

## **Discovery and Optimization of Imidazopyridine-based Inhibitors of Diacylglycerol Acyltransferase 2 (DGAT2).**

Kentaro Futatsugi,<sup>†\*</sup> Daniel W. Kung,<sup>†\*</sup> Suvi T. M. Orr,<sup>†</sup> Shawn Cabral,<sup>†</sup> David Hepworth,<sup>†</sup> Gary Aspnes,<sup>†</sup> Scott Bader,<sup>∇</sup> Jianwei Bian,<sup>†</sup> Markus Boehm,<sup>†</sup> Philip A. Carpino,<sup>†</sup> Steven B. Coffey,<sup>†</sup> Matthew S. Dowling,<sup>†</sup> Michael Herr,<sup>†</sup> Wenhua Jiao,<sup>†</sup> Sophie Y. Lavergne,<sup>†</sup> Qifang Li,<sup>†</sup> Ronald W. Clark,<sup>‡</sup> Derek M. Erion,<sup>‡</sup> Kou Kou,<sup>‡</sup> Kyuha Lee,<sup>⊥</sup> Brandon A. Pabst,<sup>‡</sup> Sylvie M. Perez,<sup>‡</sup> Julie Purkal,<sup>‡</sup> Csilla C. Jorgensen,<sup>#</sup> Theunis C. Goosen,<sup>#</sup> James R. Gosset,<sup>§</sup> Mark Niosi,<sup>#</sup> John C. Pettersen,<sup>°</sup> Jeffrey A. Pfefferkorn,<sup>‡</sup> Kay Ahn,<sup>‡</sup> and Bryan Goodwin<sup>‡</sup>

<sup>†</sup>Worldwide Medicinal Chemistry, <sup>‡</sup>Cardiovascular, Metabolic and Endocrine Diseases Research Unit, and <sup>§</sup>Pharmacokinetics, Dynamics and Metabolism, Pfizer Worldwide Research & Development, Cambridge, Massachusetts 02139, United States.

<sup>†</sup>Worldwide Medicinal Chemistry, <sup>⊥</sup>Cardiovascular, Metabolic and Endocrine Diseases Research Unit, <sup>#</sup>Pharmacokinetics, Dynamics and Metabolism, <sup>∇</sup>Pharmaceutical Sciences, and <sup>°</sup>Drug Safety Research & Development, Pfizer Worldwide Research & Development, Groton, Connecticut 06340, United States.

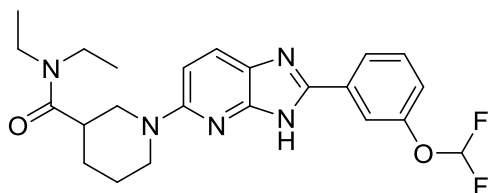
### **Supporting Information**

#### **Procedures for synthesis of compounds 1-4 and 6-8.**

**General Experimental Methods.** All chemicals, reagents, and solvents were purchased from commercial sources and used without further purification unless otherwise noted. <sup>1</sup>H NMR data are reported relative to residual solvent signals, and are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz), and integration. The multiplicities are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; sept, septet; m, multiplet; br s, broad singlet; app, apparent. Silica gel chromatography was performed using a medium pressure Biotage or

ISCO system and columns pre-packaged by various commercial vendors including Biotage and ISCO. Whatman pre-coated silica gel plates (250  $\mu\text{m}$ ) were used for analytical thin-layer chromatography (TLC). The terms “concentrated” and “evaporated” refer to the removal of solvent at reduced pressure on a rotary evaporator with a water bath temperature not exceeding 60  $^{\circ}\text{C}$ . Purity of final compounds was assessed by reversed-phase HPLC with UV detection at 215 nm; all tested compounds were >95% purity, unless otherwise noted. *Safety note:* Sodium hydride and dimethylsulfoxide can react explosively, please take appropriate precautions in using this reagent/solvent combination.

**1-(2-(3-(Difluoromethoxy)phenyl)-3H-imidazo[4,5-b]pyridin-5-yl)-N,N-diethylpiperidine-3-carboxamide (1)**



Step 1: 1-(6-Amino-5-nitropyridin-2-yl)-N,N-diethylpiperidine-3-carboxamide. To a solution of 6-chloro-3-nitropyridin-2-amine (470 mg, 2.71 mmol, 1 equiv) and N,N-diethylpiperidine-3-carboxamide (500 mg, 2.71 mmol, 1.0 equiv) in DMSO (5.0 mL) was added triethylamine (756  $\mu\text{L}$ , 5.43 mmol, 2.0 equiv) at room temperature. The reaction mixture was heated at 110  $^{\circ}\text{C}$  overnight. The mixture was cooled to room temperature and partitioned between ethyl acetate (50 mL) and water (50 mL). The organic layer was separated, washed with brine, dried over sodium sulfate, and concentrated. The crude product was purified by Isco CombiFlash (40 g RediSep cartridge, eluted with 0-70% ethyl acetate in heptane) to afford 1-(6-amino-5-nitropyridin-2-yl)-N,N-diethylpiperidine-3-carboxamide as a yellow solid (855 mg, 98%).  $^1\text{H}$

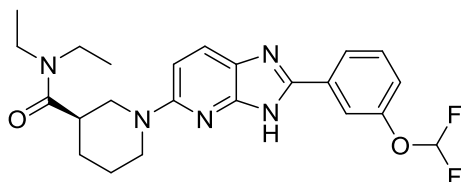
NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.14 (t,  $J$ =8.0 Hz, 3H), 1.23 (t,  $J$ =8.0 Hz, 3H), 1.50-1.62 (m, 1H), 1.84-1.96 (m, 3H), 2.61-2.68 (m, 1H), 3.00-3.20 (m, 2H), 3.31-3.48 (m, 4H), 4.38 (br s, 1H), 4.60 (br s, 1H), 6.12 (d,  $J$ =9.6 Hz, 1H), 8.19 (d,  $J$ =9.4 Hz, 1H). MS (M+H)<sup>+</sup> = 322.

Step 2: 1-(5,6-Diaminopyridin-2-yl)-*N,N*-diethylpiperidine-3-carboxamide. To a solution of 1-(6-amino-5-nitropyridin-2-yl)-*N,N*-diethylpiperidine-3-carboxamide (500 mg, 1.56 mmol) in ethanol (20 mL) was added 10% Pd-on-carbon (50% wet, 100 mg) after purging with nitrogen. The resulting suspension was left on a Parr shaker at room temperature at 50 psi H<sub>2</sub> for 4 h. The mixture was filtered through Celite, and solvent evaporated to afford the crude 1-(5,6-diaminopyridin-2-yl)-*N,N*-diethylpiperidine-3-carboxamide (450 mg, 99% yield) as a dark green oil, which was used in the next step without further purification. MS (M+H)<sup>+</sup> = 292.

Step 3: 1-(2-(3-(Difluoromethoxy)phenyl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)-*N,N*-diethylpiperidine-3-carboxamide (**1**). To a suspension of the crude 1-(5,6-diaminopyridin-2-yl)-*N,N*-diethylpiperidine-3-carboxamide (400 mg, 1.37 mmol, 1 equiv) in anhydrous *N,N*-dimethylformamide (5.0 mL) was added 3-(difluoromethoxy)benzaldehyde (473 mg, 2.75 mmol, 2.0 equiv) and a suspension of sulfur (88 mg, 2.75 mmol, 2.0 equiv) in toluene (1 mL). The resulting mixture was heated at 85 °C under nitrogen for 48 h. The mixture was concentrated under reduced pressure and residual *N,N*-dimethylformamide was removed by azeotropic evaporation with toluene (100 mL). The residue was taken up in dichloromethane (50 mL) and filtered through a pad of Celite. The filtrate was washed with 1N aqueous sodium hydroxide (50 mL) and brine (50 mL). The organic layer was dried over sodium sulfate and concentrated. The crude product was purified by Isco CombiFlash (40 g RediSep cartridge, 5-15% methanol in dichloromethane) to afford a light yellow solid, which was further purified by Isco CombiFlash

(12 g RediSep cartridge, 50-100% ethyl acetate in heptane) to give **1** as an off-white foam (320 mg, 53%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.16 (t, *J*=7.1 Hz, 3H), 1.21 (t, *J*=7.1 Hz, 3H), 1.55-1.67 (m, 1H), 1.72-1.79 (m, 1H), 1.82-1.95 (m, 2H), 2.72-2.82 (m, 1H), 2.86-2.96 (m, 1H), 3.04-3.14 (m, 1H), 3.27-3.58 (m, 5H), 4.13 (d, *J*=12.7 Hz, 1H), 4.65 (d, *J*=12.9 Hz, 1H), 6.60 (t, *J*=73.7 Hz, 1H), 6.67 (d, *J*=9.2 Hz, 1H), 7.17 (dd, *J*=8.2, 2.1 Hz, 1H), 7.44 (t, *J*=8.0 Hz, 1H), 7.86 (d, *J*=9.0 Hz, 1H), 7.92 (s, 1H), 7.98 (d, *J*=8.0 Hz, 1H). MS (M+H)<sup>+</sup> = 444.

**(*R*)-1-(2-(3-(difluoromethoxy)phenyl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)-*N,N*-diethylpiperidine-3-carboxamide ((*R*)-**1**)**

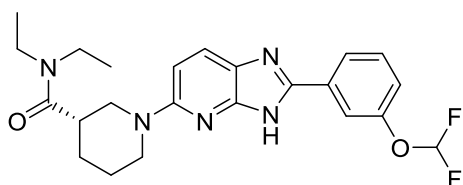


Step 1: To a solution of (*R*)-*N,N*-diethylpiperidine-3-carboxamide in *N,N*-dimethylformamide (0.6 M, 0.15 mmol, 0.25 mL) was added a solution of 6-chloro-3-nitropyridin-2-amine in *N,N*-dimethylformamide (0.6M, 0.15 mmol, 0.25 mL, 1 equiv) and triethylamine (0.05 mL, 0.416 mmol, 2.7 equiv), and the resulting mixture was heated at 80 °C for 16 h. The solvent was removed by Speedvac and the residue was used for the next step without any further purifications.

Step 2: To the residue from step 1 was added a solution of 3-(difluoromethoxy)benzaldehyde in ethanol (0.16 M, 0.15 mmol, 0.95 mmol, 1.0 equiv) and water (50 μL). Sodium dithionite (100 mg, 0.574 mmol, 3.8 equiv) was added to the mixture under nitrogen, and the resulting mixture was heated at 110 °C for 18 h. The reaction mixture was concentrated by Speedvac and the

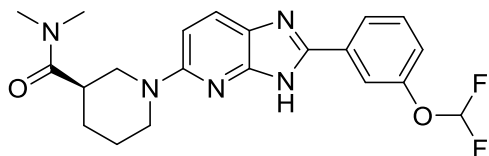
residue was purified by HPLC (Column: Kromasil Eternity-5-C18 150 x 30 mm, 5 $\mu$ m; Mobile Phase A: acetonitrile; Mobile Phase B: water (0.225% formic acid); Gradient (%A): 30% to 60% over 10 min. 100%A for 1 min; Flow rate: 30 mL/min; UV detector) to afford (**R**)-**1** as a formate salt. MS (M+H)<sup>+</sup> = 444. HPLC retention time 2.54 min (Welch XB C18 2.1 x 50 mm, 5  $\mu$ m; Mobile Phase A: 0.0375% trifluoroacetic acid in water; Mobile Phase B: 0.01875% trifluoroacetic acid in acetonitrile; Gradient: Time (min)/%B: 0.00/10, 0.50/10, 4.00/100, 4.30/10, 4.70/10; Flow Rate: 0.8 mL/min; Column Temperature: 50 °C).

**(S)-1-(2-(3-(difluoromethoxy)phenyl)-3H-imidazo[4,5-b]pyridin-5-yl)-N,N-diethylpiperidine-3-carboxamide ((S)-1)**



The title compound was synthesized through an analogous procedure to (**R**)-**1** using (*S*)-*N,N*-diethylpiperidine-3-carboxamide as a starting material, and was purified by HPLC (Column: Kromasil Eternity-5-C18 150 x 30 mm, 5 $\mu$ m; solvent: Mobile Phase A: acetonitrile; Mobile Phase B: water (0.225% formic acid); Gradient (%A): 31% to 61% over 10 min. 100% for 1 min; Flow rate: 30 mL/min; UV detector), affording (**S**)-**1** as a formate salt. MS (M+H)<sup>+</sup> = 444. HPLC retention time 2.55 min (Welch XB C18 2.1 x 50 mm, 5  $\mu$ m; Mobile Phase A: 0.0375% trifluoroacetic acid in water; Mobile Phase B: 0.01875% trifluoroacetic acid in acetonitrile; Gradient: Time (min)/%B: 0.00/10, 0.50/10, 4.00/100, 4.30/10, 4.70/10; Flow Rate: 0.8 mL/min; Column Temperature: 50 °C).

**(*R*)-1-(2-(3-(difluoromethoxy)phenyl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)-*N,N*-dimethylpiperidine-3-carboxamide (2)**



Step 1: (*R*)-*tert*-Butyl 3-(dimethylcarbamoyl)piperidine-1-carboxylate. To a solution of (*R*)-1-(*tert*-butoxycarbonyl)piperidine-3-carboxylic acid (10.0 g, 43.6 mmol, 1 equiv) in *N,N*-dimethylformamide (150 mL) was added 1,1'-carbonyldiimidazole (14.3 g, 87.2 mmol, 2.0 equiv). The reaction mixture was stirred at room temperature for 10 min. Triethylamine (18.3 mL, 130 mmol, 3.0 equiv) and dimethylamine hydrochloride (7.1 g, 87.2 mmol, 2.0 equiv) were added sequentially. The reaction mixture was stirred at room temperature for 16 h. The solvent was evaporated and the residue was treated with water and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified via flash chromatography to afford (*R*)-*tert*-butyl 3-(dimethylcarbamoyl)piperidine-1-carboxylate (9.0 g, 80%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 1.45 (s, 9H), 1.63-1.80 (m, 2H), 1.84-1.96 (m, 1H), 2.58-2.78 (m, 2H), 2.80-2.91 (m, 2H), 2.95 (s, 3H), 3.11 (s, 3H), 4.05-4.20 (m, 2H). MS (M+H)<sup>+</sup> = 257.1

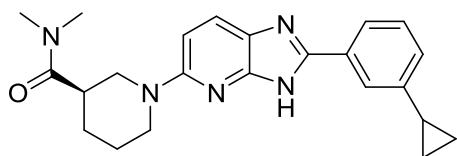
Step 2: (*R*)-*N,N*-Dimethylpiperidine-3-carboxamide hydrochloride. To a solution of (*R*)-*tert*-butyl 3-(dimethylcarbamoyl)piperidine-1-carboxylate (9.0 g, 35 mmol, 1 equiv) in methanol (20 mL) was added hydrogen chloride in methanol (50 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated to afford (*R*)-*N,N*-dimethylpiperidine-3-carboxamide hydrochloride (7.0 g, 100%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 1.63-1.80 (m, 1H), 1.88-3.10 (m, 3H), 2.97 (s, 3H), 2.98-3.05 (m, 1H), 3.11 (s, 3H), 3.15-3.28 (m, 1H), 3.35-3.48 (m, 3H), 9.28 (br s, 1H), 9.89 (br s, 1H). MS (M+H)<sup>+</sup> = 157.1.

Step 3: (*R*)-1-(6-Amino-5-nitropyridin-2-yl)-*N,N*-dimethylpiperidine-3-carboxamide. To a solution of (*R*)-*N,N*-dimethylpiperidine-3-carboxamide (1.00 g, 4.36 mmol, 1.0 equiv) and triethylamine (1.30 mL, 9.30 mmol, 2.1 equiv) in DMSO (10 mL) was added 6-chloro-3-nitropyridin-2-amine (760 mg, 4.38 mmol, 1 equiv), and the resulting reaction mixture was stirred at 100 °C overnight. After cooling to room temperature, the mixture was diluted with ethyl acetate (50 mL). The organic layer was washed with water (20 mL). The aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organic extracts were washed with brine (20 mL), dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (60%-100% ethyl acetate in heptanes) to give (*R*)-1-(6-amino-5-nitropyridin-2-yl)-*N,N*-dimethylpiperidine-3-carboxamide (1.17 g, 91% yield) as a foam. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 1.51-1.60 (m, 1H), 1.81-1.89 (m, 2H), 1.96-2.00 (m, 1H), 2.62-2.75 (m, 1H), 2.97 (s, 3H), 2.97-3.15 (m, 2H), 3.10 (s, 3H), 4.39 (br s, 1H), 4.63 (br s, 1H), 6.11 (d, *J*=9.5 Hz, 1H), 8.17 (d, *J*=9.5 Hz, 1H). MS (M+H)<sup>+</sup> = 294.1.

Step 4: (*R*)-1-(2-(3-(Difluoromethoxy)phenyl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)-*N,N*-dimethylpiperidine-3-carboxamide (**(*R*)-2**). To a vial was added 3-(difluoromethoxy)benzaldehyde (304 mg, 1.76 mmol, 0.9 equiv) and (*R*)-1-(6-amino-5-nitropyridin-2-yl)-*N,N*-dimethylpiperidine-3-carboxamide (567 mg, 1.93 mmol, 1 equiv) in ethanol (9 mL). Sodium dithionite (1.17 g, 6.70 mmol, 3.5 equiv) and water (0.5 mL) were added. The vial was sealed and the solution was heated to 110 °C and stirred overnight. After cooling to room temperature, the mixture was diluted with ethyl acetate and the organic layer was washed sequentially with water and brine (2×), dried over sodium sulfate, and concentrated. The crude residue was purified by Combiflash (60-100% ethyl acetate in dichloromethane) to afford **(*R*)-2** (383 mg, 47%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.55-1.97 (m, 4H), 2.85-3.00 (m,

2H), 3.00 (s, 3H), 3.04-3.10 (m, 1H), 3.13 (s, 3H), 4.21 (d,  $J=13.1$  Hz, 1H), 4.58 (d,  $J=13.3$  Hz, 1H), 6.59 (t,  $^2J_{HF}=73.4$  Hz, 1H), 6.68 (d,  $J=9.0$  Hz, 1H), 7.16 (dd,  $J=8.2, 2.3$  Hz, 1H), 7.46 (t,  $J=8.0$  Hz, 1H), 7.80-7.88 (m, 3H), 11.49 (br s, 1H). MS ( $M+H$ )<sup>+</sup> = 416.2.

**(*R*)-1-(2-(3-Cyclopropylphenyl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)-*N,N*-dimethylpiperidine-3-carboxamide (3)**



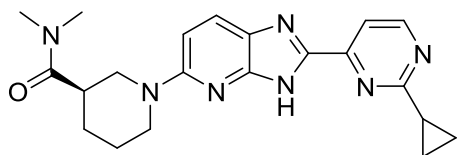
Step 1: (*R*)-1-(2-(3-Bromophenyl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)-*N,N*-dimethylpiperidine-3-carboxamide. To a stirred solution of (*R*)-1-(6-amino-5-nitropyridin-2-yl)-*N,N*-dimethylpiperidine-3-carboxamide (500 mg, 0.682 mmol, 1 equiv) in ethanol (15 mL) were added 3-bromobenzaldehyde (378 mg, 2.04 mmol, 3.0 equiv), Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> (1.12 g, 6.46 mmol, 9.5 equiv), and water (3.9 mL). The reaction vial was sealed and the mixture was heated at 110 °C for 16 h. The mixture was concentrated and the resulting residue was partitioned between water and ethyl acetate. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to afford the crude product which was purified by preparative TLC (3% methanol in dichloromethane) to yield (*R*)-1-(2-(3-bromophenyl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)-*N,N*-dimethylpiperidine-3-carboxamide (400 mg, 54%). MS ( $M+H$ )<sup>+</sup> = 429.9.

Step 2: (*R*)-1-(2-(3-cyclopropylphenyl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)-*N,N*-dimethylpiperidine-3-carboxamide (3). To a solution of (*R*)-1-(2-(3-bromophenyl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)-*N,N*-dimethylpiperidine-3-carboxamide (450 mg, 1.05 mmol, 1 equiv) in 1,4-dioxane (25 mL) was added cyclopropyl boronic acid (135 mg, 1.57 mmol, 1.5 equiv) and 1M aqueous Na<sub>2</sub>CO<sub>3</sub>



solution (5 mL), and the resulting mixture was degassed for 15 min with nitrogen. Pd(PPh<sub>3</sub>)<sub>4</sub> (73.0 mg, 0.105 mmol, 0.1 equiv) was added, and the reaction mixture was heated at 100 °C for 16 h. The mixture was concentrated, and the resulting residue was partitioned between water and ethyl acetate. The combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The residue was purified by preparative HPLC to afford **3** (60 mg, 14%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 0.77 (d, *J*=4.6 Hz, 2H), 0.98 (d, *J*=7.6 Hz, 2H), 1.20-1.35 (m, 1H), 1.56-1.66 (m, 1H), 1.72-1.79 (m, 1H), 1.80-1.88 (m, 1H), 1.90-1.95 (m, 1H), 2.83 (t, *J*=11.2 Hz, 1H), 2.90-2.96 (m, 1H), 2.99 (s, 3H), 3.04-3.08 (m, 1H), 3.12 (s, 3H), 4.19 (d, *J*=12.9 Hz, 1H), 4.56 (d, *J*=12.3 Hz, 1H), 6.67 (d, *J*=8.8 Hz, 1H), 7.14 (d, *J*=7.6 Hz, 1H), 7.33 (t, *J*=7.3 Hz, 1H), 7.81-7.85 (m, 3H). MS (M+H)<sup>+</sup> = 390.1.

**(*R*)-1-(2-(2-Cyclopropylpyrimidin-4-yl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)-*N,N*-dimethylpiperidine-3-carboxamide (**4**)**



Step 1: (*R*)-Piperidine-3-carboxylic acid. To a solution of (*R*)-1-(*tert*-butoxycarbonyl)piperidine-3-carboxylic acid (2.81 g, 12.3 mmol, 1 equiv) in dichloromethane (84 mL) was added a solution of hydrogen chloride in 1,4-dioxane (4M, 28 mL, 112 mmol, 9.1 equiv). The reaction mixture was stirred at 30 °C for 2 h. The solvent was removed under reduced pressure to afford (*R*)-piperidine-3-carboxylic acid which was used without further purification.

Step 2: (*R*)-1-(6-Amino-5-nitropyridin-2-yl)piperidine-3-carboxylic acid. A solution of 6-chloro-3-nitropyridin-2-amine (2.13 g, 12.3 mmol, 1 equiv) in *N,N*-dimethylformamide (70 mL),

and *N,N*-diisopropylethylamine (4.55 mL, 24.5 mmol, 2.0 equiv) were added to (*R*)-piperidine-3-carboxylic acid hydrochloride from Step 1 (12.3 mmol, 1.0 equiv). The reaction mixture was stirred at 80 °C for 16 h. The solvent was removed under reduced pressure to afford (*R*)-1-(6-amino-5-nitropyridin-2-yl)piperidine-3-carboxylic acid which was used without further purification.

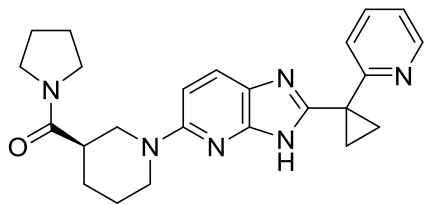
Step 3: (*R*)-1-(5,6-Diaminopyridin-2-yl)piperidine-3-carboxylic acid. To a solution of (*R*)-1-(6-amino-5-nitropyridin-2-yl)piperidine-3-carboxylic acid (12.3 mmol, 1 equiv) in *N,N*-dimethylformamide (91 mL) was added water (9.8 mL), ammonium formate (3.85 g, 61.3 mmol, 5.0 equiv), and activated zinc dust (2.1 g, 30.6 mmol, 2.5 equiv) under a nitrogen atmosphere. The reaction mixture was stirred at 45 °C for 16 h. The mixture was filtered and concentrated under reduced pressure to afford (*R*)-1-(5,6-diaminopyridin-2-yl)piperidine-3-carboxylic acid which was used without further purification.

Step 4: (*R*)-1-(2-(2-Cyclopropylpyrimidin-4-yl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)piperidine-3-carboxylic acid. To a solution of (*R*)-1-(5,6-diaminopyridin-2-yl)piperidine-3-carboxylic acid in *N,N*-dimethylformamide (49 mL) were added a solution of 2-cyclopropylpyrimidine-4-carbaldehyde (1.82 g, 12.3 mmol, 1 equiv) in *N,N*-dimethylformamide (49 mL), and acetic acid (7.35 mL, 123 mmol, 10 equiv). The reaction mixture was stirred at 60 °C for 16 h. The solvent was removed under reduced pressure and the residue was purified via HPLC to afford (*R*)-1-(2-(2-cyclopropylpyrimidin-4-yl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)piperidine-3-carboxylic acid.

Step 5: (*R*)-1-(2-(2-Cyclopropylpyrimidin-4-yl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)-*N,N*-dimethylpiperidine-3-carboxamide (**4**). To a solution of dimethylamine (7.9 mg, 175 μmol, 3.5 equiv) in *N,N*-dimethylformamide (200 μL) were added sequentially triethylamine (35 μL, 250 μmol, 5.0 equiv), a solution of (*R*)-1-(2-(2-cyclopropylpyrimidin-4-yl)-3*H*-imidazo[4,5-

b]pyridin-5-yl)piperidine-3-carboxylic acid (0.25 M, 200  $\mu$ L, 50  $\mu$ mol, 1 equiv) in *N,N*-dimethylformamide, and a solution of 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (0.4 M, 250  $\mu$ L, 100  $\mu$ mol, 2.0 equiv) in *N,N*-dimethylformamide. The reaction mixture was stirred at 50  $^{\circ}$ C for 16 h. The solvent was removed by Speedvac and the residue was purified via HPLC to afford **4** as a formate salt. MS ( $M+H$ )<sup>+</sup> = 392. HPLC retention time 2.43 min (XBridge C18 2.1 x 50 mm, 5  $\mu$ m; Mobile Phase A: 0.0375% trifluoroacetic acid in water; Mobile Phase B: 0.01875% trifluoroacetic acid in acetonitrile; Gradient: Time (min)/%B: 0.00/1, 0.60/5, 4.00/100, 4.30/1, 4.70/1; Flow Rate: 0.8 mL/min; Column Temperature: 50  $^{\circ}$ C).

**(*R*)-(1-(2-(1-(Pyridin-2-yl)cyclopropyl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone (6)**

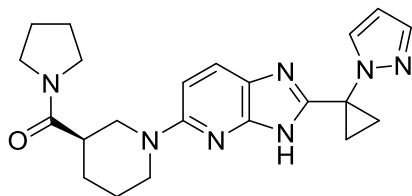


Step 1: (*R*)-*N*-(2-Amino-6-(3-(pyrrolidine-1-carbonyl)piperidin-1-yl)pyridin-3-yl)-1-(pyridin-2-yl)cyclopropane-1-carboxamide. To a solution of (*R*)-(1-(5,6-diaminopyridin-2-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone dihydrochloride (171 mg, 0.43 mmol, 1 equiv), 1-(pyridin-2-yl)cyclopropanecarboxylic acid (70 mg, 0.43 mmol, 1.0 equiv), and *N*-methylmorpholine (190  $\mu$ L, 1.7 mmol, 4.0 equiv) in *N,N*-dimethylformamide (2.0 mL) was added 1*H*-benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (278 mg, 0.47 mmol, 1.1 equiv). The reaction was stirred at 50  $^{\circ}$ C for 18 h. The mixture was cooled to room temperature and was partitioned between 1N aqueous sodium hydroxide and dichloromethane. The organic layer was

separated, washed with saturated ammonium chloride and brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was dried under high vacuum to afford (*R*)-*N*-(2-amino-6-(3-(pyrrolidine-1-carbonyl)piperidin-1-yl)pyridin-3-yl)-1-(pyridin-2-yl)cyclopropane-1-carboxamide (204 mg, >100%). The material was used without further purification.

Step 2: (*R*)-(1-(2-(1-(Pyridin-2-yl)cyclopropyl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone (**6**). To a solution of (*R*)-*N*-(2-amino-6-(3-(pyrrolidine-1-carbonyl)piperidin-1-yl)pyridin-3-yl)-1-(pyridin-2-yl)cyclopropane-1-carboxamide from Step 1 (0.43 mmol, 1 equiv) in isobutanol (0.50 mL) was added 25% sodium methoxide in methanol (87  $\mu$ L, 0.46 mmol, 1.1 equiv). The reaction mixture was stirred at 110 °C for 18 h. Additional 25% sodium methoxide in methanol (348  $\mu$ L, 1.44 mmol, 3.3 equiv) was added and the mixture was stirred for another 4 h. The mixture was concentrated and the resulting residue was partitioned between dichloromethane (50 mL) and saturated aqueous ammonium chloride solution (50 mL). The organic layer was separated, washed with brine, dried over sodium sulfate, filtered, and concentrated. The resulting residue was purified by Combiflash (0-25% methanol in dichloromethane) to afford **6** (62 mg, 31%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.53-1.69 (m, 3H), 1.75-2.04 (m, 9H), 2.61-2.70 (m, 1H), 2.85-2.95 (m, 1H), 2.98-3.08 (m, 1H), 3.42-3.49 (m, 3H), 3.60-3.66 (m, 1H), 4.26 (d,  $J=12.7$  Hz, 1H), 4.44 (d,  $J=12.9$  Hz, 1H), 6.62 (d,  $J=9.0$  Hz, 1H), 7.10 (d,  $J=7.8$  Hz, 1H), 7.15-7.20 (m, 1H), 7.60-7.73 (m, 2H), 8.59-8.61 (m, 1H). MS ( $\text{M}+\text{H}$ ) $^+$  = 417.

**(R)-(1-(2-(1-(1*H*-Pyrazol-1-yl)cyclopropyl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone (7)**



Step 1: Pyrazol-1-yl-acetic acid *tert*-butyl ester. Potassium carbonate (20.3 g, 147 mmol, 2.0 equiv) and bromoacetic acid-*tert*-butyl ester (15.9 g, 80.9 mmol, 1.1 equiv) were added to a stirred solution of pyrazole (5.00 g, 73.5 mmol, 1 equiv) in *N,N*-dimethylformamide (100 mL). The reaction mixture was allowed to stir at 23 °C for 5 h. The mixture was diluted with ethyl acetate, washed sequentially with water and brine. The organic layer was dried over sodium sulfate and concentrated. The crude product was purified by flash column chromatography (5% ethyl acetate–hexanes) to afford pyrazol-1-yl-acetic acid *tert*-butyl ester as an oil (8.0 g, 58%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.45 (s, 9H), 4.80 (s, 2H), 6.29-6.32 (m, 1H), 7.43-7.47 (m, 1H), 7.52-7.58 (m, 1H).

Step 2: 1-Pyrazol-1-yl-cyclopropanecarboxylic acid *tert*-butyl ester. To a stirred solution of pyrazol-1-yl-acetic acid *tert*-butyl ester (7.0 g, 38 mmol, 1 equiv) in tetrahydrofuran (75 mL) at -78 °C, lithium hexamethyldisilazide (1M in THF, 42.3 mL, 42.3 mmol, 1.1 equiv) was added dropwise. The reaction mixture was allowed to stir at the same temperature for 10 min. A solution of [1,3,2]dioxathiolane-2,2-dioxide (5.25 g, 42.3 mmol, 1.1 equiv) in tetrahydrofuran (20 mL) was added slowly. After complete addition the temperature was gradually raised to 0 °C and the mixture was stirred for 1 h at this temperature. The mixture was then cooled to -78 °C and another portion of lithium hexamethyldisilazide solution (1M in THF, 42.3 mL, 42.3 mmol, 1.1 equiv) was added. The temperature was gradually raised to 23 °C. After TLC showed

complete consumption of starting material and formation of a new spot, aqueous ammonium chloride solution was added. The mixture was extracted with ethyl acetate (3 × 100 mL). The organic phase was washed with water and with brine, dried over sodium sulfate and concentrated. The crude product was purified by column chromatography using 10% ethyl acetate-hexane to afford 1-pyrazol-1-yl-cyclopropanecarboxylic acid *tert*-butyl ester as an off-white solid (4.5 g, 56%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.36 (s, 9H), 1.50-1.59 (m, 2H), 1.71-1.75 (m, 2H), 6.24 (s, 1H), 7.48 (m, 2H).

Step 3: 1-Pyrazol-1-yl-cyclopropanecarboxylic acid. To a stirred solution of 1-pyrazol-1-yl-cyclopropanecarboxylic acid *tert*-butyl ester (2.0 g, 9.6 mmol, 1 equiv) in dichloromethane (40 mL) was added trifluoroacetic acid (3.68 mL, 48.0 mmol, 5.0 equiv). The reaction mixture was stirred at 23 °C for 10 h. The mixture was concentrated to remove trifluoroacetic acid, and the resulting residue was partitioned between 2N aqueous sodium hydroxide and ether. The aqueous layer was acidified with 2N aqueous hydrochloric acid, extracted with 20% isopropanol in dichloromethane. The organic layer was washed with brine, dried over sodium sulfate, and concentrated under reduced pressure to afford 1-pyrazol-1-yl-cyclopropanecarboxylic acid as an off-white solid (1.2 g, 82%). <sup>1</sup>H NMR (400 MHz, *d*-DMSO) δ 1.51-1.75 (m, 2H), 1.63-1.68 (m, 2H), 6.25 (s, 1H), 7.40 (s, 1H), 7.80 (s, 1H).

Step 4: *tert*-Butyl (*R*)-(2-amino-6-(3-(pyrrolidine-1-carbonyl)piperidin-1-yl)pyridin-3-yl)carbamate. A Parr vessel containing (*R*)-(1-(6-amino-5-nitropyridin-2-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone (3.0 g, 9.4 mmol, 1 equiv) in ethanol (40 mL) was purged with N<sub>2</sub> gas. 10% Palladium-on-carbon and di-*tert*-butyldicarbonate were added sequentially at 0 °C. The mixture was allowed to shake for 2 h under a hydrogen atmosphere of 45 psi. The mixture was filtered through Celite and the filtrate was concentrated. The crude product was purified by flash

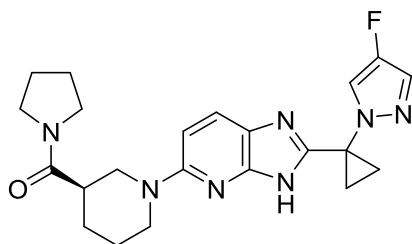
column chromatography using 1% methanol in dichloromethane to afford a light green solid (2.1 g, 57%). MS (M+H)<sup>+</sup> = 390.6.

Step 5: *tert*-Butyl (*R*)-(2-(1-(1*H*-pyrazol-1-yl)cyclopropane-1-carboxamido)-6-(3-(pyrrolidine-1-carbonyl)piperidin-1-yl)pyridin-3-yl)carbamate. To a stirred solution of *tert*-butyl (*R*)-(2-amino-6-(3-(pyrrolidine-1-carbonyl)piperidin-1-yl)pyridin-3-yl)carbamate (1.0 g, 2.57 mmol, 1 equiv) and 1-pyrazol-1-yl-cyclopropanecarboxylic acid (430 mg, 2.87 mmol, 1.1 equiv) in tetrahydrofuran (20 mL) were added *N*-methyl morpholine (1.4 mL, 12.9 mmol, 5.0 equiv) and 1-propanephosphonic acid solution (3.82 mL, 6.42 mmol, 2.5 equiv) at 0 °C. The reaction mixture was heated to reflux for 10 h. The mixture was diluted with ethyl acetate, and was washed sequentially with water and brine. The organics were dried over sodium sulfate and concentrated. Flash column chromatography afforded an off-white solid (550 mg, 41%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.47 (s, 9H), 1.50-1.54 (m, 2H), 1.58-1.72 (m, 2H), 1.78-2.08 (m, 8H), 2.49-2.56 (m, 1H), 2.82 (m, 1H), 2.93 (m, 1H), 3.38-3.57 (m, 4H), 4.09 (br d, *J*=12.5 Hz, 1H), 4.28 (br d, *J*=13.3 Hz, 1H), 6.37 (s, 1H), 6.51 (d, *J*=8.9 Hz, 1H), 7.40 (br s, 1H), 7.63 (br s, 1H), 7.67 (m, 2H), 7.78 (br s, 1H). MS (M+H)<sup>+</sup> = 524.4.

Step 6: (*R*)-(1-(2-(1-(1*H*-Pyrazol-1-yl)cyclopropyl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone (**7**). Methanesulfonic acid (1.4 mL, 21.6 mmol, 4.9 equiv) was added to a stirred solution of *tert*-butyl (*R*)-(2-(1-(1*H*-pyrazol-1-yl)cyclopropane-1-carboxamido)-6-(3-(pyrrolidine-1-carbonyl)piperidin-1-yl)pyridin-3-yl)carbamate (2.3 g, 4.4 mmol, 1 equiv) in acetic acid (25 mL). The reaction mixture was allowed to stir at 23 °C for 1 h. Sodium acetate (1.62 g, 19.8 mmol, 4.5 equiv) was then added and the mixture was stirred for another 1 h. An additional portion of sodium acetate (0.54 g, 6.60 mmol, 1.5 equiv) was added and the mixture and was stirred overnight. The mixture was partitioned between water and ethyl

acetate (3 × 50 mL). The combined organic layers were washed with aqueous NaHCO<sub>3</sub> solution and with brine, dried over sodium sulfate, and concentrated. The crude product was purified by flash column chromatography using 5% methanol-ethyl acetate to afford **7** as white solid (1.1 g, 62%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.74-2.03 (m, 12H), 2.61 (m, 1H), 2.87-2.94 (m, 1H), 3.00-3.06 (m, 1H), 3.39-3.49 (m, 3H), 3.56-3.62 (m, 1H), 4.20 (br d, *J*=12.3 Hz, 1H), 4.35 (br d, *J*=12.3 Hz, 1H), 6.32 (br s, 1H), 6.60 (d, *J*=8.9 Hz, 1H), 7.60 (br s, 1H), 7.67-7.72 (m, 2H), 9.14 (br s, 1H). MS (M+H)<sup>+</sup> = 406.2.

**(R)-(1-(2-(1-(4-Fluoro-1H-pyrazol-1-yl)cyclopropyl)-3H-imidazo[4,5-b]pyridin-5-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone (8)**



Step 1: 2-(4-Fluoro-1H-pyrazol-1-yl)acetonitrile. Sodium hydride (60% oil dispersion, 306 mg, 12.8 mmol, 1.1 equiv) was suspended in tetrahydrofuran and the suspension was cooled to 0 °C. A solution of 4-fluoro-1H-pyrazole (1.0 g, 11.6 mmol, 1.0 equiv) and 2-bromoacetonitrile (1.39 g, 11.6 mmol, 1 equiv) in tetrahydrofuran was added dropwise at 0 °C over a period of 40 min to the sodium hydride solution. The reaction mixture was stirred at room temperature for 12 h, whereupon a saturated aqueous solution of ammonium chloride was added. The mixture was extracted with diethyl ether, and the organic layer was washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude material was purified via column chromatography (100-200 mesh silica gel, 5-10% ethyl acetate in petroleum ether) to afford 2-(4-fluoro-1H-pyrazol-1-yl)acetonitrile (1.1 g, 76%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 5.0 (s, 2H), 7.42-7.48 (m, 2H).



Step 2: 1-(4-Fluoro-1*H*-pyrazol-1-yl)cyclopropanecarbonitrile. *Safety note*: Sodium hydride and DMSO can react explosively, please take appropriate precautions in using this reagent/solvent combination. Sodium hydride (60% oil dispersion, 1.26 g, 52.75 mmol, 6.0 equiv) was washed with petroleum ether under a nitrogen atmosphere. A solution of 2-(4-fluoro-1*H*-pyrazol-1-yl)acetonitrile (1.1 g, 8.8 mmol, 1 equiv) and 1,2-dibromoethane (2.28 mL, 26.4 mmol, 3.0 equiv) in DMSO (90 mL) was added at 0 °C over a period of 40 min. The reaction mixture was stirred at room temperature for 6 h. A saturated aqueous solution of ammonium chloride was added and the mixture was extracted with diethyl ether. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude material was purified via column chromatography (100-200 mesh silica gel, 5-12% ethyl acetate in petroleum ether) to afford 1-(4-fluoro-1*H*-pyrazol-1-yl)cyclopropanecarbonitrile (320 mg, 39%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 1.81-1.88 (m, 4H), 7.42 (d, *J*=4.1 Hz, 1H), 7.51 (d, *J*=4.9 Hz, 1H).

Step 3: Ethyl 1-(4-fluoro-1*H*-pyrazol-1-yl)cyclopropanecarbimide. Sodium metal (47 mg, 1.98 mmol, 3.0 equiv) was added to anhydrous ethanol (4 mL) at room temperature. The mixture was stirred for 15 min. A solution of 1-(4-fluoro-1*H*-pyrazol-1-yl)cyclopropanecarbonitrile (0.1 g, 0.66 mmol, 1 equiv) in ethanol (2 mL) was added and the reaction mixture was heated at 70 °C for 90 min. The resulting solution was used without further purification.

Step 4: (*R*)-(1-(2-(1-(4-Fluoro-1*H*-pyrazol-1-yl)cyclopropyl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone (**8**). (*R*)-(1-(5,6-Diaminopyridin-2-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone dihydrochloride (344 mg, 0.95 mmol, 1.1 equiv) in ethanol (2 mL) was added to a solution of ethyl 1-(4-fluoro-1*H*-pyrazol-1-yl)cyclopropanecarbimide (170 mg, 0.86 mmol, 1 equiv). Acetic acid (0.99 mL, 17.2 mmol, 20 equiv) and triethylamine (0.72

mL, 5.2 mmol, 6.0 equiv) were added and the reaction mixture was heated at 100 °C for 11 h. The mixture was then placed in a freezer for 65 h. The mixture was partitioned between dichloromethane (75 mL) and saturated aqueous ammonium chloride solution (70 mL). The aqueous layer was extracted with dichloromethane (3 × 75 mL) and the combined organics were washed with saturated aqueous sodium bicarbonate solution (70 mL). The organics were dried over sodium sulfate, filtered and concentrated. The crude material was purified via column chromatography (100-200 mesh silica gel, 0-7% methanol in dichloromethane) to afford **8** (290 mg, 79 %). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 1.55-1.70 (m, 1H), 1.72-2.05 (m, 11H), 2.60-2.70 (m, 1H), 2.94 (t, *J*=12.6 Hz, 1H), 3.08 (t, *J*=12.0 Hz, 1H), 3.40-3.50 (m, 3H), 3.56-3.62 (m, 1H), 4.20 (d, *J*=12.9 Hz, 1H), 4.37 (d, *J*=12.9 Hz, 1H), 6.65-6.72 (m, 1H), 7.45-7.53 (m, 2H), 7.69 (d, *J*=8.8 Hz, 1H). MS (M+H)<sup>+</sup> = 424.1.

**Generation of Constructs for DGAT1/2 and MGAT1/2/3.** All constructs were prepared to express the full length enzymes. Constructs for human DGAT2, rat DGAT2, dog DGAT2, and mouse MGAT1 were generated with N-terminal FLAG tags. Constructs for human DGAT1, human MGAT2, and human MGAT3 were generated untagged. All cDNAs except that for dog DGAT2 were custom-synthesized at Genscript (Piscataway, NJ). The cDNA for dog DGAT2 was obtained by PCR amplifications from dog adipose RNA (Zyagen, San diego, CA). The cDNAs were cloned into the pFastBac1 vector (Invitrogen, Carlsbad, CA) and all constructs were confirmed by sequencing in both directions.

**Expression and Preparation of Membrane and Microsomal Fractions.** Recombinant baculovirus for each acyltransferase construct was generated using Bac-to-Bac baculovirus

expression system (Life Technologies, Grand Island, NY) and used for infecting SF9 insect cells for 24-48 hours for the expression of each enzyme. The resulting cell paste was flash frozen in liquid N<sub>2</sub> and stored at -80 °C until needed. For DGAT2, MGAT1, MGAT2, and MGAT3 assays, detergent-solubilized membrane fractions were prepared and used as enzyme sources. For DGAT1 assay, microsomal fraction was prepared and used as a source of enzyme. All operations below were at 4 °C unless otherwise noted. The SF9 cells from above were resuspended in lysis buffer (50 mM Tris-HCl, pH 8.0, 250 mM sucrose) including 1 mM EDTA and complete protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN) at a ratio of 3 ml buffer per 1 g cell paste. The cells were lysed by dounce homogenizer. For detergent-solubilized membrane fraction preparations for DGAT2, MGAT1, MGAT2, and MGAT3, the cell debris was removed by centrifugation at 1,000 x g for 20 min and the supernatant was centrifuged at 100,000 x g for 1 hour. The resulting pellet was rinsed three times by filling ultracentrifuge tubes to the top with PBS before decanting. The washed pellet was resuspended with gentle stirring for 1 hour in lysis buffer containing 8 mM CHAPS (Calbiochem, Gibbstown, NJ) at a ratio of 1 mL buffer per 1 g of the original cell paste, and then centrifuged again at 100,000 x g for 1 hour. The resulting supernatant was aliquotted, flash frozen in liquid N<sub>2</sub>, and stored at -80 °C until use.

For DGAT1 microsomal fraction preparation, the lysed cells were centrifuged at 11,000 x g for 45 min and the supernatant was centrifuged at 100,000 x g for 1 hour. The resulting pellet was resuspended with gentle stirring for 1 hour in lysis buffer at a ratio of 1 ml buffer per 1 g of the original cell paste, aliquotted, flash-frozen in liquid N<sub>2</sub>, and stored at -80 °C until use.

### Selectivity Data of Compound 5 (supplementary to table 2)

Target Name in CEREP panel	Percent activity at 10 $\mu$ M
Sodium-dependent Serotonin transporter (SERT)	-2
Serotonin receptor 1A (5HT1A)	-3
Serotonin receptor 2A (5HT2A)	-9
Serotonin receptor 3 (5HT3)	-3
Serotonin receptor 7 (5HT7)	-5
Adenosine A2A receptor (A2A)	56 (Ki=3.7 $\mu$ M)
Angiotensin converting enzyme 1 (ACE1)	-4
Acetylcholinesterase (ACHE)	27
Alpha-2B adrenergic receptor (alpha2B)	-3
Beta 1 adrenergic receptor (BETA1)	1
Beta 2 adrenergic receptor (BETA2)	1
Cannabinoid receptor 1 (CB1)	5
Cannabinoid receptor 2 (CB2)	-15
Cholecystokinin receptor A (CCKA)	-10
Cholecystokinin receptor B (CCKB)	-1
Choline transporter (CHT1/SLC5A7)	-34
Cyclooxygenase 2 (COX2)	14
D1 dopamine receptor (D1)	-2
Sodium-dependent dopamine transporter (DAT)	2
Delta-opioid receptor (DOR-1)	18
Glucocorticoid receptor (GR)	-25
Histamine H1 receptor (H1)	-3
Histamine H3 receptor (H3)	-1
Muscarinic acetylcholine receptor 1 (M1)	89 (Ki=4.5 $\mu$ M)
Muscarinic acetylcholine receptor 3 (M3)	3
Nicotinic acetylcholine receptor, muscle-type (nAChR)	-4
Noradrenaline transporter, sodium-dependent (NAT)	6
Mitogen-activated protein kinase 14 (MAPK14 / P38-alpha)	17
D2 dopamine receptor (short)	5
Neurokinin NK2 receptor (NK2)	11
Androgen receptor (AR)	0
Cyclooxygenase 1 (COX1)	0
Rat L-type Calcium channel (verapamil site)	6
Rat L-type Calcium channel (dihydropyridine site)	-4
Rat L-type Calcium channel (diltiazem site)	-15
Rat Gamma-aminobutyric acid (GABA) receptor A	10
Rat Glutamate receptors 1-4 (AMPA)	-11
Rat GABA transporter	-16

Rat Sodium channel (site 2)	-3
Rat monoamine oxidase A (MAO-A)	2
Rat Serotonin receptor 1B (5HT1B)	-11
Rat chloride channel	-8
Urotensin II receptor (GPR14/UT-1)	6
Rat N-methyl-D-aspartate (NMDA) receptor	-12
Peroxisome proliferator activated receptor (PPAR)-gamma	1
Rat opioid receptor, kappa 1	-1
Phosphodiesterase (PDE)3B	11
Phosphodiesterase (PDE)4D2	12
5-HT2B (functional, agonist)	36
5-HT4 (functional, agonist)	-1
Endothelin-A (ET-A) (functional, agonist)	30
Histamine H2 (functional, agonist)	0
Opioid receptor, mu 1 (functional, agonist)	-39
NK1 (functional, agonist)	30
Alpha 1a receptor (functional, agonist)	62
Alpha 2b receptor (functional, agonist)	--2
Dopamine D1 receptor (functional, agonist)	0 at 1 $\mu$ M
Histamine H1 receptor (functional, agonist)	50
Epidermal growth factor receptor (EGFR) kinase	2
Lymphocyte-specific protein tyrosine kinase (LCK)	1
Muscarinic acetylcholine receptor M2 (M2)	-16
Alpha 2a receptor	-6
Adenosine A1 receptor	37
Kinase insert domain receptor (KDR) kinase (VEGFR2)	18
Nicotinic acetylcholine receptor (alpha4, beta 2 subunit containing)	-22
Cannabinoid CB1 (functional, agonist)	0
Aurora kinase-A	-14
Opioid receptor, sigma 1	18
PDE1A	42
Abl tyrosine kinase	15
Alpha 1a receptor	-10
<b>PDE selectivity Panel</b>	<b>IC<sub>50</sub> [<math>\mu</math>M]</b>
PDE 1B	3.3
PDE 3A	14
PDE 3B	21
PDE3A1	>30
PDE 7B	>26
PDE 9A	>30

PDE 8B	15
PDE2-A3	15
PDE 10A	13
PDE 4D	>19
PDE 4D3	>30
PDE 5A	1.97
PDE 5A1	>30
PDE 6A	9.4
PDE 11	1.48
PDE 4A	21
PDE 4C	>30
PDE 4B full length	14
PDE 4B supershort	>30