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Electronic Supporting Information

Leveraging kinase inhibitors to develop small molecule tools for imaging kinases by fluorescence microscopy

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Materials and Methods

Synthesis of Mps1-IN-BODIPY The synthesis of Mps1-IN-BODIPY was adapted from the synthesis of parent compound, Mps1-IN-1.[1] The chlorine in compound **1** was displaced with *tert*-butyl 4-(1-(4-amino-3-methoxyphenyl)piperidin-4-yl)piperazine-1-carboxylate to give **2**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.33 (*s*, 1H), 8.23 (d, *J* = 8.4 Hz, 1H), 7.87 (d, *J* = 7.5 Hz, 1H), 7.55 (m, 2H), 7.10 (t, *J* = 7.8 Hz, 1 H), 6.98 (d, *J* = 3.6 Hz, 1H), 6.71 (s, 1 H), 6.57 (s, 1 H), 6.39 (s, 1 H), 6.27 (d, *J* = 3.6 Hz, 1H), 5.59 (s, 2H), 4.28-4.04 (b, 2H), 3.88 (s, 3H), 3.62 (t, *J* = 8.1 Hz, 2H), 3. 31 (m, *J* = 6.9 Hz, 1H), 3. 17 (b, 4H), 2.76 (b, 4H), 2.70 (b, 2H), 2.45 (tt, 1H), 1.86 (bd, *J* = 10.8 Hz, 2H), 1.46 (s, 9H), 1.44 (b, 2H), 1.28 (*d*, *J* = 6.9 Hz, 6H), 0.95 (t, *J* = 8.1 Hz, 2H), -0.07 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 154.6, 153.2, 149.1, 148.5, 146.2, 142.2, 141.6, 134.7, 131.6, 124.5, 123.5, 122.6, 121.0, 120.0, 119.1, 108.4, 104.8, 101.0, 98.7, 90.1, 79.5, 72.9, 71.9, 66.1, 61.9, 55.6, 54.5, 50.9, 49.2, 28.6, 28.5, 17.8, 15.4, -1.3; (*m/z*): [M+1] 834.3. Deprotection to remove the BOC and SEM groups of compound **2** followed by amide coupling with BODIPY acid produced the target Mps1-IN-BODIPY. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.11 (*s*, 1H), 8.43 (*s*, 1H), 8.20 (d, *J* = 8.8 Hz, 1H), 7.78 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.67 (m, 3H), 7.56 (s, 1H), 7.19 (t, *J* = 7.2 Hz, 1H), 7.08 (d, *J* = 4.0 Hz,1H), 6.92 (dd, *J* = 3.2, 2.4 Hz, 1H), 6.59 (s, 2H), 6.41 (d, *J* = 4.0 Hz, 2H), 6.28 (s, 1H), 6.05 (dd, *J* = 3.2, 2.0Hz,1H), 4.41 (m, 1H), 3.91 (m, 1H), 3.79 (s, 3H), 3.38 (m, *J* = 7.2 Hz, 1 H), 3.06 (m, 5 H), 2.98 (t, *J* = 12.0 Hz, 2 H), 2.72 (q, *J* = 6.8 Hz, 2 H), 2.61 (b, 5 H), 2.45 (s, 3 H),

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2.24 (s, 3 H), 1.79 (b, 2 H), 1.21 (s, 1 H), 1.14 (d, *J* = 6.8 Hz, 6H); HRMS ESI calculated for $C_{46}H_{55}BF_2N_9O_4S$ [M+H$^+$] 878.4159; found 878.4157.

Synthesis of BI-BODIPY The synthesis of BI-BODIPY was adapted from the synthesis of parent compound, BI2536.[2] Amide coupling of **3** with *t*-butyl 4-((1*R*,4*R*)-4-aminocyclohexyl)piperazine-1-carboxylate afforded compound **4**. Deprotection of compound **4** followed by amide coupling with BODIPY acid produced the target BI-BODIPY. ^{1}H NMR (400 MHz, CD_3OD) δ 8.47 – 8.39 (m, 1H), 7.66 (s, 1H), 7.42–7.31 (m, 3H), 6.91 (d, *J* = 4.0 Hz, 1H), 6.23 (d, *J* = 4.0 Hz, 1H), 6.12 (s, 1H), 4.70–4.52 (m, 2H), 4.23 (dd, *J* = 7.6, 3.4 Hz, 1H), 4.10–3.91 (m, 2H), 3.90 (s, 3H), 3.71 (d, *J* = 3.7 Hz, 2H), 3.50 (d, *J* = 3.9 Hz, 2H), 3.45–3.36 (m, 2H), 3.11 (t, *J* = 7.6 Hz, 2H), 2.76–2.65 (m, 2H), 2.47 (dd, *J* = 11.9, 6.8 Hz, 2H), 2.42 (s, 4H), 2.29 (s, 1H), 2.19 (s, 3H), 1.92 (d, *J* = 1.6 Hz, 3H), 1.85 (d, *J* = 6.8 Hz, 2H), 1.85–1.74 (m, 3H), 1.68 (dd, *J* = 14.5, 7.3 Hz, 2H), 1.41 – 1.32 (m, 4H), 1.34 –1.26 (m, 6H), 1.16 (dd, *J* = 15.0, 7.8 Hz, 2H), 0.75 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 171.2, 167.6, 163.8, 160.1, 156.8, 155.5, 151.9, 147.1, 144.6, 137.9, 135.2, 133.5, 132.7, 128.2, 126.4, 124.4, 120.0, 119.9, 116.8, 116.1, 115.60, 108.7, 62.7, 60.1, 58.0, 55.2, 48.8, 48.7, 48.5, 45.3, 41.4, 31.9, 31.1, 27.4, 27.2, 26.6, 24.3, 20.0, 18.6, 13.5, 13.0, 12.6, 9.8, 7.6; HRMS ESI calculated for $C_{44}H_{57}BF_2N_{10}O_4$ [M+H$^+$]: 839.4704; found 839.4708.

Cell Culture. Hela S3 cells were grown in DMEM medium supplemented with 10% FBS and 1% penicillin/ streptomycin and incubated at 37^{o}C and 5% CO_2.

***In vitro* kinase assay.** Kinase assays for Mps1, PLK1, PLK2, and PLK3 were conducted at Life Technologies. Specifically, inhibition of Mps1 by Mps1-IN-1 and Mps1-IN-BODIPY was measured by LanthaScreen™ Kinase assay (LifeTechnologies; see http://tools.invitrogen.com/content/SFS/lanthaScreen/PV3792%20TTK%20Assay%20Validation.pdf) and inhibition of PLK1, PLK2, and PLK3 by BI2536 and BI-BODIPY were measured by

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Z´-Lyte™ kinase assay (see https://tools.invitrogen.com/content/sfs/manuals/zlyte_serthr_16_man.pdf).

Mitotic Arrest Assay. Hela S3 cells were plated at 80% confluence one day prior to the beginning of the experiment. Cells were treated with BI-2536 or BI-BODIPY for 24 hours. Cell lysates were harvested, and lysates were analyzed by Western blot for cyclin B levels using cyclin B1 antibody (1:1000, Bethyl).

Immunofluorescence. Hela S3 cells used in immunofluorescence experiments were plated on poly-D-Lysine-coated 12-mm coverslips. Cells were arrested at the G1/S transition by 24-hour treatment with thymidine. Cells were released by thymidine washout and after 8 hours cells were treated with BI-BODIPY fluorophore compound (100 nM) with and without competing unlabeled BI2536 parent compound (1 μM) for 2 hours. This concentration was predetermined to provide the best staining with the lowest background. Higher concentrations of BI-BODIPY resulted in higher background staining and were avoided for this reason as well as to avoid undesired inhibition of the kinase target. Samples were fixed with 4% paraformaldehyde in PBS at room temperature for 20 minutes. Following PBS wash, cells were permeabilized with 0.1% Triton-X in PBS (PBST). Samples were then blocked with 5% BSA in PBST for 30 minutes, incubated with mouse DM1α anti-tubulin primary antibody (1:2000, Sigma Aldrich) for 1 hour at room temperature, washed with PBST, and incubated with goat anti-mouse AlexaFluor 594 secondary antibody (1:2000, Life Technologies) for an additional 1 hour at room temperature. Coverslips were then washed and stained with PBST containing Hoechst 33342 (1:2000, Life Technologies), washed, and mounted using ProLong Antifade (Life Technologies). Images were analyzed on a Nikon Ti motorized inverted microscope with a Perfect Focus System. Wide field Images were acquired with a cooled CCD camera (Hamamatsu ORCA-R2) and Metamorph software.

References

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