Discovery of a Novel Series of Indolyl Hydrazide Derivatives as Diacylglycerol Acyltransferase-1 Inhibitors

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A novel series of hydrazide derivatives were synthesized as potential diacylglycerol acyltransferase (DGAT) inhibitors. Among them, compounds **8u** and **8v** exhibited selective and potent DGAT-1 inhibitory activities. In addition, compound **8u** dose-dependently inhibited triglyceride synthesis in HepG2 cell lines. Furthermore, treatment with compound **8u** for an oral lipid tolerance test showed a significant decrease in plasma triglyceride levels compared with vehicle-treated control animals, indicating delayed absorption of triglyceride after an acute lipid challenge.

Keywords: Diacylglycerol *O*-acyltransferase inhibitor, Triglyceride, Obesity, Hydrazide, Diacylglycerol *O*-acyltransferase

Introduction

Triglycerides (TGs), whether derived from secretion of very low density lipoproteins (VLDLs) or chylomicrons, are major contributors to the metabolic syndrome.[1] TGs are usually present in cytosolic lipid droplets in cells or in the plasma as core components of lipoproteins. However, excessive accumulation of TGs in human adipose and nonadipose tissues results in pathological conditions such as obesity, type 2 diabetes, coronary heart disease, hypertriglyceridemia, and nonalcoholic fatty liver disease.[2]

TGs are produced in the body through two pathways: (1) the major glycerol phosphate pathway and (2) the minor monoacylglycerol pathway. Diacylglycerol *O*-acyltransferase (DGAT), which catalyzes the acyl residue transfer from acyl-CoA to diacylglycerol (DAG), is an exclusive key enzyme for the final step common to both pathways.[3] In mammals, DGAT occurs in two isoforms, DGAT-1 and DGAT-2, from distinct gene families.[4] DGAT-1 is widely expressed in skeletal muscle, skin, intestine, and testis, with lower levels of expression in liver and adipose tissue. DGAT-2 is widely expressed in hepatocytes and adipocytes. This widely different distribution in tissues and their two different protein families suggest that these enzymes have specialized functions even though they catalyze the same reaction.[5–7] DGAT-1 knockouts were viable and resistant to weight gain when fed a high-fat diet, and exhibited increased insulin.[6] DGAT-1 inhibition is expected to reduce the formation of triacylglycerol (TAG) and chylomicrons in enterocytes and thus lower postprandial TAG levels in the blood.[8] Consequently, selective inhibition of DGAT-1 has become an attractive target with growing potential for the treatment of type II diabetes and obesity.

In the past few years, DGAT has emerged as an attractive target for the control of obesity and other related disorders.[9] Currently, preclinical trials are under way for the compound Pradigastat (Novartis, Switzerland),[10] compound PF-04620110 (Pfizer, USA),[11] and compound ABT-046 (Abbott, USA) [12] (Figure 1).

In earlier reports, we showed that some benzimidazole derivatives have a tendency to inhibit TG formation in HepG2 cells by acting as potent DGAT inhibitors.[3] In our research to find small organic molecules that are effective DGAT inhibitors, we have discovered a novel class of indolyl hydrazide derivatives that show potent DGAT inhibitory activities and also with the potential to control TG formation in whole cells.

Experimental

A series of indole-based hydrazide derivatives **8a–y** were prepared, as depicted in Scheme 1. Synthesis of the appropriate (*E*)-3-(indol-3-yl)acrylic acid derivatives **4a–i** involves the esterification of the carboxylic acid group of compound **1**, then *N*-protection of corresponding compound **2** by different protecting groups, and, finally, base hydrolysis of corresponding

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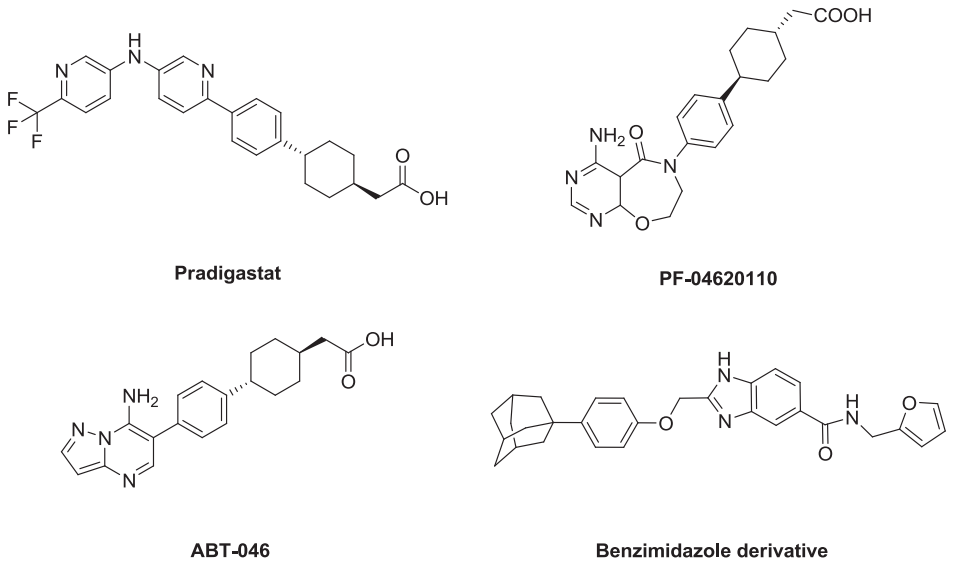


Figure 1. Selected structure of DGAT-1 inhibitors.

compounds **3a–i** to afford compounds **4a–i**. The isopropyl-hydrazide derivatives **7a–n** were synthesized in quantitative yields by performing reductive amination of hydrazide derivatives **5a–n** with acetone, using sodium cyanoborohydride as a reducing agent. Then the appropriate indole-based hydrazides **8a–y** were synthesized with good to high yields by amide coupling of isopropyl-hydrazide derivatives 7**a–n** with different (*E*)-3-(indol-3-yl)acrylic acid derivatives **4a–i** using 1-[bis (dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) and *N,N*-diisopropylethylamine (DIPEA) in dimethylformamide (DMF). Compounds **9a–d** were synthesized by the O-alkylation of hydroxyl function of **8b** and **8c** using methyl iodide or 1-bromo-2-methoxyethane (Scheme 1).

The synthesis for compounds **14a–d** is also shown in Scheme 2. First, the aldehyde moiety of different 1*H*-indole-3-carbaldehyde derivatives **10a–d** was converted to the ester moiety using ethyl (triphenylphosphoranylidene)acetate followed by hydrolysis of corresponding esters to give compounds **12a–d** in moderate to good yields. Then *N*-Boc protection of compounds **12a–d** was performed, followed by amide coupling of compounds **13a–d** with compound **7h** using standard amide coupling conditions.

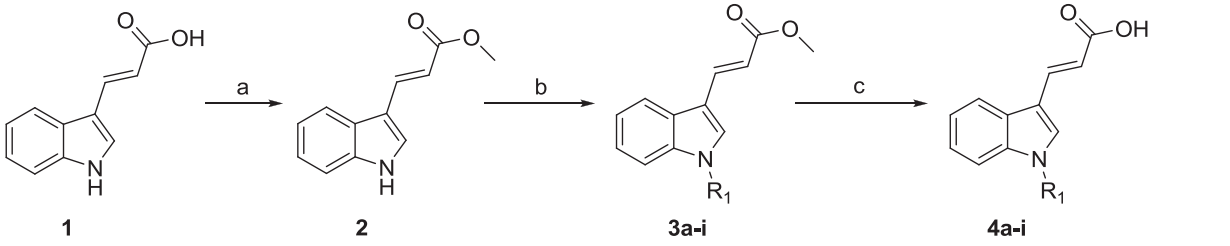
Compounds **16a–d** were synthesized through the coupling reaction of compound **1** with phenyl hydrazide derivative **7a** followed by N-acylation of compound **15** (Scheme 3).

Meanwhile, compound **19** was also synthesized based on the same strategy used for the synthesis of compounds **16a–d** starting with indolepropionic acid. Compounds **20a** and **b** were synthesized by coupling compound **4a** with benzohydrazide **7o** and benzenesulfonohydrazide **7p**, as shown in Scheme 4.

General. All reagent-grade chemicals and solvents were used without further purification. All reactions were carried out under a nitrogen atmosphere. Melting points were determined on an electrothermal capillary melting point apparatus. NMR spectra were determined on a Varian (500 MHz) spectrometer. Chemical shifts were measured in parts per million (ppm) downfield from tetramethylsilane (internal standard), with coupling constants reported in hertz (Hz). Multiplicity is indicated by abbreviations such as singlet (s), doublet (d), doublet of doublet (dd), triplet (t), multiplet (m), and broad (brs). High-resolution mass spectrometry (HRMS) was carried out on an Agilent 6530 Accurate-Mass Q-TOF LC/MS (Agilent Technologies, Korea, Seoul). The purity of the products was checked by reversed-phase high-pressure liquid chromatography (RP-HPLC), which was performed a Waters Corporation HPLC system equipped with a YMC hydrosphere C18 (HS-302) column and the UV detector set at 254 nm. The mobile phase used was a gradient of MeOH and H_2O with a flow rate of 1.0 mL/min.

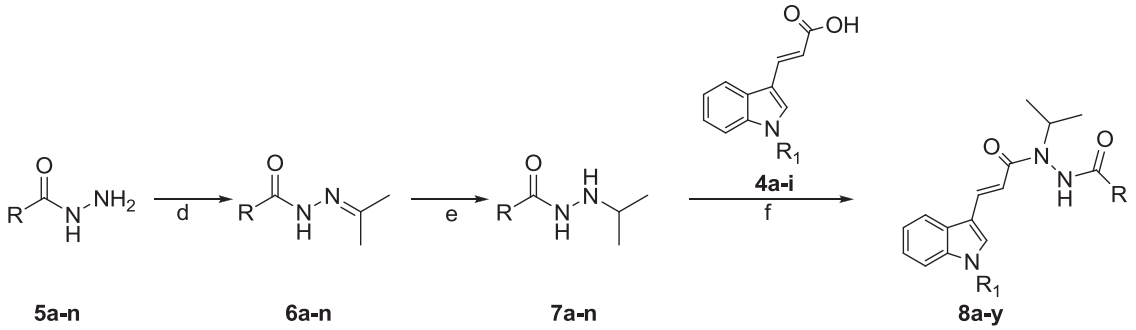
Reduction of Hydrazides. Various commercially available hydrazides (**5a–n**) were poured into a solution of acetone and hexane and stirred for 5 min. The mixture was refluxed for 12 h at 65°C, and was then cooled in an ice bath to get solid precipitates, which were filtered to afford white solids; they were then washed several times with hexane. Then the corresponding hydrazides (**6a–n**) were shifted in methanol and stirred for 20 min. Sodium cyanoborohydride and acetic acid were added to this mixture and stirred for 18 h at room temperature. After completion of reaction, a saturated sodium bicarbonate solution was added to mixture till the pH reached ~7. The mixture was extracted with ethyl acetate and water and dried over by $MgSO_4$. After evaporation under vacuum, the mixture was purified using column chromatography using *n*-hexane/EtOAc/MeOH = 6:3:1 as the eluting system. All the hydrazide derivatives obtained were colored solids with yields ranging from 85 to 100%.

Procedure of Amide Coupling. To a solution of the **4a** (1 g, 3.48 mmol), *N′*-isopropylbenzohydrazine (**7a**) (0.93 g, 5.22 mmol), and HATU (1.98 g, 5.22 mmol) in DMF (15.0 mL)



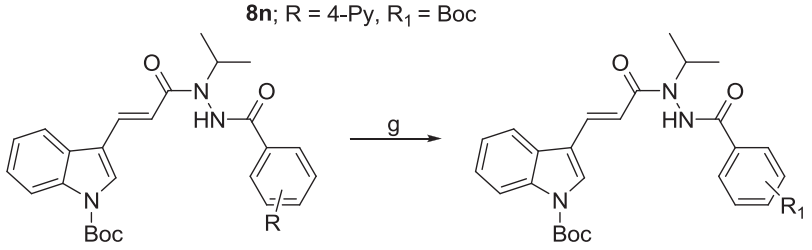
3a; R_2 = Boc
3b; R_2 = CH_3
3c; R_2 = CH_2CH_3
3d; R_2 = $CH_2CH_2CH_3$
3e; R_2 = $CH_2CH_2CH(CH_3)_2$
3f; R_2 = Bn
3g; R_2 = $CH_2CH{=}CH(CH_3)_2$
3h; R_2 = methyl(cyclopropane)
3i; R_2 = cyclopropyl

4a; R_2 = Boc
4b; R_2 = CH_3
4c; R_2 = CH_2CH_3
4d; R_2 = $CH_2CH_2CH_3$
4e; R_2 = $CH_2CH_2CH(CH_3)_2$
4f; R_2 = Bn
4g; R_2 = $CH_2CH{=}CH(CH_3)_2$
4h; R_2 = methyl(cyclopropane)
4i; R_2 = cyclopropyl



7a; R = Ph
7b; R = 3-OH-Ph
7c; R = 4-OH-Ph
7d; R = 3-Br-Ph
7e; R = 4-Br-Ph
7f; R = 3-Cl-Ph
7g; R = 4-Cl-Ph
7h; R = 3-F-Ph
7i; R = 4-F-Ph
7j; R = 3-Me-Ph
7k; R = 4-Me-Ph
7l; R = 2-Py
7m; R = 3-Py
7n; R = 4-Py

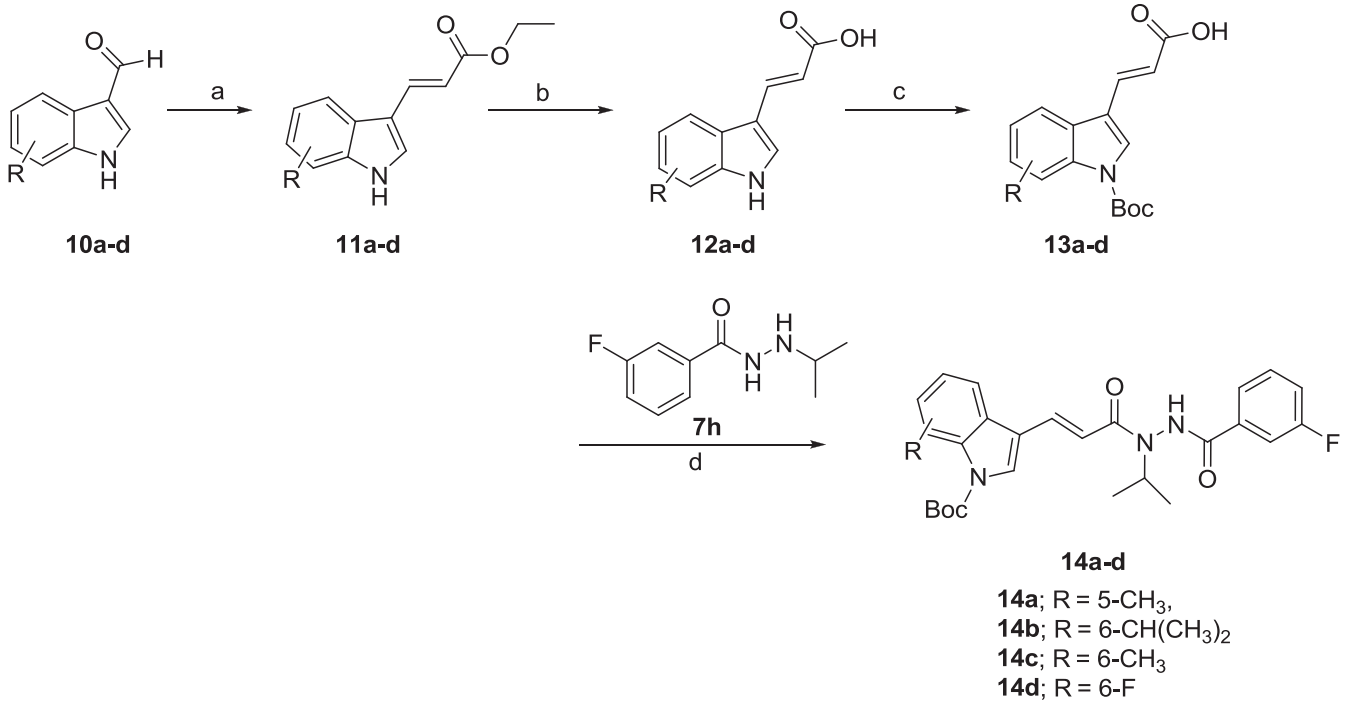
8a; R = Ph, R_1 = Boc
8b; R = 3-OH-Ph, R_1 = Boc
8c; R = 4-OH-Ph, R_1 = Boc
8d; = 3-Br-Ph, R_1 = Boc
8e; R = 4-Br-Ph, R_1 = Boc
8f; R = 3-Cl-Ph, R_1 = Boc
8g; R = 4-Cl-Ph, R_1 = Boc
8h; R = 3-F-Ph, R_1 = Boc
8i; R = 4-F-Ph, R_1 = Boc
8j; R = 3-Me-Ph, R_1 = Boc
8k; R = 4-Me-Ph, R_1 = Boc
8l; R = 2-Py, R_1 = Boc
8m; R = 3-Py, R_1 = Boc
8n; R = 4-Py, R_1 = Boc
8o; R = Ph, R_1 = Me
8p; R = Ph, R_1 = Bn
8q; R = Ph, R_1 = $CH_2CH{=}CH(CH_3)_2$
8r; R = Ph, R_1 = Methl(cyclopropane)
8s; R = Ph, R_1 = cyclopropyl
8t; R = 3-F-Ph, R_1 = $CH_2CH{=}CH(CH_3)_2$
8u; R = 4-F-Ph, R1 = $CH_2CH{=}CH(CH_3)_2$
8v; R = 3-F-Ph, R_1 = $CH_2CH_2CH(CH_3)_2$
8w; R = 4-F-Ph, R_1 = $CH_2CH_2CH(CH_3)_2$
8x; R = 3-F-Ph, R_1 = CH_2CH_3
8y; R = 3-F-Ph, R_1 = $CH_2CH_2CH_3$



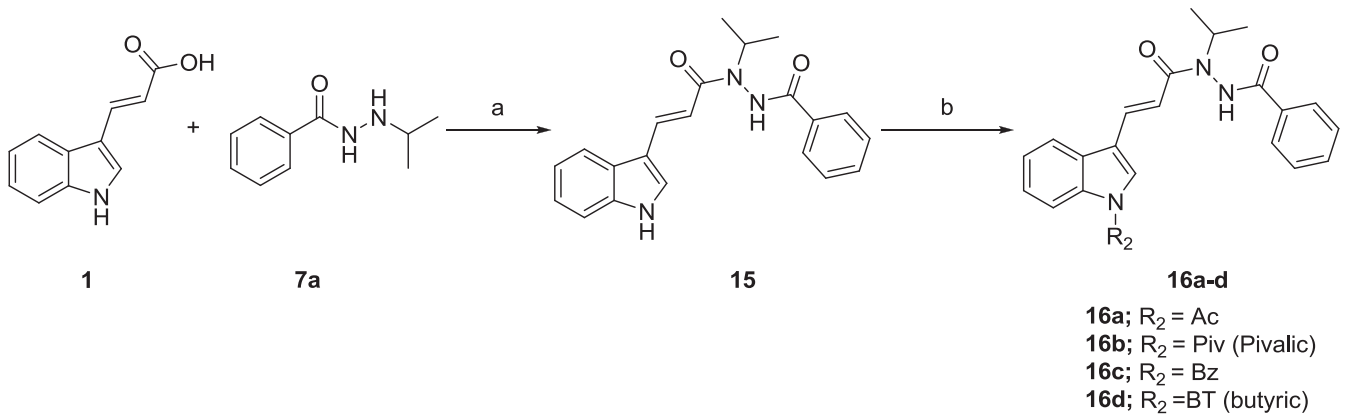
8b; R = 3-OH
8c; R = 4-OH

9a; R_1 = 3-OCH_3
9b; R_1 = 4-OCH_3
9c; R_1 = 3-$OCH_2CH_2OCH_3$
9d; R_1 = 4-$OCH_2CH_2OCH_3$

Scheme 1. Reaction and conditions: (a) MeI, K_2CO_3, acetone, 60 °C, 72 h, (90–98%); (b) $(Boc)_2O$, K_2CO_3, THF, 60 °C for **3a**, (80–95%); R_1–X (X = Br or Cl or I), NaH, DMF, 80 °C, 12 h for **3b–h**, (35–80%); cyclopropyl boronic acid, DMAP, $Cu(CO_2CH_3)_2$, NaHMDS, toluene, 95 °C, 12 h for **3i**, (45–50%); (c) NaOH, THF/MeOH/H_2O = 2:1:1, 50 °C, 12 h, (70–98%); (d) acetone, hexane, 65 °C, 12 h, (85–90%); (e) $NaBH_3CN$, AcOH, MeOH, room temperature, 12 h, (85–95%); (f) HATU, DIPEA, DMF, room temperature, 12 h, (34–70%); (g) MeI, K_2CO_3, acetone, 60 °C, 72 h for the synthesis of **9a** and **9b**, (36–50%); 1-bromo-2-methoxyethane, K_2CO_3, acetone 60 °C, 2 h for the synthesis of **9c** and **9d**, (30–55%).



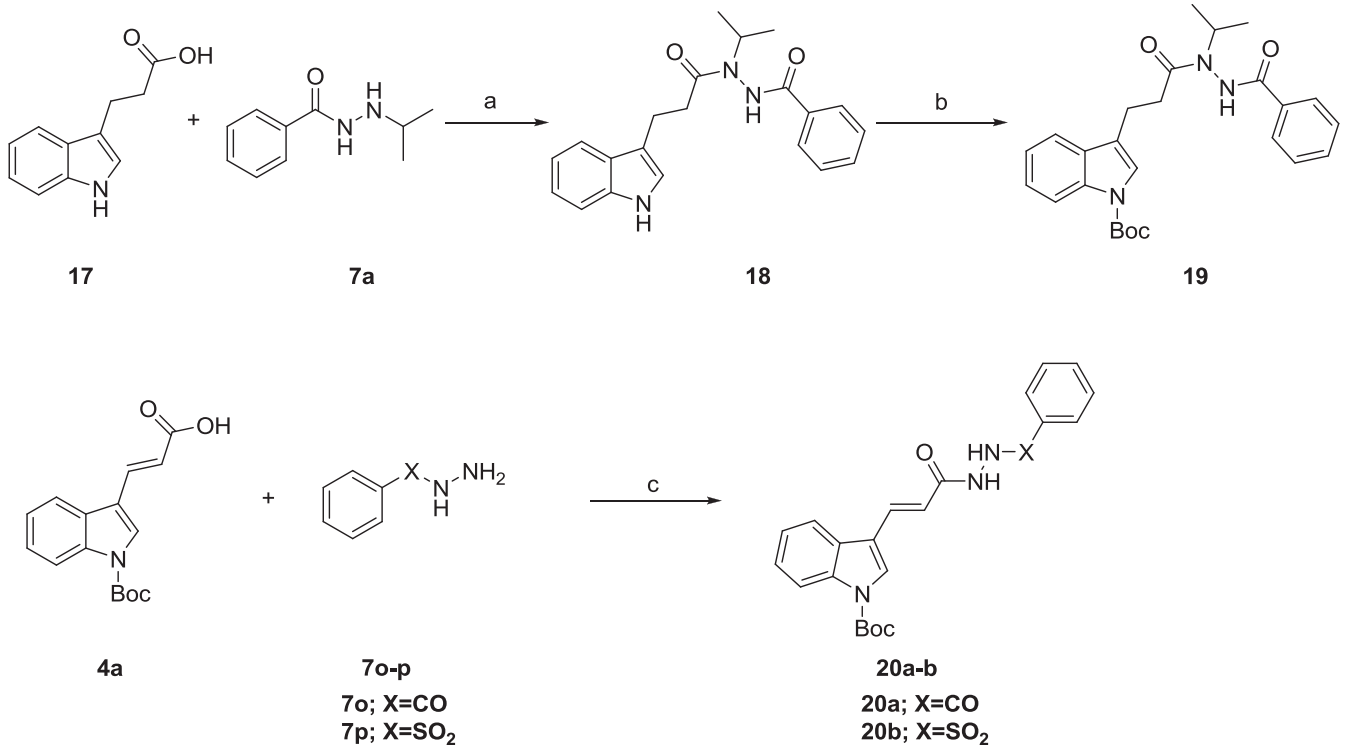
Scheme 2. Reaction and conditions: (a) ethyl(triphenylphosphoranylidene)acetate, benzene, 80 °C, 12 h, (81–89%); (b) NaOH, THF/MeOH/H_2O = 2:1:1, 50 °C, 12 h, (95–98%); (c) $(Boc)_2O$, DMAP, TEA, CH_3CN, THE, H_2O, room temperature, 4 h, (82–97%); (d) HATU, DIPEA, DMF, 80 °C, 12 h, (45–48%).



Scheme 3. Reaction and conditions: (a) HATU, DIPEA, DMF, room temperature, 12 h; (b) Ac_2O, DMAP, pyridine, DCM, room temperature, 12 h for **16a**; R_2–Cl, DIPEA, DMAP, DCM, room temperature, 12 h for **16b** and **16c**; Piv-Cl, K_2CO_3, DMAP, DMF, room temperature, 12 h for **16d**.

was added DIPEA (0.90 mL, 5.22 mmol). The reaction mixture was then stirred at room temperature overnight, and then partitioned between ethyl acetate and water. The organic phase was washed with brine, dried over $MgSO_4$, and concentrated. Purification by silica gel column chromatography using *n*-hexane/EtOAc = 3:1 as eluting solvents gave compound **8a** as a white solid. Compounds **8a–y**, **14a–d**, **15**, **18**, **20a**, and **20b** were also prepared using same procedure.

O-Alkylation of 8b. To a solution of the (*E*)-*tert*-butyl-3-(3-(2-(3-hydroxybenzoyl)-1-isopropylhydrazinyl)-3-oxoprop-1-enyl)-1*H*-indole-1-carboxylate (**8b**) (100 mg, 0.22 mmol) and potassium carbonate (35.8 mg, 0.26 mmol) in anhydrous acetonitrile (10.0 mL) was added iodomethane (0.0673 mL, 1.08 mmol) drop by drop at 0°C. The reaction mixture was refluxed at 85°C overnight. The remaining of the solvent was evaporated. Purification by silica gel column chromatography (*n*-hexane/EtOAc = 2:1) gave (*E*)-*tert*-butyl-3-(3-(1-isopropyl-2-(3-methoxybenzoyl)hydrazinyl)-3-oxo-prop-1-enyl)-1*H*-indole-1-carboxylate (**9a**) as a yellow solid. Compounds **9b–d** were also prepared using same procedure. Data for all prepared compounds can be found in Supporting Information.



Scheme 4. Reaction and conditions: (a) HATU, DIPEA, DMF, 80 °C 12 h, (60–73%); (b) $(Boc)_2O$, K_2CO_3, THF, 60 °C, 12 h, (92–98%); (c) HATU, DIPEA, DMF, 80 °C, 12 h, (60–75%).

(*E*)-4-fluoro-*N*′-isopropyl-*N*′-(3-(1-(3-methyl-but-2-enyl)-1*H*-indol-3-yl)acryloyl)benzohydrazide (8u): White solid; m. p. = 196 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.16 (1H, brs, —NH), 7.96 (2H, m, aromatic), 7.83 (1H, d, *J* = 15.4 Hz, indole—CH═CH—), 7.52 (1H, s, aromatic), 7.24 (1H, d, *J* = 10.5 Hz, aromatic), 7.11 (4H, m, aromatic), 6.93 (1H, s, aromatic), 6.75 (1H, d, *J* = 15.7 Hz, indole—CH═CH—), 5.22 (1H, s, —N–CH_2—CH═$(CH_3)_2$), 4.89 (1H, m, —N–CH—$(CH_3)_2$), 4.49 (2H, s, —N–CH_2—CH═$(CH_3)_2$), 1.74 (6H, d, *J* = 12.5 Hz, —N–CH_2—CH═$(CH_3)_2$), 1.18 (6H, s, —N–CH—$(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 166.5, 166.1, 164.1, 137.3, 131.8, 130.1, 126.2, 122.4, 120.9, 120.2, 118.8, 115.9, 115.7, 112.4, 110.2, 44.3, 25.5, 18.0; HRMS $[M + H]^+$ calcd. $[C_{26}H_{28}FN_3O_2 + H]$ 434.2239, found: 434.2233; HPLC purity = 100.0% area.

(*E*)-3-fluoro-*N*′-(3-(1-isopentyl-1*H*-indol-3-yl)acryloyl)-*N*′-isopropylbenzohydrazide (8v): Yellow solid; m.p. = 96 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.53 (1H, brs, —NH), 7.82 (1H, d, *J* = 15.4 Hz, indole—CH═CH—), 7.75 (1H, d, *J* = 7.6 Hz, aromatic), 7.70 (1H, d, *J* = 6.6 Hz, aromatic), 7.55 (1H, s, aromatic), 7.38 (1H, d, *J* = 5.4 Hz, aromatic), 7.24 (1H, d, *J* = 9.3 Hz, aromatic), 7.15 (3H, m, aromatic), 6.94 (1H, s, aromatic), 6.78 (1H, d, *J* = 15.7 Hz, indole—CH═CH—), 4.91 (1H, m, —N–CH—$(CH_3)_2$), 3.91 (2H, m, —N–CH_2—CH_2—CH—$(CH_3)_2$), 1.58 (2H, d, *J* = 6.8 Hz, –N–CH_2—CH_2—CH—$(CH_3)_2$), 1.49 (1H, m, —N–CH_2—CH_2—CH—$(CH_3)_2$), 1.19 (6H, m, —N–CH—$(CH_3)_2$), 0.89 (6H, m, —N–CH_2—CH_2—$(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 166.4, 163.6, 161.6, 137.1, 134.5, 132.0, 126.0, 123.1, 122.4, 120.7, 120.3, 115.0, 114.8, 112.4, 109.9, 60.4, 44.7, 38.5, 25.6, 22.2, 19.8, 14.1; HRMS $[M + H]^+$ calcd. $[C_{26}H_{30}FN_3O_2 + H]$ 436.2395, found: 436.2392; HPLC purity = 92.7% area.

Results and Discussion

All the newly synthesized compounds were evaluated for their ability to inhibit DGAT activity with human recombinant DGAT-1 and DGAT-2 (Table 1). The inhibitory effects of these compounds were compared with **T863** (IC_{50} = 0.068 μM)[13] as a positive control. First, structural modification of hydrazide derivatives was focused on derivatization of the phenyl ring replaced with different substituted phenyl rings. We initially checked the DGAT inhibitory activity of *N*-Boc-protected compound **8a** (containing phenyl group as a substituent). This compound showed potent DGAT inhibitory activity with an IC_{50} value of 9.6 μM for DGAT-1 and 7.5% inhibition for DGAT-2 at 10 μM (Table 1, entry 2). Compound **8a** also inhibited the biosynthesis of TG in HepG2 cells with treatment of 10 μM of **8a** (Table 1, entry 2). Thus compound **8a** might be considered to be a selective inhibitor of DGAT-1. Based on these results, derivatives of compound **8a** were screened to search for more potent compounds, and

Table 1. DGAT inhibitory activity of hydrazide derivatives.

Entry	Compound	hDGAT-1 IC_{50} (μM)	hDGAT-2 (% inhibition) (10 μM)	[^{14}C]TG synthesis (% of control) (10 μM), HepG2
1	**T863**[14]	0.068	17.1	51.1
2	**8a**	9.6	7.5	60.1
3	**8b**	>100	<0.3	86.7
4	**8c**	22.5	<0.3	62.2
5	**8d**	6.8	3.9	93.5
6	**8e**	20.4	2.3	87.1
7	**8f**	2.2	28.7	97.9
8	**8g**	8.8	<0.3	84.4
9	**8h**	20.4	<0.3	71.2
10	**8i**	2.1	28.9	94.8
11	**8j**	2.9	34.1	74.7
12	**8k**	9.8	22.1	96.7
13	**8l**	>100	<0.3	64.2
14	**8m**	>100	<0.3	66.8
15	**8n**	4.0	0.6	81.3
16	**9a**	>100	0.3	74.8
17	**9b**	>100	<0.3	85.8
18	**9c**	36.4	8.3	62.0
19	**9d**	>100	<0.3	72.6
20	**14a**	3.2	2.0	57.5
21	**14b**	>100	3.6	75.0
22	**14c**	>100	2.9	79.2
23	**14d**	>100	<0.3	85.7
24	**16a**	65.3	<0.3	89.0
25	**16b**	9.3	4.4	93.3
26	**16c**	5.7	11.0	91.4
27	**16d**	5.3	<0.3	78.2
28	**15**	>100	<0.3	115.6

Notes: hDGAT-1 and -2, human recombinant DGAT-1 and -2 *in vitro* enzymatic inhibition.

various results were obtained from compounds **8b–k** (Table 1, entries 3–12).

Although we observed good IC_{50} values for compounds **8f**, **8i**, and **8j**, those compounds showed poor control on biosynthesis of TG, suggesting that replacing the phenyl group with 2-pyridine and 3-pyridine groups (compounds **8l** and **8m**) resulted in complete loss of activity (Table 1, entries 13, 14). Although potent activity was observed with compound **8n** containing the 4-pyridine moiety, there was poor control on TG formation (Table 1, entry 15). Compounds **9a–d**, where —OH group of the phenyl ring was alkylated with methyl and 2-methoxyethane groups, also showed no activity (Table 1, entries 16–19). Compound **14a** containing 5-methyl substituent on the indole ring showed potent DGAT-1 inhibitory activity (IC_{50} = 3.2 μM) with 42.5% inhibition of TG biosynthesis at the cellular level (Table 1, entry 20). However, compounds **14b–d** containing 6-isopropyl, 6-methyl, and 6-fluoro substituents on the indole ring did not show any DGAT-1 inhibitory activity and exhibited poor control on TG formation (Table 1, entries 21–23). We speculated that the undesirable result of these examples was due to the elimination of the Boc group under physiological conditions, which led to the release of the corresponding Boc-free compounds. Notably, compound **15** containing no protecting group was found to be inactive in our preliminary studies (Table 1, entry 28); thus, we replaced the Boc group with other groups to check the role of the *N*-protecting groups. Compound **16a** with *N*-acyl protection did not show DGAT inhibitory activity (Table 1, entry 24). Although compounds **16b–d**, which was protected by pivalic, benzoyl, and butyric groups, showed good DGAT-1 inhibitory activities, they also showed poor control on TG formation at the cellular level (Table 1, entries 25–27). The loss of the activities in cellular assay would be speculated to result from the elimination of these labile groups under physiological conditions. Next, we focused on protection of the indole moiety with some stronger protecting groups in order to minimize the possibility of elimination in whole cells.

In compound **8o**, an *N*-Boc was replaced with an *N*-methyl group, but it did not show any improvement in activity (Table 2, entry 2). Although compound **8p**, which contains an *N*-benzyl protecting group, showed a moderate IC_{50} value of 13.6 μM, it presented excellent control on TG formation at the cellular level (Table 2, entry 3). Replacing the benzyl group with a 3-methyl-2-butene group (compound **8q**) improved the IC_{50} value to 11.8 μM and showed increased inhibition of TG (Table 2, entry 4). Methylcyclopropyl (compound **8r**) and cyclopropyl (compound **8s**) as *N*-protecting groups resulted in decreased level of the IC_{50} values with 41.6 and 24.6 μM and decreased inhibition of TG in 35.6 and 40.4%, respectively (Table 2, entries 5, 6). Based on these results, compound **8q** containing *N*-(3-methyl-2-butenyl) as a protecting group emerged as the better potent compound.

It has been observed in many cases that replacement of the hydrogen atom of organic compounds by a fluoro group imparts substantial effects on their characteristics.[14] Therefore, we made fluoro derivatives of the best molecule found so far in this study, namely compound **8q**. Compounds **8t** and **8u** containing 3-fluoro and 4-fluoro phenyl rings, respectively, were synthesized and screened. As expected, both compounds showed better IC_{50} values of 7.2 and 1.5 μM, respectively, and were more potent than compound **8q** (IC_{50}, 11.8 μM). Also, compound **8u** showed improvement in control of TG synthesis, with 44.3% inhibition in whole cells compared with compound **8q** (Table 2, entries 7, 8). With compound **8w**, which contains a 4-fluoro-phenyl ring and *O*-isopentyl group, a potent IC_{50} value of 1.0 μM was observed, but it only showed moderate control on TG formation with 36.5% inhibition (Table 2, entry 10). Changing the position of the fluoro group from 3 to 4 (compound **8v**) resulted in excellent control on TG formation in whole cells, with 49.2% inhibition (Table 2, entry 9). Compounds **8x** and **8y** with *O*-ethyl and *O*-*n*-propyl group, respectively, showed moderate DGAT-1 inhibitory activity with poor control on TG (Table 2, entries 11, 12).

Table 2. DGAT inhibitory activity of hydrazide derivatives.

Entry	Compound	hDGAT-1 IC_{50} (μM)	hDGAT-2 (% inhibition) (10 μM)	[^{14}C]TG synthesis (% of control) (10 μM), HepG2
1	**T863**[14]	0.068	17.1	51.1
2	**8o**	28.3	<0.3	88.6
3	**8p**	13.6	5.0	59.8
4	**8q**	11.8	1.2	58.5
5	**8r**	41.6	<0.3	64.4
6	**8s**	24.6	<0.3	59.6
7	**8t**	7.2	18.5	58.0
8	**8u**	1.5	28.0	55.7
9	**8v**	1.8	42.3	50.8
10	**8w**	1.0	64.0	63.5
11	**8x**	8.1	20.3	79.4
12	**8y**	3.6	32.4	79.9
13	**19**	>100	<0.3	60.7
14	**20a**	>100	3.7	61.7
15	**20b**	>100	<0.3	81.7

Notes: hDGAT-1 and -2, human recombinant DGAT-1 and -2 *in vitro* enzymatic inhibition.

The saturated analog **19** was synthesized to check the role of the double bond of **8a**. It did not show any DGAT-1 inhibitory activity (Table 2, entry 13) and was less potent (IC_{50} = 9.6 μM) than compound **8a**. This suggests that the rigid conformation with the double bond plays some role in controlling the activities of these compounds. Moreover, the isopropyl group on hydrazide nitrogen has also some role in enhancing the activities of these compounds because compound **20a** (without *N*-isopropyl group) did not show any DGAT-1 inhibitory activity while compound **8a** showed moderate activity (Table 1, entry 14). Replacing the carbonyl group with sulfonyl (compound **20b**) was also not useful (Table 1, entry 14). This structure–activity relationship study clearly showed that compounds with the indole moiety protected by long-chain alkyl groups such as isopentyl and 3-methyl-2-butene generally show better activities than the same compounds protected by labile or short-chain groups. Also, compounds with fluoro-substituted phenyl rings generally show better activities than compounds without fluoro substituents (Table 2). Thus, from this screening, compounds **8u** and **8v** have emerged as potent compounds that show high DGAT inhibitory activities and have a tendency to control effectively TG biosynthesis in whole cells.

Next, we chose compound **8u** for an oral lipid tolerance test (OLTT) (an acute lipid challenge model measuring TG change after corn oil bolus dosing). Mice were treated with a 10 or 30 mg/kg dose, respectively, of compounds **8u** and **T863**. **T863** had been previously reported to inhibit TG absorption through DGAT1 inhibition and was used as a positive control. After 4 h, both compounds showed good control over absorption of TG (Figure 2 and Table 3). After 4 h, compound **8u** (10 mg/kg) showed a significant decrease in plasma TG levels compared with vehicle-treated control animals, indicating delayed absorption of TG after an acute lipid challenge; however, this compound was less potent than **T863**, which showed a dynamic drop of serum TG level.

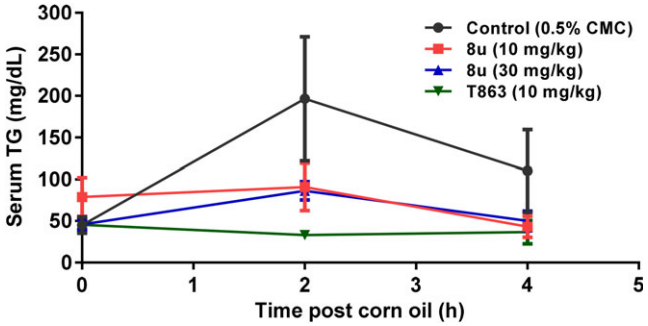


Figure 2. Oral lipid tolerance test (OLTT) of **8u**.

Table 3. Oral lipid tolerance test (OLTT) of **8u**.

Group (n = 7)	Doses (mg/kg)	Serum TG (mg/dL) 0 h	2 h	4 h
Control (0.5% CMC)	0	45.2 ± 9.8	196.6 ± 74.6	110.2 ± 49.5
8u	10	78.8 ± 23.2	90.8 ± 28.2*	43.2 ± 12.9*
	30	45.8 ± 6.1	86.4 ± 11.1*	50.0 ± 11.9*
T863	10	45.4 ± 7.4	33.2 ± 5.9**	36.6 ± 14.1*

Significant figures (t-test): $^{*}p < 0.05$, $^{**}p < 0.001$ *vs.* (vehicle control).

Conclusion

In summary, by using human recombinant DGAT-1 and DGAT-2, a novel series of hydrazide derivatives were found to exhibit selective DGAT-1 inhibitory activities. Among all the compounds screened, compounds **8u** and **8v** were found to be potent DGAT-1 inhibitors. In addition, compound **8u** exhibited an excellent dose-dependent inhibitory effect on TG synthesis in HepG2 cells. Furthermore, treatment with compound **8u** for an OLTT showed a significant decrease in plasma TG levels compared with vehicle-treated control animals, indicating delayed absorption of TG after an acute lipid challenge. Accordingly, this study demonstrates that very simple and small organic molecules can be efficient leads for treatment of metabolic disorders such as obesity.

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Supporting Information. Additional supporting information is available in the online version of this article.

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