacylglycerol synthesis. Although triacylglycerol is essential for normal physiology, excess triacylglycerol (TG) accumulation results in obesity and is associated with insulin resistance. Inhibition of TG synthesis may ameliorate obesity and its related medical consequences. One of the key enzymes in TG synthesis is acyl coenzyme A (CoA): diacylglycerol acyltransferase (DGAT), which catalyzes the final step of the TG synthesis pathway in mammalian cells by using diacylglycerol and fatty acyl CoA as substrates. TGs synthesized by DGAT enzymes are either stored in cytosolic lipid droplets or secreted as components of lipoproteins in organs such as the liver and small intestine. Two DGAT enzymes, DGAT-1 and DGAT-2, have been identified and both DGAT enzymes are ubiquitously expressed, but higher level of expression are found in tissues that are active in TG synthesis, such as white adipose tissue, small intestine and liver.[2,3)] The phenotype of DGAT-1 deficient mice has already been described.[4—6)] These animals are viable, resistant to diet-induced obesity (DIO), and have increased sensitivity to insulin and leptin. These findings suggest and encourage our exploration of novel DGAT-1 inhibitors, since pharmacological inhibition of DGAT-1 may be a feasible therapeutic strategy for human obesity and type 2 diabetes. Recent studies[7—9)] have demonstrated that small molecule DGAT-1 inhibitors (I, II) showed the similar anti-obesity effects previously demonstrated in DGAT-1 deficient mice. Both of compounds (I, II) have a similar linear structure with a terminal carboxylic acid group. In this report, we present a new class of compounds without a carboxylic acid group and with potent DGAT-1 inhibitory activity and biological properties.

Research Strategy Screening of our in-house chemical library was carried out to discover new small molecule DGAT-1 inhibitors. Thus, an amide derivative **1a** (IC_{50}=5.2 μM) was identified as a new class of DGAT-1 inhibitor (Fig. 1). Initially we examined a lead library consisting of **1a** derivatives with modified aromatic amine moieties, synthesized by high throughput synthesis technology. In this library, compound **1j** (IC_{50}=150 nM) exhibited moderate DGAT-1 inhibitory activity. We designed compounds of type **2** and initiated chemical modification, focusing on the aromatic amine moieties (NHAr), the phenyl ring substituents (R) and the linker, with the goal of improving DGAT-1 inhibitory activity (Fig. 1). In this paper we describe the synthesis, structure–activity relationships (SAR) and biological properties of this novel series of *N*-heteroaryl carboxamide derivatives.

Chemistry The target compounds **1a**—**j**, **l**, **m** and **3**—**10** were synthesized as illustrated in Chart 1. The nucleophilic amine fragments **12a**—**e**, **g**—**j**, **l**,[10)] **m**[11)] were condensed with appropriate carboxylic acid fragments **11i**—**viii** using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) to yield the corresponding amides (Method A). The glycine derivative **10** was prepared from 3,4-diethoxybenzoic acid **13** and the glycine unit by the usual manner. However, the condensation reaction of carboxylic acids **11i**—**iii**, **v**—**viii** with less nucleophilic amines frequently gave amides in low yield due to self-condensation. Self-condensation was apparently occurring through the labile benzylic carbonyl group, thus we modified the reaction conditions to

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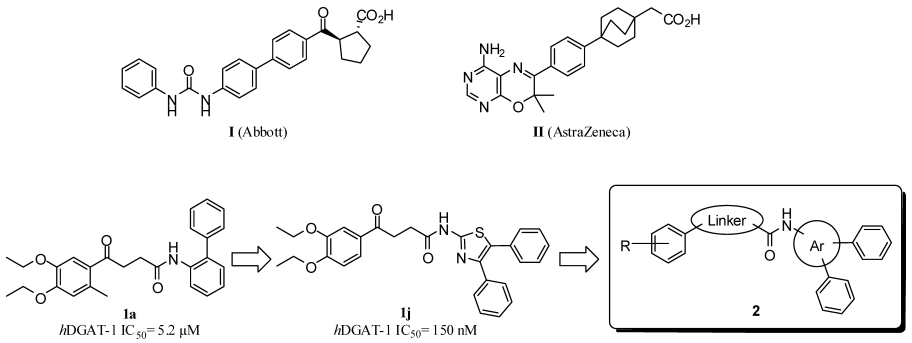
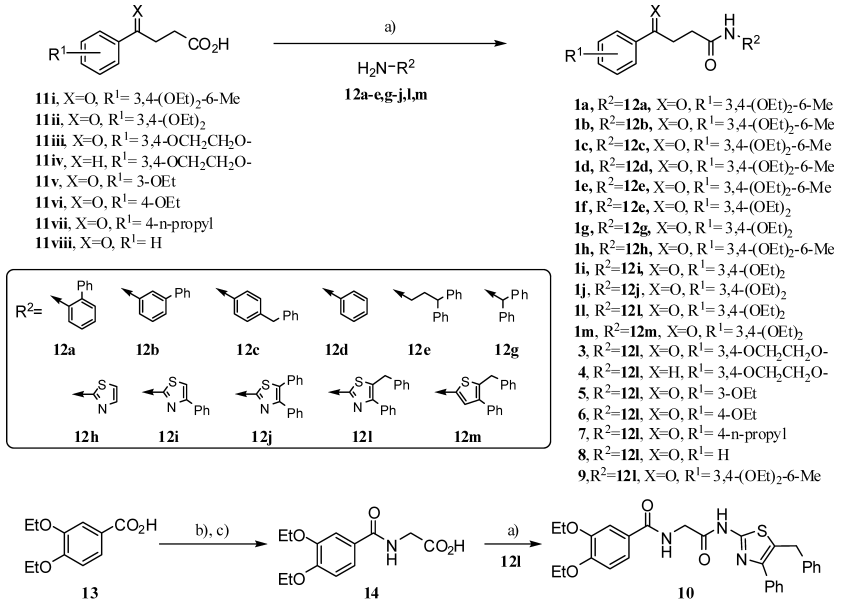
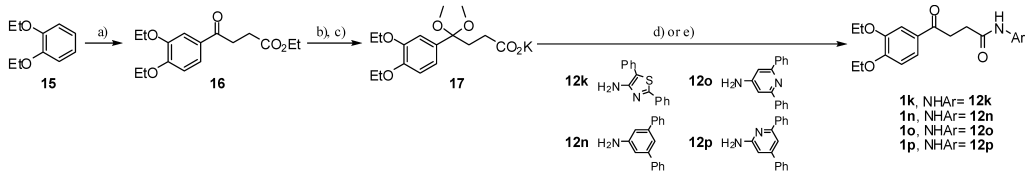


Fig. 1. Structure of Known Compounds (I, II), Lead Compounds **1a**, **j** and the General Design **2**



Reagents: a) EDCI, HOBt, DMF; b) i) $(COCl)_2$, DMF, THF, ii) methyl glycinate HCl salt, Et_3N, $CHCl_3$; c) NaOH, EtOH.

Chart 1. Synthesis of the 4-Phenylbutanamide Derivatives and the Glycine Derivative: Method A



Reagent: a) $AlCl_3$, ethyl succinyl chloride, DCM; b) cH_2SO_4, $CH(OMe)_3$, MeOH; c) KOH, MeOH; d) EDCI, HOBt, DMF; e) 2,4,6-trichlorobenzoyl chloride, Et_3N, THF.

Chart 2. Synthesis of the 4-Phenylbutanamide Derivatives: Method B

avoid formation of the by-products as shown in Chart 2 (Method B).

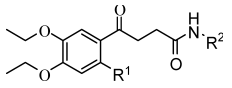
γ-Ketoester **16** was prepared from 1,2-diethoxybenzene **15** and ethyl succinyl chloride by Friedel–Crafts acylation.[12] The key intermediate **17** was obtained by treatment with orthoester in methanol under acidic condition and subsequent treatment potassium hydroxide in methanol. Using the Yamaguchi condensation[13] and subsequent acidic work-up, target compounds **1k**, **o**, **p** were prepared from **17** and the corresponding heterocyclic amine **12k**,[14] **o**,[15] **p**.[16] On the other hand, amide **1n**[17] was obtained by treatment of **17** with EDCI and subsequent removal of acetal.

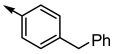
Results and Discussion

The compounds synthesized in this study were evaluated for their inhibitory activity against human DGAT-1 enzyme (Tables 1—3), measured as % inhibition at 10 μM and/or as IC_{50} values.

We initially examined the **1a**-derived lead library focusing on the amino moiety as illustrated in Table 1. The 3-biphenylamine derivative **1b** and 4-benzylaniline derivative **1c** maintained inhibitory activity; however the unsubstituted aniline derivative **1d** showed decreased potency. Aralkyl amine derivatives **1e**—**g** showed moderate inhibitory activity. As shown in the marginal difference between **1e** and **1f** on DGAT-1 inhibitory activity, it is a fair assumption that the methyl group at R^1 position can be replaced with hydrogen in

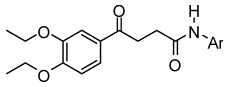
Table 1. Focus Library of Amide Derivatives



Compd.	R^1	R^2	Enzyme	
			% of inhibition at 10 μM	IC_{50} (nM)
1a	Me		69	5200
1b	Me		77	3700
1c	Me		84	3300
1d	Me		15	NT[a]
1e	Me		72	1100
1f	H		95	510
1g	H		92	770
1h	Me		0	NT[a]
1i	H		74	NT[a]
1j	H		90	150

a) Not tested.

Table 2. SAR around the Aromatic Ring



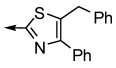
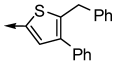
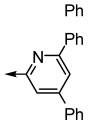
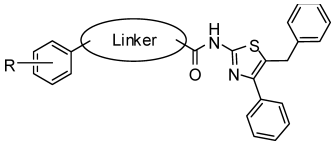
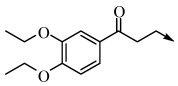
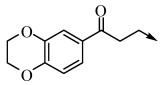
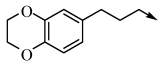
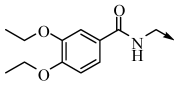
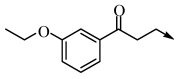
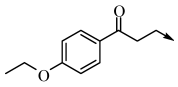
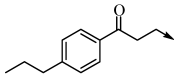
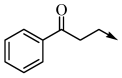
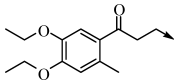
Compd.	Ar	Enzyme IC_{50} (nM)
1j		150
1k		310
1l		3
1m		14
1n		130
1o		830
1p		22

Table 3. SAR of the Phenyl and Linker Moieties



Compd.	Carbamoyl moieties	Enzyme IC_{50} (nM)
11		3
3		86
4		3700
10		140
5		3
6		6
7		290
8		220
9		8.5

rest of the SAR studies. In comparison to compound **1f**, compound **1g** did not show significant change in potency. Similar tendency was observed in other aralkyl amine derivatives (data not shown). Thiazole derivative **1h** did not show enzymatic activity at a concentration of 10 μM. However, insertion of a phenyl ring in thiazole moiety of **1h** improved inhibitory activity, and this effect is also shown in the case of **1a**—**c**. Furthermore, compound **1j** exhibited an even greater improvement in inhibitory activity by insertion of an additional phenyl ring (IC_{50}=150 nM).

Based on the discovery of the moderate DGAT-1 inhibitor **1j**, we continued further modification of di-substituted aromatic amine derivatives described in the general structure **2**, and the results are shown in Table 2. Thiazole derivative **1k**, which is a regioisomer of **1j**, showed no improvement in potency. On the other hand, 5-benzyl derivative **1l** (IC_{50}=3 nM) exhibited 50-fold greater potency than **1j**. Thiophene derivative **1m** (IC_{50}=14 nM), containing the same ring substituents as thiazole compound **1l**, showed good inhibitory activity; however the potency of **1m** was found to be 4-fold weaker than **1l**. This result suggested that the nitrogen atom in the thiazole ring might interact with the target protein. In common with **1i** and **1j**, the six-membered aromatic amine derivative **1n**, with an additional phenyl substituent on the right-hand phenyl of **1b**, exhibited a 28-fold enhancement in po-

tency as compared to **1b**. We then examined the introduction of a nitrogen atom into **1n**. The 2,6-diphenyl-4-pyridyl derivative **1o** showed 6-fold decreased inhibitory activity as compared to **1n**. In contrast, 4,6-diphenyl-2-pyridyl derivative **1p** (IC_{50}=22 nM) showed a 6-fold enhanced potency, as in the case of **1l** and **1m**. These results suggested that high potency could be obtained by the incorporation of a heterocycle nitrogen atom next to the amide group (*i.e. ortho* or 2-position).

Due to the discovery of the potent DGAT-1 inhibitor **1l**, our attention focused on the optimization of the phenyl ring substituents and the linker of **1l** (Table 3). Benzodioxine derivative **3** (IC_{50}=86 nM), which is a derivative of **1l** cyclized at the ethoxy subsituents, demonstrated reduced potency. Benzodioxine derivative **4**, without a benzylic carbonyl group, showed drastically decreased potency compared to **3**. Derivative **10**, containing an additional amide group, exhibited moderate activity. Both mono-ethoxyphenyl derivatives **5** (IC_{50}=3 nM) and **6** (IC_{50}=6 nM) maintained potent inhibitory activity. These results suggested that the benzylic carbonyl group significantly increased the inhibitory activity and that straight-chain substituents at the 3- or 4-positions of the phenyl ring are more suitable than a fused ring. Removal of an oxygen atom from the ethoxy group of **6** results in a 48-fold decrease in activity (**7**, IC_{50}=290 nM). Unsubstituted compound **8** showed similarly decreased potency. These results suggested that the alkoxy substituent is needed to achieve potent inhibitory activity. The 2-methyl derivative **9** (IC_{50}=8.5 nM) was found to be an equipotent inhibitor to **1l**.

From these SAR studies, three moieties were identified as key elements of a pharmacophore with potent DGAT-1 inhibitory activity: the alkoxyphenyl group, the carbonyl group at the benzylic position and the heterocycle nitrogen atom alpha to the amide group. This suggested that there might be at least three key interactions involved in binding to DGAT-1.

Before examining the *in vivo* efficacy of these compounds, we evaluated their activity in a cell-based assay and their selectivities against DGAT-1 related enzymes. We also tested their pharmacodynamic properties and efficacy in an *ex vivo* assay, in which the compound was orally administered to mice and plasma sample was taken after 2 h to assay reduction of TG synthesis *in vitro* [using recombinant DGAT-1]. Among the various compounds tested, compound **9** showed good potency in cell-based assay and good enzyme selectivities (Table 4). Furthermore, compound **9** significantly inhibited TG synthesis in our *ex vivo* assay (Fig. 2).

The anti-obesity efficacy of potent DGAT-1 inhibitor **9** was examined in spontaneously diabetic and obese KKA^y mice. Assessed by a four-week study in KKA^y mice (3 mg/kg/d as 0.003% food admixture), **9** reduced body weight gain (−74 %) and white adipose tissue weight in visceral and subcutaneous tissue (visc: −16%, subc: −18%) without affecting total food intake normalized with body weight.[18] These results suggested that the small molecule DGAT-1 inhibitor might have potential in the treatment of obesity and metabolic syndrome.

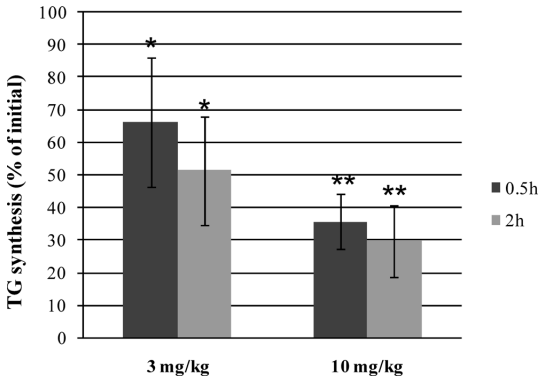


Fig. 2. Reduction of *in Vitro* TG Synthesis by Using Plasma after Oral Administration of Compound **9**

Data expressed as mean±S.D. for 3 KKA^y mice. Compound dosed at 3 and 10 mg/kg/4 ml in Labrasol solution. $*p<0.05$, $*p<0.01$ *vs.* initial value by paired *t*-test.

Table 4. Enzyme and Cellular Inhibitory Potencies[a] of Compound **9**

*h*DGAT1	*h*DGAT2	*h*ACAT1	*m*DGAT1	C_2C_{12} cell
8.5	>10000	8400	16	8.9

a) Compound **9** potencies measured as IC_{50} values (nM).

Table 5. Quantity, Purity and Mass Spectroscopic Data of the Compounds in Hit Lead Library

Compd.	Amount (mg)	Yield (%)	Purity (%)	LC/MS (*m/z*)
1a	38	44	92	432
1b	40	46	89	432
1c	37	42	91	446
1d	31	44	99	356
1e	35	91	85	474
1f	15	16	93	460
1g	16	19	94	432
1h	10	14	100	363
1i	6	7	99	425
1j	7	7	97	501

Conclusion

Modification of the *N*-hetero-aryl amide derivative **1a** led to potent DGAT-1 inhibitor **9**, which showed approximately 600-fold more potent *in vitro* activity than **1a**. The SAR data obtained in this study revealed the importance of the alkoxy substituent on the terminal phenyl ring, the presence of a benzylic carbonyl group and a heterocycle nitrogen atom located next to the amide group. Compound **9** has a unique structure, and in contrast to many known DGAT-1 inhibitors, does not possess carboxylic acid or amino functionalities. Compound **9** exhibited significant anti-obesity effects in KKA^y mice. Further *in vivo* characterization of this compound will be disclosed in due course.

Experimental

Chemistry All melting points were determined on a Yanagimoto micromelting point apparatus or Stanford Research Systems OptiMelt automated melting point system and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian Mercury-300 (300 MHz), Bruker DPX-300 (300 MHz) or Bruker-400 (400 MHz) with tetramethylsilane as internal standard. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet and br=broad. Coupling constants (*J* values) are given in hertz (Hz). LC/MS (ESI-MS) analyses were carried out using a Waters Open-Lynx system. Elemental analyses were carried out by Takeda Analytical Laboratories Ltd. and were within ±0.4% of the theoretical values for the elements indicated unless otherwise noted.

Reactions were carried out at room temperature unless otherwise noted and monitored by thin-layer chromatography (TLC) on silica gel 60 F254 precoated TLC plates (E. Merck) or by HPLC using an octadecyl silica (ODS) column (A-303, 4.6 mm i.d.×250 mm, YMC Co., Ltd.). Standard workup procedures were as follows. The reaction mixture was partitioned between the indicated solvent and water. Organic extracts were combined and washed in the indicated order using the following aqueous solutions: water, 5% aqueous sodium hydrogen carbonate solution (aqueous $NaHCO_3$), saturated sodium chloride (NaCl) solution (brine), 1 N aqueous sodium hydroxide solution (1 N NaOH) and 1 N hydrochloric acid (1 N HCl). Extracts were dried over anhydrous magnesium sulfate ($MgSO_4$), filtered, and evaporated *in vacuo*. Chromatographic separations were carried out on Silica gel 60 (0.063—0.200 mm, E. Merck) on Purif-Pack (SI 60 μm or NH 60 μm, Fuji Silysia, Ltd.) or ODS (CPO-273L, prepacked column, 22—300 mm, Kusano Kagaku Kikai Co.) using the indicated eluents. Yields were not maximized.

Amide derivatives **1a**—**j** were prepared by using high throughput synthesis technology.

General Method of Hit Lead Library FlexChem reaction block with 48 well was placed in a rack where the following reagents were added: 1) dichloromethane (DCM) (0.30 ml), 2) a solution of EDCI/HOBt coupling reagent (1.0 ml, 0.20 M/0.20 M in DCM, 0.20 mmol/0.20 mmol, 2.0 eq), 3) carboxylic acids **11i** and **11ii** (1.0 ml, 0.18 M in DCM, 0.18 mmol) and 4) amines **12a**—**j** (0.50 ml, 0.20 M in DCM, 0.10 mmol). The FlexChem block was transported to the incubator module (FlexChem Oven) and heated to 35 ° and stirred overnight. To each well, DCM and water were added, and the two phases were mixed. The mixture was allowed to settle, and the aqueous phase was removed. Further washings with a portion of aqueous hydrochloric acid and brine were carried out in a similar way, respectively. The organic phase was concentrated in a GeneVac Atlas vacuum centrifuge. The residue was dissolved in DMSO and purified using a Gilson automated preparative HPLC instrument to give the products (Table 5).

Method A. *N*-(5-Benzyl-4-phenyl-1,3-thiazol-2-yl)-4-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-oxobutanamide (3) To a mixture of 4-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-oxobutanoic acid **11iii** (354 mg, 1.50 mmol), 5-benzyl-4-phenyl-1,3-thiazol-2-amine **12l** (266 mg, 1.00 mmol) and 1-hydroxybenzotriazole monohydrate (HOBt) (230 mg, 1.50 mmol) in *N*,*N*-dimethylformamide (DMF) (20 ml) was added EDCI (345 mg, 1.80 mmol), and the mixture was stirred for 2 d. The mixture was diluted with ethyl acetate and aqueous $NaHCO_3$. The precipitate was collected by filtration, and the solid was recrystallized from tetrahydrofurane (THF)–methanol to give **3** (287 mg, 59%) as a white solid. mp 245—246 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.74 (2H, t, J=6.2 Hz), 3.27 (2H, t, J=6.2 Hz), 4.22 (2H, s), 4.26—4.30 (2H, m), 4.31—4.35 (2H, m), 6.98 (1H, d, J=8.3 Hz), 7.18—7.25 (3 H, m), 7.27—7.40 (3H, m), 7.42—7.48 (3H, m), 7.51 (1H, dd, J=8.6, 2.0 Hz), 7.60—7.65 (2H, m), 12.24 (1H, s). LC/MS *m*/*z*: 485 (MH^+). *Anal.* Calcd for $C_{28}H_{24}N_2O_4S$: C, 69.40; H, 4.99; N, 5.78. Found: C, 69.14; H, 4.99; N, 5.69.

Following compounds **1j**, **l**, **m** and **4**—**9** were prepared from carboxylic acids **11i**—**viii** and amines **12j**, **l**, **m** in a manner similar to that described for **3**. The carboxylic acid **11i** was obtained by a manner as described in Murata *et al.*, *Euro. J. Med. Chem.*, **12**, 17—20 (1977). The carboxylic acids **11ii**—**viii** were commercially available.

***N*-(4,5-Diphenyl-1,3-thiazol-2-yl)-4-(3,4-diethoxyphenyl)-4-oxobutanamide (1j)** Compound **1j** was synthesized by method A. Yield 40%. mp 217—219 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.28—1.41 (6H, m), 2.82 (2H, t, J=6.0 Hz), 3.31—3.43 (2H, m), 4.03—4.17 (4H, m), 7.07 (1H, d, J=8.3 Hz), 7.25—7.40 (8H, m), 7.40—7.49 (3H, m), 7.66 (1H, d, J=8.3 Hz), 12.44 (1H, br s). LC/MS *m*/*z*: 501 (MH^+). *Anal.* Calcd for $C_{29}H_{28}N_2O_4S$: C, 69.58; H, 5.64; N, 5.60. Found: C, 69.42; H, 5.61; N, 5.48.

***N*-(5-Benzyl-4-phenyl-1,3-thiazol-2-yl)-4-(3,4-diethoxyphenyl)-4-oxobutanamide (1l)** Yield 45%. mp 213—215 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (3H, t, J=7.2 Hz), 1.49 (3H, t, J=7.2 Hz), 2.40 (2H, t, J=6.6 Hz), 3.15 (2H, t, J=6.6 Hz), 4.10—4.17 (4H, m), 4.20 (2H, s), 6.86 (1H, d, J=8.1 Hz), 7.19—7.31 (6H, m), 7.38 (2H, m), 7.48—7.60 (4H, m), 10.72 (1H, br s). LC/MS *m*/*z*: 515 (MH^+). *Anal.* Calcd for $C_{30}H_{30}N_2O_4S$: C, 70.01; H, 5.88; N, 5.44. Found: C, 69.77; H, 5.97; N, 5.54.

***N*-(5-Benzyl-4-phenylthiophen-2-yl)-4-(3,4-diethoxyphenyl)-4-oxobutanamide (1m)** Yield 43%. mp 178—179 °C. ^{1}H-NMR (CDCl3) δ: 1.47 (6H, m), 2.80 (2H, t, J=6.6 Hz), 3.41 (2H, d, J=6.6 Hz), 4.10 (2H, s), 4.16 (4H, m), 6.64 (1H, s), 6.87 (1H, d, J=8.4 Hz), 7.16—7.39 (10H, m), 7.51 (1H, d, J=1.8 Hz), 7.59 (1H, dd, J=8.4, 1.8 Hz), 8.63(1H, br s). *Anal.* Calcd for $C_{31}H_{31}NO_4S$: C, 72.49; H, 6.08; N, 2.73. Found: C, 72.31; H, 6.05; N, 2.54.

***N*-(5-Benzyl-4-phenyl-1,3-thiazol-2-yl)-4-(2,3-dihydro-1,4-benzodioxin-6-yl)butanamide (4)** Yield 90% as amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 1.64—1.74 (2H, m), 1.75—1.82 (2H, m), 2.29 (2H, t, J=7.3 Hz), 4.17—4.24 (6H, m), 6.53 (1H, dd, J=8.1, 2.0 Hz), 6.58 (1H, d, J=1.7 Hz), 6.70—6.74 (1H, m), 7.20—7.25 (3H, m), 7.28—7.39 (3H, m), 7.39—7.45 (2H, m), 7.57 (2H, d, J=7.1 Hz), 11.26 (1H, br s.). LC/MS *m*/*z*: 471 (MH^+). *Anal.* Calcd for $C_{28}H_{26}N_2O_3S\cdot 0.3H_2O$: C, 70.65; H, 5.63; N, 5.89. Found: C, 70.74; H, 5.72; N, 5.93.

***N*-(5-Benzyl-4-phenyl-1,3-thiazol-2-yl)-4-(3-ethoxyphenyl)-4-oxobutanamide (5)** Yield 15%. mp 179 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.34 (3H, t, J=6.9 Hz), 2.78 (2H, t, J=6.1 Hz), 3.33—3.38 (2H, m), 4.09 (2H, q, J=7.0 Hz), 4.22 (2H, s), 7.18—7.25 (4H, m), 7.28—7.34 (2H, m), 7.36—7.48 (5H, m), 7.54—7.65 (3H, m), 12.24 (1H, s). LC/MS *m*/*z*: 471 (MH^+). *Anal.* Calcd for $C_{28}H_{26}N_2O_3S$: C, 71.46; H, 5.57; N, 5.95. Found: C, 71.42; H, 5.54; N, 5.93.

***N*-(5-Benzyl-4-phenyl-1,3-thiazol-2-yl)-4-(4-ethoxyphenyl)-4-oxobutanamide (6)** Yield 6%. mp 193—195 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.35 (3H, t, J=7.0 Hz), 2.76 (2H, t, J=6.4 Hz), 3.27—3.34 (2H, m), 4.12 (2H, q, J=7.0 Hz), 4.22 (2H, s), 7.03 (2H, d, J=8.7 Hz), 7.18—7.25 (2H, m), 7.34 (3H, ddd, J=16.2, 7.3, 7.1 Hz), 7.45 (2H, t, J=7.4 Hz), 7.62 (2H, d, J=7.2 Hz), 7.94 (2H, d, J=8.9 Hz), 12.22 (1H, s). LC/MS *m*/*z*: 471 (MH^+). *Anal.* Calcd for $C_{28}H_{26}N_2O_3S$: C, 71.46; H, 5.57; N, 5.95. Found: C, 71.42; H, 5.63; N, 5.95.

***N*-(5-Benzyl-4-phenyl-1,3-thiazol-2-yl)-4-oxo-4-(4-propylphenyl)butanamide (7)** Yield 38%. mp 198—199 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.94 (3H, t, J=7.5 Hz), 1.65 (2H, m), 2.42 (2H, t, J=6.6 Hz), 2.64 (2H, t, J=7.5 Hz), 3.18 (2H, t, J=6.6 Hz), 4.20 (2H, s), 7.19—7.30 (8H, m), 7.38 (2H, t, J=7.2 Hz), 7.59 (2H, d, J=7.2 Hz), 7.85 (2H, d, J=7.8 Hz), 10.68 (1H, br s). *Anal.* Calcd for $C_{29}H_{28}N_2O_2S\cdot 0.1H_2O$: C, 74.04; H, 6.04; N, 5.95. Found: C, 73.85; H, 5.99; N, 6.01.

***N*-(5-Benzyl-4-phenyl-1,3-thiazol-2-yl)-4-phenyl-4-oxobutanamide (8)** Yield 76%. mp 234—236 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.79 (2H, t, J=6.4 Hz), 3.37 (2H, t, J=6.4 Hz), 4.22 (2H, s), 7.17—7.25 (3H, m), 7.27—7.40 (3H, m), 7.42—7.48 (2H, m), 7.51—7.57 (2H, m), 7.60—7.68 (3H, m), 7.96—8.01 (2H, m), 12.27 (1H, s). LC/MS *m*/*z*: 427 (MH^+). *Anal.* Calcd for $C_{26}H_{22}N_2O_2S$: C, 73.21; H, 5.20; N, 6.57. Found: C, 73.11; H, 5.28; N, 6.60.

***N*-(5-Benzyl-4-phenyl-1,3-thiazol-2-yl)-4-(4,5-diethoxy-2-methylphenyl)-4-oxobutanamide (9)** Yield 38%. mp 134—137 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.34 (3H, t, J=7.0 Hz), 1.32 (3H, t, J=7.0 Hz), 2.36 (3H, s), 2.73 (2H, t, J=6.4 Hz), 3.26 (2H, t, J=6.2 Hz), 4.02—4.12 (4H, m), 4.23 (2H, s), 6.86 (1H, s), 7.18—7.26 (3H, m), 7.28—7.40 (3H, m), 7.41 (1H, s), 7.43—7.48 (2H, m), 7.60—7.65 (2H, m), 12.23 (1H, s). LC/MS *m*/*z*: 529 (MH^+). *Anal.* Calcd for $C_{31}H_{32}N_2O_4S$: C, 70.43; H, 6.10; N, 5.30. Found: C, 69.93; H, 6.17; N, 5.55.

***N*-[(3,4-Diethoxyphenyl)carbonyl]glycine (14)** To a solution of 3,4-diethoxybenzoic acid **13** (5.0 g, 0.0238 mol) in THF (100 ml) was added cat. DMF (3 drops) and oxalyl chloride (2.5 ml, 0.0286 mol) at room temperature (rt). The mixture was stirred for 1 h and then concentrated *in vacuo*. The residue was dissolved with chloroform (50 ml) and added methyl glycinate hydrochloride (3.0 g, 0.0239 mol). To the mixture was added triethylamine (6.7 ml, 0.0476 mol) at 0 °C and stirred at same temperature. The mixture was diluted with water and extracted with chloroform. The extracts were washed with diluted hydrochloric acid, aqueous $NaHCO_3$ and water, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was dissolved in methanol (100 ml), added 2 N NaOH (50 ml, 0.100 mol) and the mixture was stirred overnight. The reaction mixture was concentrated *in vacuo*. The residue was acidified by 1 N HCl and extracted with ethyl acetate. The extracts were washed with water, dried over anhydrous magnesium sulfate and concentrated *in vacuo* to give **14** (4.5 g, 71%) as a white solid. mp 167—168 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (6H, m), 4.15 (6H, m), 6.87 (1H, d, J=8.4 Hz), 7.01 (1H, br s), 7.37 (1H, m), 7.44 (1H, m). *Anal.* Calcd for $C_{13}H_{17}NO_5$: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.34; H, 6.43; N, 5.18.

***N*-{2-[(5-Benzyl-4-phenyl-1,3-thiazol-2-yl)amino]-2-oxoethyl}-3,4-diethoxybenzamide (10)** To a mixture of *N*-[(3,4-diethoxyphenyl)carbonyl]glycine **14** (481 mg, 1.80 mmol), 5-benzyl-4-phenyl-1,3-thiazol-2-amine **12l** (266 mg, 1.00 mmol), HOBt (306 mg, 2.00 mmol) and acetonitrile (20 ml) was added EDCI (0.351 ml, 2.00 mmol) and the mixture was stirred at 50 °C for 4 h. The precipitate was collected by filtration to give **10** (281 mg, 54%) as a white solid. mp 221—222 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.40 (3H, t, J=6.9 Hz), 1.46 (3H, t, J=6.9 Hz), 4.08 (6H, m), 4.21 (2H, s), 6.80 (1H, d, J=8.4 Hz), 6.90 (1H, br s), 7.21—7.55 (11H, m), 7.56 (1H, m), 10.85 (1H, br s). *Anal.* Calcd for $C_{29}H_{28}N_3O_4S$: C, 67.55; H, 5.67; N, 8.15. Found: C, 67.49; H, 5.78; N, 8.20.

Method B. Ethyl 4-(3,4-Diethoxyphenyl)-4-oxobutanoate (16) To a

mixture of 1,2-diethoxybenzene (20.0 g, 120 mmol) and ethyl 4-chloro-4-oxobutyrate (18.0 ml, 126 mmol) in dichloromethane (200 ml) in an ice bath was added aluminum chloride (33.6 g, 252 mmol) portionwise and the mixture stirred for 45 min. The reaction mixture was poured into ice, added conc. hydrochloric acid (100 ml) and stirred for 30 min. The mixture was extracted with chloroform, washed with brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was purified by silica gel column chromatography and recrystallized from ethyl acetate–hexane to give **16** (24.2 g, 69%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, J=7.2 Hz), 1.47 (3H, t, J=7.1 Hz), 1.49 (3H, t, J=7.2 Hz), 2.74 (2H, t, J=6.7 Hz), 3.27 (2H, t, J=6.7 Hz), 4.11—4.21 (6H, m), 6.88 (1H, d, J=8.5 Hz), 7.53 (1H, d, J=2.1 Hz), 7.59 (1H, dd, J=8.4, 2.0 Hz). LC/MS *m/z*: 317 (MNa^+).

Potassium 4-(3,4-Diethoxyphenyl)-4,4-dimethoxybutanoate (17) To a solution of ethyl 4-(3,4-diethoxyphenyl)-4-oxobutanoate **16** (24.2 g, 82.2 mmol) in methanol (250 ml) were added trimethyl orthoformate (45.0 ml, 41.1 mmol) and conc. sulfuric acid (0.1 ml), and the reaction mixture was stirred at 50 °C overnight. To the reaction mixture was added sodium hydrogen carbonate (5.0 g) and the reaction mixture was concentrated *in vacuo*. The residue was extracted with ethyl acetate, and washed with saturated aqueous sodium hydrogen carbonate solution and brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo* to give methyl 4-(3,4-diethoxyphenyl)-4,4-dimethoxybutanoate (27.0 g, quant.) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (6H, t, J=7.0 Hz), 1.99—2.10 (2H, m), 2.13—2.24 (2H, m), 3.15 (6H, s), 3.57 (3H, s), 4.05—4.16 (4H, m), 6.80—6.90 (1H, m), 6.92—6.99 (2H, m).

To a solution of methyl 4-(3,4-diethoxyphenyl)-4,4-dimethoxybutanoate (27.0 g, 82.7 mmol) in methanol (150 ml) was added potassium hydroxide (4.60 g, 69.7 mmol) and the mixture was stirred at 30 °C for 18 h. The reaction mixture was poured into diethyl ether (300 ml) and stirred for 30 min. The precipitate was collected by filtration to give **17** (11 g, 37%) as a solid. ^{1}H-NMR ($DMSO_6$) δ: 1.31 (3H, t, J=7.0 Hz), 1.32 (3H, t, J=6.8 Hz), 1.35—1.43 (2H, m), 1.87—1.97 (2H, m), 2.97—3.05 (6H, m), 3.94—4.05 (4H, m), 6.82—6.92 (3H, m).

***N*-(2,5-Diphenyl-1,3-thiazol-4-yl)-4-(3,4-diethoxyphenyl)-4-oxobutanamide (1k)** To a suspension of **17** (310 mg, 0.885 mmol) in THF (5 ml) were added triethylamine (0.247 ml, 1.76 mmol) and 2,4,6-trichlorobenzoyl chloride (0.152 ml, 0.973 mmol). The mixture was stirred for 4 h. To the reaction mixture was added a solution of **12k** (200 mg, 0.793 mmol) in THF (5 ml) and the reaction mixture was stirred at 50 °C overnight. The reaction mixture was diluted with 1 N HCl and extracted with ethyl acetate. The extracts were washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was recrystallized from acetonitrile to give **1k** (227 mg, 57%) as a white solid. mp 164—165 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.30—1.38 (6H, m), 2.69 (2H, br s), 3.25 (2H, t, J=5.6 Hz), 4.04—4.15 (4H, m), 7.06 (1H, d, J=8.6 Hz), 7.32—7.39 (1H, m), 7.41—7.49 (3H, m), 7.49—7.57 (3H, m), 7.57—7.67 (3H, m), 7.90—7.97 (2H, m), 10.24 (1H, br s). LC/MS *m/z*: 501 (MH^+). *Anal.* Calcd for $C_{29}H_{28}N_2O_4S$: C, 69.58; H, 5.64; N, 5.60. Found: C, 69.49; H, 5.63; N, 5.59.

4-(3,4-Diethoxyphenyl)-4-oxo-*N*-1,1′:3′,1″-terphenyl-5′-ylbutanamide (1n) Compound **1n** was prepared from **17** and **12n** in a manner similar to that described for **3**. White solid (yield 57%); mp 164 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (6H, dt, J=8.1, 7.0 Hz), 2.85 (2H, t, J=6.3 Hz), 3.45 (2H, t, J=6.3 Hz), 4.09—4.20 (4H, m), 6.88 (1H, d, J=8.5 Hz), 7.33—7.47 (6H, m), 7.51—7.58 (2H, m), 7.60—7.66 (5H, m), 7.77 (2H, d, J=1.3 Hz), 7.98 (1H, s). LC/MS *m/z*: 494 (MH^+). *Anal.* Calcd for $C_{32}H_{31}NO_4$: C, 77.87; H, 6.33; N, 2.84. Found: C, 77.60; H, 6.42; N, 2.76.

4-(3,4-Diethoxyphenyl)-*N*-(2,6-diphenylpyridin-4-yl)-4-oxobutanamide (1o) Yield (10 mg, 17%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 1.49 (6H, q, J=6.8 Hz), 2.87 (2H, t, J=5.4 Hz), 3.47 (2H, t, J=5.4 Hz), 4.16 (6H, t, J=6.8 Hz), 6.90 (1H, d, J=7.2 Hz), 7.42—7.65 (8H, m), 7.92 (2H, s), 8.15 (4H, d, J=7.2 Hz), 8.18 (1H, br s). LC/MS *m/z*: 495 (MH^+).

4-(3,4-Diethoxyphenyl)-*N*-(4,6-diphenylpyridin-2-yl)-4-oxobutanamide (1p) Compound **1p** was prepared from **17** and **12p** in a manner similar to that described for **1k**. Yield (300 mg, 51%) as a pale-brown solid. mp 140—141 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.30—1.41 (6H, m), 2.85 (2H, t, J=6.1 Hz), 3.28—3.37 (2H, m), 4.03—4.17 (4H, m), 7.08 (1H, d, J=8.5 Hz), 7.43—7.58 (7H, m), 7.67 (1H, dd, J=8.5, 2.1 Hz), 7.79—7.85 (2H, m), 7.93 (1H, d, J=1.3 Hz), 8.18—8.24 (2H, m), 8.34 (1H, s), 10.71 (1H, s). *Anal.* Calcd for $C_{31}H_{30}N_2O_4$: C, 75.28; H, 6.11; N, 5.66. Found: C, 75.25; H, 6.18; N, 5.60.

Assay of hDGAT1, hDGAT2, Human Acyl-CoA Cholesterol Acyltransferase 1 (hACAT1) and mDGAT1 Inhibitory Activity *in Vitro* Human DGAT-1 (hDGAT-1), hDGAT-2, hACAT-1 and mouse DGAT-1 (mDGAT-1) were expressed in Sf9 insect cells using a baculovirus expression system. The microsome of the Sf9 cells was obtained as an enzyme source.

The hDGAT1 reaction mixtures contained 100 mM Tris–HCl (pH 7.5), 250 mM sucrose, 150 mM $MgCl_2$, 0.01% bovine serum albumin (BSA fatty acid free), 1% acetone, 5 μg/ml the microsome of Sf9 expressing hDGAT-1, and 25 μM 1,2-dioleoyl-*sn*-glycerol at the final concentration. For inhibitor testing, serial dilutions of the compounds were added to the reaction mixture in the final concentration of 0.1% DMSO. The reaction was initiated by adding 25 μM [^{14}C]-oleoyl-CoA at the final concentration. The reactions were performed in a 96-well plate in a final volume of 100 μl and were terminated after 15 min at 32 °C by addition of 300 μl of chloroform/methanol (1/2 by vol) mixed solvent. After mixing the solution, 200 μl of phosphate buffer saline (PBS) was added to facilitate phase separation. After centrifugation (3000 rpm, 3 min), 50 μl of the chloroform layer was spotted on a thin layer chromatography (TLC) plate. Lipids were separated by TLC with a solvent system of *n*-hexane : diethyl ether : ethyl acetate : acetic acid (74 : 15 : 15 : 1 by vol). The radioactivity of [^{14}C]-triglyceride was counted with BAS-2500 imaging system (Fujifilm) to calculate the hDGAT-1 activity or inhibition rates of the compounds. The hDGAT-2, hACAT-1 and mDGAT-1 assays were performed as described above with the following modifications. The hDGAT-2 assay contained: 100 mM Tris–HCl (pH 7.5), 250 mM sucrose, 20 mM $MgCl_2$, 0.01% BSA, 1% acetone, 40 μg/ml the microsome of Sf9 expressing hDGAT-2, and 40 μM 1,2-dioleoyl-*sn*-glycerol, 20 μM [^{14}C]-Oleoyl-CoA. The hDGAT-2 reaction was allowed to proceed for 20 min at 32 °C, terminated, and quantified as described above. The hACAT-1 assay contained: 100 mM phosphate buffer (pH 7.2), 1 mM EDTA, 0.01% BSA, 1% acetone, 200 μg/ml the microsome of Sf9 expressing hACAT-1, and 100 μM cholesterol, 40 μM [^{14}C]-oleoyl-CoA. The hACAT-1 reaction was allowed to proceed for 25 min at 32 °C, terminated, and quantified [^{14}C]-oleoyl-cholesterol as described above. The mDGAT-1 assay contained: 100 mM Tris–HCl (pH 7.5), 250 mM sucrose, 150 mM $MgCl_2$, 0.01% BSA, 1% acetone, 5 μg/ml the microsome of Sf9 expressing mDGAT-1, and 25 μM 1,2-dioleoyl-*sn*-glycerol, 25 μM [^{14}C]-oleoyl-CoA. The mDGAT-1 reaction was allowed to proceed for 20 min at 32 °C, terminated, and quantified as described above.

Assay of Intracellular DGAT Inhibitory Activity in Mouse Myoblast C_2C_{12} Cell Line *in Vitro* C_2C_{12} cells were cultured with Dulbecco's Modified Eagle Medium (D-MEM) supplemented with 10% FBS, 50 U/ml of penicillin and 50 μg/ml of streptomycin for 2 d as myoblast cells without differentiation to myotube and were treated by trypsine-EDTA, following were suspended in phosphate buffer saline (PBS). The serial dilutions of compound were added in the C_2C_{12} cells suspension and incubated at 37 °C for 20 min. Then, [^{14}C]-oleic acid was added as a substrate of DGAT reaction. The reactions were performed in a 96-well plate in a final volume of 100 μl at the concentration of 10^6 C_2C_{12} cells/ml, 5 μM [^{14}C]-oleic acid, 0.001% BSA (fatty acid free) and 0.2% ethanol and 0.1% DMSO. The reactions were terminated after 20 min at 37 °C by addition of 300 μl of chloroform/methanol (1/2 by vol) mixed solvent. After mixing the solution, 200 μl of DW was added to facilitate phase separation. After centrifugation (2000 rpm, 2 min), 45 μl of the chloroform layer was spotted on a thin layer chromatography (TLC) plate. Lipids were separated by TLC with a solvent system of *n*-hexane : diethyl ether : ethyl acetate : acetic acid (255 : 30 : 15 : 0.6 by vol). The radioactivity of [^{14}C]-triglyceride was counted with BAS-2500 imaging system (Fujifilm) to calculate intracellular DGAT inhibitory activity of the compounds.

Measurement of DGAT1 Inhibitory Activity after Oral Administration Compounds were administered orally as a Labrasol solution to female KKA^y mice at 13—15 weeks of age. Half an hour, 1 and 2 h after drug administration, blood was collected *via* orbital venous plexus and prepared plasma. Forty microliters of 62.5 μM 1,2-dioleoylglycerol buffered solution (2.5% acetone solution in 250 mM sucrose, 150 mM $MgCl_2$ and 100 mM Tris buffer; pH 7.5), 10 μl of 10% plasma and 25 μl of 20 μg/ml human DGAT1 microsome fraction in which human DGAT1 was overexpressed in SF9 insect cells by genetic engineering method, were premixed and incubated at 37 °C for 10 min. And then 25 μl of 100 μM [^{14}C]-oleoyl-CoA (0.23 MBq/ml) was added and further incubated for 15 min. The reaction was terminated by adding 300 μl of chloroform : MeOH (1 : 2) solution, and then 200 μl of phosphate buffered saline was added, extracted and separated to organic and aqueous layer. Fifty microliters of organic layer was applied on TLC plate and separated TG with *n*-hexane : diethyleter : acetic acid (85 : 15 : 0.5), and the radioactivity incorporated in TG spot was measured by BAS2500.

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