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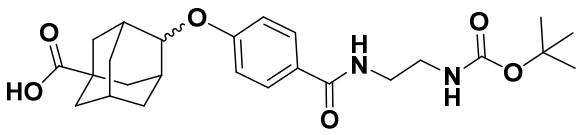
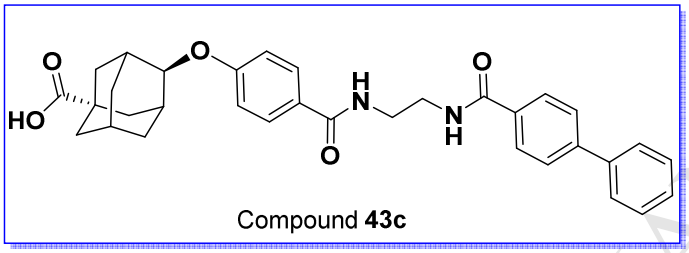


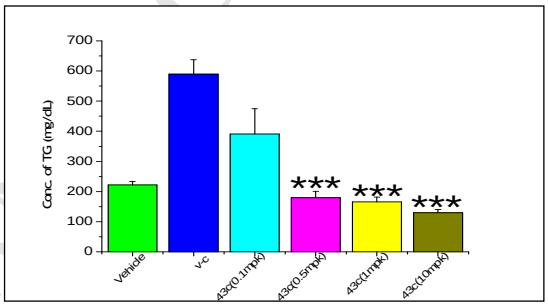
Hit compound **7**
hDGAT1 IC_{50} = 539 nM
mDGAT1 IC_{50} = 2651 nM

Optimization



Compound **43c**

hDGAT1 IC_{50} = **5 nM**
mDGAT1 IC_{50} = **5 nM**



OLTT dose dependency in C57BL/6 mice

Discovery and Optimization of Adamantane Carboxylic Acid Derivatives as Potent Diacylglycerol Acyltransferase 1 Inhibitors for the Potential Treatment of Obesity and Diabetes

Suvarna H Pagire,[†, ‡, ⊥] Haushabhau S. Pagire,[†, ‡, ⊥] Gwi Bin Lee,[†] Seo-Jung Han,[†] Hyun Jung Kwak,[†] Ji Young Kim,[†] Ki Young Kim,[†] Sang Dal Rhee,[†] Jeong Im Ryu,[†] Jin Sook Song,[†] Myung Ae Bae,[†] Mi-jin Park,[§] Dooseop Kim,[§] Duck Hyung Lee, [∥] Jin Hee Ahn[†, ‡,*]

[†] *Drug Discovery Division, Korea Research Institute of Chemical Technology, Daejeon, 305-600, Korea,*

[‡] *Department of Medicinal and Pharmaceutical Chemistry, University of Science and Technology, 305-333, Korea,*

[§] *Handok Pharmaceuticals Co., Ltd. Seoul 135-755, Republic of Korea,*

[∥] *Department of Chemistry, Sogang University, Seoul 121-742, Republic of Korea.*

1. INTRODUCTION

Inhibitors of diacylglycerol acyltransferase 1 (DGAT1) have come to the fore in recent years as potential therapies for obesity, diabetes and other elements of metabolic syndrome [1] Acyl CoA: diacylglycerol acyltransferase (DGAT) enzymes catalyze the final and the only committed step in triglyceride synthesis; the joining of 1, 2-diacylglycerol and fatty acyl CoA at the endoplasmic reticulum [2, 3]. Two members of this enzyme family have been reported; DGAT1 and DGAT2. Although both reported enzymes catalyze the formation of triglycerides (TG) from diacylglycerol and fatty acyl CoA and share limited homology. Mice lacking the DGAT1 gene were shown to be viable and resistant to the effects of diet-induced obesity (DIO) and hepatic steatosis when fed a high-fat diet. Additionally, these animals were reported to have an increased sensitivity to both insulin and leptin, decreased levels of tissue triglycerides, and increased energy expenditure. Further studies demonstrated that DGAT1$^{-/-}$ mice had dramatically reduced levels of intestinal triglyceride synthesis and chylomicron secretion following an oral lipid

challenge [4-6]. These studies have spurred research efforts to determine whether selective small molecule inhibitors of DGAT1 can produce the same improved metabolic profile observed in the $DGAT1^{-/-}$ animals.

A number of small molecule inhibitors [7] of DGAT1 have been reported and the progress of some of these compounds into clinical development supports the potential for their therapeutic use (Fig. 1).

The DGAT1 inhibitor discovered by Novartis, pradigastat (LCQ908) is the most advanced clinical candidate and is currently being evaluated in phase II and phase III studies [8a]. Novartis has recently disclosed that pradigastat lowered the fasting plasma TG level in patients with familial chylomicronemia syndrome [8b,c]. Also, AstraZeneca and Pfizer candidates [9] have been advanced into clinical trials and showed markedly reduced postprandial TG excursion with gastrointestinal side effects.

Fig. 1.

In an attempt to identify additional pharmacophores that could serve as inhibitors of DGAT1, we conducted a high-throughput screen (HTS) against DGAT1. Adamantane containing compound **7** (Fig. 2) was identified as a hit with IC_{50} values of 539 nM and 2651 nM against human and mouse DGAT1, respectively.

Fig. 2.

The adamantane group also exists in several other drugs (Fig. 3). The first adamantane derivative used as a drug was amantadine in 1967, which initially used as an antiviral drug against various strains of flu [10] and then to treat parkinson's disease (adapalene, dopamantine, memantine, rimantadine and tromantadine) as well as dipeptidyl peptidase-4 (DPP-4) inhibitors for diabetes (saxagliptin, and vildagliptin) [11, 12]. Also, polymers of adamantane have been patented as antiviral agents against HIV [13] and Choi's group reported adamantane containing DGAT1 inhibitor [7]

Fig. 3.

We herein describe the synthesis, structure-activity relationships (SAR), and optimization of DGAT1 inhibitors based on the adamantane carboxylic acid core structure **7**.

2. Chemistry

Compounds **7, 8, 10, 12** and **19a-e** were prepared as described in Scheme 1. The commercially available 4-oxo-adamantane-1-carboxylic acid methyl ester **1** was reduced using sodium borohydride ($NaBH_4$) to afford adamantanol **2,** which subsequently underwent the Mitsunobu reaction with benzyl 4-hydroxybenzoate (**3**) followed by hydrogenation to give acid intermediate **5**. The acid intermediate **5** was coupled with 1-(*tert*-butyloxycarbonyl)ethylenediamine **14,** which is synthesized from ethylenediamine **13,** to give the coupled product **6**. Subsequent hydrolysis or *tert*-butyl deprotection gave compounds **7** and **9,** respectively. Amine intermediate **9** was treated with phenylisocyanate and benzenesulfonyl chloride followed by ester hydrolysis

to give the desired acid derivatives **10** and **12**. Also, Boc-deprotection of **7** afforded amine hydrochloride **8**.

Condensation of the intermediate **14** with the appropriate carboxylic acids **15a-e** followed by acidic deprotection afforded amine hydrochlorides **17a-e**. Subsequent condensation with acid intermediate **5** followed by hydrolysis, afforded desired acid derivatives **19a-e.**

A Horner-Wadsworth-Emmons (HWE) reaction between 5-hydroxy-2-adamantanone **20** and trimethyl phosphonoacetate **21** provided α, β-unsaturated ester **22,** which was hydrogenated to give saturated alcohol **23**. Compound **24** synthesized by Mitsunobu reaction of alcohol **23** with **3,** subsequent debenzylation gave acid **25.** This was further coupled with amine intermediates **17b, c** and hydrolyzed to give desired acid **27b,c**.

In order to obtain three carbon linked adamantane derivatives, (3-aminopropyl)carbamic acid *tert*-butyl ester **28** was condensed with carboxylic acids **15b, c** followed by acidic deprotection to afford amine hydrochlorides **30b, c**. Further condensation with acid intermediates **5** followed by hydrolysis gave desired derivatives **32b, c** (Scheme 2).

Z-isomer **37** and *E*-isomer **39** adamantane carboxylic acids were synthesized as described in Scheme 3. Esterification of 4-hydroxybenzoic acid (**33**) gave *tert*-butyl ester **34**. Ester **34** underwent Mitsunobu reaction with adamantanol **2** to afforded *Z*- and *E*-mixture of **35.** *Z*-isomer **36** and *E*-isomer **38** were separated by silica gel column chromatography from mixture **35**, and both compounds were crystallized from methanol giving off white needle-like single crystals, suitable for X-ray crystallographic analysis. Fig. 4 and 5 illustrate the molecular structures of the *Z*-isomer **36** and *E*-isomer **38**, which were subsequently deprotected with trifluoroacetic acid (TFA) to give the individual *Z*-isomer and *E*-isomer carboxylic acids **37** and **39,** respectively.

Condensation of the intermediate **14** with the appropriate carboxylic acids **44a-g,** followed by acidic deprotection afforded amine hydrochlorides **46a-g**. The resulting amine hydrochlorides **46a-g** were condensed with the corresponding acid intermediates **39** and hydrolyzed to give the desired acid derivatives **48a-g** (Scheme 5).

3. RESULTS AND DISCUSSION

A high-throughput screening (HTS) of our small molecule library resulted in the identification of compound **7** as a novel and moderately potent DGAT1 inhibitor (Fig. 2). From initial implementation, we considered **7** to be a good candidate for lead optimization based on its moderate inhibitory activity. As a next step, we attempted to replace the *tert*-butyl carbamate group with the parent amine hydrochloride **8,** but this change resulted in the loss of DGAT1 potency. We next tried to replace *tert*-butyl carbamate group with urea **10**, sulfonamide **12**, benzamide **19a** and napthamide **19b**. Of these, **19b** turned out to be successful exhibiting an enhanced potency (IC_{50} = 34 nM) as compared to carbamate **7**. Additionally, we synthesized a biphenyl analogue **19c** with improved potency (IC_{50} = 12 nM). Replacement of the phenyl ring with a heteroaromatic ring (to decrease the overall lipophilicity) afforded the oxazole derivative **19d** (IC_{50} = 89 nM). When we changed oxazole into pyrazole (analogue **19e**), DGAT1 inhibitory activity was lost (Table 1). From these data, we further investigated the SAR of napthamide **19b** and biphenyl analogue **19c**.

Scheme 1

Table 1.

As shown in Table 2, introduction of adamantane acetic acid derivatives (**27b,** IC_{50} > 500 nM) possessing naphthamide analogue resulted in greatly diminished DGAT1 potency and **27c (**IC_{50} = 159 nM) biphenyl substituent appeared moderate tolerated at the DGAT1inhibitory activity. Extension of the carbon chain i.e., ethylene group to a propylene group (compound **32b** and **32c**) resulted in loss of DGAT1 inhibitory activity.

Scheme 2:

Table 2.

As shown in Table 1, we found potent naphthalene analogue **19b** and biphenyl analogue **19c**, which are mixtures of *Z*-isomer and *E*-isomer showing nanomolar *in vitro* potency respectively. Therefore, we synthesized the individual isomers of **19b** and **19c** as shown in Table 3. In each case, the *E*-isomer showed better *in vitro* potency as compared to *Z*-isomer (compounds **43b** and **43c**). Compound **43c** showed the most potent in vitro potency therefore, it was further modified.

Scheme 3:

Fig. 4.

Fig. 5.

Condensation of the amine hydrochloride intermediates **17b, c** with acid intermediates **37** and **39** followed by hydrolysis to give the desired acid derivatives **41b, c,** and **43b, c** (Scheme 4).

Scheme 4:

Table 3.

As a next step we attempted to investigate further SAR for substituent on the biphenyl ring. As shown in Table 4, we incorporated methyl group on biphenyl ring. **48a** showed an improvement in mouse DGAT1 inhibitory activity with an IC_{50} value of 2 nM, but at the same time a 6-fold reduction in human DGAT1 potency. Also we introduced trifluoromethyl and chloro substituents on biphenyl ring. Replacement of methyl group with trifluoromethyl and chloro resulted in loss of DGAT1 inhibitory activity. Further we investigated disubstituted derivatives on aromatic ring. As an insertion of one more methyl group on **48a** (compound **48e,** IC_{50} = 105 nM), showed decrease in the human DGAT1 potency. In addition, dichloro (**48f,** IC_{50} = 280 nM) and difluoro analogue (**48g,** IC_{50} = 170 nM) (electron withdrawing group) showed no improvement. Compound **43c** showed the most potent in vitro potency than substituted biphenyl ring analogues therefore, it was further evaluated for its druggability and in vivo efficacy.

Scheme 5:

Table 4

The epimerization of carboxylic acid is known and has been previously studied in detail in the case of ibuprofen and DGAT inhibitor [14, 15, 7a,b].

Fig. 6.

Usually epimerization occurs at the α-proton on the carbon atom attached to the carboxylic acid group but in our case, the carboxylic acid group is attached to a tertiary carbon that eliminating the possibility of epimerization. Discovery of biphenyl adamantane carboxylic acid analogue **43c** provided a molecule that could not undergo epimerization while improved DGAT1 potency by an order of magnitude compared to hit **7**.

As shown in Table 5, **43c** exhibited good selectivity against DGAT-2 and stability in human liver microsome, and showed no significant inhibition in any of the major CYP isoforms (1A2, 2C9, 2C19, 2D6, and 3A4). Moreover, **43c** did not inhibit the hERG channel, has reasonable permeability and no cytotoxicity.

Table 5.

To evaluate the *in vivo* efficacy of **43c**, the oral lipid tolerance test (OLTT), an acute lipid challenge model measuring TG change after a corn oil bolus dosing was performed. Normal (C57BL/6 mice) and diabetic mice (TallyHo mice) [16] were treated with vehicle (0.5% carboxymethyl cellulose, CMC) or **43c** (3 mg/kg) at 30 min prior to corn oil dosing, blood samples for TG measurement were taken at 2 h post-lipid challenge. As can be seen in Fig. 7, compound **43c** reduced plasma TG levels in the blood by over 95% compared to vehicle at an

oral dose of 3 mg/kg in normal (A) and diabetic mice (B). Also, Fig 7 (C) shows that compound **43c** was highly efficacious in reducing plasma TG, exhibiting ~100% inhibition at doses of 0.5, 1 and 10 mg/kg.

Fig. 7.

Recently, we reported lipid staining dyes "LipidGreen" and "LipidGreen2", [17] which effectively stained fat deposits in live zebrafish. We performed TG reduction efficacy after **43c** treatment *in vivo* for 3 days. As shown in Fig. 8, **43c** significantly decreased the lipid deposits compared to an untreated control in live zebrafish.

Fig. 8.

We examined the PK profile of compound **43c**. As shown in Table 6, compound **43c** the maximal plasma concentration (Cmax) of 0.24±0.14 μg·hr/mL appeared and the AUC value was 0.12 μg/mL after oral administration. The oral bioavailability was 13.4 %. To investigate PK and PD correlation, we examined intestinal disposition of **43c**. DGAT1 is mainly expressed in intestine [18]. Whole mouse small intestine was collected to estimate the total intestinal concentration of **43c** could represent total amount in the intestinal area including lumen. Separately, intestinal tissue was prepared by flushing with phosphate buffered saline (PBS) to remove the drug which had not penetrated the enterocyte. The drug concentration in whole intestine and enterocyte were 430 and 104-fold, respectively, higher than the plasma at Tmax demonstrating that most of the dosed **43c** tended to remain locally in the intestine (Table 7).

These results also propose that the characteristics of **43c** expressing local exposure to the target organ, intestine could be critical in achieving its therapeutic efficacy.

Table 6.

Table 7.

Compound **43c** was evaluated for *in vivo* efficacy in body weight gain reduction and glucose lowering efficacy in a diet-induced obesity (DIO) mouse model (Fig. 9). Treatment with 43c for 4 weeks resulted in a trend towards reduced in body weight gain by ~6% without affecting food intake (Fig. 9A) compared to the control mice at a dose of 10 mg/kg. After 4 weeks of treatment with 43c, an oral glucose tolerance test (OGTT) was performed, and **43c** significantly reduced the glucose AUC by 54% compared to DIO vehicle control (Fig. 9B).

Fig. 9.

4. Conclusions

In this study, a new series of adamantane carboxylic acid derivatives was identified and evaluated for their ability to inhibit DGAT1. Compound **43c** showed good *in vitro* activity against human and mouse DGAT1, liver microsomal stability, safety profiles such as CYP inhibition, hERG and cytotoxicity. Compound **43c** significantly reduced blood TG level after a corn oil challenge in mice and zebrafish, and also reduced body weight gain and glucose AUC after 4 weeks treatment in DIO mice.

5. Experimental

5.1. Materials and methods

Melting points were determined on an MEL-TEMP apparatus and are uncorrected. IR Spectra were obtained on a Smith ATR-FT-IR spectrometer and the absorption frequencies are reported in wavelength (cm^{-1}). ^{1}H NMR spectra were run on Bruker AVANCE-500, Bruker AVANCE-300 and Varian OXFORD-300 spectrometers at 500 and 300 MHz. Chemical shifts (δ) are expressed in ppm downfield from TMS as internal standard. The letters s, d, t, q, and m are used to indicate singlet, doublet, triplet, quadruplet, and multiplet, respectively. High-resolution mass spectra were obtained on the Autospec Magnetic sector mass spectrometer (Micromass, Manchester, UK). The reactions were monitored by thin-layer chromatography (TLC) and column chromatography was performed using Chromatorex GS60-40/75. X-ray diffraction crystal structure analysis was obtained on Bruker SMART APEX II.

5.2. Chemistry

Unless mentioned otherwise all reactions were performed under atmosphere. All anhydrous solvents (stored over molecular sieves) and chemicals were obtained from standard commercial vendors and were used without any further purification.

*5.2.1. E- and Z-4-Hydroxyadamantane-1-carboxylic acid methyl ester (**2**)*

To a stirred solution of 4-oxoadamantane-1-carboxylic acid methyl ester **1** (6 g, 28.811 mmol), in MeOH (60 mL), powdered $NaBH_4$ (1.19 g, 31.692 mmol) was added cautiously in three portions at room temperature. The mixture was stirred for 4 h and concentrated under reduced pressure to remove the bulk of the methanol. The residue was diluted with 5% aqueous HCl (20 mL) extracted with dichloromethane (2 X 200 mL). The combined organic layers were washed

with brine (50 mL), dried over anhydrous sodium sulfate. Evaporation of solvents gave ~60/40 (E/Z) mixture of the epimeric alcohols **2** as a colorless oil (5.9 g, 97%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.92-3.80 (m, 1H), 3.69-3.61 (m, 3H), 2.30-1.36 (m, 13 H); LC-MS (*m*/*z*): 211 $[M+H]^+$.

*5.2.2. E- and Z-4-(4-Benzyloxycarbonyl-phenoxy)-adamantane-1-carboxylic acid methyl ester (**4**)*

To a solution of benzyl 4-hydroxybenzoate **3** (3 g, 13.144 mmol) and triphenyl phospine (4.14 g, 15.77 mmol), dissolved in THF (50 mL) was added *E*- and *Z*-4-hydroxy-adamantane-1-carboxylic acid methyl ester **2** (3.317 g, 15.773 mmol). The reaction mixture was heated to reflux and DIAD (3.096 mL, 15.773 mmol) was added drop wise at reflux over 2 h. The resulting mixture was stirred under reflux for 16 h. The volume was reduced by evaporation and the resulting mixture was added water (50 mL) followed by extraction with DCM (3 x 200 mL). The combined organic phases were washed with brine (100 mL), dried over Na_2SO_4, filtered and the volatiles evaporated in vacuo. Crude product was purified by column Chromatography using hexane followed by a mixture of EtOAc/hexanes (1:10) .Combined fractions were evaporated in vacuum afforded *E*- and *Z*-4-(4-benzyloxycarbonyl-phenoxy)-adamantane-1-carboxylic acid methyl ester **4** as an oil (3.3 g, 60 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05-7.97 (m, 2H), 7.47-7.29 (m, 5H), 6.96-6.87 (m, 2H), 5.33 (s, 2H), 4.52-4.41 (m, 1H), 3.70-3.63 (m, 3H), 2.33-1.45 (m, 13H); LC-MS (*m*/*z*): 421 $[M+H]^+$.

*5.2.3. E- and Z -4-(4-Carboxyphenoxy)adamantane-1-carboxylic acid methyl ester (**5**)*

To a solution of *E*- and *Z*-4-(4-benzyloxycarbonylphenoxy)adamantane-1-carboxylic acid methyl ester **4** (3.2 g, 7.61 mmol) dissolved in ethyl acetate (100 mL) was added 10% (w/w) palladium

on carbon (0.32 g) and resulting mixture was hydrogenated using hydrogen balloon. The catalyst filtered off through celite and the filtrate evaporated in vacuo afforded *E*- and *Z*-4-(4-carboxyphenoxy)adamantane-1-carboxylic acid methyl ester **5** as a white solid (2.5 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04 (d, *J* = 7.83 Hz, 2H), 6.95 (d, *J* = 8.19 Hz, 2H), 4.55-4.42 (m, 1H), 3.73-3.62 (m, 3H), 2.35-2.66 (m, 12H), 1.57-1.45 (m, 1H); LC-MS (*m*/*z*): 331 $[M+H]^+$.

*5.2.4. (2-Aminoethyl)carbamic acid tert-butyl ester (**14**)*

A solution of di-*tert*-butyl dicarbonate (3.63 g, 16.639 mmol) in dichloromethane (200 mL) was added dropwise to a solution of ethylenediamine (10 g, 166.39 mmol) in dichloromethane (50 mL) over 6 h with vigorous stirring. Stirring was continued for 24 h at room temperature. Removed undissolved solid through filtration and washed with dichloromethane. Collected filtrate were washed with aqueous sodium carbonate and brine , dried over anhydrous sodium sulfate and evaporated under reduced pressure to offered the crude product which was purified by silica gel column chromatography to give (2-aminoethyl)carbamic acid *tert*-butyl ester **14** as a colorless viscous liquid (5 g, 19 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.88 (bs, 1H), 3.17 (q, J = 5.76 Hz, 2H), 2.79 (t, J = 6 Hz, 2H), 1.44 (s, 9H), 1.13 (s, 2H); LC-MS (*m*/*z*): 161.02 $[M+H]^+$.

*5.2.5. E- and Z- 4-[4-(2-tert-Butoxycarbonylaminoethylcarbamoyl)phenoxy]adamantane-1-carboxylic acid methyl ester (**6**)*

A mixture of *E*- and *Z*-4-(4-carboxyphenoxy)adamantane-1-carboxylic acid methyl ester **5** (300 mg, 0.908 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (435.19 mg, 2.27 mmol), *tert*-butyl (2-aminoethyl)carbamate **14** (160.03 mg, 0.99 mmol) and 1-hydroxybenzotriazole monohydrate (HOBt) (184.06 mg, 1.362 mmol) in dichloromethane (7 mL) was stirred for 24 h at room temperature. The reaction mixture was diluted with aqueous

$NaHCO_3$ and extracted with dichloromethane. The extracts were washed with brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography to give *E*- and *Z*- 4-[4-(2-*tert*-butoxycarbonylaminoethylcarbamoyl)phenoxy]adamantane-1-carboxylic acid methyl ester **6** (386 mg, 89%) as a white solid**.** ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75 (d, *J* = 8.34 Hz, 2H), 6.99 (bs, 1H), 6.92 (d, *J* = 8.15 Hz, 2H), 4.96 (bs, 1H), 4.51-4.38 (m, 1H), 3.71-3.63 (m, 3H), 3.58-3.48 (m, 2H), 3.45-3.33 (m, 2H), 2.33-1.46 (m, 13H), 1.42 (s, 9H); LC-MS (*m*/*z*): 473 $[M+H]^+$.

*5.2.6. E- and Z-4-[4-(2-tert-Butoxycarbonylaminoethylcarbamoyl)phenoxy]adamantane-1-carboxylic acid (**7**)*

A solution of *E*- and *Z*-4-[4-(2-*tert*-butoxycarbonylaminoethylcarbamoyl)phenoxy]adamantane-1-carboxylic acid methyl ester **6** (100 mg, 0.212 mmol) in THF (35 mL) and MeOH (35 mL) was treated with aqueous 1M sodium hydroxide solution (42.32 mg, 1.058 mmol) at 40 °C for 12 h. After this time, the solution was cooled to room temperature, and the volume was reduced by rotary evaporation to 35 mL. The resulting solution was acidified with 1N hydrochloric acid to pH 4-5. More water was added (50 mL) and the aqueous solution was extracted with EtOAc (3 x 50 mL). The combined organic layer was washed with brine, dried over sodium sulfate and concentrated. A crude product was isolated form acetonitrile to afford the title compound *E*- and *Z*-4-[4-(2-*tert*-butoxycarbonylaminoethylcarbamoyl)phenoxy]adamantane-1-carboxylic acid **7** (83 mg, 85%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.11 (s, 1H), 8.27 (t, *J* = 5.36 Hz, 1H), 7.78 (d, *J* = 8.55 Hz, 2H), 7.02 (d, *J* = 8.80 Hz, 2H), 6.88 (t, *J* = 5.65 Hz, 1H), 4.63-4.53 (m, 1H), 3.25 (q, *J* = 6.03 Hz, 2H), 3.07 (q, *J* = 6.03 Hz, 2H), 2.20-1.40 (m, 13H), 1.36 (s, 9H); LC-MS (*m*/*z*): 459 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3442, 3322, 2928, 1715, 1649, 1545,

1497, 1289, 1246, 1233, 1179, 1156, 681; Anal. Calcd for $C_{25}H_{34}N_2O_6$: C, 65.48; H,7.47; N,6.11. Found: C,65.26; H,7.54; N,6.02.

*5.2.7. E- and Z-4-[4-(2-Aminoethylcarbamoyl)phenoxy]adamantane-1-carboxylic acid, hydrochloride (**8**)*

To a mixture of *E*- and *Z*-4-[4-(2-*tert*-butoxycarbonylaminoethylcarbamoyl)phenoxy]-adamantane-1-carboxylic acid **7** (30 mg, 0.076 mmol) in ethyl acetate (2 mL) was added hydrogen chloride 4.0 M solution in 1,4-dioxane (1 mL) and the mixture was stirred for 12 h. The mixture was concentrated to minimum volume and the residue was collected by filtration to give *E*- and *Z*-4-[4-(2-aminoethylcarbamoyl)phenoxy]adamantane-1-carboxylic acid hydrochloride **8** (30 mg, 99%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.43 (s, 1H), 7.84 (d, J = 6.61 Hz, 2H), 7.05 (d, J = 6.89 Hz, 2H), 4.65-4.55 (m, 1H), 3.51-3.44 (m, 2H), 2.96 (t, J = 6.14 Hz, 2H), 2.21-1.39 (m, 13H); LC-MS (*m*/*z*): 359 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3217, 2924, 2858, 16.70, 1606, 1560, 1506, 1302, 1251, 1018, 850; Anal. Calcd for $C_{20}H_{27}ClN_2O_4$: C,60.83; H,6.89; N,7.09. Found: C,60.9; H,6.93; N,6.85.

*5.2.8. E- and Z-4-[4-(2-Aminoethylcarbamoyl)phenoxy]adamantane-1-carboxylic acid methyl ester, hydrochloride salt (**9**)*

To a solution of *E*- and *Z*-4-[4-(2-*tert*-butoxycarbonylaminoethylcarbamoyl)phenoxy]-adamantane-1-carboxylic acid methyl ester 6 (65 mg, 0.138 mmol) in ethyl acetate (6 mL) was added 4.0 M hydrogen chloride solution in 1,4-dioxane (0.5 mL) and stirring continued for 24 h. The reaction mixture was concentrated in vacuo and the residue was collected by filtration to give *E*- and *Z*-4-[4-(2-aminoethylcarbamoyl)phenoxy]adamantane-1-carboxylic acid methyl ester, hydrochloride salt **9** (55 mg, 98 %) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6):

δ 8.58 (t, *J* = 5.45 Hz, 1H), 8.01 (s, 3H), 7.86 (d, *J* = 8.67 Hz, 2H), 7.09-7.00 (m, 2H), 4.66-4.54 (m, 1H), 3.62-3.57 (m, 3H), 3.49 (q, *J* = 5.73 Hz, 2H), 3.02-2.90 (m, 2H), 2.21-1.39 (m, 13H); LC-MS (*m*/*z*): 373 $[M+H]^+$.

*5.2.9. E- and Z-4-{4-[2-(3-Phenylureido)ethylcarbamoyl]phenoxy}adamantane-1-carboxylic acid (**10**)*

To a solution of *E*- and *Z*-4-[4-(2-aminoethylcarbamoyl)phenoxy]adamantane-1-carboxylic acid methyl ester, hydrochloride **9** (56 mg, 0.137 mmol) in dichloromethane (5 mL) was added phenylisocyanate (16.13 mg, 0.137 mmol) and triethyl amine (27.72 mg, 0.274 mmol). The reaction mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with dichloromethane. Organic layer was separated, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give *E*- and *Z*-4-{4-[2-(3-phenylureido)ethylcarbamoyl]phenoxy}-adamantane-1-carboxylic acid methyl ester (61 mg, 91 %).

In which added THF (30 mL) and MeOH (30 mL) was treated with aqueous 1M sodium hydroxide solution (24.82 mg, 0.62 mmol) at 40 °C for 12 h. After this time, the solution was cooled to room temperature, and the volume was reduced by rotary evaporation. The resulting solution was acidified with 1N hydrochloric acid to pH 2. More water was added (50 mL) and the aqueous solution was extracted with EtOAc (3 x 50 mL). The combined organic layer was washed with brine, dried over sodium sulfate and concentrated. A crude product was isolated form ethyl acetate/ hexane to afford the title compound *E*- and *Z*-4-{4-[2-(3-phenylureido)ethylcarbamoyl]phenoxy}adamantane-1-carboxylic acid **10** (50 mg, 84%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.08 (s, 1H), 8.53 (s, 1H), 8.37 (t, *J* = 4.12 Hz,

1H), 7.81 (d, *J* = 8.54 Hz, 2H), 7.38 (d, *J* = 7.98 Hz, 2H), 7.20 (t, *J* = 7.89 Hz, 2H), 7.02 (dd, *J* = 9.00, 2.78 Hz, 2H), 6.87 (t, *J* = 7.24 Hz, 1H), 6.25 (t, *J* = 5.10 Hz, 1H), 4.63-4.52 (m, 1H), 3.38-3.19 (m, 4H), 2.21-1.38 (m, 13H). LC-MS (*m*/*z*): 478 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3311, 2929, 2860, 1690, 1653, 1625, 1552, 1499, 1293, 1233, 1029, 750; Anal. Calcd for $C_{27}H_{31}N_3O_5$: C,67.91; H,6.54; N,8.58. Found: C,67.72; H,6.57; N,8.58.

*5.2.10. E- and Z-4-[4-(2-Benzenesulfonylaminoethylcarbamoyl)phenoxy]adamantane-1-carboxylic acid (**12**)*

To a solution of *E*- and *Z*-4-[4-(2-aminoethylcarbamoyl)phenoxy]adamantane-1-carboxylic acid methyl ester, hydrochloride **9** (50 mg, 0.122 mmol) in dichloromethane (5 mL) was added benzenesulfonyl chloride (21.6 mg, 0.122 mmol) and triethyl amine (24.75 mg, 0.245 mmol) stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with dichloromethane. Organic layer was separated, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give *E*- and *Z*-4-[4-(2-benzenesulfonylaminoethylcarbamoyl)phenoxy]-adamantane-1-carboxylic acid methyl ester **11** (49 mg, 70 %).

In which added THF (30 mL) and MeOH (30 mL) was treated with 1M sodium hydroxide solution (19.12 mg, 0.478 mmol) at 40 °C for 12 h. After this time, the solution was cooled to room temperature, and the volume was reduced by rotary evaporation. The resulting solution was acidified with 1N hydrochloric acid to pH 2. More water was added (50 mL) and the aqueous solution was extracted with EtOAc (3 x 50 mL). The combined organic layer was washed with brine, dried over sodium sulfate and concentrated. A crude product was isolated form ethyl acetate/ hexane to afford the title compound *E*- and *Z*-4-[4-(2-benzenesulfonylamino-

ethylcarbamoyl)phenoxy]adamantane-1-carboxylic acid **12** (39 mg, 81%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.10 (s, 1H), 8.32-8.26 (m, 1H), 7.80-7.70 (m, 5H), 7.64-7.54 (m, 3H), 7.02-6.97 (m, 2H), 4.61-4.52 (m, 1H), 3.26 (q, J = 6.34 Hz, 2H), 2.87 (q, J = 6.56 Hz, 2H), 2.18-1.39 (m, 13H); LC-MS (m/z): 499 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3249, 2913, 2859, 1603, 1499, 1320, 1243, 1155, 1091, 1019, 951, 843, 753, 688; Anal. Calcd for $C_{26}H_{30}N_2O_6S$: C,62.63; H,6.07; N,5.62. Found: C,62.75; H,6.30; N,5.35.

*5.2.11. Naphthalene-2-carboxylic acid (2-aminoethyl)amide hydrochloride (**17b**)*

A mixture of (2-aminoethyl)carbamic acid *tert*-butyl ester **14** (100 mg, 0.624 mmol), EDCI (299.13 mg, 1.56 mmol), naphthalene-2-carboxylic acid (107.47 mg, 0.624 mmol), HOBt (126.52 mg, 0.936 mmol) and hünig's base (283.01 mg, 2.185 mmol) in dichloromethane (6 mL) was stirred for 2 days. The reaction mixture was diluted with aqueous $NaHCO_3$ and extracted with dichloromethane. The extracts were washed with brine, dried over anhydrous sodium sulfate and concentrated in vacuum. The residue was purified by silica gel column chromatography to give {2-[(naphthalene-2-carbonyl)amino]ethyl}carbamic acid *tert*-butyl ester **16b** (157 mg, 80%).

To a mixture of {2-[(naphthalene-2-carbonyl)amino]ethyl}carbamic acid *tert*-butyl ester **16b** (157 mg, 1.23 mmol) in ethyl acetate (7 mL) was added hydrogen chloride 4.0 M solution in 1,4 dioxane (1 mL) and the mixture was stirred for 12 h. The mixture was concentrated to minimum volume and the residue was collected by filtration to obtained naphthalene-2-carboxylic acid (2-amino-ethyl)-amide hydrochloride **17b** (119 mg, 95%) as a white solid. Product was used for next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.94 (t, J = 5.36 Hz,

1H), 8.55 (s, 1H), 8.11 (s, 3H), 8.05-7.94 (m, 4H), 7.66-7.55 (m, 2H), 3.58 (q, *J* = 5.87 Hz, 2H), 3.03 (q, *J* = 5.97 Hz, 2H); LC-MS (*m*/*z*): 215 $[M+H]^+$.

The following compounds 17a, c- e prepared from the corresponding starting materials in a similar manner to that described for 17b.

*5.2.12. N-(2-Aminoethyl)benzamide hydrochloride (**17a**)*

Using the procedure for **17b** with benzoic acid provided the title compound as a white solid in 88% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.83-8.70 (m, 1H), 8.21-7.99 (m, 3H), 7.96-7.88 (m, 2H), 7.58-7.42 (m, 3H), 3.53 (q, *J* = 5.86 Hz, 2H), 2.98 (t, *J* = 6.19 Hz, 2H); LC-MS (*m*/*z*): 165 $[M+H]^+$.

*5.2.13. Biphenyl-4-carboxylic acid (2-aminoethyl)-amide hydrochloride (**17c**)*

Using the procedure for **17b** with biphenyl-4-carboxylic acid provided the title compound as a white solid in 84% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.83 (t, *J* = 5.40 Hz, 1H), 8.10 (s, 3H), 8.02 (d, *J* = 8.28 Hz, 2H), 7.83-7.70 (m, 4H), 7.54-7.37 (m, 3H), 3.55 (q, *J* = 5.94 Hz, 2H), 3.06-2.93 (m, 2H); LC-MS (*m*/*z*): 241 $[M+H]^+$.

*5.2.14. 2-Phenyl-5-trifluoromethyloxazole-4-carboxylic acid (2-aminoethyl)amide hydrochloride (**17d**)*

Using the procedure for **17b** with 2-phenyl-5-trifuoromethyloxazole-4-carboxylic acid provided the title compound as a white solid in 79% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.94 (t, *J* = 5.72 Hz, 1H), 8.13-8.01 (m, 5H), 7.52-7.59 (m, 3H), 3.55 (q, *J* = 6.00 Hz, 2H), 3.00 (t, *J* = 6.35 Hz, 2H); LC-MS (*m*/*z*): 300 $[M+H]^+$.

*5.2.15. 1-Phenyl-3-trifluoromethyl-1H-pyrazole-4-carboxylic acid (2-aminoethyl)amide hydrochloride (**17e**)*

Using the procedure for **17b** with 1-phenyl-3-trifuoromethyl-1H-pyrazole-4-carboxylic acid provided the title compound as a white solid in 82% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.46 (s, 1H), 8.84 (t, J = 5.36 Hz, 1H), 8.05 (s, 3H), 7.86-7.79 (m, 2H), 7.65-7.57 (m, 2H), 7.52-7.44 (m, 1H), 3.49 (q, J = 5.78 Hz, 2H), 2.99 (q, J = 6.21 Hz, 2H); LC-MS (*m*/*z*): 299 $[M+H]^+$.

*5.2.16. E- and Z-4-[4-(2-Benzoylaminoethylcarbamoyl)phenoxy]adamantane-1-carboxylic acid (**19a**)*

A mixture of *E*-and *Z*-4-(4-carboxyphenoxy)adamantane-1-carboxylic acid methyl ester **5** (80 mg, 0.225 mmol), EDCI (108.06 mg, 0.564 mmol), N-(2-amino-ethyl)-benzamide hydrochloride **17a** (49.77 mg, 0.248 mmol), HOBt (45.7 mg, 0.338 mmol) and hünig's base (102.24 mg, 0.789 mmol) in dichloromethane (8 mL) was stirred for 24 h. The reaction mixture was diluted with aqueous $NaHCO_3$ and extracted with dichloromethane. The extracts were washed with brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography to give *E*- and *Z*-4-[4-(2-benzoylaminoethylcarbamoyl)-phenoxy]adamantane-1-carboxylic acid methyl ester **18a** (75 mg, 70 %).

To a solution of 4-[4-(2-benzoylaminoethylcarbamoyl)phenoxy]adamantane-1-carboxylic acid methyl ester **18a** (75 mg, 0.157 mmol) in THF/water (20 mL, 3:1) was added NaOH (31.48 mg, 0.787 mmol). The reaction mixture was stirred at ambient temperature for 24 h. The THF was removed in vacuo and resulting solution was acidified with 1N hydrochloric acid to pH 2-3. More water was added (100 mL) and the aqueous solution was extracted with EtOAc (3 x 50

mL). The combined organic layer was washed with brine, dried over sodium sulfate and concentrated. A crude product was isolated form acetonitrile to afford *E*- and *Z*-4-[4-(2-benzoylaminoethylcarbamoyl)phenoxy]adamantane-1-carboxylic acid **19a** (69 mg, 95%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.10 (s, 1H), 8.83-8.70 (m, 2H), 7.86-7.70 (m, 4H), 7.62-7.49 (m, 3H), 6.99-6.90 (m, 2H), 3.61 (s, 4H), 4.63-4.52 (m, 1H), 2.21-1.38 (m, 13H); LC-MS (*m*/*z*): 463 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3400, 3311, 2910, 2860, 1690, 1630, 1550, 1499, 1242, 1233, 1180, 750; Anal. Calcd for $C_{27}H_{30}N2O_5$: C,71.11; H,6.54; N,6.06. Found: C,69.93; H,6.44; N,5.91.

The following compounds 19b, c, d, e was prepared from the corresponding starting materials in a similar manner to that described for 19a.

*5.2.17. E-andZ-4-(4-{2-[(Naphthalene-2-carbonyl)amino]ethylcarbamoyl}phenoxy)adamantane-1-carboxylic acid (**19b**)*

Using the procedure for **19a** with naphthalene-2-carboxylic acid (2-aminoethyl)amide hydrochloride **17b** provided the title compound as a white solid in 86% yield.; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.11 (s, 1H), 8.84-8.72 (m, 1H), 8.54-8.41 (m, 2H), 8.06-7.89 (m, 4H), 7.81 (d, J = 8.28 Hz, 2H), 7.65-7.54 (m, 2H), 7.10-6.97 (m, 2H), 4.65-4.52 (m, 1H), 3.54-341(m, 2H), 3.40-3.25 (m, 2H), 2.21-1.36 (m, 13H); LC-MS (*m*/*z*): 513 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3397, 3308, 2918, 2858, 1707, 1618, 1541, 1535, 1499, 1288, 1240, 1229, 1183, 1085, 764; Anal. Calcd for $C_{31}H_{32}N_2O_5$: C,72.64; H,6.29; N,5.47. Found: C,72.24; H,6.43; N,5.31.

*5.2.18. E-andZ-4-(4-{2-[(Biphenyl-4-arbonyl)amino]ethylcarbamoyl}phenoxy)adamantane-1-carboxylic acid (**19c**)*

Using the procedure for **19a** with biphenyl-4-carboxylic acid (2-aminoethyl)amide hydrochloride **17c** provided the title compound as a white solid in 88% yield. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.10 (s, 1H), 8.69-8.61 (m, 1H), 8.50-8.42 (m, 1H), 7.94 (d, J = 8.28 Hz, 2H), 7.83-7.70 (m, 6H), 7.49 (t, J = 7.82 Hz, 2H), 7.40 (t, J = 7.26 Hz, 1H), 7.05-7.00 (m, 2H), 4.63-4.53 (m, 1H), 3.44 (s, 4H), 2.21-1.39 (m, 13H); LC-MS (*m*/*z*): 539 $[M+H]^+$. IR (ATR) ν_{max} cm^{-1}: 3402,3311, 2912, 2857, 1707, 1629, 1538, 1499, 1242, 1231, 1180, 745; Anal. Calcd for $C_{33}H_{34}N_2O_5$: C,73.59; H,6.36; N,5.20. Found: C,73.26; H,6.37; N,5.06.

*5.2.19.E-andZ-4-(4-{2-[(2-Phenyl-5-trifluoromethyloxazole-4-carbonyl)amino]ethylcarbamoyl}-phenoxy)adamantane-1-carboxylic acid (**19d**)*

Using the procedure for **19a** with 2-phenyl-5-trifluoromethyl-oxazole-4-carboxylic acid (2-aminoethyl)amide hydrochloride **17d** provided the title compound as a white solid in 81% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.12 (s, 1H), 8.93-8.81 (m, 1H), 8.49-8.39 (m,1H), 8.07 (d, J = 6.39 Hz, 2H), 7.80 (d, J = 8.56 Hz, 2H), 7.71-7.58 (m, 3H), 7.03 (d, J = 8.56 Hz, 2H), 4.65-4.52 (m, 1H), 3.44 (s, 4H), 2.21-1.38 (m, 13H); LC-MS (*m*/*z*): 598 $[M+H]^+$. IR (ATR) ν_{max} cm^{-1}: 3407, 2933, 2861, 1683, 1502, 1372, 1243, 1182, 1145, 1093, 1016, 715, 689; Anal. Calcd for $C_{31}H_{30}F_3N_3O_6$: C,62.31; H,5.06; N,7.03. Found: C,62.21; H,5.11; N,7.29.

*5.2.20. E-and Z-4-(4-{2-[(1-Phenyl-3-trifluoromethyl-1H-pyrazole-4-carbonyl)amino]-ethylcarbamoyl}phenoxy)adamantane-1-carboxylic acid (**19e**)*

Using the procedure for **19a** with 1-phenyl-3-trifluoromethyl-1H-pyrazole-4-carboxylic acid (2-aminoethyl)amide hydrochloride **17e** provided the title compound as a white solid in 81% yield. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.10 (s, 1H), 9.06 (s, 1H), 8.47 (d, J = 25.59 Hz, 2H), 7.80 (d, J = 7.59 Hz, 4H), 7.64-7.57 (m, 2H), 7.50-7.44 (m, 1H), 7.06-6.99 (m, 2H), 4.64-4.53 (m,

1H), 3.40 (s, 4H), 2.21-1.39 (m, 13H); LC-MS (*m*/*z*): 597 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3338, 2925, 2858, 1692, 1632, 1501, 1237, 1142, 760, 690; Anal. Calcd for $C_{31}H_{31}F_3N4O5$: C,62.41; H,5.24; N,9.39. Found: C,62.35; H,5.25; N,8.98.

5.2.21. (5-Hydroxyadamantan-2-ylidene)acetic acid methyl ester **(22)**

To a solution of trimethyl phosponoacetate **21** (699.58 mg, 3.842 mmol) in anhydrous tetrahydrofuran (15 mL) 60% suspension in minral oil of sodium hydride (200 mg, 4.802 mmol) was added at 0 °C under nitrogen atmosphere and stirred for 1 h at the same temperature. Added solution of 5-hydroxy-2-adamantanone **20** (500 mg, 3.201mmol) in tetrahydrofuran (5 mL) slowly at room temperature. The reaction mixture was stirred for 5 h, quenched with water and extracted with ethyl acetate. The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to provide (5-hydroxy-adamantan-2-ylidene)acetic acid methyl ester **22** (641 mg, 90%). ^1H NMR (300 MHz, DMSO-d_6): δ 5.60 (s, 1H), 4.53 (s, 1H), 3.58 (s, 3H), 2.67-2.54 (m, 1H), 2.16-2.08 (m, 1H), 1.80-1.52 (m, 11H); LC-MS (*m*/*z*): 223 $[M+H]^+$.

5.2.22. E- and Z-(5-Hydroxyadamantan-2-yl)acetic acid methyl ester **(23)**

To a solution of (5-hydroxyadamantan-2-ylidene)acetic acid methyl ester **22** (600 mg, 12.77 mmol) in ethyl acetate (25 mL), under nitrogen, was added 10% (w/w) palladium on carbon (60 mg). The atmosphere was replaced with hydrogen and the mixture stirred at room temperature for 17 h. The reaction mixture was filtered and evaporated to obtained (5-hydroxyadamantan-2-yl)acetic acid methyl ester **23** (590 mg, 97%) a colorless oil. ^1H NMR (300 MHz, $CDCl_3$): δ 3.66 (s, 3H), 2.48-2.37 (m, 2H), 2.23-1.88 (m, 4H), 1.86-1.33 (m, 10H); LC-MS (*m*/*z*): 225 $[M+H]^+$.

5.2.23. E-and Z-4-(4-Methoxycarbonylmethyladamantan-1-yloxy)benzoic acid benzyl ester (24)

To a solution of benzyl 4-hydroxybenzoate **3** (1 g, 4.381 mmol) and triphenyl phospine (1.379 g, 5.258 mmol), dissolved in benzene (100 mL) was added *E*/*Z* mixture (5-hydroxyadamantan-2-yl)acetic acid methyl ester **23** (982.71 mg, 4.381 mmol). The reaction mixture was heated to reflux and diisopropyl azodicarboxylate (DIAD) (1.032 mL, 5.258 mmol) was added drop wise at reflux over 2 h. The resulting mixture was stirred under reflux for 16 h. The volume was reduced by evaporation and the resulting mixture was added water (100 mL) followed by extraction with Methylene chloride (3 x 200 mL). The combined organic phases were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered and the volatiles evaporated in vacuo. Crude product was purified by column chromatography (elution gradient of 0−10% EtOAc in hexane). Combined fractions were evaporated in vacuo afforded *E*- and *Z*- 4-(4-methoxycarbonylmethyladamantan-1-yloxy)benzoic acid benzyl ester **24** (1.35 g, 71%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.99 (d, *J* = 8.22 Hz, 2H), 7.48-7.29 (m, 5H), 7.01 (d, *J* = 8.35 Hz, 2H), 5.34 (s, 2H), 3.66 (s, 3H), 2.47-2.34 (m, 2H), 2.22-2.07 (m, 2H), 2.06-1.81 (m, 7H), 1.81-1.62 (m, 4H), 1.40 (d, *J* = 13.05 Hz, 1H). LC-MS (*m*/z): 435 $[M+H]^+$.

*5.2.24. E-and Z-4-(4-Methoxycarbonylmethyladamantan-1-yloxy)benzoic acid (**25**)*

25 was prepared from intermediate **24** (1.2 g, 2.76 mmol) according to the procedure described for compound **5.** The crude product was purified through column chromatography, eluting with ethyl acetate in hexanes to give *E*- and *Z*-4-(4-methoxycarbonylmethyladamantan-1-yloxy)-benzoic acid **25** (910 mg, 96%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.59 (s, 1H), 7.87 (d, *J* = 8.62 Hz, 2H), 6.99 (d, *J* = 8.86 Hz, 2H), 4.04 (t, *J* = 6.57 Hz, 2H), 3.57 (s, 3H), 3.39

(t, *J* = 6.31 Hz, 2H), 2.43 (d, *J* = 7.60 Hz, 1H), 2.38 (d, *J* = 7.47 Hz, 1H), 2.09-1.29 (m, 10H). LC-MS (*m*/*z*): 345 $[M+H]^+$.

*5.2.25.E-and Z-[5-(4-{2-[(Naphthalene-2-carbonyl)amino]ethylcarbamoyl}phenoxy)adamantan-2-yl]acetic acid (**27b**)*

A mixture of *E*- and *Z*-4-(4-methoxycarbonylmethyladamantan-1-yloxy)benzoic acid **25** (40 mg, 0.116 mmol), EDCI (55.66 mg, 0.290 mmol), naphthalene-2-carboxylic acid (2-amino-ethyl)-amide hydrochloride **17b** (32.03 mg, 0.128 mmol), HOBt (23.54 mg, 0.174 mmol) and hünig's base (52.66 mg, 0.407 mmol) in dichloromethane (8 mL) was stirred for 24 h. The reaction mixture was diluted with aqueous $NaHCO_3$ and extracted with dichloromethane. The extracts were washed with brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography to give *E*- and *Z*-[5-(4-{2-[(naphthalene-2-carbonyl)amino]ethylcarbamoyl}phenoxy)adamantan-2-yl]-acetic acid methyl ester **26b** (54 mg, 86 %).

To a solution of **26b** (54 mg, 0.1 mmol) in THF /water (20 mL, 3:1) was added NaOH (16 mg, 0.4 mmol). The reaction mixture was stirred at room temperature for 16 h. The THF was removed in vacuo and resulting solution was acidified with 1N hydrochloric acid to pH 2-3. More water was added (100 mL) and the aqueous solution was extracted with EtOAc (3 x 50 mL). The combined organic layer was washed with brine, dried over sodium sulfate and concentrated. A crude product was isolated form acetonitrile to afford *E*- and *Z*-[5-(4-{2-[(naphthalene-2-carbonyl)amino]ethylcarbamoyl}phenoxy)adamantan-2-yl]acetic acid **27b** (49 mg, 93%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.05 (s, 1H), 8.79-8.74 (m, 1H), 8.57-8.51 (m,1H), 8.44 (s, 1H), 8.03-7.91 (m, 4H), 7.82-7.77 (m, 2H), 7.63-7.56 (m, 2H),

7.03 (dd, *J* = 8.37, 1.67 Hz, 2H), 3.52-3.43 (m, 4H), 2.33 (d, *J* = 7.52 Hz, 1H), 2.26 (d, *J*= 7.30 Hz, 1H), 2.14-1.84 (m, 6H), 1.82-1.77 (m, 2H), 1.71 (d, *J* = 12.82 Hz, 1H), 1.65-1.57 (m, 4H), 1.33 (d, *J* =12.58 Hz, 1H); LC-MS (*m*/*z*): 527 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3280, 2914, 2859, 1706, 1627, 1546, 1498, 1294, 1226, 1173, 860, 680; Anal. Calcd for $C_{32}H_{34}N_2O_5$: C,72.98; H,6.51; N,5.32. Found: C,72.66; H,6.47; N,5.30.

*5.2.26. E-and Z-[5-(4-{2-[(Biphenyl-4-carbonyl)amino]ethylcarbamoyl}phenoxy)adamantan-2-yl]-acetic acid (**27c**)*

Using the procedure for **27b** with biphenyl-4-carboxylic acid (2-aminoethyl)amide hydrochloride **17c** and *E*- and *Z*-4-(4-methoxycarbonylmethyladamantan-1-yloxy)benzoic acid **25** provided the title compound as a white solid (56 mg, 84%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.08 (s, 1H), 8.68-8.62 (m, 1H), 8.55-8.49 (m,1H), 7.94 (d, *J* = 8.32 Hz, 2H), 7.81-7.70 (m, 6H), 7.51-7.46 (m, 2H), 7.42-7.38 (m, 1H), 7.03 (dd, *J* = 8.53, 1.58 Hz, 2H), 3.48-3.42 (m, 4H), 2.33 (d, *J* = 7.52 Hz, 1H), 2.26 (d, *J* = 7.30 Hz, 1H), 2.14-1.83 (m, 7H), 1.82-1.77 (m, 2H), 1.71 (d, *J* = 12.93 Hz, 1H), 1.66-1.57 (m, 3H), 1.33 (d, *J* =12.93 Hz, 1H); LC-MS (*m*/*z*): 553 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3319, 2915, 2859, 1701,1699, 1630, 1534, 1498, 1223, 1067, 753, 697; Anal. Calcd for $C_{34}H_{36}N_2O_5$: C,73.89; H,6.57; N,5.07. Found: C,73.72; H,6.59; N,4.98.

*5.2.27. Naphthalene-2-carboxylic acid (3-aminopropyl)amide hydrochloride (**30b**)*

A mixture of naphthalene-2-carboxylic acid **15b** (150 mg, 0.871 mmol), EDCI (417.51 mg, 2.178 mmol), (3-aminopropyl)carbamic acid *tert*-butyl ester **28** (159.39 mg, 0.915 mmol), HOBt (176.58 mg, 1.307 mmol) and hünig's base (395.02 mg, 3.049 mmol) in dichloromethane (10 mL) was stirred for 2 days. The reaction mixture was diluted with aqueous $NaHCO_3$ and extracted with dichloromethane. The extracts were washed with brine, dried over anhydrous

sodium sulfate and concentrated in vacuum. The residue was purified by silica gel column chromatography to give {3-[(naphthalene-2-carbonyl)amino]propyl}carbamic acid *tert*-butyl ester **29b.**

To a mixture of {3-[(naphthalene-2-carbonyl)amino]propyl}carbamic acid tert-butyl ester **29b** in ethyl acetate (7 mL) was added hydrogen chloride 4.0 M solution in 1,4-dioxane (1 mL) and the mixture was stirred for 12 h. The mixture was concentrated to minimum volume and the residue was collected by filtration to obtained naphthalene-2-carboxylic acid (3-amino-propyl)-amide hydrochloride **30b** (181 mg, 78%, over two steps) as a white solid. Product was used for next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.92 (t, J = 5.66 Hz, 1H), 8.49 (s, 1H), 8.07-7.93 (m, 7H), 7.65-7.55 (m, 2H), 3.39 (q, J = 5.88 Hz, 2H), 2.86 (t, J = 7.18 Hz, 2H), 1.93-1.80 (m, 2H); LC-MS (m/z): 229 $[M+H]^+$.

The following compound 30c was prepared from the corresponding starting materials in a similar manner to that described for 30b.

*5.2.28. Biphenyl-4-carboxylic acid (3-aminopropyl)amide hydrochloride (**30c**)*

Using the procedure for **30b** with biphenyl-4-carboxylic acid **15c** provided the title compound as a white solid (179 mg, 76%, over two steps). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.79 (t, J = 5.65 Hz, 1H), 8.03-7.89 (m, 5H), 7.81-7.69 (m, 4H), 7.53-7.36 (m, 3H), 3.41-3.30 (m, 2H), 2.84 (t, J = 7.48 Hz, 2H), 1.91-1.77 (m, 2H); LC-MS (m/z): 255 $[M+H]^+$.

*5.2.29. E- and Z-4-(4-{3-[(Naphthalene-2-carbonyl)amino]propylcarbamoyl}phenoxy)-adamantane-1-carboxylic acid (**32b**)*

A mixture of *E*- and *Z*-4-(4-carboxyphenoxy)adamantane-1-carboxylic acid methyl ester **5** (40 mg, 0.116 mmol), EDCI (58.02 mg, 0.303 mmol), naphthalene-2-carboxylic acid (3-amino-propyl)amide hydrochloride **30b** (35.26 mg, 0.133 mmol), HOBt (24.54 mg, 0.182 mmol) and hünig's base (54.9 mg, 0.424 mmol) in dichloromethane (8 mL) was stirred for 24 h. The reaction mixture was diluted with aqueous $NaHCO_3$ and extracted with dichloromethane. The extracts were washed with brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography to give *E*- and *Z*-4-(4-{3-[(naphthalene-2-carbonyl)amino]propylcarbamoyl}phenoxy)adamantane-1-carboxylic acid methyl ester **31b** (60 mg, 91 %).

To a solution of **31b** (60 mg, 0.111 mmol) in THF /water (20 mL, 3:1) was added NaOH (22.20 mg, 0.555 mmol). The reaction mixture was stirred at room temperature for 16 h. The THF was removed in vacuo and resulting solution was acidified with 1N hydrochloric acid to pH 2-3. More water was added (100 mL) and the aqueous solution was extracted with EtOAc (3 x 50 mL). The combined organic layer was washed with brine, dried over sodium sulfate and concentrated. A crude product was isolated form acetonitrile to afford *E*-and *Z*-4-(4-{3-[(naphthalene-2-carbonyl)amino]propylcarbamoyl}phenoxy)adamantane-1-carboxylic acid **32b** (49 mg, 84%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.07 (s, 1H), 8.67 (t, J = 5.60 Hz, 1H), 8.43 (s, 1H), 8.36 (t, J = 5.45 Hz, 1H), 8.04-7.95 (m, 3H), 7.93 (d d, J = 8.63,1.32 Hz, 1H), 7.80 (d, J = 8.63 Hz, 2H), 7.63-7.56 (m, 2H), 7.05-6.99 (m, 2H), 4.62-4.52 (m, 1H), 3.41-3.33 (m, 4H), 2.21-1.40 (m, 15H); LC-MS (*m*/*z*): 527 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3337, 2920, 2857, 1686, 1625, 1640, 1501, 1293, 1236, 1185, 1025, 766; Anal. Calcd for $C_{32}H_{34}N_2O_5$: C,72.98; H,6.51; N,5.32. Found: C,73.11; H,6.72; N,4.99.

*5.2.30. E- and Z-4-(4-{3-[(Biphenyl-4-carbonyl)amino]propylcarbamoyl}phenoxy)adamantane-1-carboxylic acid (**32c**)*

Using the procedure for **32b** with biphenyl-4-carboxylic acid (3-aminopropyl)amide hydrochloride **(30c)** provided the title compound as a white solid (54 mg, 85%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.05 (s, 1H), 8.55 (t, J = 5.61 Hz, 1H), 8.35 (t, J = 5.44 Hz, 1H), 7.93 (d, J = 8.32 Hz, 2H), 7.82-7.70 (m, 6H), 7.49 (t, J = 7.84 Hz, 2H), 7.40 (t, J = 7.27 Hz, 1H), 7.05-7.00 (m, 2H), 4.63-4.53 (m, 1H), 3.38-3.25 (m, 4H), 2.21-1.41 (m, 15H); LC-MS (m/z): 553 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3317, 2925, 2859, 1690, 1626, 1606, 1545, 1501, 1292, 1239, 1025, 746; Anal. Calcd for $C_{32}H_{36}N_2O_5$: C,73.89; H,6.57; N,5.07. Found: C,73.57; H,6.68; N,4.82.

*5.2.31. 4-Hydroxybenzoic acid tert-butyl ester (**34**)*

To a solution of 4-hydroxybenzoic acid **33** (4 g, 28.96 mmol), 4-DMAP (176.9 mg , 1.448 mmol) and *tert*-butanol (100 mL) in dry THF (100 mL) under N_2 atmosphere, a solution of DCC in dry THF (40 mL) was added dropwise at room temperature for 30 min. The reaction mixture was stirred at room temperature under N_2 atmosphere for 20 h. The residue mixture was filtered and the filtrate was reduced by rotary evaporation to ~25 mL. The filtrate was washed with 0.3 M Na_2CO_3 solution, dried over anhydrous sodium sulfate and concentrated in vacuo. The pale yellow crude product was purified by silica gel chromatography (0 to 30% EtOAc in hexane) to afford **34** (4.3 g, 76 %) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.21 (s, 1H), 7.74 (d, J = 8.50 Hz, 2H), 6.81 (d, J = 8.50 Hz, 2H), 1.50 (s, 9H); LC-MS (m/z): 195 $[M+H]^+$.

*5.2.32. E- and Z-4-(4-tert-Butoxycarbonylphenoxy)adamantane-1-carboxylic acid methyl ester (**35**)*

Compound **35** was prepared from intermediate **34** (3 g, 15.446 mmol) according to the procedure described for compound **4** to obtain *E*- and *Z*- 4-(4-*tert*-butoxycarbonylphenoxy)adamantane-1-carboxylic acid methyl ester **35** (4.5 g, 75%) as a white solid **.** The isomer was separated through silica gel column chromatography, eluting with ethyl acetate in hexanes to give *Z*-4-(4-*tert*-butoxycarbonyl-phenoxy)-adamantane-1-carboxylic acid methyl ester **36** (1.2 g, 20%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.91 (d, *J* = 8.70 Hz, 2H), 6.90 (d, *J* = 8.70 Hz, 2H), 4.43 (s, 1H), 3.66 (s, 3H), 2.33-2.23 (m, 4H), 2.07-1.66 (m, 9H), 1.57 (s, 9H); LC-MS (*m*/*z*): 387 $[M+H]^+$ and *E*-4-(4-*tert*-butoxycarbonylphenoxy)adamantane-1-carboxylic acid methyl ester **38** (910 mg, 15%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) : δ 7.92 (d, J= 8.74 Hz, 2H), 6.89 (d, *J* = 8.61 Hz, 2H), 4.52-4.45 (m, 1H), 3.68 (s, 3H), 2.25 (s, 2H), 2.14 (d, *J* = 12.59 Hz, 2H), 2.09-1.88 (m, 7H), 1.57 (s, 9H), 1.50 (d, *J* = 12.59 Hz, 2H); LC-MS (*m*/*z*): 387 $[M+H]^+$.

(Note isomer was confirmed by X –ray crystal)

*5.2.33. Z-4-(4-Carboxyphenoxy)adamantane-1-carboxylic acid methyl ester (**37**)*

A solution of *Z*-4-(4-*tert*-butoxycarbonyl-phenoxy)adamantane-1-carboxylic acid methyl ester **36** (710 mg, 1.837 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (7 mL) and stirred at room temperature for 6 h. The reaction mixture was evaporated to a solid which was purified by recrystallization from water to afford *Z*-4-(4-carboxyphenoxy)adamantane-1-carboxylic acid methyl ester **37** (595 mg, 98%) as a white crystalline solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04 (d, *J* = 8.44 Hz, 2H), 6.95 (d, *J* = 8.12 Hz, 2H), 4.51-4.42 (m, 1H), 3.67 (s, 3H), 2.38-2.21 (m, 4H), 2.11-2.00 (m, 1H), 1.97-1.65 (m, 8H); LC-MS (*m*/*z*): 331 $[M+H]^+$.

*5.2.34. E-4-(4-Carboxyphenoxy)adamantane-1-carboxylic acid methyl ester (**39**)*

A solution of *E*-4-(4-*tert*-butoxycarbonylphenoxy)adamantane-1-carboxylic acid methyl ester **38** (900 mg, 2.329 mmol) in dichloromethane (15 mL) was added trifluoroacetic acid (9 mL) and stirred at room temperature for 6 h. The reaction mixture was evaporated to a solid which was purified by recrystallization from water to afford *E*-4-(4-carboxyphenoxy)adamantane-1-carboxylic acid methyl ester **39** (750 mg, 97%) as a white crystalline solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04 (d, *J* = 8.77 Hz, 2H), 6.94 (d, *J* = 8.90 Hz, 2H), 4.55-4.48 (m, 1H), 3.68 (s, 3H), 2.27 (s, 2H), 2.15 (d, *J* = 12.81 Hz, 2H), 2.10-1.88 (m, 7H), 1.52 (d, *J* = 12.26 Hz, 2H); LC-MS (*m*/*z*): 331 $[M+H]^+$.

*5.2.35. Z-4-(4-{2-[(Naphthalene-2-carbonyl)amino]ethylcarbamoyl}phenoxy)adamantane-1-carboxylic acid (**41b**)*

A mixture of *Z*-4-(4-carboxyphenoxy)adamantane-1-carboxylic acid methyl ester **37** (40 mg, 0.121 mmol), EDCI (58.03 mg, 0.303 mmol), naphthalene-2-carboxylic acid (2-aminoethyl)-amide hydrochloride **17b** (31.87 mg, 0.127 mmol), HOBt (24.54 mg, 0.182 mmol) and hünig's base (54.9 mg, 0.424 mmol) in dichloromethane (8 mL) was stirred for 24 h. The reaction mixture was diluted with aqueous $NaHCO_3$ and extracted with dichloromethane. The extracts were washed with brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography to give *Z*-4-(4-{2-[(naphthalene-2-carbonyl)amino]ethylcarbamoyl}phenoxy)adamantane-1-carboxylic acid methyl ester **40b** (61 mg, 95 %).

To a solution of **40b** (61 mg, 0.111 mmol) in THF /water (20 mL, 3:1) was added NaOH (22.20 mg, 0.555 mmol). The reaction mixture was stirred at 35 °C for 16 h. The THF was removed in vacuo and resulting solution was acidified with 1N hydrochloric acid to pH 2-3. More water was

added (100 mL) and the aqueous solution was extracted with EtOAc (3 x 50 mL). The combined organic layer was washed with brine, dried over sodium sulfate and concentrated. A crude product was isolated form acetonitrile to afford *Z*-4-(4-{2-[(naphthalene-2-carbonyl)amino]-ethylcarbamoyl}phenoxy)adamantane-1-carboxylic acid **41b** (56 mg, 94%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.09 (s, 1H), 8.77 (t, J = 5.16 Hz, 1H), 8.48 (t, J = 5.16 Hz, 1H), 8.44 (s, 1H), 8.03-7.91 (m, 4H), 7.81 (d, J = 8.71 Hz, 2H), 7.63-7.56 (m, 2H), 7.02 (d, J = 8.80 Hz, 2H), 4.57-4.53 (m, 1H), 3.51-3.43 (m, 4H), 2.19-2.14 (m, 2H), 2.09 (d, J = 12.20 Hz, 2H), 1.96-1.92 (m, 1H), 1.81-1.73 (m, 6H), 1.59 (d, J = 12.34 Hz, 2H); LC-MS (*m*/*z*): 513 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3297, 2918, 2854, 1685, 1629, 1545, 1502, 1328, 1294, 1234, 1185, 1025, 950, 845, 673; Anal. Calcd for $C_{31}H_{32}N_2O_5$: C,72.64; H,6.29; N,5.47. Found: C,72.62; H,6.52; N,5.20.

The following compounds 41c, 43b and 43c were prepared from the corresponding starting materials in a similar manner to that described for 41b

*5.2.36. Z-4-(4-{2-[(Biphenyl-4-carbonyl)amino]ethylcarbamoyl}phenoxy)adamantane-1-carboxylic acid (**41c**)*

Using the procedure for **41b** with biphenyl-4-carboxylic acid (2-aminoethyl)amide hydrochloride **17c** provided the title compound as a white solid (41 mg, 93%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.08 (s, 1H), 8.68-8.62 (m, 1H), 8.50-8.43 (m,1H), 7.94 (d, J = 8.33 Hz, 2H), 7.83-7.70 (m, 6H), 7.49 (t, J = 7.45 Hz, 2H), 7.40 (t, J = 7.32 Hz, 1H), 7.02 (d, J = 8.71 Hz, 2H), 4.57-4.53 (m, 1H), 3.47-3.41 (m, 4H), 2.19-2.14 (m, 2H), 2.09 (d, J = 12.25 Hz, 2H), 1.96-1.92 (m, 1H), 1.81-1.73 (m, 6H), 1.59 (d, J = 12.25 Hz, 2H); LC-MS (*m*/*z*): 539 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3397,

3306, 2912, 2857, 1699, 1627, 1608, 1539, 1499, 1333, 1298, 1242, 1231, 1183, 1023, 835; Anal. Calcd for $C_{33}H_{34}N_2O_5$: C,73.59; H,6.36; N,5.20. Found: C,73.12; H,6.44; N,4.96.

*5.2.37. E-4-(4-{2-[(Naphthalene-2-carbonyl)amino]ethylcarbamoyl}phenoxy)adamantane-1-carboxylic acid (**43b**)*

Using the procedure for **41b** with naphthalene-2-carboxylic acid (2-aminoethyl)amide hydrochloride **17b** and *E*-4-(4-carboxyphenoxy)adamantane-1-carboxylic acid methyl ester **39** provided the title compound as a white solid (41 mg, 90%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.13 (s, 1H), 8.77 (t, *J* = 5.10 Hz, 1H), 8.48 (t, *J* = 5.10 Hz, 1H), 8.45 (s, 1H), 8.03-7.91 (m, 4H), 7.81 (d, *J* = 8.76 Hz, 2H), 7.63-7.56 (m, 2H), 7.03 (d, *J* = 8.78 Hz, 2H), 4.63-4.59 (m, 1H), 3.51-3.43 (m, 4H), 2.17-2.10 (m, 2H), 2.03-1.85 (m, 7H), 1.84-1.77 (m, 2H), 1.44 (d, *J* = 11.76 Hz, 2H); LC-MS (*m*/*z*): 513 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3402,3355, 2931, 2856, 1712, 1650, 1641, 1537, 1503, 1192, 1245, 1211, 767; Anal. Calcd for $C_{31}H_{32}N_2O_5$: C,72.64; H,6.29; N,5.47. Found: C,72.64; H,6.31; N,5.22.

*5.2.38. E-4-(4-{2-[(Biphenyl-4-carbonyl)amino]ethylcarbamoyl}phenoxy) adamantane-1-carboxylic acid (**43c**)*

Using the procedure for **41b** with biphenyl-4-carboxylic acid (2-aminoethyl)amide hydrochloride **17c** and *E*-4-(4-carboxyphenoxy)adamantane-1-carboxylic acid methyl ester **39** provided the title compound as a white solid (41 mg, 91%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.10 (s, 1H), 8.67-8.61 (m, 1H), 8.48-8.40 (m,1H), 7.94 (d, *J* = 8.31 Hz, 2H), 7.81 (d, *J* = 8.77 Hz, 2H), 7.76 (d, *J* = 8.31 Hz, 2H), 7.72 (d, *J* = 7.47 Hz, 2H), 7.49 (t, *J* = 7.47 Hz, 2H), 7.40 (t, *J* = 7.40 Hz, 1H), 7.03 (d, *J* = 8.77 Hz, 2H), 4.62-4.59 (m, 1H), 3.48-3.41 (m, 4H), 2.16-2.11 (m, 2H), 2.03-1.85 (m, 7H), 1.83-1.79 (m, 2H), 1.44 (d, *J* = 11.92 Hz, 2H). LC-MS (*m*/*z*): 539 $[M+H]^+$; IR

(ATR) ν_{max} cm^{-1}: 3297, 2918, 2854, 1685, 1629, 1545, 1502, 1328, 1294, 1234, 1185, 1025, 950, 845, 673; Anal. Calcd for $C_{33}H_{34}N_2O_5$: C,72.64; H,6.29; N,5.47. Found: C,72.62; H,6.52; N,5.20.

*5.2.39. 2'-Methylbiphenyl-4-carboxylic acid (2-aminoethyl)amide hydrochloride (**46a**)*

Using the procedure for **30b** with 2'-methylbiphenyl-4-carboxylic acid **44a** provided the title compound as a white solid (155 mg, 85%, over two steps)**.** ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.81 (t, *J* = 5.47 Hz, 1H), 8.08 (s, 3H), 7.99 (d, *J* = 8.20 Hz, 2H), 7.44 (d, *J* = 8.20 Hz, 2H), 7.34-7.18 (m, 4H), 3.55 (q, *J* = 5.70 Hz, 2H), 3.06-2.94 (m, 2H), 2.23 (s, 3H); LC-MS (*m*/*z*): 255 $[M+H]^+$.

*5.2.40. 2'-Trifluoromethylbiphenyl-4-carboxylic acid (2-aminoethyl)amide hydrochloride (**46b**)*

Using the procedure for **30b** with 2'-trifluoromethylbiphenyl-4-carboxylic acid **44b** provided the title compound as a white solid (134 mg, 69%, over two steps)**.** ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.83 (t, *J* = 5.33 Hz, 1H), 8.05 (s, 3H), 7.98 (d, *J* = 8.39 Hz, 2H), 7.86 (d, *J* = 7.75 Hz, 1H), 7.79-7.60 (m, 2H), 7.42 (d, *J* = 7.99 Hz, 3H), 3.55 (q, *J* = 5.81 Hz, 2H), 3.01 (t, *J* = 6.08 Hz, 2H); LC-MS (*m*/*z*):309 $[M+H]^+$.

*5.2.41. 2'-Chlorobiphenyl-4-carboxylic acid (2-aminoethyl)amide hydrochloride (**46c**)*

Using the procedure for **30b** with 2'-chlorobiphenyl-4-carboxylic acid **44c** provided the title compound as a white solid (143 mg, 71%, over two steps)**.** ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.82 (t, *J* = 4.57 Hz, 1H), 8.14-7.96 (m, 5H), 7.64-7.57 (m, 1H), 7.54 (d, *J* = 8.27, Hz, 2H), 7.50-7.39 (m, 3H), 3.55 (q, *J* = 5.66 Hz, 2H), 3.07-2.94 (m, 2H); LC-MS (*m*/*z*): 275 $[M+H]^+$.

*5.2.42. 2-Chlorobiphenyl-4-carboxylic acid (2-aminoethyl)amide hydrochloride (**46d**)*

Using the procedure for **30b** with 2-chlorobiphenyl-4-carboxylic acid **44d** provided the title compound as a white solid (144 mg, 71%, over two steps)**.** ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.93 (t, J = 5.28 Hz, 1H), 8.17-8.01 (m, 4H), 7.95 (dd, J = 8.13, 1.58 Hz, 1H), 7.57-7.40 (m, 6H), 3.55 (q, J = 5.88 Hz, 2H), 3.07-2.94 (m, 2H). LC-MS (m/z): 275 $[M+H]^+$.

*5.2.43. N-(2-aminoethyl)-2', 6'-dimethylbiphenyl-4-carboxamide hydrochloride (**46e**)*

Using the procedure for **30b** with 2',6'-dimethylbiphenyl-4-carboxylic acid **44e** provided the title compound as a white solid (156 mg, 77%, over two steps). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.79 (t, J = 5.38 Hz, 1H), 8.12-7.95 (m, 5H), 7.26 (d, J = 8.26 Hz, 2H), 7.21-7.10 (m, 3H), 3.55 (q, J = 5.82 Hz, 2H), 3.07-2.94 (m, 2H), 1.96 (s, 6H); LC-MS (m/z): 269 $[M+H]^+$.

*5.2.44. N-(2-aminoethyl)-2', 6'-dichlorobiphenyl-4-carboxamide hydrochloride (**46f**)*

Using the procedure for **30b** with 2',6'-dichlorobiphenyl-4-carboxylic acid **44f** provided the title compound as a white solid (156 mg, 80%, over two steps)**.**^{1}H NMR (300 MHz, DMSO-d_6): δ 8.84 (t, J = 5.40 Hz, 1H), 8.14-7.96 (m, 5H), 7.61 (d, J = 8.33 Hz, 2H), 7.51-7.42 (m, 1H), 7.39 (d, J = 8.06 Hz, 2H), 3.55 (q, J = 5.68 Hz, 2H), 3.07-2.94 (m, 2H); LC-MS (m/z): 310 $[M+H]^+$.

*5.2.45. N-(2-aminoethyl)-2', 6'-difluorobiphenyl-4-carboxamide hydrochloride (**46g**)*

Using the procedure for **30b** with 2',6'-difluorobiphenyl-4-carboxylic acid **44g** provided the title compound as a white solid (147 mg, 73%, over two steps)**.** ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.85 (t, J = 5.35 Hz, 1H), 8.14-7.98 (m, 5H), 7.56 (d, J = 8.25 Hz, 2H), 7.53-7.46 (m, 1H), 7.31-7.19 (m, 2H), 3.55 (q, J = 5.73 Hz, 2H), 3.07-2.94 (m, 2H); LC-MS (m/z): 277 $[M+H]^+$.

*5.2.46. E-4-(4-{2-[(2'-Methylbiphenyl-4-carbonyl)amino]ethylcarbamoyl}phenoxy)adamantane-1-carboxylic acid (**48a**)*

A mixture of *E*-4-(4-carboxyphenoxy)adamantane-1-carboxylic acid methyl ester **39** (40 mg, 0.121 mmol), EDCI (58.03 mg, 0.303 mmol), 2'-methylbiphenyl-4-carboxylic acid (2-amino-ethyl)amide hydrochloride **46a** (38.73 mg, 0.133 mmol), HOBt (24.54 mg, 0.182 mmol) and hünig's base (54.9 mg, 0.424 mmol) in dichloromethane (8 mL) was stirred for 24 h. The reaction mixture was diluted with aqueous $NaHCO_3$ and extracted with dichloromethane. The extracts were washed with brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography to give *E*-4-(4-{2-[(2'-Methyl-biphenyl-4-carbonyl)amino]ethylcarbamoyl}phenoxy)adamantane-1-carboxylic acid methyl ester **47a** (59 mg, 86%).

To a solution of **47a** (59 mg, 0.104 mmol) in THF /water (20 mL, 3:1) was added NaOH (20.82 mg, 0.521 mmol). The reaction mixture was stirred at 35 °C for 16 h. The THF was removed in vacuo and resulting solution was acidified with 1N hydrochloric acid to pH 2-3. More water was added (100 mL) and the aqueous solution was extracted with EtOAc (3 x 50 mL). The combined organic layer was washed with brine, dried over sodium sulfate and concentrated. A crude product was isolated form acetonitrile to afford *E*-4-(4-{2-[(2'-methylbiphenyl-4-carbonyl)-amino]ethylcarbamoyl}phenoxy)adamantane-1-carboxylic acid **48a** (52 mg, 90%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.11 (s, 1H), 8.67-8.60 (m, 1H), 8.47-8.41 (m,1H), 7.91 (d, J= 8.22 Hz, 2H), 7.81 (d, *J* = 8.77 Hz, 2H), 7.42 (d, *J* = 8.28 Hz, 2H), 7.33-7.25 (m, 3H), 7.23-7.19 (m, 1H), 7.03 (d, *J* = 8.83 Hz, 2H), 4.62-4.59 (m, 1H), 3.48-3.41 (m, 4H), 2.23 (s, 3H), 2.16-2.11 (m, 2H), 2.04-1.85 (m, 7H), 1.83-1.79 (m, 2H), 1.44 (d, *J* = 12.17 Hz, 2H); LC-MS (*m*/*z*): 553 $[M+H]^+$. IR (ATR) ν_{max} cm^{-1}: 3402, 3303, 2911, 2857, 1706, 1697, 1606, 1500, 1448,

1289, 1244, 1227, 1183, 1089, 680; Anal. Calcd for $C_{34}H_{36}N_2O_5$: C,73.89; H,6.57; N,5.07. Found: C,73.57; H,6.67; N,4.91.

The following compounds, 48b-g, were prepared from the corresponding starting materials in a similar manner to that described for 48a.

*5.2.47. E-4-(4-{2-[(2'-Trifluoromethylbiphenyl-4-carbonyl)amino]ethylcarbamoyl}phenoxy)-adamantane-1-carboxylic acid (**48b**)*

Using the procedure for **48a** with 2'-trifluoromethylbiphenyl-4-carboxylic acid (2-aminoethyl)-amide hydrochloride **46b** and *E*-4-(4-carboxyphenoxy)adamantane-1-carboxylic acid methyl ester **39** provided the title compound as a white solid (60 mg, 90%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.11 (s, 1H), 8.71-8.65 (m, 1H), 8.48-8.42 (m,1H), 7.90 (d, *J* = 8.16 Hz, 2H), 7.85 (d, *J* = 7.74 Hz, 1H), 7.81 (d, *J* = 8.79 Hz, 2H), 7.74 (t, *J* = 7.53 Hz, 1H), 7.64 (t, *J* = 7.74 Hz, 1H), 7.44-7.38 (m, 3H), 7.03 (d, *J* = 8.79 Hz, 2H), 4.63-4.59 (m, 1H), 3.48-3.41 (m, 4H), 2.16-2.11 (m, 2H), 2.03-1.85 (m, 7H), 1.83-1.79 (m, 2H), 1.44 (d, J= 12.14 Hz, 2H). LC-MS (*m*/*z*): 607 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3393, 3312, 2941, 2856, 1706, 1649, 1622, 1607, 1548, 1501, 1448, 1317, 1245, 1176, 1123, 772; Anal. Calcd for $C_{34}H_{33}F_3N_2O_5$: C,67.32; H,5.48; N,4.62. Found: C,67.20; H,5.49; N,4.44.

*5.2.48. E-4-(4-{2-[(2'-Chlorobiphenyl-4-carbonyl)amino]ethylcarbamoyl}phenoxy)adamantane-1-carboxylic acid (**48c**)*

Using the procedure for **48a** with 2'-chlorobiphenyl-4-carboxylic acid (2-aminoethyl)amide hydrochloride **46c** and *E*-4-(4-carboxyphenoxy)adamantane-1-carboxylic acid methyl ester **39** provided the title compound as a white solid (60 mg, 90%). ^{1}H NMR (500 MHz, DMSO-d_6):

δ 12.11 (s, 1H), 8.74-8.68 (m, 1H), 8.52-8.46 (m, 1H), 7.94 (d, *J* = 8.21 Hz, 2H), 7.82 (d, *J* = 8.74 Hz, 2H), 7.60-7.57 (m, 1H), 7.51 (d, *J* = 8.29 Hz, 2H), 7.45-7.41 (m,3H), 7.03 (d, *J* = 8.89 Hz, 2H), 4.62-4.59 (m, 1H), 3.48-3.41 (m, 4H), 2.16-2.11 (m, 2H), 2.03-1.85 (m, 7H), 1.83-1.79 (m, 2H), 1.44 (d, *J* = 11.96 Hz, 2H); LC-MS (*m*/*z*): 574 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3399, 3295, 2930, 2858, 1707, 1647, 1606, 1550, 1500, 1335, 1289, 1245, 1227, 1183, 755, 681; Anal. Calcd for $C_{33}H_{33}ClN_2O_5$: C,69.16; H,5.80; N,4.89. Found: C,68.82; H,5.45; N,4.45.

*5.2.49. E-4-(4-{2-[(2-Chlorobiphenyl-4-carbonyl)amino]ethylcarbamoyl}phenoxy)adamantane-1-carboxylic acid (**48d**)*

Using the procedure for **48a** with 2-chlorobiphenyl-4-carboxylic acid (2-aminoethyl)amide hydrochloride **46d** and *E*-4-(4-carboxyphenoxy)adamantane-1-carboxylic acid methyl ester **39** provided the title compound as a white solid (54 mg, 82%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.14 (s, 1H), 8.80-8.74 (m, 1H), 8.48-8.42 (m,1H), 8.02 (d, *J* = 1.55 Hz, 1H), 7.87 (dd, *J* = 8.10, 1.56 Hz, 1H), 7.80 (d, *J* = 8.74 Hz, 2H), 7.53-7.41 (m, 6H), 7.03 (d, *J* = 8.80 Hz, 2H), 4.63-4.59 (m, 1H), 3.47-3.41 (m, 4H), 2.17-2.10 (m, 2H), 2.03-1.85 (m, 7H), 1.83-1.78 (m, 2H), 1.44 (d, *J* = 11.97 Hz, 2H); LC-MS (*m*/*z*): 574 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3390, 3301, 2932, 2860, 1697, 1625, 1605, 1541, 1501, 1290, 1253, 1182, 1085, 1015, 837; Anal. Calcd for $C_{33}H_{33}ClN_2O_5$: C,69.16; H,5.80; N,4.89. Found: C,68.92; H,5.77; N,4.83.

*5.2.50. E-4-(4-(2-(2',6'-Dimethylbiphenyl-4-ylcarboxamido)ethylcarbamoyl)phenoxy)-3,7-dimethylbicyclo[3.3.1]nonane-1-carboxylic acid (**48e**)*

Using the procedure for **48a** with N-(2-aminoethyl)-2',6'-dimethylbiphenyl-4-carboxamide hydrochloride **46e** and *E*-4-(4-carboxyphenoxy)adamantane-1-carboxylic acid methyl ester **39** provided the title compound as a white solid (54 mg, 82%). ^{1}H NMR (500 MHz, DMSO-d_6):

δ 12.12 (s, 1H), 8.68-8.63 (m, 1H), 8.49-8.44 (m, 1H), 7.93 (d, *J* = 8.13 Hz, 2H), 7.81 (d, *J* = 8.81 Hz, 2H), 7.23 (d, *J* = 8.13 Hz, 2H), 7.19-7.10 (m, 3H), 7.03 (d, *J* = 8.81 Hz, 2H), 4.63-4.59 (m, 1H), 3.47-3.41 (m, 4H), 2.16-2.11 (m, 2H), 2.03-1.85 (m, 13H), 1.83-1.78 (m, 2H), 1.44 (d, *J* = 12 Hz, 2H); LC-MS (*m*/*z*): 567 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3394, 3300, 2913, 2857, 1706, 1649, 1606, 1546, 1501, 1134, 1288, 1244, 1228, 1183, 1088, 1019, 768; Anal. Calcd for $C_{35}H_{38}N_2O_5$: C,74.18; H,6.76; N,4.94. Found: C,73.88; H,6.75; N,4.92.

*5.2.51. E-4-(4-(2-(2',6'-Dichlorobiphenyl-4-ylcarboxamido)ethylcarbamoyl)phenoxy)-3,7-dimethylbicyclo[3.3.1]nonane-1-carboxylic acid (**48f**)*

Using the procedure for **48a** with N-(2-aminoethyl)-2',6'-dichlorobiphenyl-4-carboxamide hydrochloride **46f** and *E*-4-(4-carboxyphenoxy)adamantane-1-carboxylic acid methyl ester **39** provided the title compound as a white solid (49 mg, 83%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.13 (s, 1H), 8.73-8.67 (m, 1H), 8.49-8.43 (m,1H), 7.94 (d, *J* = 8.12 Hz, 2H), 7.81 (d, *J* = 8.75 Hz, 2H), 7.60 (d, *J* = 8.07Hz, 2H), 7.48-7.43 (m, 1H), 7.36 (d, *J* = 8.12 Hz, 2H), 7.03 (d, *J* = 8.75 Hz, 2H), 4.63-4.59 (m, 1H), 3.48-3.40 (m, 4H), 2.16-2.10 (m, 2H), 2.03-1.85 (m, 7H), 1.83-1.78 (m, 2H), 1.44 (d, *J* = 12.10 Hz, 2H); LC-MS (*m*/*z*): 608 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3401, 3291, 2925, 2860, 1707, 1651, 1607, 1501, 1287, 1244, 1225, 1183, 680; Anal. Calcd for $C_{33}H_{32}Cl_2N_2O_5$: C,65.24; H,5.31; N,4.61. Found: C,65.31; H,5.46; N,4.49.

*5.2.52. E-4-(4-(2-(2',6'-Difluorobiphenyl-4-ylcarboxamido)ethylcarbamoyl)phenoxy)-3,7-dimethylbicyclo[3.3.1]nonane-1-carboxylic acid (**48g**)*

Using the procedure for **48a** with N-(2-aminoethyl)-2',6'-difluorobiphenyl-4-carboxamide hydrochloride **46g** and *E*-4-(4-carboxyphenoxy)-adamantane-1-carboxylic acid methyl ester **39** provided the title compound as a white solid (53 mg, 89%).^{1}H NMR (500 MHz, DMSO-d_6):

δ 12.15 (s, 1H), 8.73-8.66 (m, 1H), 8.49-8.41 (m, 1H), 7.95 (d, *J* = 8.20 Hz, 2H), 7.80 (d, *J* = 8.79 Hz, 2H), 7.55 (d, *J* = 7.97 Hz, 2H), 7.53-7.47 (m, 1H), 7.28-7.21 (m, 2H), 7.03 (d, *J* = 8.67 Hz, 2H), 4.63-4.59 (m, 1H), 3.47-3.40 (m, 4H), 2.16-2.10 (m, 2H), 2.03-1.85 (m, 7H), 1.83-1.78 (m, 2H), 1.44 (d, *J* = 11.91 Hz, 2H); LC-MS (*m*/*z*): 575 $[M+H]^+$; IR (ATR) ν_{max} cm^{-1}: 3402, 3306, 2932, 2912, 2858, 1706, 1697, 1645, 1619, 1606, 1559, 1500, 1465, 1449, 1289, 1245, 1228, 1182, 1000, 786; Anal. Calcd for $C_{33}H_{32}F_2N_2O_5$: C,68.98; H,5.61; N,4.88. Found: C,69.13; H,5.73; N,4.65.

5.3. Biology

5.3.1. In vitro DGAT-1 enzyme activity assay

The identification of compounds as DGAT-1 inhibitors was readily achieved using a FlashPlate assay. In this assay, pCMV6-recombinant human DGAT-1 plasmid (Origene #RC220595) transfected for 24 hours in Hep3B cells. After transfection of the plasmid, human DGAT-1 expression was analyzed by Western blot or RT-PCR using whole cell extracts. For establishment of a human DGAT-1 overexpressed stable cell line, cells were grown and maintained in Dulbecco's modified Eagle's medium high glucose containing 10% fetal bovine serum and 200 μg/ml G-418 in a 5% CO_2 environment for 4 weeks. Cell pellets were resuspended in homogenization buffer [250 mM sucrose, 10 mM Tris-HCl (pH 7.4), 1 mM EDTA] and lysed using a homogenization apparatus and then cell debris was removed by centrifugation at 600 x g for 15 min.

Human and mouse DGAT-1 activities were determined as follows: Assay buffer [20 mM HEPES (pH 7.4), 100 mM $MgCl_2$, 0.04% BSA] containing 500 μM of enzyme substrate (didecanoyl glycerol) and 7.5 μM radiolabeled acyl-CoA substrate ([14C]decanoyl-CoA) was added to each

well of a phospholipid FlashPlate (PerkinElmer Life Sciences). A small aliquot of lysate (5 μg/well) or mouse liver microsome (0.1 mg/well) was added to start the reaction, which was allowed to proceed for 60 min. The reaction was terminated upon the addition of an equal volume (100 μL) of isopropanol. The plates were sealed, incubated overnight and counted the next morning on a Wallac 1450 Microbeta Trilux Liquid Scintillation Counter and Luminometer (PerkinElmer Life Science). DGAT-1 catalyzes the transfer of the radiolabeled decanoyl group onto the sn-3 position of didecanoyl glycerol. The resultant radiolabeled tridecanoyl glycerol (tricaprin) preferentially binds to the hydrophobic coating on the phospholipid FlashPlate. The proximity of the S15 radiolabeled product to the solid scintillant incorporated into the bottom of the FlashPlate induced fluorescence release from the scintillant, which was measured in the Wallac 1450 Microbeta Trilux Liquid Scintillation Counter and Luminometer. Various concentrations (e.g. 0.0008 μM, 0.004 μM, 0.02 μM, 0.1 μM, 0.5 μM, 2.5 μM, 10.0 μM) of the representative compounds were added to individual wells prior to the addition of lysates. IC50 values of compounds were determined from concentration-dependent inhibition curves of triplicate experiments by GraphPad Prism software (GraphPad Software Inc., La Jolla, CA).

5.3.2. The oral lipid tolerance test (OLTT) assay

Animals were weighed regularly to allow accurate dosing with drugs. All mice (8 week-old male C57BL/6 or TallyHo) were fasted for 16 hours and basal blood samples were collected from ophthalmic venous plexus using heparin-coated capillary tubes. The test compound 43c was formulated as a suspension in 0.5% carboxymethyl cellulose (CMC) and orally treated with chemical dosing (10 mL/kg). A 30 min later, a bolus dose of oil (5 mL/kg, 0.5% CMC) was given to the mice. After oil treatment, blood samples were collected at 2 hours. The blood samples were immediately centrifuged for 10 minutes at 1000 x g and resulting plasma samples

were stored at -20 ^{o}C until assayed. Plasma concentration of TG was measured by a colometric assay using an automatic biochemical analyzer, the Selectra 2 (Vital Scientific N. V., The Netherlands).

5.3.3. Maintenance of zebrafish

Zebrafish were maintained under standard laboratory conditions at 28.5 °C with a 14 h light/10 h dark cycle. Embryos were obtained by natural spawning. Embryos were fixed at specific days post fertilization (dpf).

5.3.4. Treatment and staining of LipidGreen2 with zebrafish

Compounds were dissolved in dimethyl sulfoxide (DMSO) as 10 mM concentration and diluted with egg water at a final concentration of 10 μM. Compounds treatment started at 2 dpf until 5 dpf. 5 dpf zebrafish embryos were incubated with 5 μM LipidGreen2 in the dark for 15 min. After staining of LipidGreen2, zebrafish embryos washed with egg water for 30 min. After washing, embryos were anaesthetized with 0.016% tricaine and mounted in 3% methylcellulose. The photographs were performed on a fluorescence stereomicroscope.

5.3.5. Imaging and Quantitative analysis

Zebrafish images were captured by using a fluorescence stereomicroscope (MZ10F, Leica Microsystems, Wetzlar, Germany) with a coupled device camera (DFC425C, Leica Microsystems, Wetzlar, Germany). The fluorescence stereomicroscope equipped with the filter set GFP Plant (470/40nm excitation, 525/50nm emission). The Fluorescence intensity analysis was performed by fluorescent microscopy and LAS v3.8 Leica Microsystems software.

5.3.6. In vivo efficacy test in diet-induced obesity (DIO)

C57BL/6 mice (4 weeks of age) were purchased from Orient Bio Inc. in Korea. After 1 week acclimation, the mice were fed with high fat diet (40% fat, 60% of total energy), purchased from Research Diet (Cat No., USA), for 8 weeks. The DIO mice were acclimated with vehicle (0.5% carboxymethyl cellulose, CMC) for 1 week and 10 mg/kg of **43c** was orally administered by daily for 4 weeks. The body weight and food intake rate were monitored biweekly and weekly, respectively. After final administration, the mice were fasted for 16 hours and oral glucose tolerance test (OGTT) was done. In OGTT, 2 g/kg of glucose was orally gavaged and blood samples were obtained from the retro-orbital plexus at time points of 0, 15, 30, 60, and 120 minutes. The blood glucose levels were measured with automated biochemical analyzer (Response 920, Diasys, Germany). Data are expressed as means ± S.E.M. (n = 10 mice). *P < 0.05, and **P < 0.01 *versus* vehicle by Student's t-test.

AUTHOR INFORMATION

Corresponding Author

* Phone: +82-42-860-7076. Fax: +82-42-860-7160. E-mail: jhahn@krict.re.kr

Author Contributions

⊥These authors contributed equally to the work presented in this manuscript.

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ABBREVIATIONS USED

DGAT, acyl CoA:diacylglycerol acyltransferase; TG, triglyceride; DIPEA, *N*,*N*-diisopropylethylamine; OLTT, oral lipid tolerance test; DIO, diet-induced obesity ; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole monohydrate; DIAD, diisopropyl azodicarboxylate; DMAP, 4-dimethylaminopyridine; DCC, N,N'-dicyclohexylcarbodiimide; TFA, Trifluoroacetic acid; TEA, triethyl amine; EtOAc, ethyl acetate; DCM, dichloromethane; THF, tetrahydrofuran.

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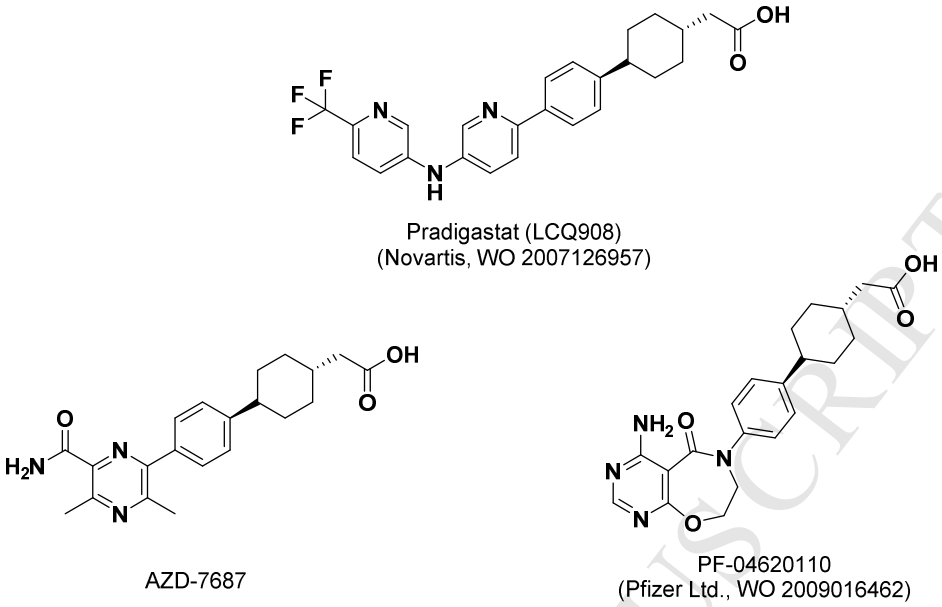


Fig. 1. Clinically studied DGAT1 inhibitors.

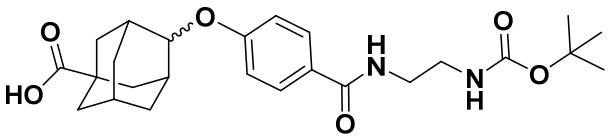


Fig. 2. Hit compound **7** (IC_{50} = 539 nM and 2651 nM against human and mouse DGAT1, respectively).

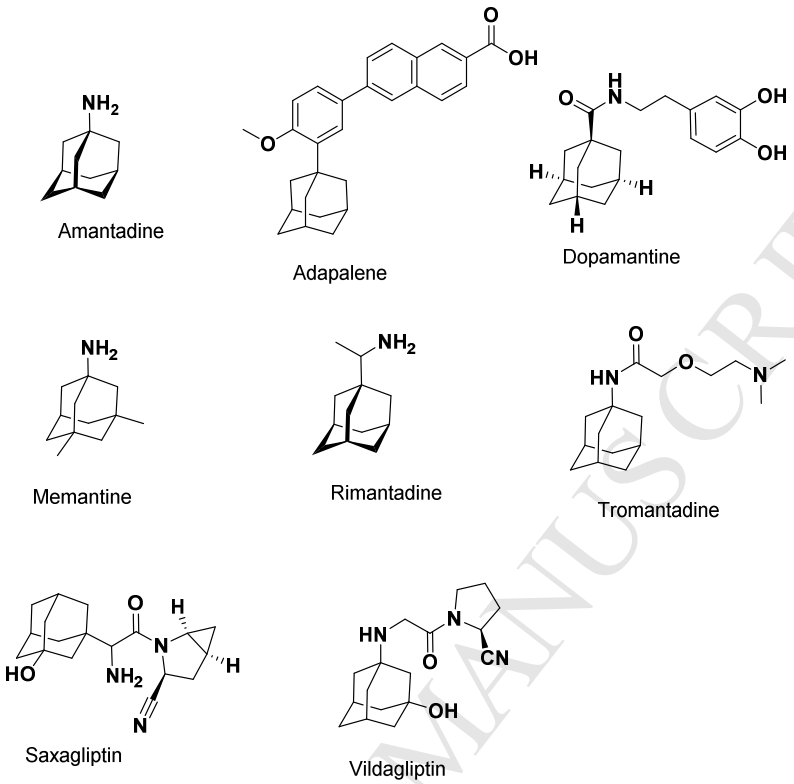
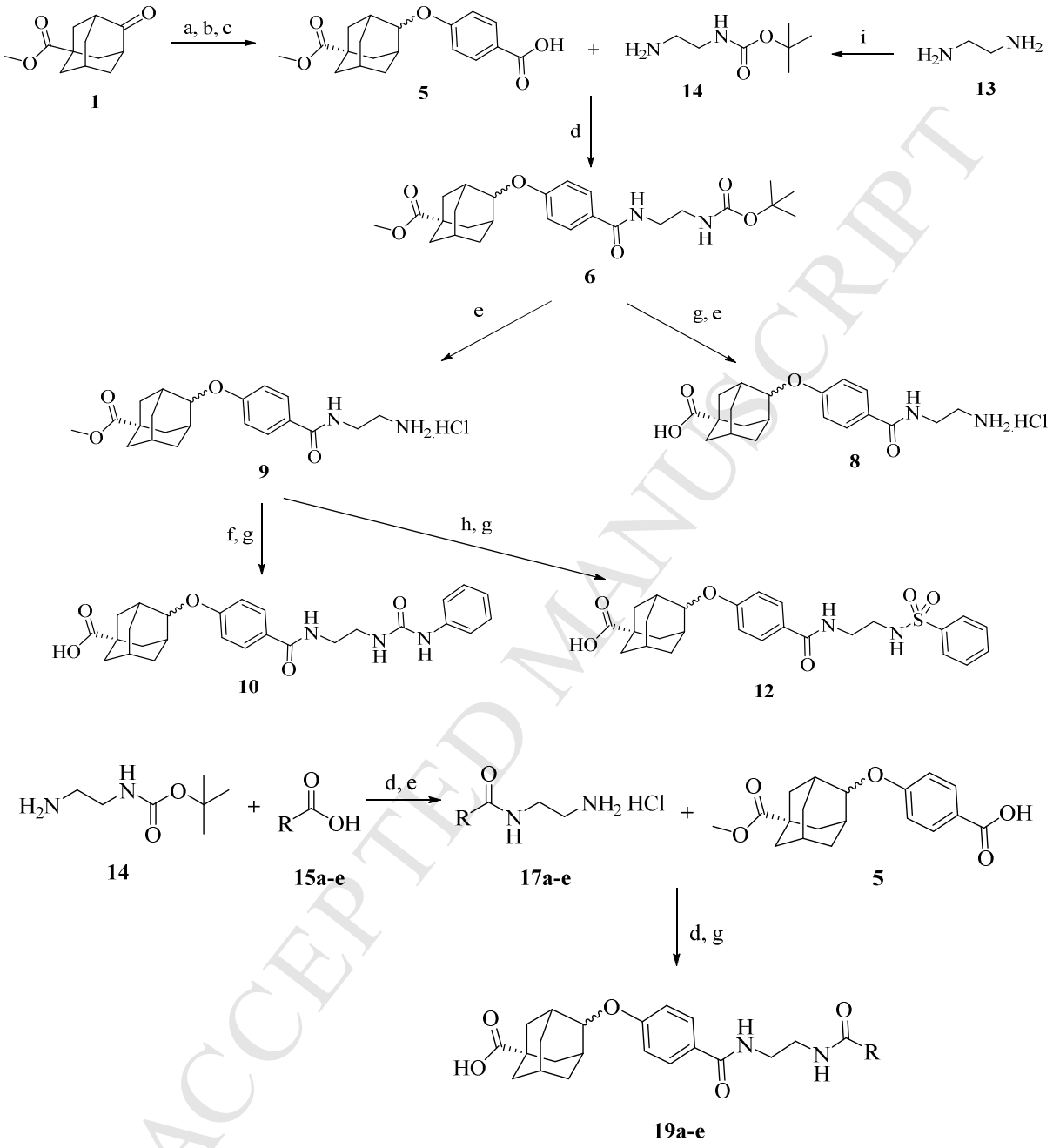
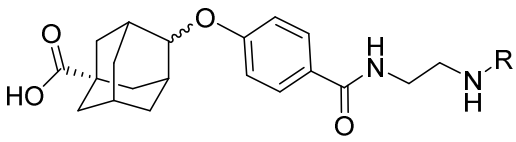


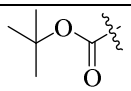
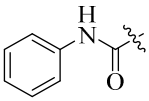
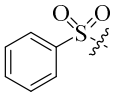
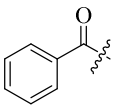
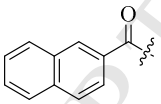
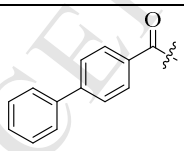
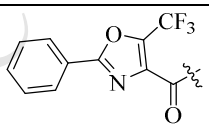
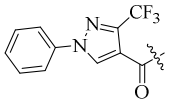
Fig. 3. Adamantane containing drugs.



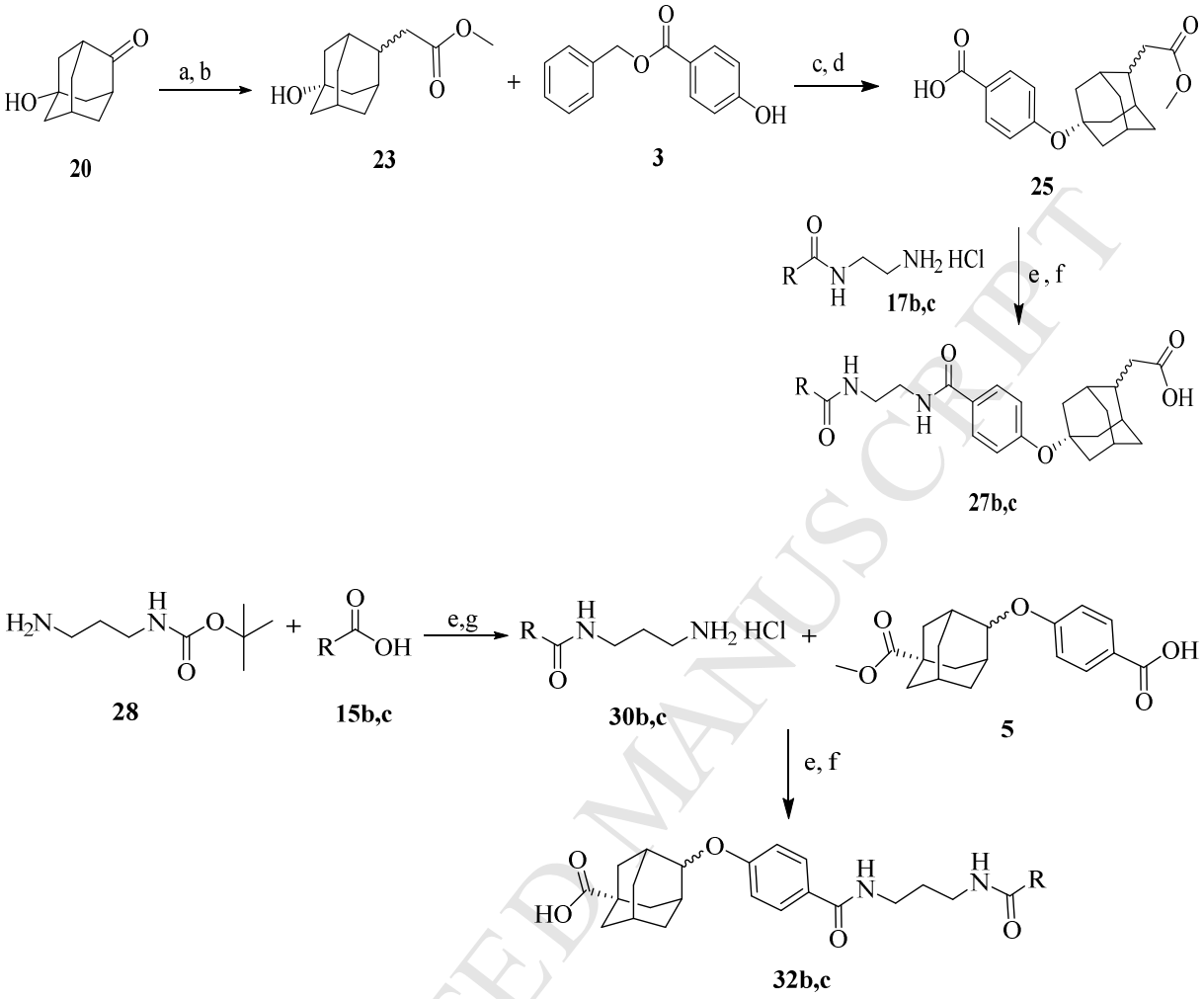
Scheme 1: Reagents and conditions: (a) $NaBH_4$, MeOH, rt, 97%; (b) benzyl 4-hydroxybenzoate **3** , PPh_3, DIAD, THF, reflux, 60%; (c) H_2, Pd/C, EtOAc, rt, 99%; (d) EDCI, HOBt, DIPEA, DCM, rt; (e) 4M-HCl/1,4-dioxane, EA, rt; (f) phenylisocyanate, TEA, DCM, rt, 91%; (g) NaOH, H_2O/THF, rt; (h) benzenesulfonyl chloride, TEA, DCM, rt, 70%; (i) $(Boc)_2O$, DCM, rt, 19%.

Table 1. DGAT1 IC_{50} Values of N-Substituted Analogs.



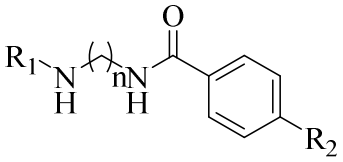
Compound	R	Human DGAT1 IC_{50} (nM)[a]	Mouse DGAT1 IC_{50} (nM)[a]
7		539	2651
8	H.HCl	>50 μM	>50 μM
10		201	491
12		>50 μM	>50 μM
19a		>50 μM	>50 μM
19b		34	22
19c		12	8
19d		89	5
19e		>10 μM	>10 μM

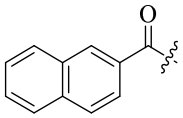
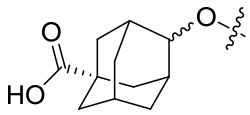
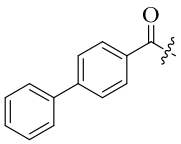
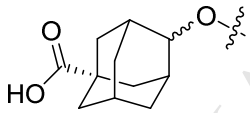
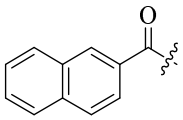
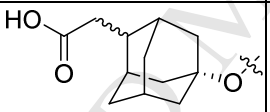
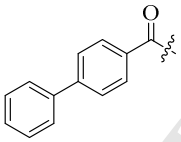
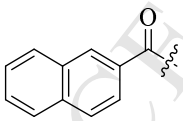
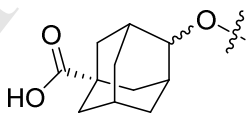
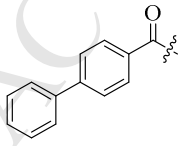
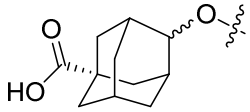
[a]IC_{50} values were determined from concentration-dependent inhibition curves of triplicate experiments by the GraphPad Prism software.



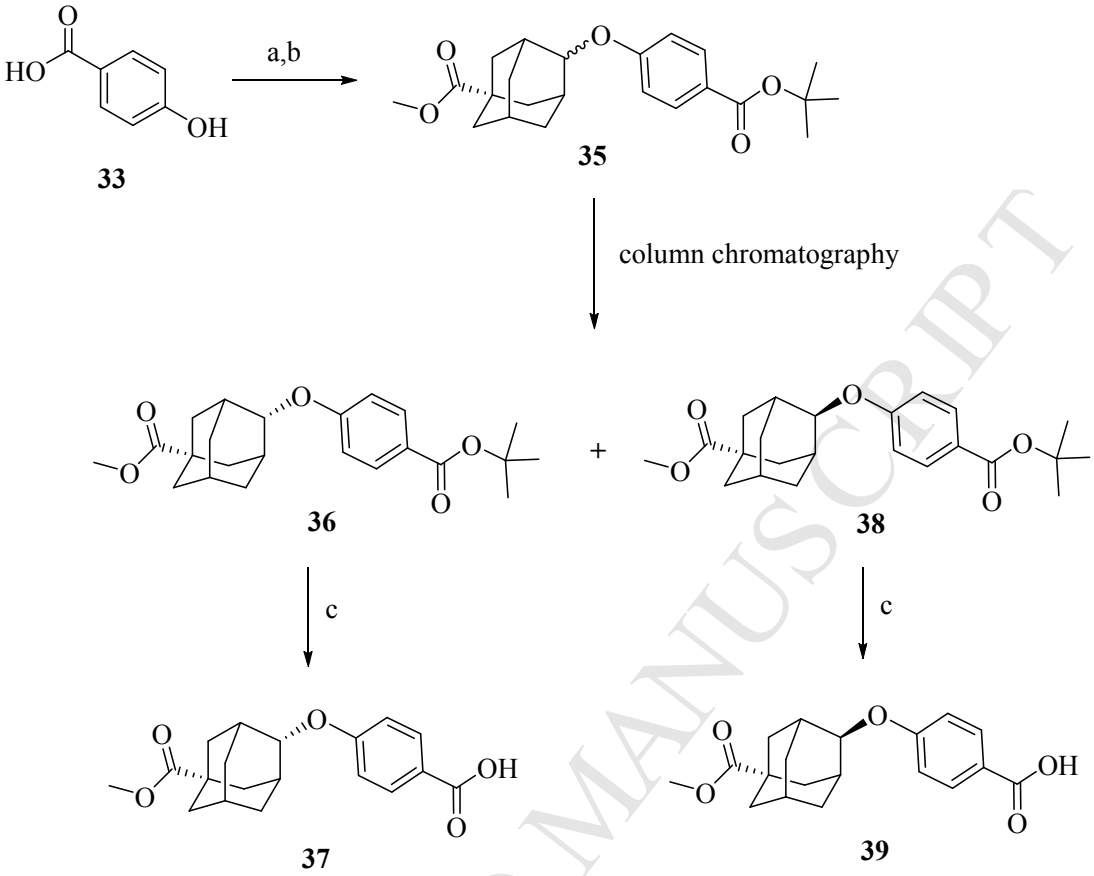
Scheme 2: Reagents and conditions: (a) trimethyl phosphonoacetate (**21**), NaH, THF, rt, 90%; (b) H_2, Pd/C, EtOAc, rt, 97%; (c) benzyl 4-hydroxybenzoate **3** , PPh_3, DIAD, benzene, reflux, 71%; (d) H_2, Pd/C, EtOAc, rt, 96%; (e) EDCI, HOBt, DIPEA, DCM, rt; (f) NaOH, H_2O/THF, rt; (g) 4M-HCl/1,4-dioxane, EA, rt.

Table 2. Adamantane Carboxylic Acid Variation.



Compound	R_1	R_2	n	Human DGAT1 IC_{50} (nM)[a]	Mouse DGAT1 IC_{50} (nM)[a]
19b			2	34	22
19c			2	12	8
27b			2	>500	ND
27c			2	159	69
32b			3	>10 μM	ND
32c			3	>10 μM	ND

ND = Not Determined; [a]IC_{50} values were determined from concentration-dependent inhibition curves of triplicate experiments by the GraphPad Prism software.



Scheme 3: Reagents and conditions: (a) DMAP, DCC, THF, *tert*-butanol, rt, 76%; (b) **2**, PPh_3, DIAD, THF, reflux, 75%; (c) TFA, DCM, rt.

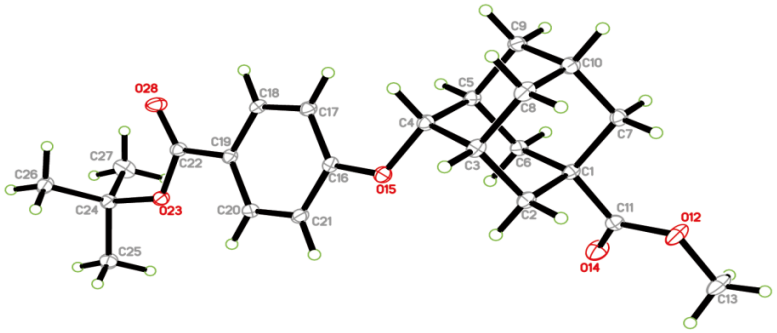


Fig. 4. X-ray crystal structure of *Z*-isomer **36.**

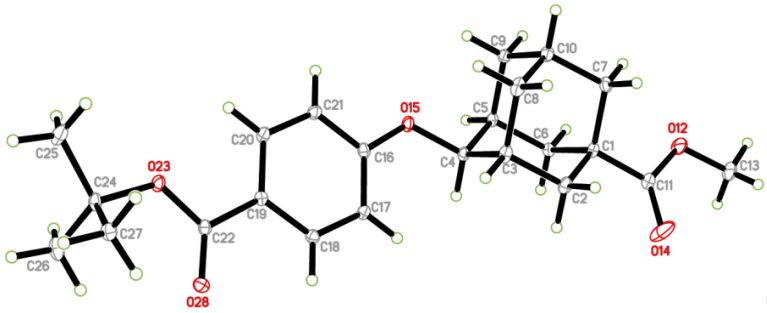
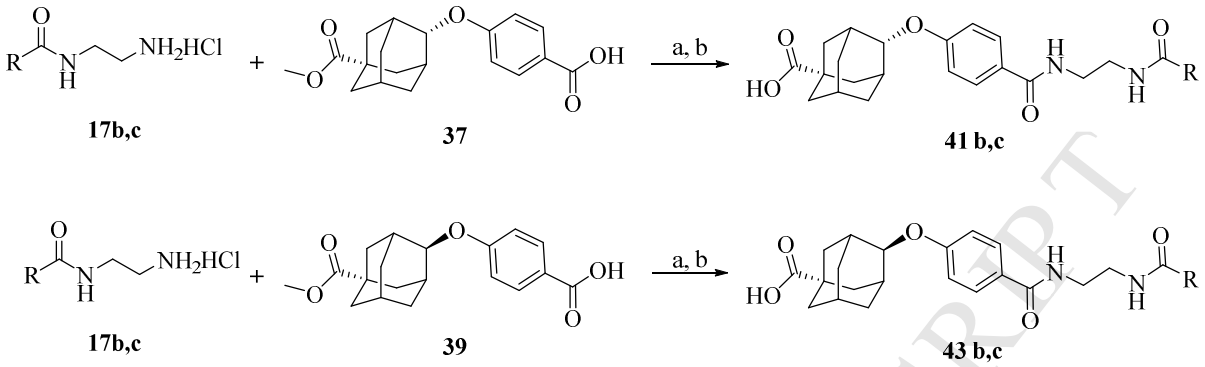
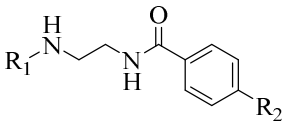


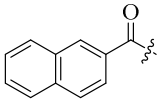
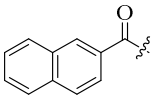
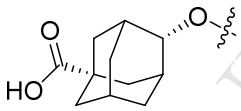
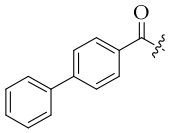
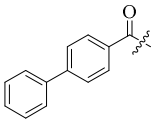
Fig. 5. X-ray crystal structure of *E*-isomer **38.**



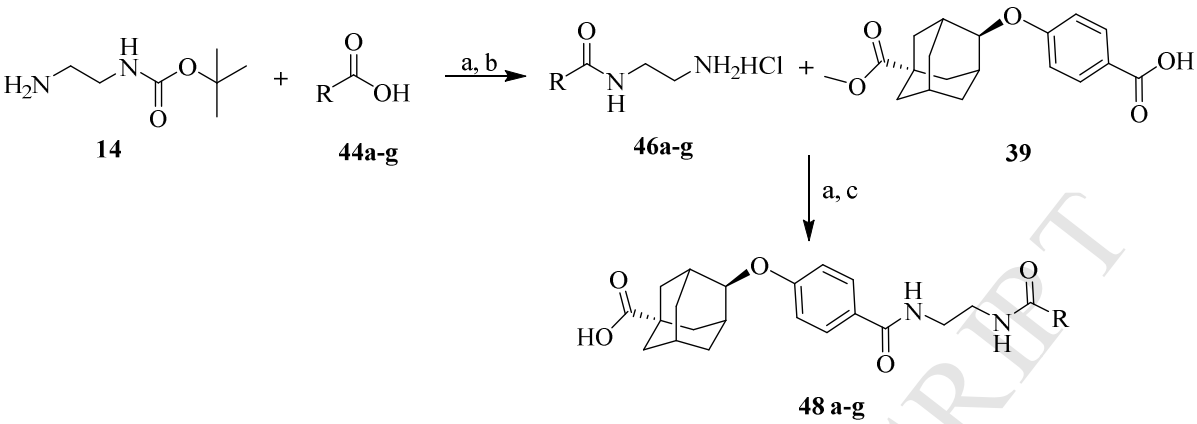
Scheme 4: Reagents and conditions: (a) EDCI, HOBt, DIPEA, DCM, rt; (b) NaOH, H_2O/THF, rt.

Table 3. Activity evaluation between *Z*-isomer and *E*-isomer.



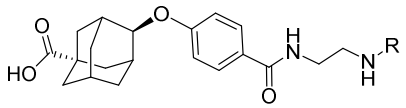
Compound	R_1	R_2	Human DGAT1 IC_{50} (nM)[a]	Mouse DGAT1 IC_{50} (nM)[a]
43b			44	8
41b			48	19
43c			5	5
41c			25	10

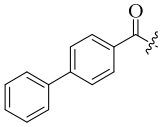
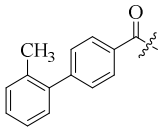
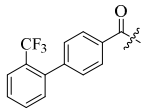
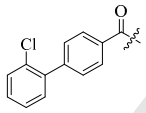
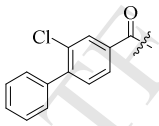
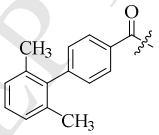
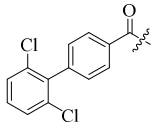
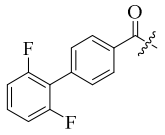
[a]IC_{50} values were determined from concentration-dependent inhibition curves of triplicate experiments by the GraphPad Prism software



Scheme 5: Reagents and conditions: (a) EDCI, HOBt, DIPEA, DCM, rt; (b) 4M-HCl/1,4-dioxane, EtOAc, rt; (c) NaOH, H_2O/THF, rt.

Table 4. DGAT1 IC_{50} values of substituted biphenyl analogs 48a-g.



Compound	R	Human DGAT1 IC_{50} (nM)[a]	Mouse DGAT1 IC_{50} (nM)[a]
43c		5	5
48a		32	2
48b		>500	ND
48c		>500	ND
48d		300	ND
48e		105	5
48f		280	ND
48g		170	ND

ND = Not Determined; [a]IC_{50} values were determined from concentration-dependent inhibition curves of triplicate experiments by the GraphPad Prism software.

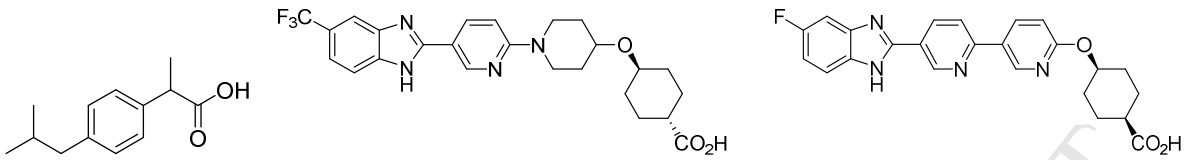


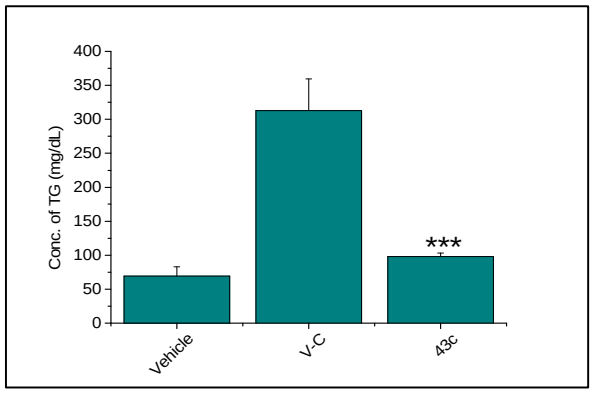
Fig. 6. Structure of Ibuprofen and DGAT1 inhibitors epimerized

Table 5. Selectivity, stability, CYP, hERG and cytotoxicity of compound 43c.

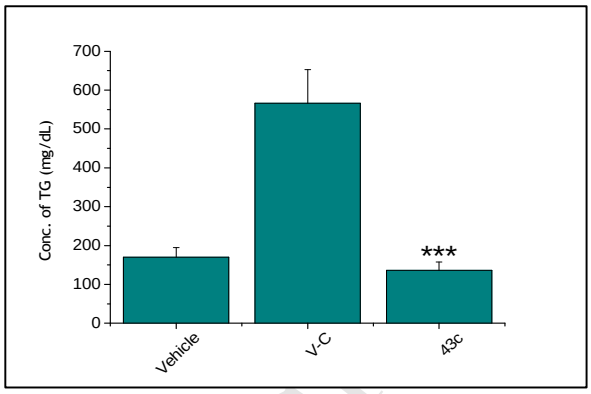
Assay	Results
human DGAT2 (IC_{50})	>100 μM
Liver microsomal stability (Human)	>99% parent remained after 30 min incubation
Liver microsomal stability (rat)	>62% parent remained after 30 min incubation
CYP inhibition	1A2: 4.56% inhibition at 10 μM 2C19: 9.53% inhibition at 10 μM 3A4: 10.9% inhibition at 10 μM 2C9: 41.9% inhibition at 10 μM 2D6: 8.09% inhibition at 10 μM
hERG[a]	< 1% at 10 μM
Permeability (PAMPA)	- 4.72 ± 0.0864
Cytotoxicity[b]	VERO: IC_{50} = >100 μM NIH 3T3: IC_{50} = >100 μM L929: IC_{50} = >100 μM HFL-1: IC_{50} = >100 μM CHO-K1: IC_{50} = >100 μM

[a]hERG: ligand binding assay; [b]VERO: African green monkey kidney cell line; NIH 3T3: mouse embryonic fibroblast cell line; L929: NCTC clone 929, mouse fibroblast cell line; HFL-1: human embryonic lung cell line; CHO-K1: Chinese hamster ovary cell line.

(A) OLTT in C57BL/6 mice (3 mg/kg)



(B) OLTT in TallyHo mice (3 mg/kg)



(C) OLTT dose dependency in C57BL/6 mice

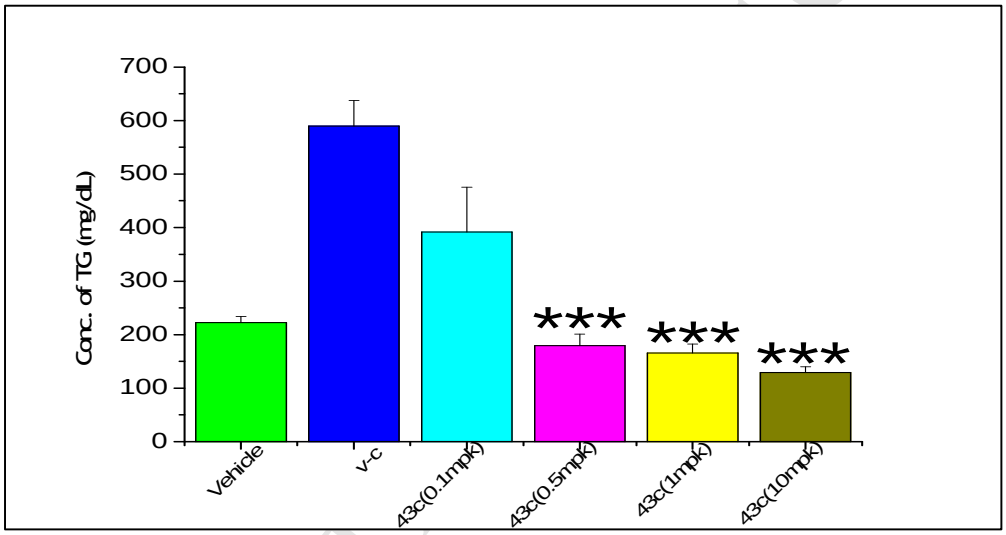


Fig. 7. OLTT (normal and diabetic mice) and OLTT dose dependency (normal mice). Data are expressed as means ± S.E.M. (*n* = 5 mice). Vehicle (0.5% carboxymethyl cellulose), V-C (corn oil, 5 mL/kg). ***P <0.001 versus vehicle by Student's t-test.

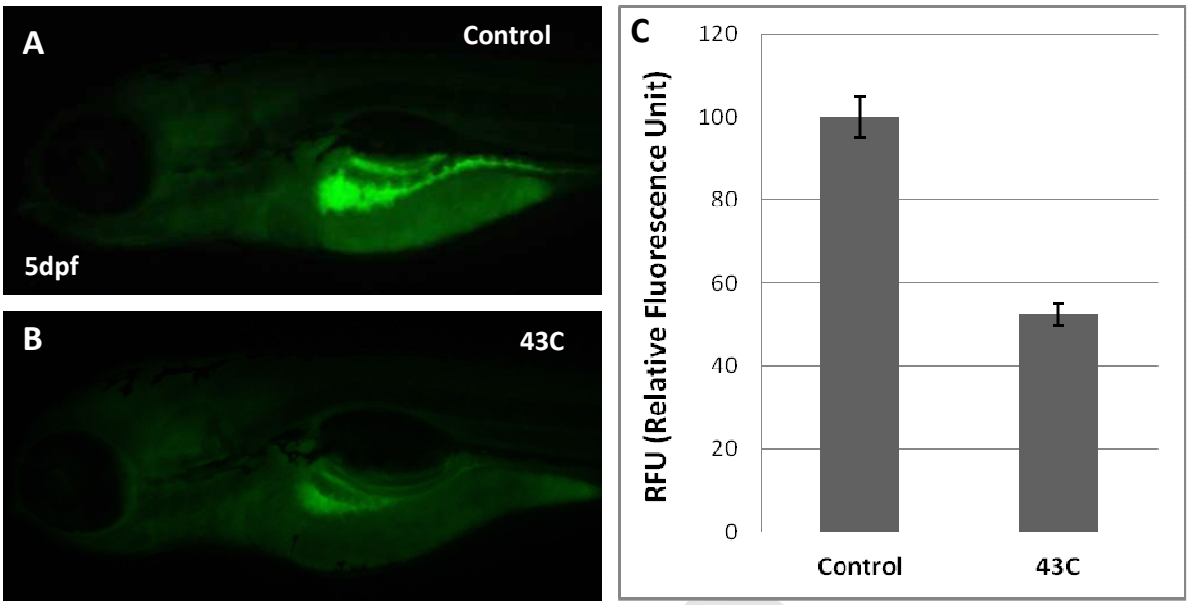


Fig. 8. Detection of lipid accumulation in zebrafish treated with a DGAT1 inhibitor. Zebrafish embryos were incubated with 10 μM of **43c** for 3 days. Lipid deposits in the yolk stained with LipidGreen2 (A and B). Quantitative analysis of LipidGreen2 fluorescence in zebrafish (C).

Table 6. Pharmacokinetic properties of 43c in rat.

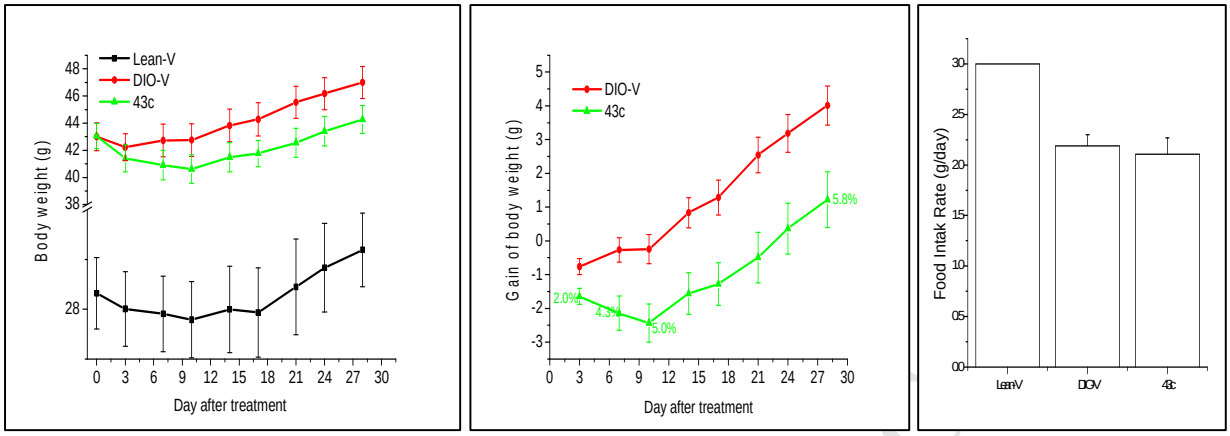
Parameters	IV (5 mg/kg)[a]	PO (5 mg/kg)[a]
T_{max} (h)	-	1.17±0.76
C_{max} (μg/mL)	-	0.12±0.04
$t_{1/2}$ (h)	1.07±0.13	1.71±0.51
$AUC_{0\text{-}8\ h}$ (μg·h/mL)	1.85±0.20	0.24±0.14
CL (L/kg/h)	2.71±0.33	-
V_{SS} (L/kg)	3.47±1.34	-
F (%)		13.4%

[a] Data are expressed as mean±S.D (n = 3).

Table 7. Concentration of 43c in blood, intestine, and enterocyte after oral dosing at 5 mg/kg in Mice.

Sample	Concentration (μg/mL or μg/g)	Tissue/plasma ratio
Plasma	0.21±0.33	1
Whole small intestine	93±31	434
Enterocyte	22.2±11.4	104

(A) Body weight change and food intake (DIO mice, 10 mg/kg)



(B) OGTT (DIO mice, 10 mg/kg)

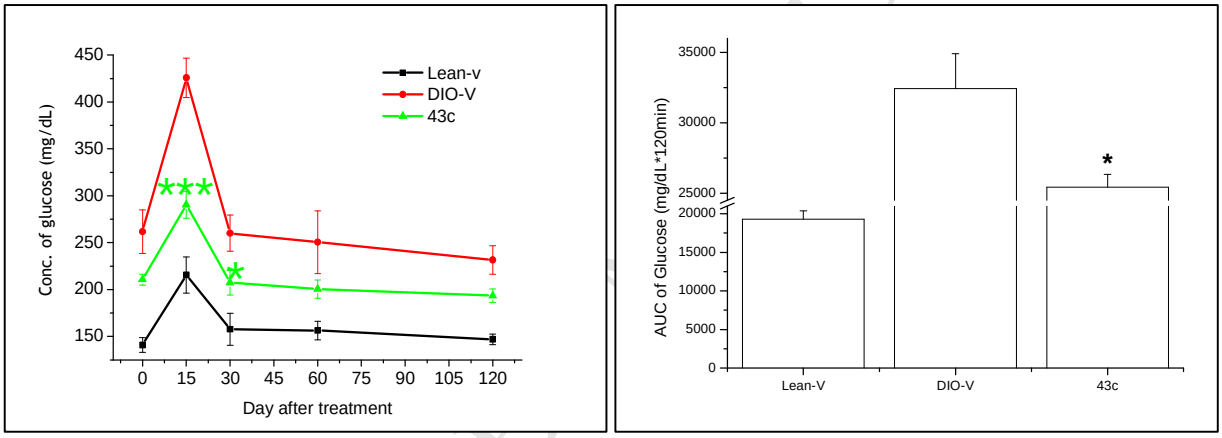
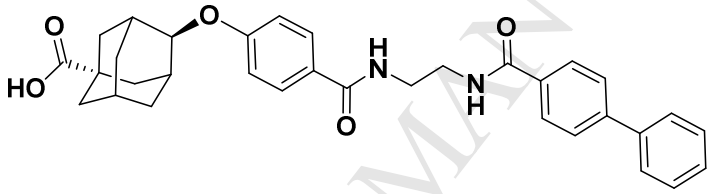


Fig. 9. *In vivo* study of compound **43c** in DIO mice for 4 weeks at 10 mg/kg. (A) Percent reduction in body weight compared to start weight in DIO mice. (B) Oral glucose tolerance test (OGTT); Data are expressed as means ± S.E.M. (*n* = 10 mice). *P <0.05, and ***P <0.001 versus vehicle by Student's t-test.

ABSTRACT: We have developed a series of adamantane carboxylic acid derivatives exhibiting potent diacylglycerol acyltransferase 1 (DGAT1) inhibitory activities. Optimization of the series led to the discovery of *E*-adamantane carboxylic acid compound **43c**, which showed excellent *in vitro* activity with an IC_{50} value of 5 nM against human and mouse DGAT1, also good druggability as well as microsomal stability and safety profiles such as hERG, CYP and cytotoxicity. Compound **43c** significantly reduced plasma triglyceride levels *in vivo* (in rodents and zebrafish) and also showed bodyweight gain reduction and glucose area under curve (AUC) lowering efficacy in diet-induced obesity (DIO) mice.



43c

Highlights

- A series of adamantane carboxylic acid derivatives has been identified and synthesized.
- Diacylglycerol acyltransferase 1 (DGAT1) inhibitory activities of the compounds were evaluated.
- The compound 43c showed good in vitro activity and druggability.
- The compound 43c showed good in vivo efficacy (lipid lowering efficacy etc.)
- The compound 43c may be a promising lead to develop as an anti-obesity/diabetes drug.