Discovery of Novel Imidazo[4,5-*b*]pyridines as Potent and Selective Inhibitors of Phosphodiesterase 10A (PDE10A)

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SUPPORTING INFORMATION

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Chemistry, Materials, and General Methods.

Unless otherwise noted, all reagents and solvents were obtained from commercial suppliers such as Aldrich, Sigma, Fluka, Acros, EDM Sciences, etc., and used without further purification. Dry organic solvents (dichloromethane, acetonitrile, DMF, etc.) were purchased from Aldrich packaged under nitrogen in Sure/Seal bottles. All reactions involving air or moisture sensitive reagents were performed under a nitrogen or argon atmosphere. Silica gel chromatography was performed using prepacked silica gel cartridges (Biotage or RediSep). Microwave assisted reactions were performed in Biotage Initiator Sixty microwave reactor. ^{1}H NMR spectra were recorded on a Bruker DRX 300 MHz, Bruker DRX 400 MHz, Bruker AV 400 MHz, Varian 300 MHz, or a Varian 400 MHz spectrometer at ambient temperature. Chemical shifts are reported in parts per million (ppm, δ units). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants, and number of protons. Reactions were monitored using Agilent 1100 series LC/MSD SL high performance liquid chromatography (HPLC) systems with UV detection at 254 nm and a low resonance electrospray mode (ESI). All final compounds were purified to > 95% purity, as determined by high performance liquid chromatography (HPLC). HPLC methods used the following: Agilent 1100 spectrometer, Zorbax SB-C18 column (50 mm × 3.0 mm, 3.5 μm) at 40 °C with a 1.5 mL/min flow rate; solvent A of 0.1% TFA in water, solvent B of 0.1% TFA in acetonitrile; 0.0−3.0 min, 5−95% B; 3.0−3.5 min, 95% B; 3.5−3.51 min, 5% B. Flow from UV detector was split (50:50) to the MS detector, which was configured with APIES as ionizable source. All high resolution mass spectrometry (HRMS) data were acquired on a Synapt G2 HDMS instrument (Waters Corporation, Manchester, UK) operated in positive electrospray ionization mode. The sample was diluted to 10 μM in 50% acetonitrile (v/v), 0.1% formic acid (v/v), and infused into the mass spectrometer at a flow rate of 5 μL/min through an electrospray ionization source operated with a capillary voltage of 3 kV. The sample cone

was operated at 30 V. The time-of-flight analyzer was operated at a resolution (fwhm) of 30000 at m/z 785 and was calibrated over the m/z range 50−1200 using a 1 μM sodium iodide (NaI) solution (50% v/v acetonitrile solution). To obtain accurate mass measurements, an internal lock-mass correction was applied using leucine enkephalin (m/z 556.2771). Collision induced fragmentation (CID) was performed using an injection voltage of 28 V. Instrument control was performed through the software suite Mass-Lynx, version 4.1.

(1*H*-benzo[*d*]imidazol-2-yl)(4-(2-methoxy-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)methanone (**2**).

Step 1. To a solution of 1*H*-Benzoimidazole **13** (1.0 g, 8.5 mmol), in 3,4-dihydro-2*H*-pyran (40 mL) was added catalytic amount of p-toluenesulfonic acid. The reaction mixture was refluxed at 130 ^{0}C overnight. The reaction mixture was diluted with water (50 mL) and extracted with EtOAc (200 mL). The organic layer was washed with more water (2 x 100mL), dried over Na_2SO_4 and purified over silica gel to get 1.5 g of 1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-benzo[*d*]imidazole product (88% yield). MS (ES+ve): calculated for $C_{12}H_{14}N_2O_5$ [M] = 202.25, observed $C_{12}H_{14}N_2O_5$ [M+1] = 203.25.

Step 2. To a solution of 1-(tetrahydro-2*H*-pyran-2-yl)-1H-benzo[*d*]imidazole (1.0 g, 4.9 mmol) in THF (13 mL) was added methyl 4-iodobenzoate **14** (1.3 g, 4.9 mmol). The resulting mixture was cooled to -78 ^{0}C. LiHMDS (1.0 mL, 6.2 mmol) was added drop wise while maintaining the internal temperature at -78 ^{0}C. The reaction mixture was allowed to gradually warm to room temperature. The reaction mixture was diluted with water (50 mL) and extracted with EtOAc (2 x 150 mL). The combined organic layer was dried over Na_2SO_4 and purified using silica gel to give 0.7 g (33% yield) of the desired product (4-iodophenyl)(1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)methanone **15**. MS (ES+ve): calculated for $C_{19}H_{17}IN_2O_2$ [M] = 432.26, observed $C_{19}H1_{I7}IN_2O_2$ [M+1] = 433.26.

Step 3. To a solution of (4-iodophenyl)(1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)methanone **15** (5.0 g, 11.6 mmol) in dimethyl acetamide (25 mL) was added Cs_2CO_3 (5.6 g, 17.4

mmol) and 3-nitropyridin-2-amine **16** (1.6 g, 11.6 mmol). The reaction mixture was degassed for 15 min before addition of $Pd_2(dba)_3$ (0.4 g, 0.4 mmol) and xantphos (0.5 g, 0.9 mmol). The resulting mixture was kept under nitrogen and heated to 100 ^{0}C overnight. The reaction mixture was diluted with water and extracted with EtOAc (300 mL). The organic layer was further washed with water (2 x 50 mL), and purified over silica gel to afford 5.0 g (98% yield) of the desired product (4-((3-nitropyridin-2-yl)amino)phenyl)(1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)methanone. MS (ES+ve): calculated for $C_{24}H_{21}N_5O_4$ [M] = 443.45, observed $C_{24}H_{21}N_5O_4$ [M+1] = 444.45.

Step 4. To a solution of (4-((3-nitropyridin-2-yl)amino)phenyl)(1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)methanone (1.5 g, 3.4 mmol) in ethanol (20 mL) was added molybdenum hexacarbonyl (0.9 g, 3.4 mmol) followed by DBU (1.0 mL, 6.8 mmol). The resulting mixture was heated to 150 ^{0}C for 6 h in a sealed tube. The reaction mixture was evaporated to remove the volatile solvent. Purification by silica gel afforded 1.0 g (77% yield) of desired product (4-((3-aminopyridin-2-yl)amino)phenyl)(1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)methanone **17**. MS (ES+ve): calculated for $C_{24}H_{23}N_5O_2$ [M] = 413.47, observed $C_{24}H_{23}N_5O_2$ [M+1] = 414.47.

Step 5. To a solution of (4-((3-aminopyridin-2-yl)amino)phenyl)(1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)methanone **17** (0.1 g, 0.2 mmol) in tetramethyl orthoformate (10 mL) was added catalytic amount of propionic acid. The resulting mixture was refluxed at 100 ^{0}C for 2 h. Volatile solvents were removed by rotovap. The residue was purified by silica gel to give 50 mg (50% yield) of (4-(2-methoxy-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)(1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)methanone. MS (ES+ve): calculated for $C_{26}H_{23}N_5O_3$[M] = 453.49, observed $C_{26}H_{23}N_5O_3$ [M+1] = 454.49.

Step 6. To the solution of (4-(2-methoxy-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)(1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)methanone (100 mg, 0.2 mmol) in EtOAc and THF (1:1) (6 mL) was added sulfuric acid (0.1 mL) at –20 ^{o}C. The reaction mixture was stirred at –20 ^{o}C for 15 min.

The reaction mixture was neutralized by aqueous sodium bicarbonate solution and extracted with EtOAc. The organic layer was separated, dried over sodium sulfate and concentrated to get crude product as solid. The product was purified by washing with DCM to give 23 mg (23% yield) desired product (1H-benzo[*d*]imidazol-2-yl)(4-(2-methoxy-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)methanone **2**. MS (ES+ve): calculated for $C_{21}H_{15}N_5O_2$ [M] = 369.3, observed $C_{26}H_{23}N_5O_3$ [M+1] = 370.6. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.21 (dd, *J*=1.46, 4.97 Hz, 1H), 8.09 (d, *J*=2.34 Hz, 1H), 7.85 (dd, *J*=1.39, 7.82 Hz, 1H), 7.49 (s, 4H), 7.38 (dd, *J*=2.27, 8.40 Hz, 1H), 7.19 (dd, *J*=4.97, 7.89 Hz, 1H), 6.91 (d, *J*=8.33 Hz, 1H), 6.79 (s, 1H), 4.24 (s, 3H), 2.27 (s, 3H)

General procedures for synthesis of compounds 3-7, 9-12b as illustrated by synthesis of N-(4-(2-ethyl-3H-imidazo[4,5-b]pyridin-3-yl)phenyl)-5-methylpyridin-2-amine (**11b**).

Step 1. 2-Chloro-3-nitro-pyridine **19** (52.7 g, 333.3 mmol), anhydrous dimethylsulfoxide (450 mL), N-(4-aminophenyl)acetamide **18** (50 g, 333.3 mmol) and anhydrous triethylamine (50.5 g, 500 mmol) were combined with stirring under nitrogen. The reaction mixture was heated to 90 ^{o}C overnight. The mixture was poured into water and the resulting orange solid was filtered off to give the desired compound *N*-[4-(3-nitro-pyridin-2-ylamino)-phenyl]-acetamide (82 g, 301.5 mmol, 90% yield) which was used in the next step without further purification.

Step 2. A mixture of *N*-[4-(3-nitro-pyridin-2-ylamino)-phenyl]-acetamide (27.3 g,100 mmol) and 5 N HCl solution (100 mL) in 1,4-dioxane (200 mL) was stirred at 70 ^{o}C overnight. Volatile solvents were removed by rotovap. The residue was diluted with water (100 mL), basified to pH = 7 with solid Na_2CO_3 then extracted with CH_2Cl_2 (3 x 100 mL). The organic layer was separated, dried over Na_2SO_4, filtered and evaporated to give *N*-(3-nitro-pyridin-2-yl)-benzene-1,4-diamine **20** (20.8 g, 90 mmol, 90% yield).

Step 3. A mixture of *N*-(3-nitro-pyridin-2-yl)-benzene-1,4-diamine **20** (1 g, 4.3mmol) and 2-chloro-5-methyl-pyridine (5 mL) was heated to 180 ^{o}C by microwave for 2 h. Volatile solvents was re-

moved by rotovap. The residue was partitioned between CH_2Cl_2 and H_2O. The organic layer was dried over Na_2SO_4 and concentrated to give the crude product which was purified by column chromatography (3: 1 EtOAc/petroleum ether) to give N^1-(5-methylpyridin-2-yl)-N^4-(3-nitropyridin-2-yl)benzene-1,4-diamine (1 g, 3.1 mmol, 72% yield).

Step 4. A mixture of N^1-(5-methylpyridin-2-yl)-N^4-(3-nitropyridin-2-yl)benzene-1,4-diamine (1 g, 3.1 mmol) and 50% Pd/C (0.5 g) in MeOH (15 mL) was stirred under H_2 at 30 psi for 2 h. Residual Pd/C was removed by filtration and the filtrate was concentrated to give N^2-(4-((5-methylpyridin-2-yl)amino)phenyl)pyridine-2,3-diamine (0.8 g, 2.8 mmol, 90% yield) which was used in the next step without further purification.

Step 5. A mixture of N^2-(4-((5-methylpyridin-2-yl)amino)phenyl)pyridine-2,3-diamine (200 mg, 0.7 mmol), propionic acid (51 mg, 0.7 mmol), HATU (313 mg, 0.8 mmol) and triethylamine (139 mg, 1.4 mmol) in dry CH_2Cl_2 (10 mL) was stirred at room temperature overnight. Reaction mixture was diluted with water (10 mL), extracted with CH_2Cl_2 (3 x 10 mL) and separated. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated to give crude product which was purified by column chromatography to give *N*-(2-((4-((5-methylpyridin-2-yl)amino)phenyl)amino)pyridin-3-yl)propionamide (120 mg, 0.34 mmol, 50% yield).

Step 7. The mixture of *N*-(2-((4-((5-methylpyridin-2-yl)amino)phenyl)amino)pyridin-3-yl)propionamide (120 mg, 0.3 mmol) and acetic acid (5 mL) was heated to 90 ^oC for 2 h. The mixture was then concentrated to give the crude product which was purified by column chromatography to afford *N*-(4-(2-ethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)-5-methylpyridin-2-amine **11b** (35 mg, 0.11 mmol, 31% yield).

The following compounds were prepared according to this general route:

***N*-(4-(2-methoxy-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)-1*H*-benzo[*d*]imidazol-2-amine (3).** ESI-

MS [M+1] = 357 calculated for $C_{20}H_{16}N_6O$ [M] = 356. ^{1}H-NMR (400MHz, MeOD): δ (ppm) 8.09-8.07 (dd, J =1.6, 3.2 Hz, 1H); 7.86-7.83 (dd, J = 1.6, 3.2 Hz, 1H); 7.60 (d, J = 1.6 Hz, 2H); 7.58 (s, 2H); 7.37 (s, 2H); 7.26-7.23 (m, 1H); 7.10-7.08 (m, 2H); 4.18 (s, 3H).

***N*-(4-(2-methoxy-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)benzo[*d*]thiazol-2-amine (4).** ESI-MS [M+1] = 374 calculated for $C_{20}H_{15}N_5S$ [M] = 373. HRMS calculated for $C_{20}H_{16}N_5S$ [M+1] = 374.1076, found 374.1067. ^{1}H-NMR (400MHz, CHLOROFORM-d): δ (ppm) 8.18 (s, 1H); 7.83 (d, J = 7.6 Hz, 1H); 7.58-7.53 (m, 4H); 7.38 (s, 2H); 7.28 (s, 1H); 7.10 (s, 2H), 4.16 (s, 3H). ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.2, 158.1, 152.0, 147.9, 141.3, 140.8, 133.6, 130.4, 127.5, 126.9, 126.0, 125.3, 122.5, 120.6, 119.9, 119.3, 118.7, 57.3

***N*-(4-(2-methoxy-3*H*-imidazo[4,5-b]pyridin-3-yl)phenyl)quinolin-2-amine (5).** ESI-MS [M+1] = 368 calculated for $C_{22}H_{17}N_5O$ [M] = 367. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.67 (s, 1H), 8.06-8.25 (m, 4H), 7.88 (d, J=7.60 Hz, 1H), 7.68-7.81 (m, 2H), 7.47-7.65 (m, 3H), 7.32 (t, J=7.31 Hz, 1H), 7.23 (dd, J=4.97, 7.75 Hz, 1H), 7.12 (d, J=8.92 Hz, 1H), 4.16 (s, 3H).

5-chloro-N-(4-(2-methoxy-3H-imidazo[4,5-b]pyridin-3-yl)phenyl)pyridin-2-amine (6). ESI-MS [M+1] = 352 calculated for $C_{18}H_{14}ClN_5O$ [M] = 351. ^{1}H-NMR (400MHz, MeOD): δ (ppm) 8.09 (dd, J = 0.8, 2.0 Hz, 1H), 8.06 (dd, J = 1.6, 3.6 Hz, 1H), 7.84 (dd, J = 1.6, 8.4 Hz, 1H), 7.78 (d, J = 9.2 Hz, 1H), 7.69 (d, J = 8.8 Hz, 1H), 7.54 (dd, J = 2.8, 6.0 Hz, 1H), 6.85-7.25 (m, 1H), 6.40 (dd, J = 4.0, 8.4 Hz, 1H), 4.18 (s, 3H).

***N*-(4-(2-methoxy-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)-5-methylpyridin-2-amine (7).** ESI-MS [M+1] = 332 calculated for $C_{19}H_{17}N_5O$ [M] = 331. HRMS calculated for $C_{19}H_{18}N_5O$ [M+1] = 332.1511, found 332.1506. ^{1}H NMR (*d*-DMSO, 400 MHz): δ (ppm) 9.11(s, 1H); 8.07 (m, 1H); 7.97 (s, 1H); 7.85 (m, 1H); 7.77 (m, 1H); 7.45 (m, 1H); 7.37 (d, J = 8.8 Hz, 2H); 7.17 (m, 1H); 4.10 (s, 3H);

2.16 (s, 3H). ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 157.9, 153.2, 148.1, 147.7, 141.8, 141.1, 138.7, 133.1, 127.3, 126.6, 124.8, 124.6, 119.4, 118.5, 108.9, 57.2, 17.6

***N*-(4-(3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)benzo[*d*]thiazol-2-amine (9).** ESI-MS [M+1] = 344 calculated for $C_{19}H_{13}N_5S$ [M] = 343. ^{1}H NMR (MeOD, 400 MHz): δ (ppm) 8.26-8.24 (m, 1H); 8.00-7.98 (m, 2H); 7.84-7.82 (m, 2H); 7.72-7.70 (m, 1H); 7.62-7.60 (d, J = 4.2 Hz, 1H); 7.56-7.52 (m, 1H); 7.37-7.33 (m, 1H); 7.21-7.17 (m, 1H).

***N*-(4-(2-methyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)benzo[*d*]thiazol-2-amine (10a).** ESI-MS [M+1] = 358 calculated for $C_{20}H_{15}N_5S$ [M] = 357. ^{1}H-NMR (400MHz, MeOD): δ (ppm) 8.24-8.24 (d, J = 1.2 Hz, 1H); 8.11 (s, 1H); 8.09 (s, 2H); 7.74-7.72 (m, 1H); 7.64-7.57 (m, 4H); 7.38-7.34 (m, 1H); 7.22-7.18 (m, 1H); 2.77 (s, 3H).

5-methyl-*N*-(4-(2-methyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)pyridin-2-amine (10b). ESI-MS [M+1] = 316 calculated for $C_{19}H_{17}N_5$ [M] = 315. ^{1}H-NMR (400MHz, MeOD): δ (ppm) 8.55-8.53 (m, 1H); 8.32-8.30 (m, 1H); 7.84-7.80 (m, 2H); 7.59-7.54(m, 5H); 7.39-7.36 (m, 1H); 2.83 (s, 3H); 2.34 (s, 3H).

***N*-(4-(2-ethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)benzo[*d*]thiazol-2-amine (11a).** ESI-MS [M+1] = 372 calculated for $C_{21}H_{17}N_5S$ [M] = 371. ^{1}H NMR (MeOD, 400 MHz): δ (ppm) 8.22 (m, 1H); 8.04-8.01 (m, 3H); 7.69 (m, 1H); 7.60 (m, 1H); 7.43 (d, J = 8.8 Hz, 2H); 7.34-7.32 (m, 2H); 7.17 (m, 1H); 2.87-2.85 (m, 2H); 1.34-1.30 (m, 3H).

***N*-(4-(2-ethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)-5-methylpyridin-2-amine (11b).** ESI-MS [M+1] = 330 calculated for $C_{20}H_{19}N_5$ [M] = 329. ^{1}H-NMR (400MHz, MeOD): δ (ppm) 8.49-8.47 (d, J = 8.0 Hz,1H); 8.25-8.23 (d, J = 8.0 Hz,1H); 8.02-8.00 (d, J = 8.0 Hz, 1H); 7.86-7.85 (d, J = 4.0 Hz ,1H); 7.72-7.66 (m, 4H); 7.60-7.56 (m, 1H); 7.35- 7.33 (d, J = 8.0 Hz, 1H); 3.09-3.07 (m, 2H); 2.33 (s, 1H); 1.44-1.40 (m, 3H).

***N*-(4-(2-cyclopropyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)benzo[*d*]thiazol-2-amine (12a).** ESI-MS [M+1] = 384 calculated for $C_{22}H_{17}N_5S$ [M] = 383. ^{1}H-NMR (400MHz, MeOD): δ (ppm) 8.18-8.17 (m, 1H); 8.02 (d, J = 6.8 Hz, 2H); 7.94 (d, J = 8.0 Hz, 1H); 7.68 (d, J = 8.4 Hz, 1H); 7.60 (d, J = 8.0Hz, 1H); 7.50 (d, J = 7.2 Hz, 2H); 7.33-7.27 (m, 2H); 7.17-7.13 (m, 1H); 1.97 (m, 1H); 1.24 (s, 2H); 1.11 (m, 2H).

***N*-(4-(2-cyclopropyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)-5-methylpyridin-2-amine (12b).** ESI-MS [M+1] = 342 calculated for $C_{21}H_{19}N_5$ [M] = 341. HRMS calculated for $C_{21}H_{20}N_5$ [M+1] = 342.1719, found 342.1716. ^{1}H-NMR (400MHz, MeOD): δ (ppm) 8.36 (s, 1H); 8.08-8.07 (d, J = 4.0 Hz, 1H); 8.01-8.00 (d, J = 4.0 Hz, 1H); 7.85 (s, 1H); 7.77-7.74 (d, J = 12.0 Hz, 2H); 7.69-7.67 (d, J = 8.0 Hz, 2H); 7.48-7.45 (m, 1H); 7.31 (m, 1H); 2.34 (s, 3H); 2.15-2.13 (m, 1H); 1.39-1.29 (m, 4H). ^{13}C NMR (75 MHz, CHLOROFORM-d): δ (ppm) 163.2, 162.7, 158.5, 151.0, 146.6, 146.3, 144.8, 138.8, 136.0, 130.1, 130.0, 129.3, 126.2, 124.6, 124.2, 120.6, 111.0, 22.5, 17.3, 11.6, 8.5

N-(3-(2-methoxy-3*H*-imidazo[4,5-*b*]pyridin-3-yl)cyclobutyl)benzo[*d*]thiazol-2-amine (**24**).

Step 1. Benzylamine (751 mg, 7.0 mmol), *tert*-butyl (3-oxocyclobutyl)carbamate **25** (500 mg, 2.7 mmol), and sodium cyanoborohydride (237 mg, 3.8 mmol) were dissolved in 25 mL of anhydrous methanol. The pH of the solution was lowered to 5 by addition of acetic acid. The resulting reaction mixture was stirred for 24 h at room temperature. The pH of the solution then was increased to 8 by addition of 1 M solution of $NaHCO_3$. The solvents were removed under reduced pressure. The crude product was partitioned between EtOAc (80 mL) and H_2O (25 mL). The organic layer was washed with water and brine. The residue was purified using flash column chromatography with hexanes/EtOAc (2:1) as eluant to obtain *tert*-butyl (3-(benzylamino)cyclobutyl)carbamate (600 mg, 2.2 mmol, 80% yield). ESI-MS [M+1] = 277 calculated for $C_{16}H_{24}N_2O_2$ [M] = 276.

Step 2. A mixture of *tert*-butyl (3-(benzylamino)cyclobutyl)carbamate (600 mg, 2.2 mmol) and wet $Pd(OH)_2/C$ (50%, 200 mg) in MeOH (30 mL) was stirred under H_2 (30 psi) at room temperature for

3 h. The reaction mixture was filtered through a cake of Celite and washed with MeOH. The filtrate was concentrated *in vacuo* to give tert-butyl (3-aminocyclobutyl)carbamate **26** (388 mg, 2.1 mmol, 95% yield). ESI-MS [M+1] = 187 calculated for $C_9H_{18}N_2O_2$ [M] = 186.

Step 3. To a mixture of 2-chlorobenzo[*d*]thiazole **27** (355 g, 2.1 mmol) and tert-butyl (3-aminocyclobutyl)carbamate **26** (388 g, 2.1 mmol) was added NMP (8 mL) and ethyl-diisopropyl-amine (668 mg, 5.2 mmol). The solution was heated to 180 °C by microwave for 2 h. The mixture was then diluted with water (40 mL) and extracted with EtOAc (2 x 50 mL). The combined organic extracts were combined and washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4 and filtered. The filtrate was evaporated *in vacuo* and the residue was purified by flash column chromatography on silica gel (20% to 50% EtOAc/hexanes) to give tert-butyl (3-(benzo[*d*]thiazol-2-ylamino)cyclobutyl)carbamate (319 mg, 1.0 mmol, 50% yield). ESI-MS [M+1] = 320 calculated for $C_{16}H_{21}N_3O_2S$ [M] = 319.

Step 4. To tert-butyl (3-(benzo[*d*]thiazol-2-ylamino)cyclobutyl)carbamate (319 mg, 1.0 mmol) was added 4 M HCl in MeOH (20 mL). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated to give *N*1-(benzo[*d*]thiazol-2-yl)cyclobutane-1,3-diamine **28** as an HCl salt (255 mg, 1.0 mmol, 100% yield) which was used in the next step without further purification. ESI-MS [M+1] = 220 calculated for $C_{11}H_{13}N_3S$ [M] = 219.

Step 5. To a solution of *N*1-(benzo[*d*]thiazol-2-yl)cyclobutane-1,3-diamine **28** (255 mg, 1.0 mmol) in DMF (15 mL) was added Na_2CO_3 (320 mg, 3.0 mmol) and 2-chloro-3-nitro-pyridine **19** (159 mg, 1.0 mmol). The reaction mixture was stirred and heated at reflux overnight. The reaction mixture was then diluted with water (25 mL) and extracted with EtOAc (2 x 30 mL). The combined organic extracts were washed with water (30 mL), brine (30 mL), dried over Na_2SO_4 and filtered. The filtrate was evaporated *in vacuo* and the residue was purified by column chromatography on silica gel (20% to 40% EtOAc/petroleum ether) to give *N*1-(benzo[*d*]thiazol-2-yl)-*N*3-(3-nitropyridin-2-yl)cyclobutane-1,3-

diamine (239 mg, 0.70 mmol, 70% yield for two steps). ESI-MS [M+1] = 343 calculated for $C_{16}H_{16}N_5O_2S$ [M] = 342.

Step 6. A mixture of *N*1-(benzo[*d*]thiazol-2-yl)-*N*3-(3-nitropyridin-2-yl)cyclobutane-1,3-diamine (239 mg, 0.7 mmol) and wet Pd/C (50%, 60 mg) in MeOH (30 mL) was stirred under H_2 (balloon) at room temperature for 3 h. The reaction mixture was then filtered through a cake of Celite and washed with methanol. The filtrate was concentrated *in vacuo* to give *N*2-(3-(benzo[*d*]thiazol-2-ylamino)cyclobutyl)pyridine-2,3-diamine **29** (211 mg, 0.7 mmol, 97% yield). ESI-MS [M+1] = 312 calculated for $C_{16}H_{17}N_5S$ [M] = 311.

Step 7. *N*2-(3-(benzo[*d*]thiazol-2-ylamino)cyclobutyl)pyridine-2,3-diamine **29** (105 mg, 0.3 mmol) was combined with tetramethyl orthocarbonate (1 mL) and propionic acid (8 mg). The resulting mixture was heated to 90 ^{o}C for 2 h. The reaction solution was then concentrated under reduced pressure and the residue was purified by column chromatography on silica gel to give *N*-(3-(2-methoxy-3*H*-imidazo[4,5-*b*]pyridin-3-yl)cyclobutyl)benzo[*d*]thiazol-2-amine **24** (70 mg, 0.2 mmol, 63% yield). ESI-MS [M+1] = 352 calculated for $C_{18}H_{17}N_5OS$ [M] = 351. ^{1}H NMR (CHLOROFORM-d, 400 MHz): δ (ppm) 8.21-8.20 (m, 1 H); 7.79-7.76 (m, 1 H); 7.61-7.56(m, 2 H); 7.30-7.26 (m, 1 H); 7.16-7.09 (m, 2 H); 4.87-4.85 (m, 1 H); 4.32-4.31 (m, 1 H); 4.21 (s, 3 H); 3.15-3.11 (m, 4 H).

Cis and trans isomers of *N*-(3-(2-methoxy-3*H*-imidazo[4,5-*b*]pyridin-3-yl)cyclobutyl)benzo[*d*]thiazol-2-amine (24a and 24b). *N*-(3-(2-methoxy-3*H*-imidazo[4,5-*b*]pyridin-3-yl)cyclobutyl)benzo[*d*]thiazol-2-amine **24** (800 mg, 2.27 mmol) was separated by chiral *prep*. HPLC (Column: Chiralcel OD-H 250 x 30 mm, 5u; Mobile phase: 85% hexane in EtOH with 0.05% diethyl amine; Flow rate: 30 mL/min) to give the cis and trans isomers.

Cis isomer *N*-((1*r*,3*r*)-3-(2-methoxy-3*H*-imidazo[4,5-*b*]pyridin-3-yl)cyclobutyl)benzo[*d*]thiazol-2-amine **24a** (0.342 g, 0.97 mmol, 42% yield). Absolute stereospecificity was determined by NOE.

HRMS calculated for $C_{18}H_{18}N_5OS$ [M+1] = 352.1232, found 352.1226. ^{1}H NMR (CHLOROFORM-d, 400 MHz): δ (ppm) 8.22-8.20 (m, 1 H); 7.80-7.78 (m, 1 H); 7.62-7.58(m, 2 H); 7.32-7.29 (m, 1 H); 7.17-7.08 (m, 2 H); 6.94 (brs, 1H); 4.92-4.84 (m, 1 H); 4.37-4.33 (m, 1 H); 4.21 (s, 3 H); 3.16-3.12 (m, 4 H). ^{13}C NMR (75 MHz, CHLOROFORM-d): δ (ppm) 166.3, 158.0, 152.5, 147.1, 140.8, 133.3, 130.5, 126.0, 124.6, 121.7, 120.8, 119.0, 117.8, 57.1, 44.3, 41.3, 36.8.

Trans isomer N-((1*s*,3*s*)-3-(2-methoxy-3*H*-imidazo[4,5-*b*]pyridin-3-yl)cyclobutyl)benzo[*d*]thiazol-2-amine **24b** (0.2 g, 0.5 mmol, 23% yield). Absolute stereospecificity was determined by NOE. HRMS calculated for $C_{18}H_{18}N_5OS$ [M+1] = 352.1232, found 352.1230. ^{1}H NMR (CHLOROFORM-d, 400 MHz): δ (ppm) 8.21-8.19 (m, 1 H); 7.79-7.77 (m, 1 H); 7.66-7.61 (m, 2 H); 7.33-7.31 (m, 1 H); 7.16-7.11 (m, 2 H); 6.28 (brs, 1H); 5.46-5.41 (m, 1 H); 4.63-4.61 (m, 1 H); 4.26 (s, 3 H); 3.58-3.50 (m, 2 H); 2.66-2.59 (m, 2H). ^{13}C NMR (75 MHz, CHLOROFORM-d): δ (ppm) 166.9, 158.2, 152.3, 147.2, 141.1, 133.0, 130.7, 126.0, 124.4, 121.7, 120.9, 119.1, 117.8, 57.1, 46.6, 44.7, 35.4.

PHARMACOLOGY

PDE10A Biochemical Assay.

Functional inhibition of human recombinant PDE10A was measured as described in the IMAP TR-FRET (time-resolved fluorescence energy transfer) assay kit protocol (Molecular Devices, Sunnyvale, CA. cat. nos. R8160, R8176, or R8159). Compound was serially diluted in 100% DMSO from a 10 mM stock and further diluted in Complete reaction buffer (10 mM Tris-HCl, pH 7.2, 10 mM $MgCl_2$, 0.05% NaN_3, 0.01% Tween 20, and 1 mM freshly added DTT) to a 4× concentration. Recombinant PDE10A enzyme and cAMP substrate were diluted in Complete reaction buffer. The optimized binding buffer was 70% binding buffer A and 30% binding buffer B. Binding reagent (1:800) and terbium donor (1: 400) were added to the binding buffer. All incubations were carried out at room temperature. Testing or

control compounds (5 μL of each 4× concentration per well, 12-point dose−response curve ranging from 5.1 pM to 10 μM, tested in quadruplicate) were incubated with 5 μL of recombinant human PDE10A (0.06 units per well) in a 384-well microplate. After 30 min, 10 μL of cAMP substrate was added to each well for a final substrate concentration of 100 nM. After 1 h, 60 μL of binding buffer was added to each well. The plate was then incubated from 3 h to overnight before reading on an EnVision plate reader. The IMAP binding reagent binds to the nucleotide monophosphate generated from cyclic nucleotides (cAMP/cGMP) through phosphodiesterases and enables measurement of substrate turnover.

Permeability and Transcellular Transport.

Materials. Digoxin and mannitol were purchased from Sigma-Aldrich (St. Louis, MO). ^{3}H-Digoxin and ^{14}C-mannitol were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). Transport buffer was prepared using Hank's Balanced Salt Solution (HBSS) supplemented with 10 mM Hepes, pH 7.4, and 0.1% BSA (HHBSS, Invitrogen, Grand Island, NY, BSA, Bovine Serum Albumin, Calbiochem, La Jolla, CA). Cell Lines and Cultures. Cultures were incubated at 37 °C in a humidified (95% relative humidity) atmosphere of 5% CO_2/95% air. The parental cell line LLC-PK1 (porcine renal epithelial cells) was purchased from American Type Culture Collection (ATCC, Manassas, VA). Human MDR1 and Sprague−Dawley rat mdra1 transfectants in LLC-PK1 were generated at Amgen (Thousand Oaks, CA). Cells were cultured in Medium 199 supplemented with 2 mM L-glutamine, penicillin (50 units/mL), streptomycin (50 μg/mL), and 10% (v/v) fetal bovine serum (all from Invitrogen).[1] Permeability and Transcellular Transport of Test Compounds. LLC-PK1, MDR1-LLC-PK1, and mdr1a-LLC-PK1 cell monolayers were seeded onto porous (1.0 μm) polycarbonate 96-well transwell membrane filters (Millipore Corp., Billerica, MA) and cultured for six days with one media replacement on day four prior to transwell experiments. Cells were washed once with warmed HHBS prior to transwell experiments. Experiments were initiated by replacing the buffer in each compartment with 0.15 mL of HBSS containing 0.1% BSA with and without 5 μM of test compound in triplicate wells. The plates

were incubated for 2 h at 37 °C in an EVO incubator with shaking. Aliquots (100 μL) from both donor and receiver chambers were transferred to 96-well plates or scintillation vials. Protein was precipitated by addition of 200 μL of acetonitrile containing 0.1% formic acid and prazosin (25 ng/mL) as internal standard. After vortexing and centrifugation at 3000 rpm for 20 min, 150 μL supernatant samples were transferred to a new plate containing 50 μL of water for LC-MS/MS analysis. Transcellular transport of ^{3}H-digoxin was used as a positive control for P-gp. Paracellular permeability of ^{14}C-mannitol was used to measure the integrity of the monolayer. Sample radioactivity was measured using a liquid scintillation counter (Packard Tri-Carb 2910TR, PerkinElmer).

The apparent permeability coefficient (Papp) of all tested agents was estimated from the slope of cumulative amount (dQ) of the agent vs time (dt), and the equation:

$$P\text{app} = (\mathrm{d}Q/\mathrm{d}t)/(A \times C0)$$

where dQ/dt is the penetration rate of the agent (μm/s), A is the surface area of the cell layer on the Transwell (0.11 cm2), and C0 is the initial concentration of the test compound (μM).

Rat and Human Liver Microsomal Assays.

Liver microsomal stability was measured at 37 °C in phosphate buffer (66.7 mM, pH 7.4). Test compounds (1 μM) were incubated with pooled human or rat liver microsomes at 0.25 mg/mL of protein, with or without NADPH (1 mM). After 30 min, the reaction was stopped by the addition of acetonitrile containing 0.5% formic acid and internal standard. The quenched samples were centrifuged at 1650g for 20 min. The supernatants were analyzed directly for unchanged test compound using liquid chromatography and tandem mass spectrometric detection (LC-MS/MS). Intrinsic clearance was calculated based on substrate disappearance rate, assuming first-order elimination of compound over the 30 min incubation.

Ex vivo RO assay

Animals. Adult male Sprague Dawley rats® weighing 180-225 g (Harlan, San Diego) were cared for in accordance to the Guide for the Care and Use of Laboratory Animals, 8th Edition. Animals were group-housed at an Association for Assessment and Accreditation of Laboratory Animal Committee, internationally- accredited facility in non-sterile ventilated micro-isolator housing on corn cob bedding. All research protocols were approved by the Amgen, Thousand Oaks Institutional Animal Care and Use Committee (IACUC). Animals had *ad libitum* access to pelleted feed (Harlan Teklad 2020X, Indianapolis, IN) and water (on-site generated reverse osmosis) via automatic watering system. Animals were maintained on a 12:12 hr light: dark cycle in rooms at (70 ± 5 °F, 50 ± 20 % RH) and had access to enrichment opportunities (nesting materials and plastic domes). All animals were sourced from approved vendors who meet or exceed animal health specifications for the exclusion of specific pathogens (i.e., mouse parvovirus, Helicobacter). Rats were allowed at least 3 days of acclimation prior to any procedures.

Ex vivo RO assay with po administration. PDE10A inhibitors were dissolved in 2% Hydroxypropylmethylcellulose (HPMC), 1% Tween-80, pH 2.2 with methanesulfonic acid. 4 rats per group were dosed orally with either vehicle or 3 mg/kg PDE10A inhibitors and then returned to their home cage to allow for absorption of the compounds. After four hours rats were sacrificed by CO_2 inhalation.

Sample Analysis and Receptor Occupancy Determination. Blood was obtained by heart puncture and plasma was frozen and stored at -80°C for exposure analysis. Brains were removed and immediately frozen in chilled methylbutane, and stored at -80°C until cutting. Three coronal brain slices per brain containing the striatum were cut at 20 µm using a cryostat and placed onto microscope slides, air-dried and stored at -20 ^{o}C. For radioligand binding experiments, slides were thawed at room temperature and

then incubated with 1nM ^{3}H-AMG7980 in binding buffer (150 mM Phosphate-buffered saline containing 2 mM $MgCl_2$ and 100 mM DTT, pH 7.4) for 1 minute at 4 °C. To assess non-specific binding, slides containing adjacent brain sections were incubated in the same solution with addition of 10 mM of test compound. Afterwards slides were washed 3 times in ice-cold binding buffer, dipped into distilled water to remove buffer salts, and dried under a stream of cold air. Emission of beta particles from the sections was counted for 8 hours in a Beta Imager 2000 (Biospace, Paris, France) and digitized and analyzed using M3 Vision software (Biospace, Paris, France). Total binding radioactivity in the striatum was measured as cpm/mm^2 in hand-drawn regions of interest and averaged across the three sections per brain. Non-specific binding was subtracted to obtain specific binding values and percent occupancy was calculated by setting vehicle specific binding as 0% occupancy.

LC-MS/MS RO Assay

Animals. All experiments were conducted under approved research protocols by Amgen's Animal Care and Use Committee (IACUC) and in accordance with National Institutes of Health Guide for Care and Use of Laboratory Animals guidelines in facilities accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AALAC). Adult male Sprague-Dawley® (SD) rats (250-280 g) were purchased from Harlan (Harlan, Indianapolis). Rats were group housed on a filtered, forced air isolation rack, and maintained on sterile wood chip bedding in a quiet room on a 12 hour light-dark cycle, with food and water available *ad libitum*. Animals were allowed for minimum 3 days of adaptation to the laboratory conditions prior to being utilized in the experiments.

LC-MS RO assay with po administration. Sprague Dawley rats were pretreated with vehicle or compound via po dosing. Fifty minutes after dosing, our PDE10A tracer AMG7980[2] was administered by bolus IV injection via lateral tail vein. Ten minutes after tracer injection, animals under isoflurane anesthesia were euthanized by decapitation; samples of blood and brain samples were collected for analysis.

Sample Analysis and Receptor Occupancy Determination. Striatum was used as the target tissue due to high endogenous expression of PDE10A protein, and thalamus was chosen as the reference tissue due to lower expression of PDE10A.[3]

Brain samples were weighed and HPLC-grade water was added (20% weight/volume), followed by homogenization using a Covaris E110 Acoustic Homogenizer (Model: E210, Covaris, Inc., Woburn, MA). Covaris settings: 4 ^{o}C, Duty Cycle 20%, Intensity 8, Cycles per Burst 500, Treatment time 6 x 10 seconds. Homogenized samples were stored at -20 °C. Extraction and analysis of compound and AMG7980 tracer concentrations by API 4000 LC-MS/MS (Applied Biosystems, Carlsbad, CA).

PDE10A occupancy based on the reference tissue model was determined using the following equations:

$BP = (STR - THA)/THA$

$RO\% = 100 \times [1 - (BP_{drug}/BP_{veh})]$

where "BP" refers to binding potential, "RO" refers to receptor occupancy, "BP_{drug}" refers to the binding potential of the test article dosed and "BP_{veh}" refers to the binding potential of the vehicle.

Note: Animals regarded as "mis-dosed" were excluded from data analysis due to experimental error during day of experiment.

Plasma Sample Preparation and Analysis. Blood was collected individually in Microtainer EDTA tubes, mixed and centrifuged at 4 ^{o}C for 10 min. The plasma sample (top aqueous phase) was then pipetted into a 96 well V-bottom plate. Plasma samples were stored at -20 ^{o}C. Extraction and analysis of compound and AMG7980 tracer concentrations by API 4000 LC-MS/MS (Applied Biosystems, Carlsbad, CA).

Statistical Analysis

Results were expressed as the mean ± SEM (standard error of mean). Curve fit was assessed using one site-specific binding non-linear regression on GraphPad Prism software, version 5 (GraphPad Inc., San Diego, CA).

Analysis: One site – Specific binding

Equation: Y = Bmax * X / (Kd + X)

PROTEIN CRYSTALLIZATION, X-RAY DATA COLLECTION

Diffraction quality crystals were obtained by mixing 2 µL of apo protein (in 25 mM HEPES pH 7.5, 150 mM NaCl, and 1 mM TCEP) with 2 µL of reservoir solution (0.1 M MES monohydrate pH 6.5, 1.6 M Ammonium sulfate, and 10% v/v 1,4-Dioxane) in a hanging drop that was incubated at room temperature. Prism-shaped apo crystals were transferred into 5 µL of cryoprotectant solution (15% Glycerol, 1.6 M Ammonium sulfate, 10% v/v 1,4-Dioxane, and 0.1 M MES monohydrate at pH 6.5) containing 1 mM final concentration of compound **7** or compound **24** and incubated for 12 h. Crystals were then directly frozen in liquid nitrogen for data collection.

X-ray diffraction data for compound **7** and compound **24** were collected on Rigaku RAxis IV++/FR-E SuperBright. Diffraction data were processed and scaled using HKL2000.[4] The crystals belong to the space group F23 with approximate unit cell dimensions of $a = b = c = 252$ Å. Molecular replacement was performed using MOLREP.[5] The structure was refined using Refmac5,[6] and the model with ligand was built using Coot (4).[7]

Crystal	PDE10a + compound 7	PDE10a + compound 24
Data Collection [a]		
Space group	F23	F23
Unit cell parameters (Å)	a=252.97, b=252.97, c=252.97	a=253.28, b=253.28, c=253.28
Resolution (Å)	30-2.24 (2.24-2.28)	30-2.08 (2.13-2.08)
Total Reflections	63577	75363
Unique reflections	60631 (3158)	77609 (3059)
Completeness (%)	99.8 (99.5)	98.6 (76.0)
R_{merge} [b]	10.9 (39.9)	5.2 (45.7)
$I/\sigma(I)$	12.39 (3.65)	27.3 (2.77)
Refinement Statistics		
R/R_{free} (%)	15.5 / 17.4	15.9/18.2
Δbonds (Å)	0.005	0.005
Δangles (deg)	0.863	1.056

[a]Values in parentheses are for the highest resolution shell.

[b]$R_{merge} = \Sigma(|(I - < I >)|/\Sigma (I)$.

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