Discovery of a Potent, Selective, and Orally Efficacious Pyrimidinooxazinyl Bicyclooctaneacetic Acid Diacylglycerol Acyltransferase-1 Inhibitor

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Inhibition of DGAT-1 is increasingly seen as an attractive mechanism with the potential for treatment of obesity and other elements of the metabolic syndrome. We report here a bicyclooctaneacetic acid derivative in the pyrimidinooxazine structural class of DGAT-1 inhibitors that has good potency, selectivity, and pharmacokinetic characteristics across a variety of species. This compound is an effective inhibitor of DGAT-1 in both intestinal and adipose tissue, which results in a reduction in body weight or body weight gain following oral administration in both mouse and rat models of dietary-induced obesity.

Introduction

Current therapeutic options for the treatment of obesity target a reduction of energy intake either through inhibition of appetite or through reduction in lipid absorption from the small intestine.[1] The continuing need for improved therapies, which are either more efficacious or give rise to fewer side effects or both, has led to a variety of newer mechanistic targets, including those which seek to inhibit triacylglycerol synthesis and storage in adipose tissue. The diacylglycerol acyltransferase enzymes DGAT-1[a] and DGAT-2 are potential targets in such an approach,[2] as they are dedicated to the final committed step in trigylceride synthesis. Inhibitors of varying structural types of DGAT-1 are claimed and reported with increasing frequency as potential treatments for obesity and other disorders.[3] This particular interest in DGAT-1 inhibition stems from the published phenotype of DGAT-1 deficient (*Dgat1*$^{-/-}$) mice.[4] These animals are viable, resistant to weight gain when fed a high-fat diet, and show increased insulin and leptin sensitivity. Resistance to weight gain results from increased energy expenditure rather than decreased food intake (the animals are in fact hyperphagic) and is associated with loss of adipose rather than lean tissue mass. A recent paper[5] has demonstrated that most aspects of this phenotype can be reproduced in rodents by treatment with a potent and selective small molecule inhibitor of DGAT-1 such as compound **1**. We now report a further structural class of potent and selective DGAT-1 inhibitors, see Figure 1, one example of which is highly effective in rodent pharmacodynamic and disease models.

The first published patent application, which described small molecule inhibitors of DGAT-1, resulted from a collaboration between the Japan Tobacco and Tularik companies.[6] It claimed a series of pyrimido[4,5-*b*][1,4]oxazine derivatives, and one interesting example, compound **2**, proved to be a potent and selective DGAT-1 inhibitor (Table 1). It also proved to have an acceptable PK profile, in terms of bioavailability and half-life, in rodents (Table 2). We sought to improve on the overall profile of this compound, with a specific aim to reduce the clearance of the compound in rat and dog PK studies. We had evidence that the major route of metabolism of **2** in these species was via conjugation of the acid group to form the acylglucuronide. In the light of recent concerns that the reactivity of such metabolites can lead to covalent protein adducts, which may give rise to idiosyncratic toxicity,[7] we sought to introduce a greater degree of steric crowding around the acid group by replacing the cyclohexane ring with various bi- and tricyclic systems. Certain sterically hindered carboxylic acid acyl glucuronides have been shown[8] to be inherently more stable both to hydrolysis and to rearrangement to more reactive isomers, making them less likely to react with proteins in vivo. We therefore prepared a short series of adamantane, bicyclo[2.2.2]-octane, and bicyclo[3.2.2]nonane-pyrimido[4,5-*b*][1,4]oxazine derivatives, as described below.

Chemistry

The phenylbicyclo[2.2.2]octane core was constructed according to either method A or B as illustrated in Scheme 1. Method A starts with a base-catalyzed condensation of acetophenone and diethyl (ethoxymethylene)malonate, giving the intermediate diethyl (3-oxo-3-phenylpropylidene)malonate, which was converted into the pyrone **9** on treatment with acetyl chloride and DMF. The Diels−Alder reaction of **9** with ethene was effected at 75 bar rather than the reported 3000 bar[9−11] to give bicyclooctene **10**, which was hydrogenated to afford **11**.

The procedure described by Chapman et al.[12] (Method B) was also used to make the phenylbicyclo[2.2.2]octane as in **15**, and this method could be extended to the bicyclo[3.2.2]nonane system as in **19**. Selective hydrolysis of the diesters **12**[13] and **16**[14] with barium hydroxide gave the acids **13** and **17**, which were converted into the corresponding bromides **14** and **18** under Hunsdiecker conditions. The bromobicycles **14** and **18** were treated with aluminum chloride in benzene to provide **15** and **19**.

The synthesis of compounds **3**−**5** are outlined in Scheme 2. The esters **20** and **22** were obtained by using the modified Arndt−Eistert protocol,[15] and **20** was alkylated with LDA and methyl iodide to give **21**. The pyrimido ring system of the test compounds was built up via a Friedel−Crafts acylation with

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[a] Abbreviations: ACAT-1, acyl-cholesterol acyltransferase-1; AG, acyl-glucuronide; Clp, plasma clearance; DAG, diacylglycerol; DGAT-1, diacylglycerol acyltransferase-1; DGAT-2, diacylglycerol acyltransferase-2; DIO, diet-induced obesity; ED_{50}, effective dose, dose effects a 50% reduction in biological end point; GI, gastrointestinal; HuTu 80, human gastrointestinal tumor cell line; iv, intravenous; MDCK, Madin−Darby canine kidney cell line; OLTT, oral lipid tolerance test; PK, pharmacokinetic; SEM, standard error of the mean; TAG, triacylglycerol; V_{dss}, volume of distribution at steady state.

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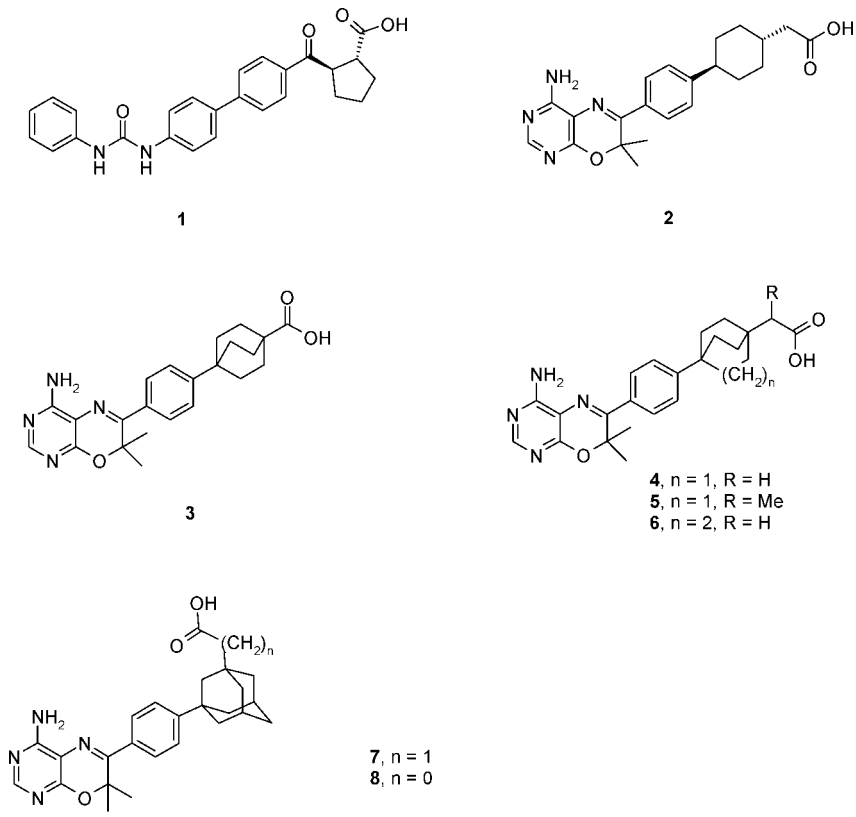


Figure 1. Literature and novel DGAT-1 inhibitors.

2-bromoisobutyryl bromide, followed by cyclization with 5,6-diaminopyrimidin-4-ol and final basic hydrolysis gave compounds **3**−**6**.

Compounds **7** and **8** were made using analogous chemistry to that described for compounds **3**−**6** (Scheme 3).

Results and Discussion

Compounds in this series showed good to very good potency (15−250 nM) as inhibitors of recombinant human DGAT-1 (Table 1). The bicyclooctane and bicyclononane compounds, which have a 1,4- relationship between the aryl and acid groups, were more potent than the adamantane derivatives that have a corresponding 1,3-relationship. All compounds showed much lower potency against human ACAT-1, the nearest enzyme in terms of structural homology and where tested were essentially inactive versus human DGAT-2, which, although serving the same function, is only distantly related to DGAT-1 structurally.[16] Where tested, compounds showed little difference between their IC_{50} values against rat microsomal and recombinant human DGAT-1. The bicyclo[2.2.2]octaneacetic acid **4** was the most potent of the new compounds, giving an equivalent IC_{50} to compound **2**. These two compounds also showed excellent potency in inhibiting triacylglycerol synthesis in human HuTu 80 cells, which are relevant to the gut as a target organ.

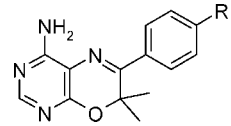
Comparison of the compounds in PK studies after oral and iv dosing to rats showed reduced clearance for **4** compared to compound **2** (Table 2). This ranking was repeated in dogs with compound **4** again showing a superior profile, with reduced clearance compared to **2**. Compound **4** also showed a very good PK profile in marmosets. Acyl glucuronidation is the major route of metabolism of **4** in hepatocytes (18%, 38%, and 17% conversion during 2 h incubation in rat, dog, and human, respectively). In alamethicin-activated microsomes, the conversion to glucuronide was 9%, 76%, 15%, and 25% in rat, dog, marmoset, and human, respectively, during 1 h incubation. These data are consistent with the relative in vivo clearance data in the rat, dog, and marmoset (Table 2). Furthermore, in dog, 82% of parent-related material was excreted as the acyl glucuronide in a 2 h urine sample.

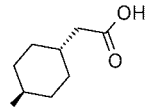
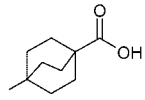
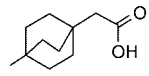
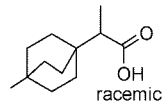
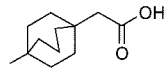
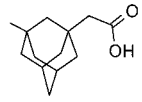
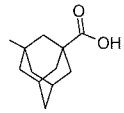
It is believed that in some circumstances the initially formed 1β-isomer of an acyl glucuronide metabolite can directly lead to drug−protein covalent conjugates through acylation, while the 2- and 4-*O* acyl rearrangement products can give rise to adducts through Schiff base formation with protein amino groups.[7] To assess the potential of the acylglucuronide metabolite of compound **4** to enter into such reactions its hydrolytic stability (as an indication of acylation potential) and propensity to form the *O*-acyl rearrangement products was investigated. Compound **4** acyl glucuronide was found to undergo hydrolytic cleavage only very slowly at 37 °C in pH 7.4 buffer (Figure 2) compared to various standard drugs reported in the literature.[8] In addition, only ∼15% rearrangement to acyl migrated isoforms was noted under these conditions. Taken together, these data suggest that compound **4** would be unlikely to give rise to idiosyncratic toxicity as a result of covalent protein binding involving its acyl glucuronide metabolite.

Compound **4** was inactive (<25% inhibition @30 μM) versus the cytochrome P450 enzymes CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Similarly, the compound showed no significant activity against the hERG encoded potassium channel. The key physical properties of **4** are given in Table 3 and are typical for a moderately lipophilic carboxylic acid. Although aqueous solubility is lower than ideal, this is counterbalanced by good cellular permeability, resulting in no issues with absorption in animal studies.

Although ubiquitously expressed, adipocytes and the enterocytes of the small intestine are two major tissue types in which DGAT-1 has significant expression and is therefore thought to

Table 1. Enzyme and Cellular Inhibitory Potencies for Target Compounds[a]



Compound	R	human DGAT-1 IC_{50} (μM)	human DGAT-2 % inhib @ 10 μM	human ACAT-1 IC_{50} (μM)	rat DGAT-1 IC_{50} (μM)	HuTu 80 cell IC_{50} (μM)[b]
2		0.015 (n = 2)	3.7	12	0.019	0.004
3		0.028	NT	18	NT	NT
4		0.015	0.0	5.2	0.009	0.003
5		0.028	NT	18	NT	NT
6		0.069	NT	28	NT	NT
7		0.120	NT	>30 (n = 2)	NT	NT
8		0.25	NT	29 (n = 2)	NT	NT

[a] Mean values from a minimum $n = 3$ experiments. [b] Inhibition of triacylglycerol synthesis in HuTu 80 cells. NT: not tested.

Table 2. Pharmacokinetic Parameters for Selected DGAT-1 Inhibitors[a]

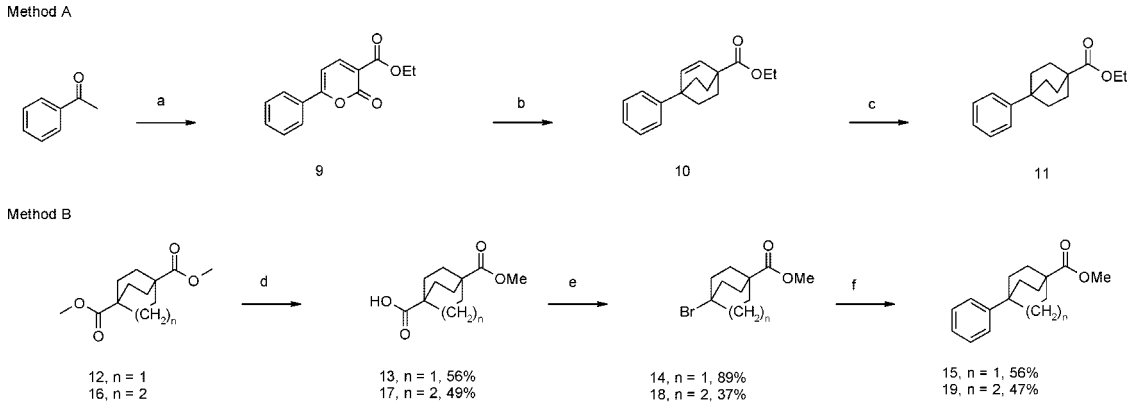
compd	species	Clp (mL/min/kg)	V_{dss} (L/kg)	iv half-life (h)	oral half-life (h)	bioavailability (%)
2	rat	5.2	0.69	3.0	5.6	>100[b]
	dog	12	2.0	3.9	2.8	>100[b]
4	rat	1.5	0.70	7.0	5.4	72
	dog	3.3	1.0	8.6	9.9	>100[b]
	marmoset	0.78	0.88	13	9.5	61

[a] Compounds were dosed at between 1 and 3 mg/kg in either solution (DMSO/hydroxy-β-cyclodextrin) or suspension (HPMC/Tween). [b] Bioavailabilities >100% are believed to reflect enterohepatic recirculation of acyl glucuronide metabolite.

play a major role in lipid metabolism. In adipocytes, DGAT-1 catalyzes the condensation of diacylglycerol (DAG) and a range of fatty acids (FA) to form triacylglycerol (TAG) for high-density energy storage. In the GI tract, dietary sources of FA and monoacylglycerol absorbed by the enterocyte are resynthesized to TAG, which is critical for the assembly and secretion of chylomicrons that transport triacylglycerol into the blood stream for utilization by high energy requiring tissues such as muscle. Inhibition of DGAT-1 in adipocytes would be expected to result in measurable effects on TAG synthesis in these cells. Inhibition of the enzyme in enterocytes should lead to reduced TAG synthesis in these cells also, which should in turn result in a measurable effect on TAG levels in the systemic circulation following an oral lipid load. Encouraged by the potency, selectivity, and PK profile of compound **4**, we tested it further in a variety of animal models designed to demonstrate the effects described above.

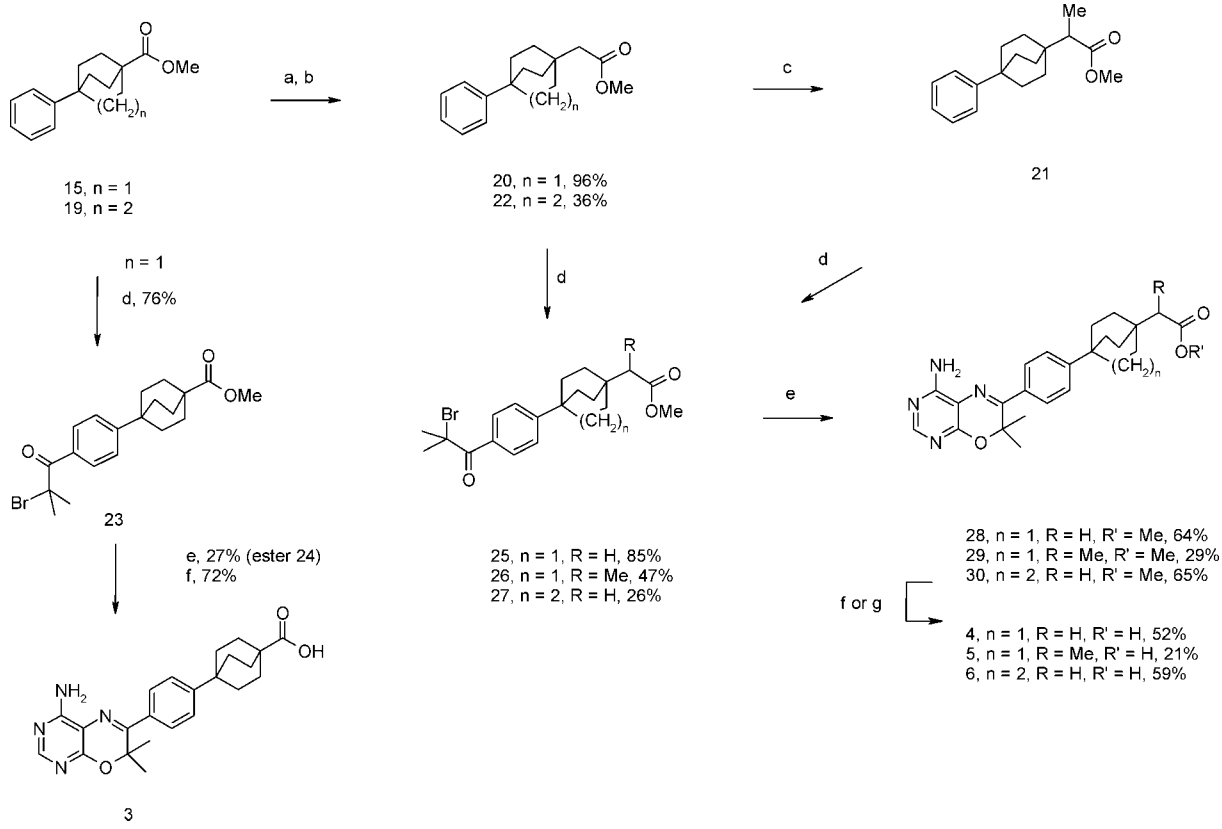
In an oral lipid tolerance test (OLTT), fasted rats were administered compound by oral gavage at a range of doses 2 h before a bolus dose of corn oil. Plasma TAG levels were measured 1.5 h later. Figure 3A shows that compound **4** was highly efficacious in reducing plasma TAG excursion in this model (statistically significant effect at 0.3 mg/kg), indicating a reduction in enterocyte lipid transport. This compound also showed good, but less potent, effects on adipose TAG synthesis (Figure 3B), giving a statistically significant decrease in the TAG to DAG ratio in adipose cells at 1 mg/kg, thereby demonstrating inhibition of DGAT-1 in these cells. The greater potency of **4** in oral lipid tolerance test is to be anticipated from its presumed relatively higher local unbound concentration in the gut from a given dose compared to that appearing in the blood, giving greater DGAT-1 inhibition in the enterocytes compared to that

Scheme 1. Synthesis of Phenylbicycloalkanecarboxylates[a]



[a] Reagents and conditions: (a) (i) NaOEt, EtOH, diethyl (ethoxymethylene)malonate, DME; (ii) AcCl, DMF, 56%. (b) Ethene, 200 °C, 15 h, 75 bar, 51%. (c) H_2, 10% Pt on carbon, EtOH, 84%. (d) $Ba(OH)_2$, $MeOH/H_2O$. (e) (i) NaOH, acetone; (ii) $AgNO_3$; (iii) Br_2, pet ether (bp 40−60 °C). (f) $AlCl_3$, PhH.

Scheme 2. Synthesis of Compounds **3**−**6**[a]



[a] Reagents and conditions: (a) NaOH, MeOH. (b) (i) Oxalyl chloride, DCM; (ii) $TMSCHN_2$/MeCN/THF/Et_3N; (iii) $PhCO_2Ag/Et_3N$/MeOH. (c) LDA, MeI, THF, −70 °C. (d) 2-Bromoisobutyryl bromide, $AlCl_3$, DCM. (e) 5,6-diaminopyrimidin-4-ol, 1 M HCl, EtOH. (f) 2 M NaOH, MeOH. (g) KOTMS, THF.

in adipocytes. These results demonstrate that DGAT-1 inhibitors can elicit a dual pharmacodynamic effect through inhibition of the enzyme in both the enterocyte and adipose tissue, with potential beneficial clinical effects on both postprandial plasma TAG and adiposity.

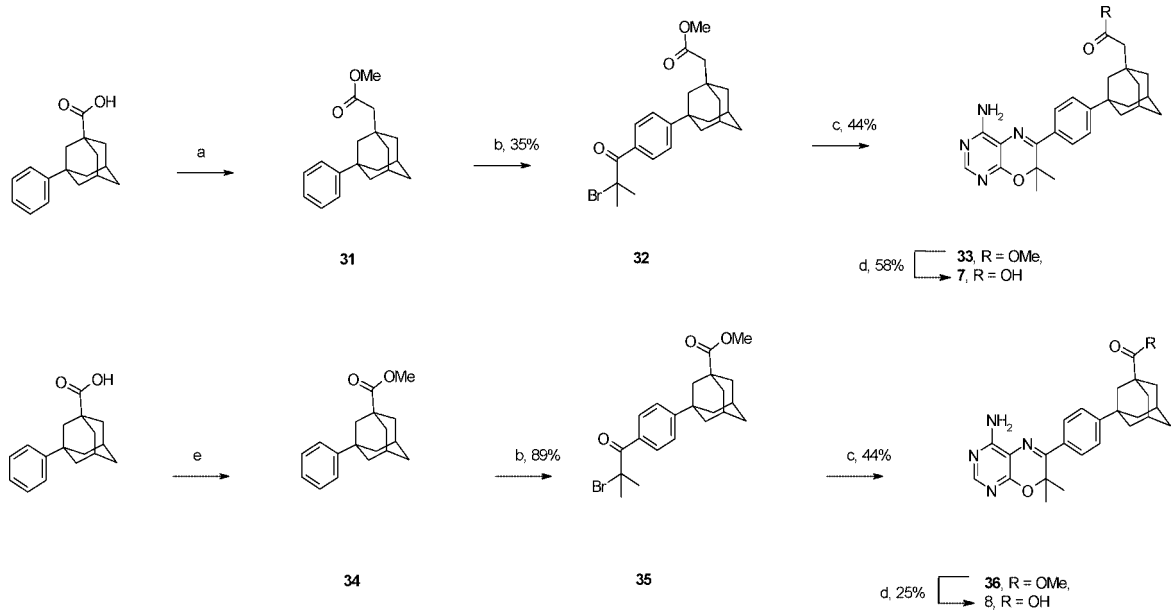
To assess its activity in a model of diet-induced obesity, compound **4** was tested for effects on body weight loss in DIO mice (Figure 4). Treatment for 3 days resulted in a modest but statistically significant reduction in body weight compared to start weight (2%) and in comparison with age-matched vehicle-treated control mice on the same diet (3%).

In summary, the novel DGAT-1 inhibitor **4** has been shown to be a potent and selective inhibitor of the enzyme, with a superior PK profile in rat and dog compared to compound **2**. Compound **4** shows potent effects in rodent pharmacodynamic models, demonstrating efficacy in both gastrointestinal and adipose tissues, and is effective in reducing body weight in a rodent model. These results provide further evidence that small molecule DGAT-1 inhibitors can reproduce key aspects of the DGAT-1 ko phenotype and have potential in the treatment of obesity and other elements of metabolic syndrome.

Experimental Section

All solvents and chemicals used were reagent grade. Anhydrous solvents tetrahydrofuran (THF), benzene, and dimethoxyethane (DME) were purchased from Aldrich. Flash column chromatogra-

Scheme 3. Synthesis of Compounds **7** and **8**[a]



[a] Reagents and conditions: (a) (i) Oxalyl chloride, DCM; (ii) $TMSCHN_2$/MeCN/THF/Et_3N, 87%; (iii) $PhCO_2Ag$/Et_3N/MeOH, 51%. (b) 2-Bromoisobutyryl bromide, $AlCl_3$, DCM. (c) 5,6-diaminopyrimidin-4-ol, 1 M HCl, EtOH. (d) 2 M NaOH, MeOH. (e) $TMSCHN_2$, MeOH, PhMe, 94%.

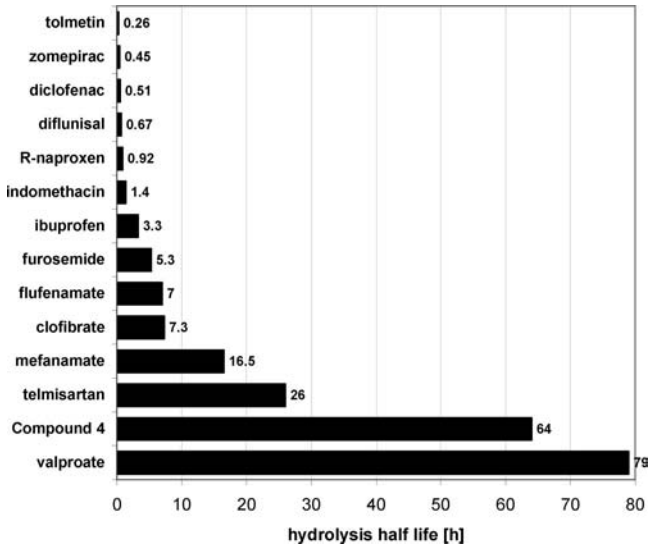


Figure 2. Hydrolysis half-life of compound **4** acyl glucuronide compared with those of standard drugs. Compound **4** acyl glucuronide was biosynthesised utilizing dog activated microsomes, followed by isolation and purification via solid phase extraction. It was then incubated at 37 °C in pH 7.4 phosphate buffer and samples taken at regular intervals to determine (via HPLC-MS) the half-life of hydrolysis.

phy was carried out using prepacked silica cartridges (from 4 g up to 330 g) from Redisep, Biotage, or Crawford and eluted using an Isco Companion system. Purity and characterization of compounds were established by a combination of liquid chromatography−mass spectroscopy (LC-MS), gas chromatography−mass spectroscopy (GC-MS), and NMR analytical techniques and was >95% for all test compounds. Combustion analysis was also employed for compound **4**. ^{1}H NMR were recorded on a Varian Gemini 2000 (300 MHz) or a Bruker Avance DPX400 (400 MHz) and were determined in $CDCl_3$ or DMSO-d_6. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) (0.00 ppm) or solvent peaks as the internal reference and coupling constant (*J*) values are reported in Hertz (Hz). Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad peak. Merck precoated thin layer chromatography (TLC) plates (silica gel 60 F_{254}, 0.25 mm, art. 5715) were used for TLC analysis. Solutions were dried over anhydrous magnesium sulfate, and the solvent was removed by rotary evaporation under reduced pressure.

Method A: Ethyl 2-Oxo-6-phenyl-2*H*-pyran-3-carboxylate (9). To a solution of sodium ethoxide (21% solution in ethanol; 116 mL, 358.33 mmol) in anhydrous DME (300 mL) was added diethyl (ethoxymethylene)malonate (66 mL, 325.75 mmol). The reaction mixture was heated to reflux, and 1-phenylethanone (38 mL, 325.75 mmol) was added dropwise and heating maintained for 1 h. The reaction mixture was allowed to cool to ambient temperature and added to a stirred solution of 5 M HCl (500 mL), and the resulting yellow solution was extracted into ether (3 × 500 mL). The organic extracts were combined, washed with brine (300 mL), dried over $MgSO_4$, and concentrated to leave a red oil. Acetyl chloride (60 mL) was added followed by DMF (2 mL), and the mixture was heated to 70 °C for 2 h, allowed to cool, and then concentrated to leave a dark residue that formed a crystalline solid on standing. This was recrystallized from absolute EtOH (50 mL) to give **9** (49.16 g, 201.2 mmol, 62%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.39 (t, J = 7.1 Hz, 3H), 4.39 (q, J = 7.1, 2H), 6.77 (d, J = 7.3, 1H), 7.47−7.56 (m, 3H), 7.89−7.91 (m, 2H), 8.29 (d, J = 7.4,1H). GC-MS EI *m/z*: ($M^{+\bullet}$) 244.

Ethyl 4-Phenylbicyclo[2.2.2]oct-2-ene-1-carboxylate (10). Ethyl 2-oxo-6-phenyl-2*H*-pyran-3-carboxylate **9** (49.1 g, 201.2 mmol) was dissolved in toluene saturated with ethylene and heated at 200 °C for 15 h at 75 bar pressure. The solvent was concentrated to leave a pale-yellow gum (49 g), which was purified by silica chromatography (ether/isohexane = 5:95) to provide **10** (26.4 g, 103 mmol, 51%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.31 (t, J = 7.1, 3H), 1.56−1.66 (m, 4H), 1.90−2.06 (m, 4H), 4.23 (q, J = 7.1, 2H), 6.40−6.43 (m, 1H), 6.59 (d, J = 8.6, 1H), 7.18−7.26 (m, 1H), 7.31−7.43 (m, 5H). GC-MS CI *m/z*: (M^+ + H) 257.

Ethyl 4-Phenylbicyclo[2.2.2]octane-1-carboxylate (11). Ethyl 4-phenylbicyclo[2.2.2]oct-2-ene-1-carboxylate **10** (26.3 g, 102.5 mmol) in absolute ethanol (600 mL) and 10% Pt on carbon (2.4 g) were stirred under a balloon of hydrogen at ambient temperature overnight. The reaction mixture was filtered and the solvent removed under reduced pressure to leave a **11** as a colorless oil, which rapidly formed a white solid on standing (22.179 g, 85.96 mmol, 84%). ^{1}H NMR ($CDCl_3$) δ: 1.25 (t, J = 7.1, 3H), 1.83−1.96 (m, 12H,), 4.12 (q, J = 7.1, 2H), 7.15−7.20 (m, 1H), 7.28−7.31 (m, 4H). GC-MS EI *m/z*: ($M^{+\bullet}$) 258.

Table 3. Physical Properties of Compound **4**

logD	aqueous solubility pH 7.4 (μM)	rat plasma protein binding[a] (% free)	dog plasma protein binding[a] (% free)	human plasma protein binding[a] (% free)	MDCK[b] permeability P_{app} ($\times 10^{-6}$ cm·s^{-1})
1.9	12 ($n = 2$)	0.91 ($n = 1$)	0.60 ($n = 2$)	1.0 ($n = 2$)	18 (A−B); 26 (B−A) ($n = 1$)

[a] PPB was assessed by equilibrium dialysis in the appropriate species plasma at 37 °C. Free and bound concentrations were quantified by LC-UVMS. [b] Compounds were incubated at 10 μM in cultured MDCK cells. Permeability was measured in both the A to B and B to A direction

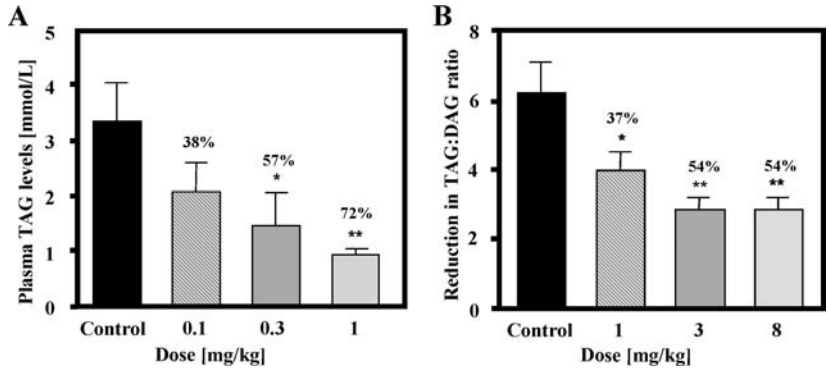


Figure 3. Pharmacodynamic effects of compound **4**. (A) Dose-related reduction in plasma triacylglycerol excursion at 1.5 h post bolus corn oil administration; $n = 6$. (B) Dose-related reduction in adipose triacylglycerol synthesis as determined by changes in the ratio of radiolabel incorporation into TAG and DAG; $n = 10$. Mean values ± SEM (* $P < 0.05$, ** $P < 0.01$).

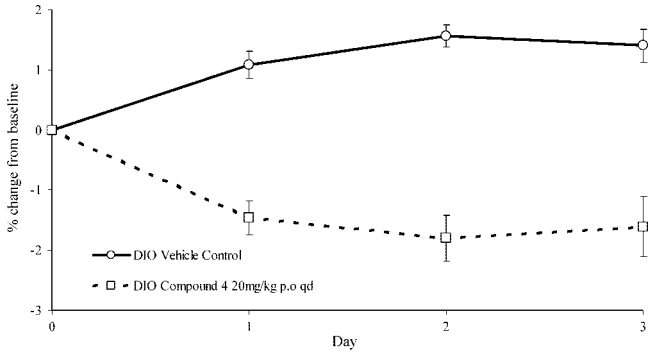


Figure 4. Percent reduction in body weight compared to start weight in DIO mice receiving high energy cafeteria diet. Day 0 is first treatment day; mice dosed daily at 16:00 and weighed at this time. In mice receiving compound **4**, statistical differences from DIO controls were seen at all time points; $n = 12$ ($P < 0.001$).

Method B: 4-(Methoxycarbonyl)bicyclo[2.2.2]octane-1-carboxylic Acid (13). A solution of dimethyl bicyclo[2.2.2]octane-1,4-dicarboxylate[13] **12** (18.36 g, 81.14 mmol), barium hydroxide octahydrate (12.8 g, 40.57 mmol) in methanol (200 mL), and water (40 mL) was stirred vigorously at ambient temperature overnight. The reaction mixture was diluted with water (100 mL) and washed with isohexane (4 × 250 mL), and the aqueous phase was acidified with 2 M HCl to pH 2 and extracted into AcOEt (5 × 300 mL). The organic extracts were combined, dried over $MgSO_4$, and concentrated to leave a white solid. This was re-dissolved in toluene (500 mL), filtered, and the filtrate evaporated to dryness to give **13** as a white solid (9.64 g, 45.41 mmol, 56%). ^{1}H NMR (DMSO) δ: 1.71 (s, 12H), 3.58 (s, 3H), 12.03 (s, 1H). GC-MS *m/z*: ($M^+ + H$) 213.

Methyl 4-Bromobicyclo[2.2.2]octane-1-carboxylate (14). To a stirred suspension of 4-(methoxycarbonyl)bicyclo[2.2.2]octane-1-carboxylic acid **13** (14.64 g, 68.98 mmol) in acetone (120 mL) was added 1 M NaOH (70 mL), and the resulting clear solution was allowed to stir at ambient temperature for 10 min. A 4 M solution of silver nitrate (12.4 g, 73.11 mmol) in water (18 mL) was added dropwise and an immediate white suspension formed, which was allowed to stir at ambient temperature for a further hour. The suspension was filtered, washed with water (100 mL), acetone (100 mL), ether (100 mL), and dried under vacuum overnight at 60 °C to leave 19.74 g (61.88 mmol, 89%) of the silver salt as a pale-brown solid. This was suspended in petroleum ether (bp 40−60 °C) (230 mL) under nitrogen, and bromine (3.2 mL, 61.88 mmol) was added dropwise. The resulting orange suspension was allowed to stir at ambient temperature for 30 min and then at 60 °C for 40 min. The reaction mixture was allowed to cool to ambient temperature, the suspension was filtered, and the solid was washed with ether (3 × 100 mL) and then 1 M Na_2CO_3 (3 × 150 mL). The organic phase was separated, washed with brine (100 mL), dried over $MgSO_4$, and concentrated to give **14** (15.3 g, 61.8 mmol, 89%) as a pale-yellow oil, which formed a solid on standing. ^{1}H NMR ($CDCl_3$) δ: 1.94−1.98 (m, 6H), 2.23−2.27 (m, 6H), 3.64 (s, 3H). GC-MS *m/z*: ($M^+ + H$) 247.

Methyl 4-Phenylbicyclo[2.2.2]octane-1-carboxylate (15). A solution of methyl 4-bromobicyclo[2.2.2]octane-1-carboxylate **14** (15.3 g, 61.91 mmol) in anhydrous benzene (50 mL) was added dropwise to an ice−water cooled suspension of aluminum chloride (30.5 g, 229.1 mmol) in benzene (100 mL) under nitrogen. The resulting reaction mixture was allowed to stir in the ice bath for 30 min and then at ambient temperature overnight. The mixture was heated to 60 °C for 4 h and then allowed to cool to ambient temperature and cautiously poured onto ice (300 g) and concentrated HCl (50 mL) and the mixture was extracted into ether (4 × 300 mL). The ether extracts were combined, washed with brine (200 mL), separated, and dried over $MgSO_4$ to leave an orange−brown solid. Purification by silica chromatography (ether/isohexane = 1:9) gave **15** (8.45 g, 34.63 mmol, 56%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.86−1.93 (m, 12H), 3.67 (s, 3H), 7.28−7.31 (m, 5H). GC-MS EI *m/z*: ($M^{+\bullet}$) 244.

Methoxycarbonylbicyclo[3.2.2]nonane-5-carboxylic Acid (17). To a stirred solution of dimethyl bicyclo[3.2.2]nonane-1,5-dicarboxylate[14] **16** (2.02 g, 8.41 mmol) and barium hydroxide octahydrate (1.33 g, 4.20 mmol) was added methanol (20 mL) and water (4 mL). The reaction mixture was stirred vigorously at ambient temperature overnight. The reaction mixture was diluted with water (20 mL) and washed with isohexane (2 × 100 mL), and the aqueous phase was acidified with 2 M HCl to pH 2 and extracted into AcOEt (2 × 100 mL). The organic extracts were combined, dried over $MgSO_4$, and concentrated to give **17** (931 mg, 4.11 mmol, 49%) as a colorless gum, which slowly formed a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.72−1.75 (m, 2H), 1.87−1.92 (m, 12H), 3.65 (s, 3H). MS *m/z*: ($M^+ + H$) 226.

Methyl 5-Bromobicyclo[3.2.2]nonane-1-carboxylate (18). To a stirred suspension methoxycarbonylbicyclo[3.2.2]nonane-5-carboxylic acid **17** (3.43 g, 15.16 mmol) in acetone (25 mL) was added 1 M NaOH (16 mL), and the resulting clear pale-yellow solution was allowed to stir at ambient temperature for 10 min. A 4 M

solution of silver nitrate (2.73 g, 16.07 mmol) in water (4 mL) was added dropwise, and an immediate thick brown suspension formed, which was allowed to stir at ambient temperature for a further 60 min. The suspension was filtered, washed with water (100 mL), acetone (100 mL), ether (100 mL), and dried under vacuum overnight at 45 °C to leave 4.42 g of a brown solid.

To a suspension of the silver salt (4.24 g, 12.73 mmol) in petroleum ether (40−60) (50 mL) under nitrogen was added bromine (0.65 mL, 12.73 mmol) dropwise. The resulting orange suspension was allowed to stir at ambient temperature for 30 min and then at 60 °C for 40 min. The reaction mixture was allowed to cool to ambient temperature, the suspension was filtered, and the solid was washed with ether (3 × 100 mL) and then 1 M Na_2CO_3 (3 × 150 mL). The organic phase was separated, washed with brine (100 mL), dried over $MgSO_4$, and concentrated to give **18** (1.22 g, 4.67 mmol, 37%) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.69−1.75 (m, 2H), 1.83−2.00 (m, 6H), 2.43−2.50 (m, 6H), 3.65 (s, 3H). MS *m*/*z*: (M^+ + H) 261.

5-Phenylbicyclo[3.2.2]nonane-1-carboxylic Acid. A solution of methyl 5-bromobicyclo[3.2.2]nonane-1-carboxylate **18** (1.22 g, 4.67 mmol) in anhydrous benzene (5 mL) was added dropwise to an ice−water cooled suspension of aluminum chloride (2.3 g, 17.28 mmol) in benzene (5 mL) under nitrogen. The resulting reaction mixture was allowed to stir in the ice bath for 30 min and then removed from the cooling bath and allowed to warm to and stir at ambient temperature overnight. Then the reaction mixture was heated to 60 °C for 4 h and allowed to cool to ambient temperature. It was cautiously poured onto ice (30 g) and concentrated HCl (5 mL), and the mixture was extracted into ether (4 × 100 mL). The ether extracts were combined, washed with brine (50 mL), separated, and dried over $MgSO_4$ to leave methyl 5-phenylbicyclo-[3.2.2]nonane-1-carboxylate **19** as an orange−brown gum (821 mg), which was submitted to the following step without purification.

To methyl 5-phenylbicyclo[3.2.2]nonane-1-carboxylate (821 mg) **19** in MeOH (20 mL) was added 2 M NaOH (8 mL) and allowed to stir at ambient temperature overnight. The solvent was removed, and the residue was washed with ether, the aqueous phase was acidified with 2 M HCl, and extracted into AcOEt. The organic extracts were dried over $MgSO_4$ and concentrated to give the title compound (534 mg, 2.18 mmol, 47%) as a brown gum. ^{1}H NMR (DMSO) δ: 1.34−1.38 (m, 6H), 1.61−1.65 (m, 6H), 2.40 (s, 2H), 7.04−7.07 (m, 2H), 7.16−7.20 (m, 1H), 7.24−7.28 (m, 2H), 11.87 (s, 1H). MS (EI) *m*/*z*: $MH^{+\bullet}$ 244. HRMS (EI) for $C_{16}H_{20}O_2$ (MH^+): calcd, 244.1463; found, 244.1478.

Methyl-4-[4-(2-bromo-2-methylpropanoyl)phenyl]bicyclo[2.2.2]-octane-1-carboxylate (23). To an ice/IPA cooled solution of methyl 4-phenylbicyclo[2.2.2]octane-1-carboxylate **15** (412 mg, 1.69 mmol) in DCM (20 mL) was added aluminum chloride (680 mg, 5.06 mmol) followed by the dropwise addition of 2-bromoisobutyryl bromide (0.21 mL, 1.69 mmol). The reaction mixture was allowed to stir at 0 °C for 30 min and then poured onto ice−water (∼50 mL). The aqueous mixture was extracted into DCM (3 × 100 mL), the organic extracts were combined, washed with brine (100 mL), dried over $MgSO_4$, and concentrated to leave crude product. Chromatography (isohexane/ether = 9:1) gave **23** (507 mg, 1.29 mmol, 76%) as an orange solid. ^{1}H NMR ($CDCl_3$) δ: 1.87−1.95 (m, 12H), 2.04 (s, 6H), 3.68 (s, 3H), 7.38 (d, J = 8.4, 2H), 8.11 (d, J = 8.4, 2H). MS *m*/*z*: MH^+ 407.

Methyl 4-[4-(4-Amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]bicyclo[2.2.2]octane-1-carboxylate (24). To a solution of methyl 4-[4-(2-bromo-2-methylpropanoyl)phenyl]bicyclo[2.2.2]octane-1-carboxylate **23** (507 mg, 1.29 mmol) in absolute EtOH (10 mL) was added 5,6-diaminopyrimidin-4-ol (179 mg, 1.42 mmol),followed by 1 M HCl (1.4 mL). The suspension was heated under reflux overnight, and the reaction mixture was allowed to cool to ambient temperature and then evaporated to dryness. The residue treated with 2 M K_2CO_3 to adjust the pH to 10, and then the mixture was extracted into AcOEt (4 × 50 mL). The organic extracts were combined, dried over $MgSO_4$, and concentrated, and the residue was purified by chromatography (DCM/MeOH = 0−5%) to provide **24** (150 mg, 0.357 mmol, 27%) as a white solid. ^{1}H NMR (DMSO) δ: 1.61 (s, 6H), 1.84 (s, 12H), 3.61 (s, 3H), 6.98 (s, 2H), 7.41 (d, J = 8.5, 2H), 7.66 (d, J = 8.4, 2H), 7.95 (s, 1H). MS *m*/*z*: (M^+ + H) 421.

4-[4-(4-Amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]bicyclo[2.2.2]octane-1-carboxylic Acid (3). To a solution of methyl 4-[4-(4-amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]bicyclo[2.2.2]octane-1-carboxylate **24** (140 mg, 0.33 mmol) in MeOH (10 mL) was added 2 M NaOH (0.82 mL, 1.66 mmol). The reaction mixture was allowed to stir at ambient temperature overnight, and then after the addition of EtOH (5 mL) and 1 M NaOH (2 mL) the reaction mixture was heated to 50 °C for 5.5 h. The organic solvent was removed by evaporation under reduced pressure, and the residue was acidified to pH 2 with 2 M HCl. AcOEt was added, and the suspension was filtered and dried to provide **3** (97 mg, 2.39 mmol, 72%) as a white solid. ^{1}H NMR (DMSO) δ: 1.60 (s, 6H), 1.82 (s, 12H), 6.97 (s, 2H), 7.40 (d, J = 8.7, 2H), 7.65 (d, J = 8.7, 2H), 7.95 (s, 1H). MS *m*/*z*: (M^+ + H) 407. HRMS (ES^+) for $C_{23}H_{27}N_4O_3$ (M^+ + H): calcd, 407.20777; found, 407.20773.

4-Phenylbicyclo[2.2.2]octane-1-carboxylic Acid. To a solution of methyl 4-phenylbicyclo[2.2.2]octane-1-carboxylate **15** (8.45 g, 34.58 mmol) in methanol (500 mL) was added 2 M NaOH (86 mL, 173.2 mmol). The reaction mixture was allowed to stir at ambient temperature overnight, the methanol was removed by evaporation, and the residue acidified to pH 2 with 2 M HCl and then extracted with AcOEt (250 mL). The organic phase was washed with brine (50 mL), separated, dried over $MgSO_4$, and concentrated to give **37** as a pale-yellow solid, (5.76 g, 25.0 mmol, 72%). ^{1}H NMR (DMSO) δ: 1.86 (s, 12H), 7.19−7.23 (m, 1H), 7.31−7.39 (m, 4H). MS EI *m*/*z*: (M^- − H) 229. HRMS (ES^-) for $C_{15}H_{17}O_2$ (M − H): calcd, 229.1229; found, 229.1218.

Methyl (4-Phenylbicyclo[2.2.2]oct-1-yl)acetate (20). To an ice−water cooled solution of 4-phenylbicyclo[2.2.2]octane-1-carboxylic acid (5.76 g, 25.00 mmol) in DCM (200 mL) was added DMF (∼5 drops) followed by oxalyl chloride (3.3 mL, 37.51 mmol). The reaction mixture was allowed to stir at 0 °C for 30 min and then at ambient temperature overnight, and the solvent was evaporated under reduced pressure to leave crude acid chloride that was used directly. This was redissolved in a 1:1 solution of MeCN and THF (100 mL) and added dropwise to an ice−water cooled solution of 2 M trimethylsilyldiazomethane (20 mL, 40 mmol), triethylamine (4.4 mL, 31.26 mmol) in a 1:1 solution of MeCN, and THF (200 mL). The reaction mixture was allowed to stir at 0 °C for 1 h and then overnight at ambient temperature. The solvent was removed under reduced pressure and the residue redissolved in AcOEt (250 mL) and washed with water (100 mL), saturated $NaHCO_3$ (150 mL), and brine (150 mL). The organic phase was dried over $MgSO_4$ and concentrated to leave a brown gum. Chromatography (isohexane/AcOEt = 8:2) gave 2-diazo-1-(4-phenylbicyclo[2.2.2]oct-1-yl)ethanone (5.74 g, 22.59 mmol, 90%) as an orange−yellow gum, which formed a solid on standing. ^{1}H NMR ($CDCl_3$) δ: 1.82−1.91 (m, 12H), 5.38 (s, 1H), 7.15−7.21 (m, 2H), 7.29−7.31 (m, 4H). GC-MS ES^+ *m*/*z*: $M^{+\bullet}$ 255. HRMS (EI) for $C_{16}H_{21}N_2O$ (M^+): calcd, 255.1497; found, 255.1490.

2-Diazo-1-(4-phenylbicyclo[2.2.2]oct-1-yl)ethanone (5.74 g, 22.59 mmol) in methanol (250 mL) and placed in an ultrasound bath, a solution of silver benzoate (1.03 g, 4.52 mmol, 0.2 equiv) in triethylamine (12.6 mL, 90.39 mmol, 4 equiv) was added dropwise, and the mixture was sonicated for 1 h. The methanol was removed by evaporation and the residue dissolved in AcOEt (200 mL) and washed with $NaHCO_3$ (100 mL), citric acid (2 M, 100 mL), brine (100 mL), dried over $MgSO_4$, and concentrated to leave **20** (5.59 g, 21.66mmol, 96%) as a yellow oil, which formed a solid on standing. ^{1}H NMR ($CDCl_3$) δ: 1.63−1.71 (m, 6H), 1.83−1.93 (m, 6H), 2.17 (s, 2H), 3.67 (s, 3H), 7.14−7.19 (m, 1H), 7.29−7.32 (m, 4H). GC-MS EI *m*/*z*: $M^{+\bullet}$ 258. HRMS (EI) for $C_{17}H_{22}O_2$ (M^+); calcd, 258.1620; found, 258.1640.

Methyl 2-(4-Phenylbicyclo[2.2.2]oct-1-yl)propanoate (21). To a solution of diisopropylamine (0.9 mL, 6.39 mmol) in anhydrous THF (10 mL) cooled to −78 °C was added butyllithium (3.2 mL, 6.4 mmol, 2 M solution in cyclohexane), and the reaction mixture was allowed to stir at −78 °C for 5 min. Methyl (4-phenylbicyclo[2.2.2]oct-

1-yl)acetate **20** (1.5 g, 5.81 mmol) in anhydrous THF (2 mL) was added, and the mixture was allowed to stir for 30 min at −78 °C. Methyl iodide (0.4 mL, 6.39 mmol) was added, and the reaction mixture was allowed to warm to ambient temperature over 4 h. Saturated NH_4Cl (50 mL) was added and the mixture extracted into AcOEt (2 × 150 mL), and the organic extracts were combined, dried over $MgSO_4$, and concentrated to leave crude product. This was an inseparable mixture of the **20** and **21** and was submitted to the following step without purification.

Methyl2-[1-[4-(2-Bromo-2-methylpropanoyl)phenyl]-4-bicyclo[2.2.2]-octanyl]propanoate (26). To an ice−water cooled solution of a mixture of **20** and **21** (961 mg) in DCM (10 mL) was added aluminum chloride (1.4 g, 10.58 mmol), followed by the dropwise addition of 2-bromoisobutyryl bromide (0.45 mL, 3.53 mmol). The reaction mixture was allowed to stir at 0 °C for 40 min, and then it was then poured onto ice−water (100 mL). The organic phase was separated and the aqueous phase washed with DCM (3 × 50 mL), the organic washings were combined, dried over $MgSO_4$, and concentrated to leave a yellow gum. This was purified by chromatography (isohexane/AcOEt = 8:2) to provide the **26** (705 mg, 1.67mmol, 47%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.09 (d, J = 7.1, 3H), 1.50−1.57 (m, 3H), 1.67−1.74 (m, 3H), 1.82−1.86 (m, 6H), 2.03 (s, 6H), 2.27 (q, J = 7.1, 1H), 3.67 (s, 3H), 7.35−7.38 (m, 2H), 8.09−8.12 (m, 2H). MS m/z: $MH^{+\bullet}$ 421.

Methyl 2-{4-[4-(4-Amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]bicyclo[2.2.2]oct-1-yl}propanoate (29). To a solution of methyl {4-[4-(2-bromo-2-methylpropanoyl)phenyl]bicyclo[2.2.2]-oct-1-yl}acetate **26** (705 mg, 1.67 mmol) in absolute EtOH (20 mL) was added 5,6-diaminopyrimidin-4-ol (233 mg, 1.84 mmol), followed by 1 M HCl (2.0 mL). The suspension was heated under gentle reflux overnight. The reaction mixture was allowed to cool to ambient temperature, evaporated to dryness, and treated with 2 M NaOH to adjust the pH to 10. The suspension was filtered to provide a yellow solid, the filtrate was extracted into AcOEt (2 × 100 mL), the organic extracts were separated, dried over $MgSO_4$, and concentrated to leave a pale-yellow gum. The solid was purified by chromatography (DCM/MeOH = 0−5%) to provide **29** (220 mg, 0.491 mmol, 29%) as a cream solid. ^{1}H NMR (DMSO) δ: 1.02 (d, J = 7.1, 3H), 1.44−1.52 (m, 3H), 1.61 (s, 6H), 1.58−1.67 (m, 3H), 1.77−1.79 (m, 6H), 2.24 (q, J = 7.1, 1H), 3.60 (s, 3H), 6.89 (s, 2H), 7.39 (d, J = 8.5, 2H), 7.64 (d, J = 8.5, 2H), 7.95 (s, 1H). MS m/z: (M^+ + H) 449.

2-{4-[4-(4-Amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]bicyclo[2.2.2]oct-1-yl}propanoic Acid (5). A mixture of methyl 2-{4-[4-(4-amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]bicyclo[2.2.2]oct-1-yl}propanoate **29** (220 mg, 0.49 mmol) and potassium trimethylsilanolate (315 mg, 2.45 mmol) in THF (5 mL) was heated in a microwave for 35 min at 100 °C and then allowed to stir at ambient temperature for 2 days. The solvent was removed under reduced pressure and the residue partitioned between 2 M HCl (20 mL) and AcOEt (50 mL) and the aqueous phase was re-extracted into AcOEt (50 mL). The organic extracts were combined, dried over $MgSO_4$, and concentrated to leave a gum. This was purified by reverse phase chromatography eluting 5−95% water−acetontrile (+0.2% TFA) to provide **5** (45 mg, 0.104 mmol, 21%) as a white solid. ^{1}H NMR (DMSO) δ: 1.01 (d, J = 7.1, 3H), 1.49−1.55 (m, 3H), 1.62 (s, 6H), 1.64−1.69 (m, 3H), 1.78−1.81 (m, 6H), 2.12 (q, J = 7.0, 1H), 7.05 (s, 1H), 7.40 (d, J = 8.5, 2H), 7.65 (d, J = 8.5, 2H), 7.99 (s, 1H). MS m/z: (M^+ + H) 435. HRMS (ES^+) for $C_{25}H_{31}N_4O_3$ (M^+ + H): calcd, 435.23907; found, 435.23889.

Methyl {4-[4-(2-Bromo-2-methylpropanoyl)phenyl]bicyclo[2.2.2]oct-1-yl}acetate (25). To an ice−water cooled solution of methyl (4-phenylbicyclo[2.2.2]oct-1-yl)acetate **20** (3.88 g, 15.02 mmol) in DCM (150 mL) was added aluminum chloride (6.01 g, 45.05 mmol), followed by the dropwise addition of 2-bromoisobutyryl bromide (1.86 mL, 15.02 mmol). The reaction mixture was allowed to stir at 0 °C for 30 min and then poured onto ice−water (20 mL). The organic phase was separated and the aqueous phase washed with DCM (2 × 150 mL), the organic washings combined, dried over $MgSO_4$, and concentrated to leave a yellow gum. This was purified by chromatography (isohexane/AcOEt = 8:2) to give **25** (5.18 g, 12.72 mmol, 85%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.64−1.69 (m, 6H), 1.83−1.88 (m, 6H), 2.03 (s, 6H), 2.18 (s, 2H), 3.66 (s, 3H), 7.35−7.38 (d, J = 8.6, 2H), 8.07−8.12 (m, 2H). Solid probe MS m/z: M^+ 407. HRMS (EI) (M-OMe) (M^+): calcd, 375.0998; found, 375.0960.

Methyl {4-[4-(4-Amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]-oxazin-6-yl)phenyl]bicyclo[2.2.2]oct-1-yl}acetate (28). To a solution of 5,6-diaminopyrimidin-4-ol (280 mg, 2.2 mmol) in water (8 mL), absolute ethanol (25 mL), and 1 M HCl (2.2 mL, 2.2 mmol) was added a solution of methyl {4-[4-(2-bromo-2-methylpropanoyl)-phenyl]bicyclo[2.2.2]oct-1-yl}acetate **25** (877 mg, 2.15 mmol) in absolute EtOH (80 mL). The solution was heated to 100 °C overnight and then allowed to cool to ambient temperature, evaporated to dryness, and the residue treated with 2 M NaOH to adjust the pH to 10. The resulting suspension was filtered to provide a yellow solid, which was purified by chromatography (DCM/MeOH = 0−5%) to provide **28** (601 mg, 1.38 mmol, 64%) as a white solid. ^{1}H NMR δ: 1.59−1.63 (m, 12H), 1.79−1.83 (m, 6H), 2.16 (s, 2H), 3.59 (s, 3H), 6.91 (s, 2H), 7.39 (d, J = 8.6, 2H), 7.64 (d, J = 8.6, 2H), 7.95 (s, 1H). MS m/z: (M^+ + H) 435.

{4-[4-(4-Amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]bicyclo[2.2.2]oct-1-yl}acetic Acid (4). To a solution of methyl {4-[4-(4-amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]bicyclo[2.2.2]oct-1-yl}acetate **28** (601 mg, 1.38 mmol) in MeOH (40 mL) and THF (40 mL) was added 2 M NaOH solution (3.5 mL, 6.9 mmol). The reaction mixture was stirred at 60 °C overnight, the organic solvent was concentrated, and the residue acidified with 2 M HCl to pH 2. The suspension was filtered and the solid purified by reverse phase chromatography 5−95% water (+0.5% NH_3)−acetonitrile to give **4** (302 mg, 0.717 mmol, 52%) as a white solid. ^{1}H NMR (DMSO) δ: 1.61 (6H, s), 1.59−1.64 (m, 6H), 1.79−1.83 (m, 6H), 2.06 (s, 2H), 6.90 (s, 2H), 7.40 (d, J = 8.4, 2H), 7.64 (d,, J = 8.4, 2H), 7.95 (s, 1H). MS m/z: (M^+ + H) 421. HRMS (ES^+) for $C_{24}H_{29}N_4O_3$ (M^+ + H): calcd, 421.22342; found, 421.22357.

Methyl (5-Phenylbicyclo[3.2.2]non-1-yl)acetate (22). To a solution of 5-phenylbicyclo[3.2.2]nonane-1-carboxylic acid (534 mg, 2.19 mmol) in DCM (10 mL) was added DMF (2 drops), followed by oxalyl chloride (0.3 mL, 3.28 mmol). The reaction mixture was allowed to stir at ambient temperature overnight, and the solvent was evaporated under reduced pressure to leave crude acid chloride, which was used directly.

This was redissolved in a 1:1 solution of MeCN and THF (10 mL) and added dropwise to an ice−water cooled solution of 2 M trimethylsilyldiazomethane (1.5 mL, 3.0 mmol) and triethylamine (0.4 mL, 2.73 mmol) in a 1:1 solution of MeCN and THF (20 mL). The reaction mixture was allowed to stir at 0 °C for 1 h and then for 5 h and ambient temperature and allowed to stand for 48 h. The solvent was removed under reduced pressure and the residue redissolved in AcOEt (250 mL) and washed with water (100 mL), saturated $NaHCO_3$ (150 mL), and brine (150 mL). The organic phase was dried over $MgSO_4$ and concentrated to leave a brown gum. The residue purified by chromatography (isohexane/AcOEt = 8:2) to provide 2-diazo-1-(4-phenylbicyclo[3.2.2]non-1-yl)ethanone (252 mg, 0.940 mmol, 43%) as an orange gum. ^{1}H NMR ($CDCl_3$) δ 1.24−1.29 (m, 2H), 1.43−1.47 (m, 6H), 1.61−1.67 (m, 6H), 5.28 (s,1H), 7.05−7.24 (m, 5H). MS m/z $MH^{+\bullet}$ 268.

A solution of 2-diazo-1-(4-phenylbicyclo[3.2.2]non-1-yl)ethanone (252 mg, 0.94 mmol) in methanol (10 mL) was placed in an ultrasound bath, a solution of silver benzoate (44 mg, 0.19 mmol, 0.2 equiv) in triethylamine (0.52 mL, 3.76 mmol, 4 equiv) was added dropwise, and the mixture was sonicated for 1 h. The methanol was removed by evaporation and the residue dissolved in AcOEt (20 mL), filtered through a pad of celite, and washed with $NaHCO_3$ (10 mL), citric acid (2M, 10 mL), brine (10 mL), dried over $MgSO_4$, and concentrated to leave **22** as a brown gum, 229 mg which was which was submitted to the following step without purification.

Methyl {5-[4-(2-Bromo-2-methylpropanoyl)phenyl]bicyclo[3.2.2]non-1-yl}acetate (27). To an ice−water cooled solution of methyl (5-phenylbicyclo[3.2.2]non-1-yl)acetate **22** (229 mg, 0.79 mmol) in DCM (10 mL) was added aluminum chloride (318 mg, 2.38 mmol), followed by the dropwise addition of 2-bromoisobutyryl bromide (0.10 mL, 0.79 mmol). The reaction mixture was allowed to stir at 0 °C for 40 min, and it was then poured onto ice−water (100 mL). The organic phase was separated and the aqueous phase washed with DCM (3 × 50 mL), the organic washings were combined, dried over $MgSO_4$, and concentrated to leave a yellow gum. The residue purified by chromatography (isohexane/AcOEt = 8:2) to provide **27** (85 mg, 0.202 mmol, 26%) as a pale-yellow gum. ^{1}H NMR ($CDCl_3$) δ: 1.41−1.51 (m, 14H), 2.03 (s, 6H), 2.45 (s, 2H), 3.67 (s, 3H), 7.37 (d, J = 8.3, 2H), 8.11 (d, J = 8.3, 2H). MS *m*/*z*: $MH^{+\bullet}$ 421. HRMS (EI) for $C_{22}H_{29}BrO_3$ ($M^{+\bullet}$): calcd, 420.1300; found, 420.1334.

Methyl{5-[4-(4-Amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)-phenyl]bicyclo[3.2.2]non-1-yl}acetate (30). To a solution of methyl {5-[4-(2-bromo-2-methylpropanoyl)phenyl]bicyclo[3.2.2]non-1-yl}acetate **27** (80 mg, 0.19 mmol) in absolute EtOH (20 mL) was added 5,6-diaminopyrimidin-4-ol (30 mg, 0.21 mmol), followed by 1 M HCl (0.21 mL). The suspension was heated under gentle reflux overnight, the mixture was allowed to cool, the solvent was removed, and the residue was treated with 2 M NaOH (2 mL). The aqueous phase was extracted into AcOEt (3 × 50 mL), the organic extracts were combined, dried over $MgSO_4$, and concentrated to leave a pale-yellow gum. The residue purified by chromatography (DCM/MeOH = 0−5−10% MeOH) to give **30** (55 mg, 0.123mmol, 65%) as a pale-yellow gum. ^{1}H NMR ($CDCl_3$) δ: 1.42−1.64 (m, 14H), 1.70 (s, 6H), 2.07 (s, 2H), 3.61 (s, 3H), 7.11 (d, J = 8.3, 2H), 7.51 (d, J = 8.3, 2H), 8.14 (1H, s). MS *m*/*z*: (M^+ + H) 449.

{5-[4-(4-Amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]bicyclo[3.2.2]non-1-yl}acetic Acid (6). To a solution of methyl {5-[4-(4-amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]bicyclo[3.2.2]non-1-yl}acetate **30** (55 mg, 0.12 mmol) in MeOH (5 mL) was added 2 M NaOH (0.3 mL) and the reaction mixture was allowed to stir at ambient temperature overnight and then at 50 °C for 8 h and ambient temperature overnight. The reaction mixture was allowed to cool, the solvent was removed under reduced pressure, the residue was acidified to pH 2 with 2 M HCl, and the filtrate was extracted into AcOEt (2 × 50 mL). The organic extracts were combined, dried over $MgSO_4$, and concentrated to leave a yellow gum. This was purified by reverse phase chromatography eluting 5−95% water−acetontrile 0.2% TFA to provide **6** (31 mg, 0.0714 mmol, 59%) as a pale-yellow solid. ^{1}H NMR (DMSO) δ: 1.34−1.56 (m, 14H), 1.63 (s, 6H), 1.95 (s, 2H), 7.15 (d, J = 8.2, 2H), 7.64 (d, J = 8.2, 2H), 8.02 (1H, s). MS *m*/*z* (M^+ + H) 435. HRMS (ES^+) for $C_{25}H_{31}N_4O_3$ (M^+ + H): calcd, 435.23907; found, 435.23892.

Methyl (3-Phenyl-1-adamantyl)acetate (31). To an ice−water cooled solution of 3-phenyladamantane-1-carboxylic acid (735 mg, 2.87 mmol) in DCM (50 mL) was added DMF (2−3 drops), followed by oxalyl chloride (0.25 mL). The reaction mixture was allowed to stir at 0 °C for 6 h, and the solvent was evaporated under reduced pressure to leave crude acid chloride that was used directly. This was redissolved in a 1:1 solution of MeCN and THF (10 mL) and added dropwise to an ice−water cooled solution of 2 M trimethylsilyldiazomethane (2.0 mL) and triethylamine (0.50 mL, 3.58 mmol) in a 1:1 solution of MeCN and THF (20 mL). The resulting yellow reaction mixture was allowed to warm to ambient temperature overnight. The solvent was removed under reduced pressure and the residue redissolved in AcOEt (50 mL) and washed with water (50 mL), $NaHCO_3$ (50 mL), and brine (50 mL). The organic phase was dried over $MgSO_4$ and concentrated to leave a red−brown gum (344 mg), which was purified by chromatography (isohexane/AcOEt = 8:2) to give 2-diazo-1-(3-phenyl-1-adamantyl)ethanone (699 mg, 2.49 mmol, 87%) as a yellow gum. ^{1}H NMR ($CDCl_3$) δ: 1.74 (s, 2H), 1.83 (s, 4H), 1.91−1.94 (m, 6H), 2.27 (s, 2H), 5.43 (s, 1H), 7.18−7.22 (m, 2H), 7.31−7.38 (m, 4H). MS *m*/*z*: MH^+ 281. HRMS (ES^+) for $C_{18}H_{21}N_2O$ (M^+ + H): calcd, 281.1654; found, 281.1626. IR ν cm^{-1} 2101 (strong, broad).

The diazoketone (699 mg, 2.49 mmol) was dissolved in methanol (25 mL) and placed in an ultrasound bath, and a solution of silver benzoate (114 mg, 0.498 mmol, 0.2 equiv) in triethylamine (1.4 mL, 9.96 mmol, 4 equiv) was added dropwise and the mixture was sonicated for 1 h. The methanol was removed by evaporation and the residue dissolved in AcOEt (50 mL) and washed with $NaHCO_3$ (40 mL), citric acid (2M, 40 mL), brine (40 mL), dried over $MgSO_4$, and concentrated to leave a yellow oil. This was purified by chromatography (isohexane/AcOEt = 8:2) to provide **31** (421 mg 1.48 mmol, 59%, from the diazoketone, 51% from the starting acid) as a pale-yellow gum. ^{1}H NMR ($CDCl_3$) δ: 1.64−1.70 (m, 5H), 1.76 (s, 2H), 1.86−1.96 (m, 5H), 2.17 (s, 2H), 2.18−2.19 (m, 2H), 3.65 (s, 3H), 7.15−7.20 (m, 1H), 7.29−7.37 (m, 4H). MS GC-MS-EI *m*/*z*: $MH^{+\bullet}$ 284.

Methyl{3-[4-(2-Bromo-2-methylpropanoyl)phenyl]-1-adamantyl}-acetate (32). To an ice−water cooled solution of methyl (3-phenyl-1-adamantyl)acetate **31** (247 mg, 0.868 mmol) in DCM (15 mL) was added aluminum chloride (350 mg, 2.61 mmol), followed by the dropwise addition of 2-bromoisobutyryl bromide (0.11 mL, 0.87 mmol). The reaction mixture was allowed to stir at 0 °C for 15 min, and it was then poured onto ice−water (20 mL). The organic phase was separated and the aqueous phase washed with DCM (2 × 50 mL), and the organic washings were combined, washed with brine (100 mL), dried over $MgSO_4$, and concentrated to leave a yellow gum. This was purified by chromatography (isohexane/AcOEt = 8:2) to provide **32** (133 mg, 0.307 mmol, 35%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.65−1.67 (m, 4H), 1.71 (s, 2H), 1.77 (s, 2H), 1.87 (s, 4H), 2.04 (s, 6H), 2.18 (s, 2H), 2.20−2.22 (m, 2H), 3.65 (s, 3H), 7.40−7.42 (m, 2H), 8.10−8.14 (m, 2H). MS *m*/*z*: (M^+ + H) 433.

Methyl {3-[4-(4-Amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]-1-adamantyl}acetate (33). Preparation according to the procedure described for methyl {4-[4-(4-amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]bicyclo[2.2.2]oct-1-yl}acetate **28** using methyl {3-[4-(2-bromo-2-methylpropanoyl)phenyl]-1-adamantyl}acetate **32** isolated the title compound in 44% yield. ^{1}H NMR (DMSO) δ: 1.62 (s, 6H), 1.65−1.69 (m, 6H), 1.71 (s, 2H), 1.83 (s, 4H), 2.15 (s, 2H), 2.17 (s, 2H), 3.58 (s, 3H), 6.92 (s, 2H), 7.42 (d, J = 8.5, 2H), 7.68 (d, J = 8.5, 2H), 7.96 (s, 1H). MS *m*/*z*: (M^+ + H) 461.

{3-[4-(4-Amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]-1-adamantyl}acetic Acid (7). To a solution of methyl {3-[4-(4-amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]-1-adamantyl}acetate **33** (292 mg, 0.633 mmol) in EtOH (20 mL) and THF (20 mL) was added 2 M NaOH (1.6 mL, 3.17 mmol). The reaction mixture was allowed to stir at ambient temperature overnight, and a further 1 mL of 1 M NaOH (1 mmol) was added and the reaction mixture was heated to 50 °C, for 6 h. The reaction mixture was allowed to cool, the solvent was removed under reduced pressure, and the residue was acidified to pH 2 with 2 M HCl and the suspension was filtered to provide a yellow solid, which was dried under vacuum over the weekend to leave 229 mg of crude product. The residue purified by reverse phase chromatography 17−40% water (+1% NH_3)−acetonitrile to give **7** (163 mg, 0.365 mmol, 58%) as a white solid. ^{1}H NMR (DMSO) δ: 1.67 (s, 6H), 1.61 (s, 6H), 1.78 (s, 2H), 1.88 (s, 4H), 2.12 (s, 2H), 2.20 (s, 2H), 6.96 (s, 2H), 7.42 (d, J = 8.5, 2H), 7.68 (d, J = 8.5, 2H), 7.95 (s, 1H). MS *m*/*z*: (M^+ + H) 447. HRMS (ES^+) for $C_{26}H_{31}N_4O_3$ (M^+ + H): calcd, 447.23907; found, 447.23907.

Methyl 3-[4-(2-Bromo-2-methylpropanoyl)phenyl]adamantane-1-carboxylate (35). Compound **35** was prepared in 89% yield according to the procedure described for methyl {4-[4-(2-bromo-2-methylpropanoyl)phenyl]bicyclo[2.2.2]oct-1-yl}acetate **25** using methyl 3-phenyladamantane-1-carboxylate **34**.[17] Isolated **35**: ^{1}H NMR ($CDCl_3$) δ: 1.91−1.93 (m, 8H), 2.04 (s, 6H), 2.23−2.27 (m, 6H), 4.52 (s, 3H), 7.42 (d, J = 8.7, 2H), 8.13 (d, J = 8.7, 2H). MS *m*/*z*: (M^+ + H) 421.

Methyl 3-[4-(4-Amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]adamantane-1-carboxylate (36). Prepared according to the procedure described for methyl {4-[4-(4-amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]bicyclo[2.2.2]oct-1-yl}acetate **28** using methyl 3-[4-(2-bromo-2-methylpropanoyl)phenyl]adamantane-1-carboxylate **35** to provide **36** in 44% yield, which was submitted to the following step without purification. MS *m*/*z* MH^+: (M^+ + H) 447.

3-[4-(4-Amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]adamantane-1-carboxylic Acid (8). Prepared according to the procedure described for {4-[4-(4-amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]bicyclo[2.2.2]oct-1-yl}acetic acid **2** using methyl 3-[4-(4-amino-7,7-dimethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)phenyl]adamantane-1-carboxylate **36** to provide **8** in 25% yield. ^{1}H NMR (DMSO) δ: 1.62 (s, 6H), 1.70−1.72 (m, 2H), 1.83−1.88 (m, 7H), 2.16−2.19 (m, 2H), 6.92 (s, 2H), 7.45 (d, J = 8.4, 2H), 7.68 (d, J = 8.4, 2H), 7.96 (s, 1H). HRMS (ES^+) for $C_{25}H_{29}O_3N_4$ (M^+ + H): calcd 433.22342; found, 433.22333.

Enzyme Assays. The in vitro assay to identify DGAT-1 inhibitors uses human DGAT-1 expressed in insect cell membranes as the enzyme source.[21] Briefly, sf9 cells were infected with recombinant baculovirus containing human DGAT-1 coding sequences and harvested after 48 h. Cells were lysed by sonication and membranes isolated by centrifuging at 28000 rpm for 1 h at 4 °C on a 41% sucrose gradient. The membrane fraction at the interphase was collected, washed, and stored at −80 °C.

DGAT-1 activity was assayed by a modification of the method described by Coleman.[18] Compound at 0.00003−10 μM (final conc) was incubated with 25 μg/mL (final conc) membrane protein, 5 mM $MgCl_2$, and 100 μM 1,2 dioleoyl-*sn*-glycerol in a total assay volume of 200 μL in a 96-deep well plate. The reaction was started by adding ^{14}C oleoyl coenzyme A (30 μM final concentration) and incubated at room temperature for 30 min. The reaction was stopped by adding 300 μL 2-propanol:heptane 7:1. Radioactive triolein product was separated into the organic phase by adding 200 μL of heptane and 200 μL of 0.1 M carbonate buffer pH 9.5. DGAT-1 activity was quantified by counting aliquots of the upper heptane layer by liquid scintillography.

DGAT-2 activity was assayed by an adaptation of the above method. Compound at 0.01−10 μM was incubated with 0.6 μg of membrane protein, 5 mM $MgCl_2$, 100 μM 1,2 dioleoyl-*sn*-glycerol, and 10 mM dithiothreitol in a total assay volume of 200 μL in 96-deep well plates. The reaction was started by adding ^{14}C oleoyl coenzyme A (30 μM final concentration) and incubated at room temperature for 30 min. The reaction was stopped by adding 0.3 mL of 2-propanol:heptane (12:1). Radioactive triolein product was separated into the organic phase by adding 0.2 mL of heptane and 0.2 mL of 1 M carbonate buffer pH 9.5. DGAT-1 activity was quantified by counting aliquots of the upper heptane layer by liquid scintillography.

ACAT-1 activity was assayed by a modification of the method described by Billheimer.[19] Compound at 0.005−30 μM was incubated with 10 μg of membrane protein, 0.25 mM cholesterol solubilized in Triton WR-1339, and 100 mM glutathione in a total assay volume of 200 μL in 96-well plates. The reaction was started by adding ^{14}C oleoyl coenzyme A (50 μM final concentration) and incubated at 37 °C for 30 min. The reaction was stopped by adding 0.3 mL of 2-propanol:heptane(12:1). Radioactive cholesteryl oleate product was separated into the organic phase by adding 0.2 mL of heptane and 0.2 mL of 1 M carbonate buffer pH 9.5. ACAT-1 activity was quantified by counting aliquots of the upper heptane layer by liquid scintillography.

Measurement of Triacylglycerol Synthesis in HuTu 80 Cells. HuTu 80 cells were cultured to confluency in 6-well plates in minimum essential media containing 10% fetal calf serum. For the experiment, the medium was changed to serum-free medium and the cells preincubated with compound solubilized in DMSO (final concentration 0.1%) for 30 min. De novo lipogenesis was measured by the addition of 0.12 mM sodium oleate plus 1 μCi/mL ^{14}C-oleic acid complexed to 0.03 mM BSA to each well for a further 2 h. The cells were washed in phosphate buffered saline and solubilized in 1% sodium dodecyl sulfate. An aliquot was removed for protein determination using a protein estimation kit (Perbio) based on the method of Lowry.[20] The lipids were extracted into the organic phase using a heptane:propan-2-ol:water (80:20:2) mixture followed by aliquots of water and heptane according to the method of Coleman.[18] The organic phase was collected and the solvent evaporated under a stream of nitrogen. The extracts solubilized in iso-hexane:acetic acid (99:1) and lipids separated via normal phase high performance liquid chromatography (HPLC) using a Lichrospher diol-5, 4 mm × 250 mm column, and a gradient solvent system of iso-hexane:acetic acid (99:1) and iso-hexane:propan-2-ol:acetic acid (85:15:1), flow rate of 1 mL/min according to the method of Silversand and Haux.[22] Incorporation of radiolabel into the triacylglycerol fraction was analyzed using a Radiomatic Flo-one Detector (Packard) connected to the HPLC machine.

Adipose Triacylglycerol Synthesis in the Rat. Male Han−Wistar rats (∼300 g) maintained on a chow diet were dosed by oral gavage with compound suspended in HPMC/Tween. After 1.5 h, animals were anaesthetised using inactin, a canula was introduced into the right jugular vein, and [^{3}H]-palmitic acid was infused as a bolus. Twenty minutes later, rats were euthanized and epididymal fat pads removed. Then 100 mg segments of fat (in duplicate) were homogenized with microbeads (Fast PrepTM) in the presence of 1 mL of methanol and addition of [^{14}C]-oleic acid internal standard. Following centrifugation, an aliquot of the organic phase was combined with 4 mL of chloroform mixed and incubated for 1 h at 40 °C, after which 1.2 mL of 0.73% sodium chloride was added. Following separation of the phases, the lower organic layer was removed and dried under nitrogen and redissolved in hexane:glacial acetic acid (99:1) to a final volume of 500 μL. Lipid classes were separated by HPLC according to the method described in HuTu 80 cells above and radiolabel incorporation into triacylglycerol measured by in-line scintillography (Radiomatic Flo-one Detector; Packard). Following correction for recovery of internal standard and fat weight, TAG synthesis was calculated as the dpm/mg tissue.

DGAT-1 inhibitory activity was calculated as the ratio of incorporation of radiolabeled FA into newly synthesized TAG and DAG. Efficacy was calculated as the percentage reduction of this ratio in animals treated with DGAT-1 inhibitor compared to vehicle control animals.

Oral Lipid Tolerance Test (OLTT) in the Rat. Male Han−Wistar rats (∼230 g), previously maintained on a chow diet, were fasted for 14 h. Animals were dosed by oral gavage with compound suspended in HPMC/Tween and 2 h later dosed with a bolus of corn oil (5 mL/kg). After a further 1.5 h, animals were euthanized and a blood sample rapidly collected from the abdominal vena cava. Plasma triacylglycerol levels were determined using a commercially available colorimetric assay (GPO-PAP, Roche). Efficacy was calculated as the percentage reduction in plasma triacylglycerol compared to vehicle-treated control animals.

Diet-Induced Obesity in the Mouse. Female C57BlJ/6 mice (∼20 g) were housed in groups of three animals and allowed free access to water and high calorie cafeteria diet consisting of chocolate, cheese, and chocolate coconut balls (Delecato, Sweden) for 15 weeks to accelerate weight gain. When mice had achieved a mean weight of ∼29 g, they were randomized by mean body weight into groups of 12 and dosed once per day by oral gavage (10 mL/kg) for 3 d, in a subacute protocol, with compound 2 h prior to start of the dark phase of the light cycle. Body weights were measured daily just prior to dose administration. Results are presented as the percentage daily increase in body weight compared to day 0 start weight.

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Supporting Information Available: Combustion analysis data for compound **4**, HPLC data for compounds **2**−**8**, HPLC traces for compounds **2**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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