Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supplementary Information

Design, Synthesis and Evaluation of N^6-Substituted 2-Aminoadenosine-5'-*N*-methylcarboxamides as A_3 Adenosine Receptor Agonists

Shane M. Devine,[a] Lauren T. May,[b] Peter J. Scammells[a*]

[a] Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia

[b] Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia

* Corresponding author. Email: peter.scammells@monash.edu

Experimental

General Experimental

All microwave reactions took place in a Biotage Initiator Microwave Synthesiser. NMR spectra (^{1}H, ^{19}F, ^{13}C) were recorded on a Bruker Avance Nanobay III 400 MHz Ultrashield Plus spectrometer at 400.13, 376.50 and 100.62 MHz, respectively coupled to a BACS 60 automatic sample changer at 25 °C or a Bruker Avance DPX 300 MHz spectrometer at 300.13 and 75.49 MHz, respectively. Chemical shifts (δ) are recorded in parts per million (ppm) by correction with reference to the chemical shift of the solvent, according to the procedure described by Gottlieb.[1] Coupling constants (*J*) are recorded in Hz, and the significant multiplicities described by singlet (s), doublet (d), triplet (t), quadruplet (q), broad (br), multiplet (m), doublet of doublets (dd), and doublet of triplets (dt). LC-MS were run to verify reaction outcome and purity using an Agilent 6100 series Single Quad coupled to an Agilent 1200 series HPLC. The following buffers were used: buffer A, 0.1% formic acid in H_2O; buffer B, 0.1% formic acid in MeCN. The following gradient was used with a Phenomenex Luna 3 μM C8(2) 15 mm × 4.6 mm column, and a flow rate of 0.5 mL/min and total run time of 12 min; 0–4 min 95% buffer A and 5% buffer B, 4–7 min 0% buffer A and 100% buffer B, 7–12 min 95% buffer A and 5% buffer B. Mass spectra were acquired in positive and negative ion mode with a scan range of 0–1000 *m*/*z* at 5 V. UV detection was carried out at 254 nm. All retention times (t_R) are quoted in minutes. All compounds were of >95% purity. Thin layer chromatography was conducted on 0.2 mm plates using Merck silica gel 60 F_{254}. Column chromatography was achieved using Merck silica gel 60 (particle size 0.063–0.200 μm, 70–230 mesh). O^6-(benzotriazol-1-yl)-2',3'-*O*-isopropylideneguanosine-5'-*N*-methylcarboxamide (**1**) and O^6-(benzotriazol-1-yl)-2-fluoro-2',3'-*O*-isopropylideneinosine-5'-*N*-methylcarboxamide (**4**) were prepared as previously reported.[2]

General procedure for N^6-substituted, 2-aminoadenosine-5'-*N*-methylcarboxamides:

To a solution of O^6-(benzotriazol-1-yl)-2',3'-*O*-isopropylideneguanosine-5'-*N*-methylcarboxamide (**1**) (200 mg, 0.43 mmol) in *t*-BuOH (3 mL), was added DIPEA (3.0 eq, 1.28 mmol) and the appropriate amine (1.5 eq, 0.64 mmol) and heated at 80 °C for 2 h in a microwave reactor. The solution was then evaporated under reduced pressure to give an orange gum. This was taken up in DCM (50 mL) and washed with H_2O (3 × 10 mL), then sat. NaCl (10 mL). The organic phase was dried with $MgSO_4$, filtered and the filtrate evaporated under reduced pressure to give a crude solid (**2**). This solid was taken up in a 4:1 mixture of MeCN/1M HCl (10 mL) and heated at 60 °C for 1 h, then evaporated under reduced pressure. The crude residue was taken up in EtOAc (50 mL) and washed with $NaHCO_3$ (50 mL, then 2 × 15 mL), then sat NaCl (10 mL). The residue was purified by column chromatography (DCM/MeOH/NH_4OH, 89:10:1) to give (**3**) typically as a colourless or beige glassy solid.

2-Amino-N^6-methyladenosine-5'-*N*-methylcarboxamide (3a)

Compound **1** (200 mg, 0.43 mmol) gave **3a** (85 mg, 63%) over 2 steps**.** ^{1}H NMR (300 MHz, D_2O): δ 8.15 (br s, 1H), 6.04 (d, *J* = 6.3 Hz, 1H), 4.70 – 4.67 (m, 1H), 4.57 – 4.53 (m, 2H), 3.10 (s, 3H), 2.78 (s, 3H). ^{13}C NMR (101 MHz, DMSO): δ 170.0, 160.3, 155.0, 150.6, 136.9, 114.0, 87.0, 84.3, 73.1, 71.4, 25.8, 25.0. HR-ESMS calcd for $C_{12}H_{18}N_7O_4^+$ (M+H) 324.1415, found 324.1414.

2-Amino-N^6-ethyladenosine-5'-*N*-methylcarboxamide (3b)

Compound **1** (200 mg, 0.43 mmol) gave **3b** (92 mg, 64%) over 2 steps. ^{1}H NMR (400 MHz, MeOD): δ 7.89 (s, 1H), 5.86 (d, *J* = 7.5 Hz, 1H), 4.91 (dd, *J* = 7.4, 5.0 Hz, 1H), 4.42 (d, *J* = 1.7 Hz, 1H), 4.32 (dd, *J* = 4.9, 1.7 Hz, 1H), 3.56 (d, *J* = 6.0 Hz, 2H), 2.87 (s, 3H), 1.27 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO): δ 170.0, 160.3, 154.9, 150.6, 136.9, 114.0, 87.0, 84.3, 73.1, 71.4, 34.3, 25.6, 15.2. HR-ESMS calcd for $C_{13}H_{20}N_7O_4^+$ (M+H) 338.1571, found 338.1577.

2-Amino-N^6-propyladenosine-5'-*N*-methylcarboxamide (3c)

Compound **1** (200 mg, 0.43 mmol) gave **3c** (90 mg, 60%) over 2 steps. ^{1}H NMR (400 MHz, MeOD): δ 7.89 (s, 1H), 5.86 (d, *J* = 7.5 Hz, 1H), 4.91 (dd, *J* = 7.4, 5.0 Hz, 1H), 4.42 (d, *J* = 1.7 Hz, 1H), 4.32 (dd, *J* = 4.9, 1.5 Hz, 1H), 3.49 (s, 2H), 2.87 (s, 3H), 1.78 – 1.60 (m, 2H), 1.01 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO): δ 170.0, 160.3, 155.1, 150.6, 136.9, 114.0, 87.0, 84.3, 73.1, 71.4, 41.2, 25.6, 22.5, 11.4. HR-ESMS calcd for $C_{14}H_{22}N_7O_4^+$ (M+H) 352.1728, found 352.1732.

2-Amino-N^6-butyladenosine-5'-*N*-methylcarboxamide (3d)

Compound **1** (200 mg, 0.43 mmol) gave **3d** (97 mg, 62%) over 2 steps. ^{1}H NMR (400 MHz, MeOD): δ 7.88 (s, 1H), 5.86 (d, *J* = 7.5 Hz, 1H), 4.91 (dd, *J* = 7.4, 5.0 Hz, 1H), 4.43 (d, *J* = 1.7 Hz, 1H), 4.33 (dd, *J* = 4.9, 1.7 Hz, 1H), 3.51 (br s, 2H), 2.87 (s, 3H), 1.73 – 1.54 (m, 2H), 1.53 – 1.33 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, MeOD): δ 173.0, 162.1, 156.7, 151.7, 138.9, 115.5, 90.0, 86.1, 74.8, 72.7, 41.1, 32.8, 26.3, 21.1, 14.2. HR-ESMS calcd for $C_{15}H_{24}N_7O_4^+$ (M+H) 366.1884, found 366.1885.

2-Amino-N^6-cyclobutyladenosine-5'-*N*-methylcarboxamide (3e)

Compound **1** (200 mg, 0.43 mmol) gave **3e** (110 mg, 71%) over 2 steps. ^{1}H NMR (400 MHz, MeOD): δ 7.89 (s, 1H), 5.86 (d, *J* = 7.5 Hz, 1H), 4.90 (dd, *J* = 7.4, 5.0 Hz, 1H), 4.67 (s, 1H), 4.42 (d, *J* = 1.7 Hz, 1H), 4.33 (dd, *J* = 4.9, 1.7 Hz, 1H), 2.86 (s, 3H), 2.46 – 2.35 (m, 2H), 2.09 – 1.97 (m, 2H), 1.83 – 1.70 (m, 2H). ^{13}C NMR (101 MHz, MeOD): δ 172.9, 162.1, 155.6, 151.9, 139.1, 115.3, 90.0, 86.1, 74.8, 72.7, 46.8, 32.1 (×2), 26.3, 15.9. HR-ESMS calcd for $C_{15}H_{22}N_7O_4^+$ (M+H) 364.1728, found 364.1717.

2-Amino-N^6-cyclopentyladenosine-5'-*N*-methylcarboxamide (3f)

Compound **1** (200 mg, 0.43 mmol) gave **3f** (126 mg, 78%) over 2 steps. ^{1}H NMR (400 MHz, MeOD): δ 7.89 (s, 1H), 5.85 (d, *J* = 7.5 Hz, 1H), 4.91 (dd, *J* = 7.4, 5.0 Hz, 1H), 4.51 (br s, 1H), 4.42 (d, *J* = 1.7 Hz, 1H), 4.32 (dd, *J* = 4.9, 1.6 Hz, 1H), 2.87 (s, 3H), 2.15 – 2.00 (m, 2H), 1.88 – 1.73 (m, 2H), 1.73 – 1.51 (m, 4H). ^{13}C NMR (101 MHz, MeOD): *δ* 173.0, 162.2, 156.2, 138.9, 115.5, 90.0, 86.1, 74.8, 72.6, 53.2, 34.0 (×2), 26.3, 24.7 (×2). HR-ESMS calcd for $C_{16}H_{24}N_7O_4^+$ (M+H) 378.1884, found 378.1889.

2-Amino-N^6-cyclohexyladenosine-5'-*N*-methylcarboxamide (3g)

Compound **1** (200 mg, 0.43 mmol) gave **3g** (125 mg, 75%) over 2 steps. ^{1}H NMR (400 MHz, MeOD): *δ* 7.89 (s, 1H), 5.85 (d, *J* = 7.5 Hz, 1H), 4.90 (dd, *J* = 7.5, 4.9 Hz, 1H), 4.42 (d, *J* = 1.7 Hz, 1H), 4.32 (dd, *J* = 4.9, 1.6 Hz, 1H), 4.07 (br s, 1H), 2.87 (s, 3H), 2.04 – 2.00 (m, 2H), 1.83–1.78 (m, 2H), 1.68 – 1.64 (m, 1H), 1.49 – 1.27 (m, 5H). ^{13}C NMR (101 MHz, MeOD): *δ* 173.0, 162.2, 155.8, 150.7, 138.9, 115.4, 90.0, 86.1, 74.8, 72.6, 49.8, 34.1 (×2), 26.8 (×2), 26.3, 26.0. HR-ESMS calcd for $C_{17}H_{26}N_7O_4^+$ (M+H) 392.2041, found 392.2053.

2-Amino-N^6-benzyladenosine-5'-*N*-methylcarboxamide (3h)

Compound **1** (200 mg, 0.43 mmol) gave **3h** (107 mg, 63%) over 2 steps. ^{1}H NMR (300 MHz, D_2O): δ 8.06 (br s, 1H), 7.42 – 7.34 (m, 5H), 6.00 (d, *J* = 6.6 Hz, 1H), 4.75 – 4.67 (m, 3H), 2.78 (s, 3H). ^{13}C NMR (101 MHz, DMSO): δ 170.0, 160.3, 155.1, 150.6, 138.1, 136.9, 128.9 (×2), 127.8 (×2), 127.3, 114.0, 87.0, 84.3, 73.1, 71.4, 48.0, 25.6. HR-ESMS calcd for $C_{18}H_{22}N_7O_4^+$ (M+H) 400.1728, found 400.1729.

2-Amino-N^6-(3-chlorobenzyl)adenosine-5'-*N*-methylcarboxamide (3i)

Compound **1** (200 mg, 0.43 mmol) gave **3i** (140 mg, 75%) over 2 steps. ^{1}H NMR (400 MHz, DMSO) δ 8.41 (d, *J* = 4.5 Hz, 1H), 8.04 (br s, 1H), 8.01 (s, 1H), 7.38 (s, 1H), 7.36 – 7.25 (m, 3H),

5.92 (br s, 2H), 5.81 (d, *J* = 7.5 Hz, 1H), 5.63 (d, *J* = 4.3 Hz, 1H), 5.49 (d, *J* = 6.5 Hz, 1H), 4.69 – 4.64 (m, 3H), 4.25 (d, *J* = 1.6 Hz, 1H), 4.18 – 4.06 (m, 1H), 2.71 (d, *J* = 4.7 Hz, 3H). ^{13}C NMR (101 MHz, DMSO): δ 169.9, 160.2, 154.8, 143.2, 137.2, 132.8, 130.1, 127.0, 126.5, 126.0, 86.9, 84.3, 73.0, 71.5, 48.6, 25.6. HR-ESMS calcd for $C_{18}H_{21}ClN_7O_4^+$ (M+H) 434.1338, found 434.1351.

2-Amino-*N*^{6}-(3-iodobenzyl)adenosine-5'-*N*-methylcarboxamide (3j)

Compound **1** (200 mg, 0.43 mmol) gave **3j** (135 mg, 60%) over 2 steps. ^{1}H NMR (400 MHz, DMSO) δ 8.41 (d, *J* = 4.2 Hz, 1H), 8.01 (br s, 2H), 7.71 (s, 1H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.37 (d, *J* = 7.8 Hz, 1H), 7.11 (t, *J* = 7.7 Hz, 1H), 5.92 (br s, 2H), 5.81 (d, *J* = 7.5 Hz, 1H), 5.63 (d, *J* = 4.2 Hz, 1H), 5.49 (d, *J* = 6.4 Hz, 1H), 4.71 – 4.64 (m, 1H), 4.60 (br s, 2H), 4.25 (d, *J* = 1.4 Hz, 1H), 4.11 (td, *J* = 4.5, 1.6 Hz, 1H), 2.71 (d, *J* = 4.7 Hz, 3H). ^{13}C NMR (101 MHz, DMSO): δ 169.9, 160.1, 154.8, 143.2, 137.1, 135.7, 135.2, 130.4, 126.7, 113.9, 94.6, 86.9, 84.2, 73.0, 71.5, 41.9, 25.5. HR-ESMS calcd for $C_{18}H_{21}IN_7O_4^+$ (M+H) 526.0694, found 526.0701.

2-Amino-*N*^{6}-phenethyladenosine-5'-*N*-methylcarboxamide (3k)

Compound **1** (200 mg, 0.43 mmol) gave **3m** (103 mg, 58%) over 2 steps. ^{1}H NMR (300 MHz, MeOD): δ 7.88 (br s, 1H), 7.28 – 7.16 (m, 5H), 5.86 (d, *J* = 7.2 Hz, 1H), 4.94 – 4.90 (m, 1H), 4.42 (s, 1H), 4.38 (d, *J* = 4.8 Hz, 1H), 3.77 (br s, 2H), 2.96 (t, *J* = 7.4 Hz, 2H), 2.87 (s, 3H). ^{13}C NMR (75 MHz, MeOD): δ 171.6, 160.7, 155.2, 139.3, 137.6, 128.5 (×2), 128.1 (×2), 126.6, 125.9, 119.6, 88.6, 84.7, 73.4, 71.2, 41.6, 35.4, 24.9. HR-ESMS calcd for $C_{19}H_{24}N_7O_4^+$ (M+H) 414.1884, found 414.1871.

2-Amino-*N*^{6}-(2,2-diphenylethyl)adenosine-5'-*N*-methylcarboxamide (3l)

Compound **1** (200 mg, 0.43 mmol) gave **3l** (154 mg, 74%) over 2 steps. ^{1}H NMR (300 MHz, DMSO): δ 8.43 (d, *J* = 4.2 Hz, 1H), 7.92 (br s, 1H), 7.35 – 7.16 (m, 10H), 5.99 (br s, 2H), 5.77 (d, *J* = 7.2 Hz, 1H), 5.64 (d, *J* = 3.9 Hz, 1H), 5.49 (d, *J* = 6.3 Hz, 1H), 4.67 – 4.57 (m, 2H), 4.23 – 4.03

(m, 4H), 2.71 (d, J = 4.5 Hz, 3H). ^{13}C NMR (75 MHz, DMSO): δ 170.4, 160.7, 155.3, 152.2, 143.5, 137.3, 128.9 (×4), 128.6 (×4), 126.7 (×2), 114.3, 87.3, 84.7, 73.5, 71.9, 50.0, 44.4, 26.0. HR-ESMS calcd for $C_{25}H_{28}N_7O_4^+$ (M+H) 490.2197, found 490.2210.

General procedure for N^6-(cyclopentyl), 2-*N*-substituted adenosine-5'-*N*-methylcarboxamides: To a solution of N^6-(cyclopentyl)-2',3'-*O*-isopropylideneguanosine-5'-*N*-methylcarboxamide (**5**) (150 mg, 0.36 mmol) in *t*-BuOH (3 mL), was added DIPEA (3.0 eq, 1.07 mmol) and the appropriate amine (5.0 eq, 1.78 mmol) and heated at 150 °C for 2 h in a microwave reactor. The solution was then evaporated under reduced pressure to give a gum. This was taken up in DCM (40 mL) and washed with H_2O (3 × 10 mL), then sat. NaCl (10 mL). The organic phase was dried with $MgSO_4$, filtered and the filtrate evaporated under reduced pressure to give a crude solid (**6**). This solid was taken up in a 4:1 mixture of MeCN/1M HCl (10 mL) and heated at 60 °C for 1 h, then evaporated under reduced pressure. The crude residue was taken up in EtOAc (30 mL) and washed with $NaHCO_3$ (50 mL, then 2 × 15 mL), then sat NaCl (10 mL). The residue was purified by column chromatography (DCM/MeOH/NH_4OH, 89:10:1) to give (**7**) typically as a colourless or beige glassy solid.

2-Methylamino-N^6-cyclopentyladenosine-5'-*N*-methylcarboxamide (7a)

Compound **4** (150 mg, 0.32 mmol) gave **7a** (82 mg, 66%) over 3 steps. ^{1}H NMR (400 MHz, MeOD): δ 7.87 (s, 1H), 5.89 (d, J = 6.8 Hz, 1H), 4.99 (dd, J = 6.7, 5.1 Hz, 1H), 4.52 (s, 1H), 4.46 (dd, J = 5.0, 2.5 Hz, 1H), 4.41 (d, J = 2.5 Hz, 1H), 2.91 (s, 3H), 2.80 (s, 3H), 2.11 – 2.02 (m, 2H), 1.82 – 1.75 (m, 2H), 1.71 – 1.54 (m, 4H). ^{13}C NMR (101 MHz, MeOD): δ 172.9, 162.2, 155.9, 152.2, 138.4, 114.9, 90.1, 85.7, 74.7, 72.9, 53.3, 34.1, 34.0, 29.0, 26.3, 24.7 (×2). HR-ESMS calcd for $C_{17}H_{26}N_7O_4^+$ (M+H) 392.2041, found 392.2054.

2-Dimethylamino-*N*^{6}-cyclopentyladenosine-5'-*N*-methylcarboxamide (7b)

Compound **4** (150 mg, 0.32 mmol) gave **7b** (93 mg, 72%) over 3 steps. ^{1}H NMR (400 MHz, MeOD) δ 7.87 (s, 1H), 5.92 (d, *J* = 6.3 Hz, 1H), 5.08 (dd, *J* = 6.2, 5.3 Hz, 1H), 4.56 (dd, *J* = 5.1, 3.1 Hz, 1H), 4.50 (s, 1H), 4.40 (d, *J* = 3.0 Hz, 1H), 3.13 (s, 6H), 2.74 (s, 3H), 2.13 – 2.03 (m, 2H), 1.81 – 1.74 (m, 2H), 1.68 – 1.55 (m, 4H). ^{13}C NMR (101 MHz, MeOD): δ 172.8, 161.5, 155.4, 152.5, 138.4, 114.1, 90.1, 85.3, 74.6, 73.0, 53.4, 37.7 (×2), 34.1, 34.0, 26.2, 24.8 (×2). HR-ESMS calcd for $C_{18}H_{28}N_7O_4^+$ (M+H) 406.2197, found 406.2208.

2-Ethylamino-*N*^{6}-cyclopentyladenosine-5'-*N*-methylcarboxamide (7c)

Compound **4** (150 mg, 0.32 mmol) gave **7c** (70 mg, 54%) over 3 steps. ^{1}H NMR (400 MHz, MeOD) δ 7.87 (s, 1H), 5.87 (d, *J* = 6.8 Hz, 1H), 4.99 (dd, *J* = 6.8, 5.0 Hz, 1H), 4.51 (br s, 1H), 4.44 (dd, *J* = 5.0, 2.5 Hz, 1H), 4.40 (d, *J* = 2.5 Hz, 1H), 3.40 (dd, *J* = 13.2, 7.2 Hz, 2H), 2.81 (s, 3H), 2.09 – 2.05 (m, 1H), 1.81 – 1.77 (m, 1H), 1.69 –1.58 (m, 2H), 1.20 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, MeOD): δ 172.9, 161.4, 155.9, 152.2, 138.4, 114.9, 90.1, 85.7, 74.7, 72.9, 53.3, 37.5, 34.2, 34.1, 26.3, 24.8 (×2), 15.4. HR-ESMS calcd for $C_{18}H_{28}N_7O_4^+$ (M+H) 406.2197, found 406.2205.

2-Diethylamino-*N*^{6}-cyclopentyladenosine-5'-*N*-methylcarboxamide (7d)

4 (150 mg, 0.32 mmol) gave **7d** (67 mg, 48%) over 3 steps. ^{1}H NMR (400 MHz, MeOD) δ 7.86 (s, 1H), 5.92 (d, *J* = 5.8 Hz, 1H), 5.05 (t, *J* = 5.5 Hz, 1H), 4.59 (dd, *J* = 5.2, 3.6 Hz, 1H), 4.50 – 4.43 (m, 1H), 4.38 (d, *J* = 3.6 Hz, 1H), 3.71 (dq, *J* = 14.0, 7.0 Hz, 2H), 3.51 (dq, *J* = 13.9, 7.0 Hz, 2H), 2.72 (s, 3H), 2.06 (dt, *J* = 18.5, 6.5 Hz, 2H), 1.84 – 1.73 (m, 2H), 1.64 (dd, *J* = 11.2, 4.6 Hz, 4H), 1.17 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, MeOD): δ 172.8, 160.0, 155.5, 152.6, 138.0, 114.0, 90.3, 85.0, 74.5, 73.4, 53.4, 43.2 (×2), 34.2, 34.1, 26.2, 24.9 (×2), 13.8 (×2). HR-ESMS calcd for $C_{20}H_{32}N_7O_4^+$ (M+H) 434.2510, found 434.2507.

cAMP Accumulation Assay

Chinese Hamster Ovary FlpIn (FlpIn-CHO) cells stably transfected with the human adenosine A_1, A_{2A}, A_{2B} and A_3 receptor were maintained in DMEM containing 10% FBS and 500 µg/mL hygromycin-B at 37°C in a humidified incubator containing 5% CO_2: 95% O_2. Cells were seeded in poly-D-lysine coated 384-well cell culture plates at 1 x 10^4 cells per well and grown until 90% confluent. Media was then replaced with 20 µL stimulation buffer (140 mM NaCl, 5 mM KCl, 0.8 mM $MgSO_4.7H_2O$, 0.2 mM $Na_2HPO_4.2H_2O$, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2.2H_2O$, 5.6 mM *D*-glucose, 5 mM HEPES, 0.1% BSA, 0.1 U/mL ADA and 10 µM rolipram, pH= 7.45) and incubated at 37 °C for 1 hour. Cells were then stimulated for 30 minutes with ligand in the absence (A_{2A}-FlpINCHO and A_{2B}-FlpINCHO) or presence (A_1-FlpINCHO and A_3-FlpINCHO) of 3 µM forskolin in a final volume of 40 µL. Buffer was then removed and cells lysed with 50 µL of 100% ethanol. Following ethanol evaporation, 20 µL of detection buffer was added (0.1% BSA, 0.3% Tween-20 and 5mM HEPES, pH= 7.45) and plates agitated for 10 minutes. Lysate (10 µL) was transferred to a 384-well Optiplate™ and 1 Unit/well of AlphaScreen™ acceptor beads, AlphaScreen™ donor beads and biotinylated cAMP were added. AlphaScreen™ donor beads and biotinylated cAMP were equilibrated for 30 mins prior to addition. Plates were incubated overnight in the dark at room temperature, followed by measurement of fluorescence by an EnVision® plate reader (PerkinElmer) using AlphaScreen™ settings. All data obtained were analysed in GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA).

References

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