Electronic Supplementary Information

Synthesis of imidazothiadiazole-benzimidazole conjugates as mitochondrial apoptosis inducers

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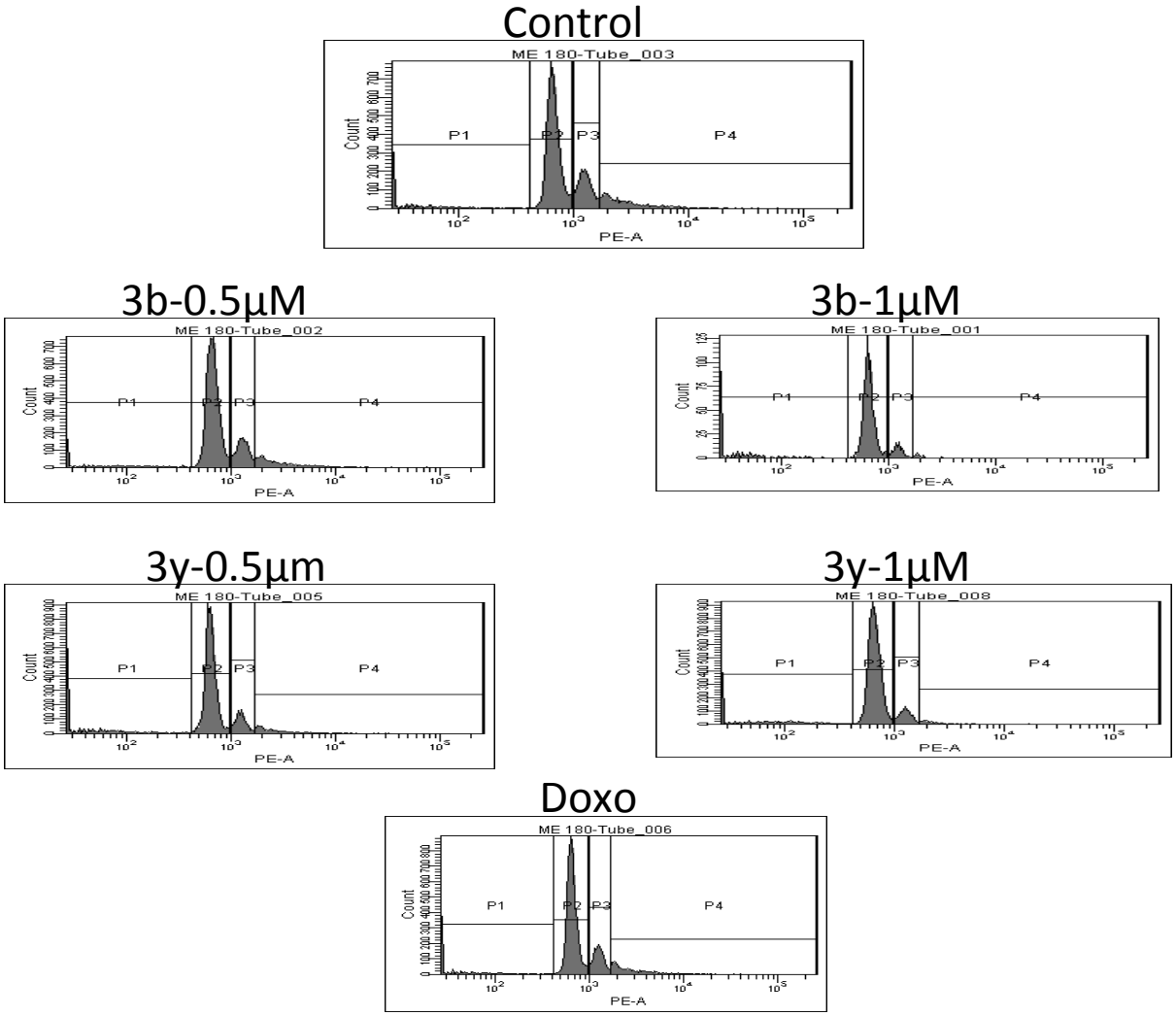
General. (**A**) **Chemistry:** All chemicals were purchased from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) or Spectrochem Pvt. Ltd (Mumbai, India) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 GF-254, and visualization on TLC was achieved by UV light or iodine indicator. Column chromatography was performed with Merck 60–120 mesh silica gel. Melting points were determined on a microscopic apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker UXNMR/XWIN-NMR (300 MHz) instruments. Chemical shifts are reported in parts per million (δ in ppm) relative to the peak for tetramethylsilane (TMS) as an internal standard and coupling constants are reported in hertz (Hz). Spectral patterns were designated as s, singlet; d, doublet; dd, double doublet; t, triplet; bs, broad singlet; m, multiplet. ESI spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. High-resolution mass spectra (HRMS) were recorded on QSTAR XL Hybrid MS/MS mass spectrometer.

(**B**) **Biology:**

(**a**) **Evaluation of cytotoxic activity:** Cell viability was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. The pale yellow coloured tetrazolium salt (MTT) reduces to a dark blue water-insoluble formazan by metabolically active cells and that is measured quantitatively after soluble in DMSO. The absorbance of the soluble formazan is directly proportional to the number of viable cells. Cells were seeded at a density of 1×10^4 cells in 200 μL of medium (DMEM), supplemented with 10% FBS in each well of 96-well micro culture plates and incubated for 24 h at 37 °C in a CO_2 incubator. All the test compounds were diluted to the desired concentrations in culture medium and then added to the wells with respective vehicle control. After 48 h of incubation, 10 ml MTT (5 mg/mL) was added to each well, and the plates were further incubated for 4 h. The supernatant was aspirated and plates were air dried and the MTT-formazon crystals dissolved in 100 μL of DMSO. The absorbance was recorded at 540 nm wavelength. Antiproliferative activity of the cancer cells to the test compounds were expressed in terms of IC_{50} value, which defines as a concentration of compound that produced 50% absorbance reduction relative to control.

(**b**) **Cell cycle analysis:** To establish the effect of compound on the stages of cell cycle, ME-180 cells (1×10^6) were seeded with trypsin EDTA in six-well plates and treated with the

compounds **3b** and **3y** at 0.5 and 1 µM for 48 h. After the incubation period, both floating and trypsinized adherent cells were collected and fixed with 70% ethanol. Then the cells were washed with PBS and stained with 50 mg/mL propidium iodide in hypotonic lysis buffer (0.1% sodium citrate, 0.1% Triton X-100) containing DNase-free RNase-A for 20 min. Stained cells were analyzed using fluorescence-activated cell sorter caliber (Becton Dickinson).



Flow cytometric analysis of **3b**, **3y** and doxorubicin in the cervical cancer cells (ME-180)

(**c**) **DNA fragmentation assay:** ME-180 cells were seeded (1×10^6) in six-well plates and incubated for 48 h with compounds **3b** and **3y** at 1 µM. After the incubation period, cells were collected and centrifuged at 2500 rpm for 5 min at 4 °C. Pellet was collected and washed with phosphate buffered saline (PBS). Then 100 µl of lysis buffer was added and centrifuged at 3000 rpm for 5 min at 4 °C. The supernant was collected and suspended in 10 mL of 10% SDS and 10 mL of (50 mg/mL) RNase-A, and incubated for 2 h at 56 °C. After

the incubation period, proteinase K (25 mg/mL) was added and further incubated at 37 °C for 2 h. Then added 65 mL of 10 M ammonium acetate and 500 mL of ice cold ethanol and mixed well. These samples were incubated at 80 °C for 1 h. After the incubation period, samples were centrifuged at 12000 rpm for 20 min at 4 °C. The pellet was washed with 80% ethanol and air dried for 10 min at room temperature. Then dissolved the pellet in 50 mL of TE buffer and DNA laddering was determined by using 2% agarose gel electrophoresis in TE Buffer.

(**d**) **Morphological analysis for apoptosis with Hoechst staining:** Cells were seeded at a density of 10,000 cells over 18-mm cover slips and incubated for 24 h. Then, the medium was replaced, and cells were treated with compounds **3b** and **3y** at 1 μM for 24 h. Cells treated with vehicle (0.001% DMSO) were included as controls for all experiments. An overnight treatment, Hoechst 33342 (Sigma–Aldrich) were added to medium at a concentration of 0.5 mg/mL containing 4% paraformaldehyde. After incubation for 30 min at 37 °C, cells from each dish were captured from randomly selected fields under fluorescent microscope (Leica, Germany) to qualitatively determine the proportion of viable and apoptotic cells based on their relative fluorescence and nuclear fragmentation.

(**e**) **Loss of mitochondrial membrane potential using JC-1:** Mitochondrial membrane potential (ΔΨm) was determined by using JC-1 dye. Cell-permeable cationic carbocyanine dye JC-1 (Invitrogen), also known as 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolyl-carbocyanine iodine, emits green fluorescence (525 nm) in its monomeric form. However, upon transfer to the membrane environment of a functionally active mitochondrion, it exhibits an aggregation dependent orange-red fluorescence (emission at 590 nm). Briefly, ME-180 cells were treated with 1 μM of test drugs for 48 h. After drug treatment the compounds **3b** and **3y** were incubated with JC-1 dye for 20 min at 37 °C. The treated cells and control were observed by using a fluorescence microscope (Nikon 50).

(**f**) **Caspase-3 activity assay:** AFC conjugated Ac-DEVD substrate was used to determine caspase-3 activity of the potent compounds **3b** and **3y**. ME-180 cells were seeded in six well plates with the confluence of per (2.5×10^6) well and are treated with the compounds at 1 μM concentration along with standard doxorubicin. After incubation for 48 h, the cells were washed with PBS and then scraped in to the PBS and centrifuged at 2000 rpm for 10 min at 4 °C. Pellet was resuspended in 80 mL of lysis buffer. Then the pellet was passed through insulin syringe followed by incubation of suspension on ice for 20–30 min centrifuged the lysate at 13,200 rpm for 20 min at 4 °C and transferred the supernatant to fresh tubes. In a 96

well black polystyrene plate, 50 mL cell lysate, 50 mL of 2X assay buffer and 2 mL of caspase-3substrate were taken. The reaction was allowed to take place for 1 h. The fluorescence generated by the release of the fluorogenic group AFC on cleavage by caspase-3 was measured by excitation at 400 nm and emission at 505 nm for every 5 min over 1 h. Protein was estimated by Bradford's method and normalized consequently.

(**g**) **Materials and methods used in docking studies:** Geometry optimization for the molecules (**3b** and **3y**) was performed by Gaussian 09 using PM3 semi-empirical method. Coordinates of protein structure were obtained from Protein Data Bank (PDB ID 2O22). Docking studies were performed using AutoDock 4.2 docking software. Images were taken by using Pymol visualization software.

Spectral Data and Procedure of Compounds (7a–c, 8a–c and **3a–z)**

2-methyl-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazole (7a): To a stirred solution of 5-methyl-1,3,4-thiadiazol-2(3*H*)-imine (**4**) (20 mmol) in acetone (100 mL), 2-bromo-1-phenylethanone (**5a**) (20 mmol, 1 equiv) was added. After the completion of addition, the mixture was stirred at 65 °C for 6-8 h under nitrogen atmosphere. After completion of reaction as indicated by TLC, reaction mixture was cooled and filtered. Then the solid was dried and used for next step without any purification. To the solid, 2N HCl (200 mL) was added and then refluxed for 1 h. The reaction mixture was poured on crushed ice and neutralized with 15% NH_4OH, in order to precipitate the crude compound (**7a**), which was crystallized from ethanol. Yield: 3.65 g, 85%; Off-white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.91 (s, 1H), 7.84 (dd, 2H, J = 1.5, 7.5 Hz), 7.52 (dd, 2H, J = 1.5, 7.5 Hz), 7.25 (s, 1H), 2.86 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.2, 146.0, 145.6, 133.9, 128.6, 127.4, 124.9, 109.1, 17.7; ESIMS: *m/z* 216 $(M+H)^+$.

6-(4-fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (7b): The compound **7b** was prepared according to the procedure of **7a** using compounds **4** and **5b**. Yield: 3.72 g, 80%; Off-white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.93 (s, 1H), 7.92 (dt, 2H, J = 2.1, 7.5 Hz), 7.34 (dt, 2H, J = 2.1, 7.5 Hz), 2.70 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 162.0, 159.2, 146.0, 130.9, 130.7, 128.3, 115.9, 115.6, 17.8; ESIMS: *m/z* 234 $(M+H)^+$.

2-methyl-6-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazole (7c): The compound **7c** was prepared according to the procedure of **7a** using compounds **4** and **5c**. Yield: 3.75 g, 82%; Off-white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.92 (S, 1H), 7.70 (d, 2H, J = 8.3 Hz), 7.22 (d, 2H, J =

8.2 Hz), 2.69 (s, 3H), 2.37 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.0, 146.0, 137.1, 131.0, 129.3, 124.8, 124.8, 108.6, 21.1, 17.7; ESIMS: *m/z* 230 $(M+H)^+$.

6-(4-methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (7d): The compound **7d** was prepared according to the procedure of **7a** using compounds **4** and **5d**. Yield: 3.87 g, 79%; Off-white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.91 (s, 1H), 7.70 (d, 2H, J = 8.3 Hz), 7.22 (d, 2H, J = 8.1 Hz), 3.80 (s, 3H), 2.69 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.1, 158.9, 126.6, 126.2, 126.2, 114.0, 114.0, 108.1, 55.2, 17.7; ESIMS: *m/z* 246 $(M+H)^+$.

2-methyl-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (8a): The Vilsmeier reagent was prepared at 0–58 °C by dropping $POCl_3$ (20 mmol) into a stirred solution of DMF (25 mmol) in $CHCl_3$ (5 mL). Compound **7a** (10 mmol) in $CHCl_3$ (60 mL) was added drop wise to the Vilsmeier reagent while maintaining cooling and stirring. The reaction mixture was kept at 25 °C for 3 h and under reflux for 1–24 h (according to a TLC test). The solvent was removed under reduced pressure, and the resulting oil was poured onto ice. The crude aldehyde **8a** thus obtained was collected by filtration and crystallized from ethanol. Yield: 3.40 g, 75%; Off-white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.0 (s, 1H), 7.84 (dd, 2H, J = 1.5, 7.5 Hz), 7.53 (dd, 2H, J = 1.5, 7.5 Hz), 7.25 (s, 1H), 2.85 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 177.5, 162.0, 156.4, 132.1, 129.7, 129.0, 129.0, 128.8, 124.0, 18.0; ESIMS: *m/z* 244 $(M+H)^+$.

6-(4-fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (8b): The compound **8b** was prepared according to the procedure of **8a** using compounds **7b**. Yield: 2.91 g, 70%; Off-white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.1 (bs, 1H), 7.81 (d, 2H, J = 8.3 Hz), 7.17 (t, 2H, J = 8.2 Hz), 2.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 177.7, 165.3, 162.0, 154.8, 130.9, 130.7, 128.3, 115.9, 115.6, 17.9; ESIMS: *m/z* 262 $(M+H)^+$.

2-methyl-6-*p*-tolylimidazo [2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (8c): The compound **8c** was prepared according to the procedure of **8a** using compounds **7c**. Yield: 3.15 g, 75%; Off-white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.0 (bs, 1H), 7.81 (d, 2H, J = 8.3 Hz), 7.16 (t, 2H, J = 8.3 Hz), 2.89 (s, 3H), 2.37 (S, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 177.5, 162.0, 156.4, 132.1, 129.7, 129.1, 129.0, 128.8, 124.0, 21.3, 18.0; ESIMS: *m/z* 258 $(M+H)^+$.

6-(4-methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (8d): The compound **8d** was prepared according to the procedure of **8a** using compounds **7d**. Yield: 3.29 g, 76%; Off-white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.0 (bs, 1H), 7.81 (d, 2H, J = 8.3 Hz), 7.16 (t, 2H, J = 8.3 Hz), 3.80 (S, 3H), 2.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ

177.4, 170.5, 165.8, 164.3, 155.0, 144.1, 144.1, 130.4, 130.4, 55.5, 16.2; ESIMS: *m/z* 276 $(M+H)^+$.

5-(5,6-dimethyl-1*H*-benzo[d]imidazol-2-yl)-2-methyl-6-phenylimidazo-[2,1-*b*][1,3,4]-thiadiazole (3a): To a solution of 2-methyl-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**8a**) (0.6 mmol, 145 mg) in EtOH (5 mL), sodium thiosulphate (0.6 mmol, 144 mg) and 4,5-dimethylbenzene-1,2-diamine (0.6 mmol, 81 mg) were added and the mixture was refluxed for 4 h. The reaction was monitored by TLC. After completion of the reaction, $Na_2S_2O_5$ was removed by filtration and the organic layer was dried with Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (50% ethyl acetate–hexane) to obtain the pure product (**3a**) as off-white solid. Yield: 168 mg, 78%; mp 279–280 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.12 (d, 2H, J = 6.9 Hz), 7.36–7.47 (m, 5H), 2.84 (s, 3H), 2.37 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 152.2, 150.1, 148.7, 133.3, 131.3, 127.9, 127.8, 127.7, 126.9, 122.6, 120.6, 115.9, 20.0, 17.7; ESIMS: *m/z* 360 $(M+H)^+$; HRMS (ESI): For $C_{20}H_{18}N_5S$ $(M+H)^+$ *m/z* calcd., 360.12774; found, 360.12685.

5-(5,6-dichloro-1*H*-benzo[*d*]imidazol-2-yl)-2-methyl-6-phenylimidazo-[2,1-*b*][1,3,4]thia-diazole (3b): The compound **3b** was prepared by following the method described for the preparation of the compound **3a**, employing **8a** (145 mg, 0.6 mmol) and 4,5-dichlorobenzene-1,2-diamine (106 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3b** as off-white solid. Yield: 180 mg, 75%; mp 267–268 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.43 (bs, 1H), 8.16 (d, 2H, J = 7.5 Hz), 7.88 (s, 1H), 7.57 (s, 1H), 7.40–7.51 (m, 3H), 2.88 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 160.2, 145.8, 145.2, 143.3, 132.7, 127.5, 127.5, 127.1, 125.1, 119.6, 113.6, 111.0, 103.7, 94.7, 17.2; ESIMS: *m/z* 400 $(M+H)^+$; HRMS (ESI): For $C_{18}H_{12}Cl_2N_5S$ $(M+H)^+$ *m/z* calcd., 400.01887; found, 400.01850.

5-(5-chloro-1*H*-benzo[*d*]imidazol-2-yl)-2-methyl-6-phenylimidazo[2,1-*b*][1,3,4]thia-diazole (3c): The compound **3c** was prepared by following the method described for the preparation of the compound **3a**, employing **8a** (145 mg, 0.6 mmol) and 4-chlorobenzene-1,2-diamine (84 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3c** as yellow solid. Yield: 166 mg, 76%; mp 209–210 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.33 (bs, 1H), 8.15–8.18 (m, 2H), 7.79 (s, 1H), 7.70 (d, 1H, J = 8.7 Hz), 7.45–7.48 (m, 2H), 7.35–7.42 (m, 1H), 7.22 (d, 1H, J = 8.8 Hz), 2.86 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.0, 144.0, 142.1,

141.1, 139.9, 128.0, 127.7, 121.0, 120.3, 116.4, 113.0, 112.9, 112.3, 109.5, 15.8; ESIMS: *m/z* 366 $(M+H)^+$; HRMS (ESI): For $C_{18}H_{13}ClN_5S$ $(M+H)^+$ *m/z* calcd., 366.05802; found, 366.05810.

5-(5-chloro-6-fluoro-1*H*-benzo[d]imidazol-2-yl)-6-(4-fluorophenyl)-2-methylimidazo-[2,1-*b*][1,3,4]thiadiazole (3d): The compound **3d** was prepared by following the method described for the preparation of the compound **3a**, employing **8b** (156 mg, 0.6 mmol) and 4-chloro-5-fluorobenzene-1,2-diamine (95 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3d** as off-white solid. Yield: 166 mg, 72%; mp 242–243 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.63 (bs, 1H), 8.27–8.31 (m, 2H), 7.81 (d, 1H, J = 6.0 Hz), 7.52–7.55 (m, 1H), 7.16 (t, 2H, J = 8.3 Hz), 2.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 161.9, 159.8, 158.6, 144.4, 142.1, 138.1, 128.1, 128.0, 125.8, 119.1, 117.9, 113.2, 112.9, 112.4, 110.8, 15.9; ESIMS: *m/z* 402 $(M+H)^+$; HRMS (ESI): For $C_{18}H_{11}ClF_2N_5S$ $(M+H)^+$ *m/z* calcd., 402.03863; found, 402.03857.

5-(4,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)-2-methyl-6-phenylimidazo-[2,1-*b*][1,3,4]-thiadiazole (3e): The compound **3e** was prepared by following the method described for the preparation of the compound **3a**, employing **8a** (145 mg, 0.6 mmol) and 3,4-dimethylbenzene-1,2-diamine (81 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3e** as off-white solid. Yield: 161 mg, 75%; mp 271–272 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.99 (bs, 1H), 8.15 (dd, 2H, J = 1.5, 6.7 Hz), 7.60 (s, 1H), 7.47 (t, 2H, J = 6.7 Hz), 7.41 (d, 1H, J = 6.7 Hz), 7.24 (s, 1H), 2.86 (s, 3H), 2.38 (s, 3H), 2.17 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 160.3, 145.7, 146.1, 141.5, 138.4, 133.5, 130.6, 128.9, 128.8, 128.5, 125.0, 122.6, 121.0, 115.0, 114.7, 21.4, 19.2, 18.0; ESIMS: *m/z* 360 $(M+H)^+$; HRMS (ESI): For $C_{20}H_{18}N_5S$ $(M+H)^+$ *m/z* calcd., 360.12685; found, 360.12774.

2-methyl-6-phenyl-5-(5-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)imidazo[2,1-*b*][1,3,4]thiadiazole (3f): The compound **3f** was prepared by following the method described for the preparation of the compound **3a**, employing **8a** (145 mg, 0.6 mmol) and 4-(trifluoromethyl)benzene-1,2-diamine (93 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3f** as off-white solid. Yield: 170 mg, 71%; mp 176–177 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.78 (bs, 1H), 8.32–8.35 (m, 2H), 8.09 (s, 1H), 7.82–7.87 (m, 1H), 7.52–7.58 (m, 2H), 7.17 (t, 2H, J = 8.5 Hz), 2.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.0, 144.1, 142.0, 141.0, 140.0, 127.9,

127.7, 121.0, 120.3, 116.4, 113.1, 112.9, 112.2, 109.6, 15.9; ESIMS: *m/z* 400 $(M+H)^+$; HRMS (ESI): For $C_{19}H_{13}F_3N_5S$ $(M+H)^+$ *m/z* calcd., 400.08299; found, 400.08383.

5-(5-chloro-6-fluoro-1*H*-benzo[*d*]imidazol-2-yl)-2-methyl-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazole (3g): The compound **3g** was prepared by following the method described for the preparation of the compound **3a**, employing **8a** (145 mg, 0.6 mmol) and 4-chloro-5-fluorobenzene-1,2-diamine (95 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3g** as white solid. Yield: 156 mg, 68%; mp 225–226 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.03–8.07 (m, 2H), 7.72–7.82 (m, 1H), 7.52–7.58 (m, 1H), 7.40 (s, 1H), 7.09 (t, 2H, J = 8.6 Hz), 2.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.5, 159.4, 152.5, 151.0, 144.2, 143.2, 141.7, 138.1, 129.1, 128.7, 126.7, 125.6, 117.7, 111.9, 110.6, 15.7; ESIMS: *m/z* 384 $(M+H)^+$; HRMS (ESI): For $C_{18}H_{12}FClN_5S$ $(M+H)^+$ *m/z* calcd., 384.04805; found, 384.04752.

5-(4,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)-6-(4-methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (3h): The compound **3h** was prepared by following the method described for the preparation of the compound **3a**, employing **8d** (163 mg, 0.6 mmol) and and 3,4-dimethylbenzene-1,2-diamine (81 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3h** as grey solid. Yield: 151 mg, 65%; mp 240–241 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.98 (d, 2H, J = 8.3 Hz), 7.85–7.88 (m, 2H), 6.98 (d, 2H, J = 6.3 Hz), 3.90 (s, 3H), 2.57 (s, 3H), 2.43 (s, 3H), 2.31 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 160.4, 159.7, 146.0, 142.0, 132.5, 129.8, 125.9, 124.1, 113.6, 55.1, 29.6, 21.6, 18.0; ESIMS: *m/z* 390 $(M+H)^+$; HRMS (ESI): For $C_{21}H_{20}N_5OS$ $(M+H)^+$ *m/z* calcd., 390.13886; found, 390.13894.

5-(5-chloro-6-fluoro-1*H*-benzo[*d*]imidazol-2-yl)-6-(4-methoxyphenyl)-2-methylimidazo-[2,1-*b*][1,3,4]thiadiazole (3i): The compound **3i** was prepared by following the method described for the preparation of the compound **3a**, employing **8d** (163 mg, 0.6 mmol) and 4-chloro-5-fluorobenzene-1,2-diamine (95 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3i** as yellow solid. Yield: 168 mg, 68%; mp 189–190 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.85 (s, 1H), 7.98 (d, 2H, J = 8.3 Hz), 7.85–7.88 (m, 1H), 6.98 (d, 2H, J = 6.3 Hz), 3.91 (s, 3H), 2.56 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 165.3, 159.2, 157.4, 150.3, 145.5, 144.1, 143.5, 142.2, 135.4, 135.4, 127.2, 127.0, 124.0, 111.5, 111.4, 53.1, 15.8; ESIMS: *m/z* 414$(M+H)^+$; HRMS (ESI): For $C_{19}H_{14}ClFN_5OS$ $(M+H)^+$ *m/z* calcd., 414.05916; found, 414.05933.

6-(4-fluorophenyl)-2-methyl-5-(5-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)imidazo-[2,1-*b*][1,3,4]thiadiazole (3j): The compound **3j** was prepared by following the method described for the preparation of the compound **3a**, employing **8b** (156 mg, 0.6 mmol) and 4-(trifluoromethyl)benzene-1,2-diamine (105 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3j** as off-white solid. Yield: 177 mg, 71%; mp 211–212 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.7 (bs, 1H), 8.33–8.35 (m, 2H), 8.09 (s, 1H), 7.82–7.87 (m, 1H), 7.58 (d, 1H, J = 7.8 Hz), 7.17 (t, 2H, J = 8.5 Hz), 2.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 198.5, 164.6, 146.2, 130.9, 130.7, 129.1, 125.2, 120.0, 119.5, 117.3, 115.2, 114.9, 110.8, 108.3, 29.6, 18.0; ESIMS: *m/z* 418 $(M+H)^+$; HRMS (ESI): For $C_{19}H_{12}F_4N_5S$ $(M+H)^+$ *m/z* calcd., 418.07441; found, 418.07271.

5-(5,6-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)-6-(4-methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (3k): The compound **3k** was prepared by following the method described for the preparation of the compound **3a**, employing **8d** (163 mg, 0.6 mmol) and 4,5-dimethylbenzene-1,2-diamine (81 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3k** as off-white solid. Yield: 165 mg, 71%; mp 240–241 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.98 (d, 2H, J = 8.3 Hz), 7.85–7.88 (m, 2H), 6.98 (d, 2H, J = 6.3 Hz), 3.90 (s, 3H), 2.57 (s, 3H), 2.35 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.5, 159.4, 152.5, 151.0, 144.2, 143.2, 141.7, 138.0, 129.2, 128.7, 126.8, 117.8, 113.7, 111.8, 110.6, 53.2, 15.8; ESIMS: *m/z* 390 $(M+H)^+$; HRMS (ESI): For $C_{21}H_{20}N_5OS$ $(M+H)^+$ *m/z* calcd., 390.13886; found, 390.13899.

5-(1*H*-benzo[*d*]imidazol-2-yl)-2-methyl-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazole (3l): The compound **3l** was prepared following the method described for the preparation of the compound **3a**, employing **8a** (145 mg, 0.6 mmol) and benzene-1,2-diamine (64 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3l** as yellow solid. Yield: 153 mg, 77%; mp 200–201 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.15 (bs, 1H), 8.12 (d, 2H, J = 6.7 Hz), 7.65 (bs, 1H), 7.36–7.48 (m, 5H), 7.10 (d, 1H, J = 6.7 Hz), 2.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 144.1, 143.1, 139.9, 139.1, 131.9, 126.5, 126.4, 126.2, 126.1, 125.8, 125.7, 120.6, 113.2, 16.1; ESIMS: *m/z* 332 $(M+H)^+$; HRMS (ESI): For $C_{18}H_{14}N_5S$ $(M+H)^+$ *m/z* calcd., 332.09699; found, 332.09811.

6-(4-methoxyphenyl)-2-methyl-5-(5-methyl-1*H*-benzo[*d*]imidazol-2-yl)-imidazo[2,1-*b*][1,3,4]thiadiazole (3m): The compound **3m** was prepared by following the method described for the preparation of the compound **3a**, employing **8d** (163 mg, 0.6 mmol) and 4-

methylbenzene-1,2-diamine (72 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3m** as off-white solid. Yield: 162 mg, 72%; mp 219–220 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.15 (bs, 1H), 8.12 (d, 2H, J = 6.7 Hz), 7.36–7.48 (m, 4H), 7.10 (d, 1H, J = 6.7 Hz), 3.90 (s, 3H), 2.84 (s, 3H), 2.48 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 157.3, 143.6, 142.7, 135.1, 134.1, 129.5, 129.0, 127.3, 126.8, 126.2, 124.3, 119.9, 112.3, 111.7, 53.2, 19.6, 15.9; ESIMS: *m/z* 376 $(M+H)^+$; HRMS (ESI): For $C_{20}H_{18}N_5OS$ $(M+H)^+$ *m/z* calcd., 376.12321; found, 376.12433.

5-(5-fluoro-1*H*-benzo[*d*]imidazol-2-yl)-2-methyl-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazole (3n): The compound **3n** was prepared by following the method described for the preparation of the compound **3a**, employing **8a** (145 mg, 0.6 mmol) and 4-fluorobenzene-1,2-diamine (75 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3n** as off-white solid. Yield: 163 mg, 78%; mp 220–221 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.45 (bs, 1H), 8.12 (d, 2H, J = 6.7 Hz), 7.81 (d, 1H, J = 6.7 Hz), 7.53 (d, 1H, J = 9.8 Hz), 7.40–7.48 (m, 3H), 7.22 (d, 1H, J = 8.3 Hz), 2.86 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.4, 144.1, 143.7, 143.0, 141.9, 140.9, 139.5, 134.7, 131.6, 126.2, 126.1, 126.0, 125.6, 112.7, 15.8; ESIMS: *m/z* 350 $(M+H)^+$; HRMS (ESI): For $C_{18}H_{13}FN_5S$ $(M+H)^+$ *m/z* calcd., 350.08702; found, 350.08626.

6-(4-fluorophenyl)-2-methyl-5-(5-methyl-1*H*-benzo[*d*]imidazol-2-yl)imidazo[2,1-*b*][1,3,4]thiadiazole (3o): The compound **3o** was prepared by following the method described for the preparation of the compound **3a**, employing **8b** (156 mg, 0.6 mmol) and 4-methylbenzene-1,2-diamine (72 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3o** as off-white solid. Yield: 163 mg, 75%; mp 214–215 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.63 (bs, 1H), 8.27–8.31 (m, 2H), 7.81 (d, 1H, J = 6.0 Hz), 7.52–7.55 (m, 2H), 7.16 (t, 2H, J = 8.3 Hz), 2.89 (s, 3H), 2.44 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.4, 161.0, 158.2, 145.6, 145.5, 141.7, 130.5, 130.1, 129.4, 127.8, 124.3, 122.5, 115.1, 114.8, 114.2, 21.6, 18.0; ESIMS: *m/z* 364 $(M+H)^+$; HRMS (ESI): For $C_{19}H_{15}FN_5S$ $(M+H)^+$ *m/z* calcd., 364.10322; found, 364.10344.

5-(5-chloro-1*H*-benzo[*d*]imidazol-2-yl)-6-(4-fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (3p): The compound **3p** was prepared by following the method described for the preparation of the compound **3a**, employing **8b** (156 mg, 0.6 mmol) and 4-chlorobenzene-1,2-diamine (85 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3p** as white solid. Yield: 161 mg, 70%; mp 209–210 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.59 (bs, 1H),

8.27–8.29 (m, 2H), 7.45–7.48 (m, 1H), 7.22 (d, 2H, *J* = 7.7 Hz), 7.11–7.14 (m, 2H), 2.85 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 161.0, 159.0, 144.1, 142.1, 141.0, 139.9, 127.9, 127.7, 121.0, 120.3, 116.4, 113.1, 112.9, 112.3, 109.6, 15.7; ESIMS: *m/z* $(M+H)^+$; HRMS (ESI): For $C_{18}H_{12}ClFN_5S$ $(M+H)^+$ *m/z* calcd., 384.04860; found, 384.04891.

5-(5-chloro-1*H*-benzo[*d*]imidazol-2-yl)-6-(4-methoxyphenyl)-2-methyl-imidazo[2,1-*b*][1,3,4]thiadiazole (3q): The compound **3q** was prepared by following the method described for the preparation of the compound **3a**, employing **8d** (163 mg, 0.6 mmol) and 4-chlorobenzene-1,2-diamine (86 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3q** as yellow solid. Yield: 154 mg, 65%; mp 209–210 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.85 (s, 1H), 7.98 (d, 2H, *J* = 8.3 Hz), 7.85–7.88 (m, 2H), 6.98 (d, 2H, *J* = 6.3 Hz), 3.90 (s, 3H), 2.57 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 160.8, 159.7, 146.6, 146.0, 143.4, 129.7, 128.1, 125.5, 123.1, 113.7, 113.4, 55.1, 29.6, 17.9; ESIMS: *m/z* 396 $(M+H)^+$; HRMS (ESI): For $C_{19}H_{15}ClN_5OS$ $(M+H)^+$ *m/z* calcd., 396.06858; found, 396.06890.

5-(5,6-dichloro-1*H*-benzo[*d*]imidazol-2-yl)-6-(4-fluorophenyl)-2-methyl-imidazo[2,1-*b*][1,3,4]thiadiazole (3r): The compound **3r** was prepared by following the method described for the preparation of the compound **3a**, employing **8b** (156 mg, 0.6 mmol) and 4,5-dichlorobenzene-1,2-diamine (105 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3r** as off-white solid. Yield: 168 mg, 67%; mp 277–278 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.65 (bs, 1H), 8.30–8.35 (m, 2H), 7.88 (s, 1H), 7.62 (m, 1H), 7.14–7.20 (m, 2H), 2.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 160.2, 144.0, 142.9, 129.0, 128.9, 128.5, 124.8, 124.8, 124.5, 124.5, 118.9, 114.0, 113.7, 113.1, 16.8; ESIMS: *m/z* 418 $(M+H)^+$; HRMS (ESI): For $C_{18}H_{11}FCl_2N_5S$ $(M+H)^+$ *m/z* calcd., 418.00835; found, 418.00908.

5-(5-chloro-1*H*-benzo[*d*]imidazol-2-yl)-2-methyl-6-p-tolylimidazo[2,1-*b*][1,3,4]thia-diazole (3s): The compound **3s** was prepared by following the method described for the preparation of the compound **3a**, employing **8c** (154 mg, 0.6 mmol) and 4-chlorobenzene-1,2-diamine (85 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3s** as yellow solid. Yield: 161 mg, 71%; mp 219–220 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.55 (bs, 1H), 7.85 (d, 2H, *J* = 8.1 Hz), 7.34–7.54 (m, 2H), 7.18 (d, 2H, *J* = 8.0 Hz), 2.82 (s, 3H), 2.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 160.9, 153.3, 152.4, 146.7, 146.1, 143.8, 143.2, 138.5, 130.1, 128.9, 128.8, 128.2,

123.1, 118.2, 114.0, 21.2, 18.0; ESIMS: *m/z* 380 $(M+H)^+$; HRMS (ESI): For $C_{19}H_{15}ClN_5S$ $(M+H)^+$ *m/z* calcd., 380.07367; found, 380.07380.

5-(1*H*-benzo[*d*]imidazol-2-yl)-6-(4-fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (3t): The compound **3t** was prepared by following the method described for the preparation of the compound **3a**, employing **8b** (156 mg, 0.6 mmol) and benzene-1,2-diamine (64 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3t** as white solid. Yield: 152 mg, 73%; mp 225–226 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.46 (bs, 1H), 8.13 (d, J = 6.7 Hz, 2H), 7.81 (d, 1H, J = 6.7 Hz), 7.54 (d, 1H, J = 9.8 Hz), 7.40–7.48 (m, 3H), 7.22 (d, 1H, J = 8.3 Hz), 2.86 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.4, 144.0, 143.5, 143.0, 141.9, 141.0, 139.5, 134.7, 131.6, 126.3, 126.2, 126.0, 125.6, 112.6, 15.9; ESIMS: *m/z* 350 $(M+H)^+$; HRMS (ESI): For $C_{18}H_{13}FN_5S$ $(M+H)^+$ *m/z* calcd., 350.08757; found, 350.08790.

2-methyl-5-(5-methyl-1*H*-benzo[*d*]imidazol-2-yl)-6-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazole (3u): The compound **3u** was prepared by following the method described for the preparation of the compound **3a**, employing **8c** (154 mg, 0.6 mmol) and 4-methylbenzene-1,2-diamine (73 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3u** as off-white solid. Yield: 163 mg, 76%; mp 255–256 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.91 (d, 2H, J = 8.3 Hz), 7.86 (m, 2H), 7.20 (d, 2H, J = 8.3 Hz), 7.07 (d, 1H, J = 8.3 Hz), 2.80 (s, 3H), 2.47 (s, 3H), 2.36 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 160.3, 145.9, 145.7, 141.7, 138.1, 130.3, 128.7, 128.0, 124.0, 114.6, 21.6, 21.1, 17.8; ESIMS: *m/z* 360 $(M+H)^+$; HRMS (ESI): For $C_{20}H_{18}N_5S$ $(M+H)^+$ *m/z* calcd., 360.12774; found, 360.12685.

5-(4,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)-2-methyl-6-*p*-tolylimidazo-[2,1-*b*][1,3,4]thiadiazole (3v): The compound **3v** was prepared by following the method described for the preparation of the compound **3a**, employing **8c** (145 mg, 0.6 mmol) and 3,4-dimethylbenzene-1,2-diamine (81 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3v** as off-white solid. Yield: 168 mg, 75%; mp 283–284 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.96–8.25 (m, 2H), 7.24 (d, 2H, J = 7.9 Hz), 7.07 (d, 2H, J = 7.7 Hz), 2.82 (s, 3H), 2.40 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 160.5, 146.1, 145.7, 141.5, 138.3, 133.5, 130.6, 128.9, 128.8, 128.5, 125.0, 122.7, 121.0, 115.0, 114.7, 25.5, 21.3, 19.3, 18.0; ESIMS: *m/z* 374 $(M+H)^+$; HRMS (ESI): For $C_{21}H_{20}N_5S$ $(M+H)^+$ *m/z* calcd., 374.14339; found, 374.14370.

5-(5,6-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)-6-(4-fluorophenyl)-2-methyl-imidazo[2,1-*b*][1,3,4]thiadiazole (3w): The compound **3w** was prepared by following the method described for the preparation of the compound **3a**, employing **8b** (156 mg, 0.6 mmol) and 4,5-dimethylbenzene-1,2-diamine (81 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3w** as white solid. Yield: 147 mg, 65%; mp 244–245 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.63 (bs, 1H), 8.27–8.31 (m, 2H), 7.81 (d, 1H, J = 6.0 Hz), 7.52–7.55 (m, 1H), 7.16 (t, 2H, J = 8.3 Hz), 2.89 (s, 3H), 2.42 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.3, 144.1, 143.1, 139.9, 131.9, 126.5, 126.4, 126.1, 125.8, 125.7, 120.6, 113.2, 19.1, 16.1; ESIMS: *m/z* 378 $(M+H)^+$; HRMS (ESI): For $C_{20}H_{17}FN_5S$ $(M+H)^+$ *m/z* calcd., 378.11832; found, 378.11738.

2-methyl-5-(5-methyl-1*H*-benzo[*d*]imidazol-2-yl)-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazole (3x): The compound **3x** was prepared by following the method described for the preparation of the compound **3a**, employing **8a** (145 mg, 0.6 mmol) and 4-methylbenzene-1,2-diamine (73 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3x** as off-white solid. Yield: 144 mg, 70%; mp 244–245 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.15 (bs, 1H), 8.12 (d, 2H, J = 6.7 Hz), 7.65 (bs, 1H), 7.36–7.48 (m, 4H), 7.10 (d, 1H, J = 6.7 Hz), 2.84 (s, 3H), 2.48 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 160.5, 145.8, 141.6, 133.3, 132.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 124.1, 114.9, 21.6, 17.9; ESIMS: *m/z* 346 $(M+H)^+$; HRMS (ESI): *m/z* calcd. for $C_{19}H_{16}N_5S$ $(M+H)^+$, 346.11209; found, 346.11227.

5-(5-chloro-6-fluoro-1*H*-benzo[*d*]imidazol-2-yl)-2-methyl-6-*p*-tolylimid-azo[2,1-*b*][1,3,4]-thiadiazole (3y): The compound **3y** was prepared by following the method described for the preparation of the compound **3a**, employing **8a** (145 mg, 0.6 mmol) and (95 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–hexane) to afford the compound **3y** as yellow solid. Yield: 173 mg, 73%; mp 235–236 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.56 (bs, 1H), 7.86 (d, 2H, J = 8.1 Hz), 7.18 (d, 2H, J = 8.0 Hz), 2.82 (s, 3H), 2.36 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.6, 159.4, 152.5, 150.9, 144.2, 143.2, 141.7, 138.0, 129.1, 128.7, 126.8, 125.6, 117.7, 111.8, 110.6, 18.9, 15.7; ESIMS: *m/z* 398 $(M+H)^+$; HRMS (ESI): For $C_{19}H_{14}ClFN_5$ $(M+H)^+$ *m/z* calcd., 398.06425; found, 398.06441.

2-(2-methyl-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzo[*d*]-imidazol-5-ol (3z): The compound **3z** was prepared by following the method described for the preparation of the compound **3a**, employing **8a** (145 mg, 0.6 mmol) and 3,4-diaminophenol (73 mg, 0.6 mmol), and the crude product was purified by column chromatography (50% ethyl acetate–

hexane) to afford the compound **3z** as off-white solid. Yield: 122 mg, 59%; mp 150–151 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.0 (s, 1H), 9.95 (bs, 1H), 7.81–7.84 (m, 2H), 7.50–7.55 (m, 5H), 2.85 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 177.5, 162.0, 156.4, 151.3, 144.1, 132.1, 129.7, 129.0, 128.9, 128.8, 128.7, 128.2, 124.0, 112.7, 18.0; ESIMS: *m/z* 348 $(M+H)^+$; HRMS (ESI): For $C_{18}H_{14}N_5OS$ $(M+H)^+$ *m/z* calcd., 348.09136; found, 348.09069.