Supplementary Information

Discovery of asymmetric pyridinium-based fluorescent probes with large Stokes shifts for live neural stem/progenitor cells

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Experimental section

1. Synthesis

General Procedure: Preparation of Pyridinium Salts using asymmetry internal alkyne (6a-6m)

Preparation of 2-cyclopropyl-3-(4-(dimethylamino)phenyl)-5-methyl-1-phenylpyridin-1-ium tetrafluoroborate (6b) and 3-cyclopropyl-2-(4-(dimethylamino)phenyl)-5-methyl-1-phenylpyridin-1-ium tetrafluoroborate (6b'): To a 5 mL reaction vial were added N-(2-methylallyl)aniline (0.2 mmol, 29.4 mg), 4-(cyclopropylethynyl)-N,N-dimethylaniline (0.3 mmol, 55.6 mg), copper acetate (0.4 mmol, 72 mg), $[Cp^*RhCl_2]_2$ (5 mol%), sodium tetrafluoroborate (0.3 mmol, 33 mg) and methanol (1 mL). The resulting solution was stirred at 100 ^oC for overnight, filtered, and the filtrate was concentrated under reduced pressure giving a residue that was subjected to prep HPLC to give **6b** and **6b'** in 26% and 24% yield respectively (yellow solid, 21.6 mg of **6b** and 20 mg of **6b'**).

^1H NMR of **6b** (400 MHz, MeOD) δ 8.51 (s, 1H), 7.77 (s, 1H), 7.38-7.30 (m, 5H), 7.01 (d, J = 8.8 Hz, 2H), 6.53 (d, J= 8.8 Hz, 2H), 2.95 (s, 6H), 2.59 (s, 3H), 1.86-1.81 (m, 1H), 1.10-1.05 (m, 2H), 0.94-0.89 (m, 2H); ^{13}C NMR of **6b** (100 MHz, MeOD) δ 155.4, 152.3, 146.0, 144.3, 143.7, 143.3, 138.1, 132.3, 131.2, 130.5, 127.5, 118.1, 112.4, 40.1, 18.0, 14.3, 11.4; LC/MS(ESI) m/z : 329 $[M+H]^+$

^1H NMR of **6b'** (400 MHz, MeOD) δ 8.48 (s, 1H), 8.21 (s, 1H), 7.67-7.62 (m, 5H), 7.51 (d, J = 8.44 Hz, 2H), 7.20 (d, J = 8.44 Hz, 2H), 3.14 (s, 6H), 2.60 (s, 3H), 2.06-2.01 (m, 1H), 0.66-0.61 (m, 2H), 0.24-0.23 (m, 2H); ^{13}C NMR of **6b'** (100 MHz, MeOD) δ 153.7, 151.8, 148.9, 145.2, 145.1, 144.0, 137.8, 132.0, 131.8, 131.1, 127.1, 125.2, 113.8, 40.9, 17.9, 16.0, 12.6; LC/MS(ESI) m/z : 329 $[M+H]^+$

Preparation of 1-([1,1'-biphenyl]-4-yl)-2-cyclopentyl-3-(4-(dimethylamino)phenyl)-5-methylpyridin-1-ium tetrafluoroborate (6c)

General procedure was used employing *N*-(2-methylallyl)-[1,1'-biphenyl]-4-amine (**1a**) and 4-(cyclopentylethynyl)-N,N-dimethylaniline (**2c**) to give the desired product **6c** (yellow solid, 6% yield). ^1H NMR (400 MHz, MeOD) δ 8.77 (s, 1H), 8.63 (s, 1H), 7.67 (d, J = 8.56 Hz, 2H), 7.60 (d, J = 1.36 Hz, 2H), 7.58-7.36 (m, 5H), 7.09 (d, J = 8.88 Hz, 2H), 6.67 (d, J = 8.88 Hz, 2H),

3.08-2.99 (m, 1H), 2.93 (s, 6H), 2.63 (s, 3H), 2.02-1.86 (m, 4H), 1.77-1.66 (m, 4H); ^{13}C NMR (100 MHz, MeOD) δ 155.1, 152.4, 148.7, 146.7, 144.6, 144.2, 143.4, 140.1, 138.5, 132.0, 130.1, 129.4, 128.7, 128.08, 128.06, 118.1, 112.4, 43.2, 40.1, 36.3, 26.8, 18.1; LC/MS(ESI) m/z : 433 $[M+H]^+$

2. WST-1 assay

Cell viability and proliferation were assessed using the WST-1 assay according to the manufacturer's instructions. Cells were seeded into 96-well plates at a density of 1×10^4 cells per well one day before treatment. For viability, cells were treated with the indicated concentrations of compounds for 1 hour, followed by replacement with 100 μL of fresh medium. For proliferation, cells were treated in the same way, then further incubated for 1, 2, or 3 days after medium replacement. On the day of the WST-1 assay, the culture medium was refreshed (100 μL per well), and 10 μL of WST-1 reagent (Cell Proliferation Reagent WST-1, Roche, 5015944001, USA) was added. After 2 hours of incubation at 37 °C, absorbance was measured at 440 nm using a SpectraMax microplate reader. Results were calculated as values relative to untreated control cells.

3. Cell fractionation for mechanistic analysis

SH-SY5Y cells were harvested and resuspended at 4×10^5 cells per tube in DMEM supplemented with 10% FBS and 100 μM **KD01**. The suspension was incubated for 1 hour at 37 °C, followed by centrifugation at 1,000 rpm for 5 minutes. The supernatant was discarded. For the non-lysed sample, the cell pellet was directly resuspended in PBS. For fractionated samples, the pellet was resuspended in PBS containing 1% Triton X-100 (PBS–T) and incubated for 15 minutes at room temperature. After centrifugation at 12,000 rpm for 15 minutes at 4 °C, the supernatant was collected as the Triton-soluble fraction, and the remaining pellet was resuspended in PBS as the Triton-insoluble fraction. The three samples (intact cells, Triton-soluble fraction, and Triton-insoluble fraction) were transferred to a black 96-well plate, and fluorescence was measured using a SpectraMax microplate reader with excitation at 405 nm.

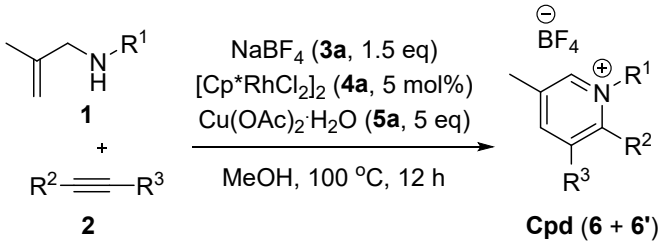
4. Quantification of Neurite Outgrowth Using Imaris Filament Tracer

Differentiated SH-SY5Y neurite outgrowth was measured using the Filament module of Imaris (Oxford Instruments, Abingdon, UK). Neurite structures were reconstructed based on Tuj1 immunostaining in the red fluorescence channel, which selectively labels neuronal processes. The number of branches was automatically quantified based on fluorescence intensity and morphology. Seed point threshold and minimal segment length were adjusted to optimize filament detection.

5. Statistical analysis

Statistical analysis was performed using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). One-way analysis of variance (ANOVA) was applied for comparisons among multiple groups, and p-values are indicated in the figure legends.

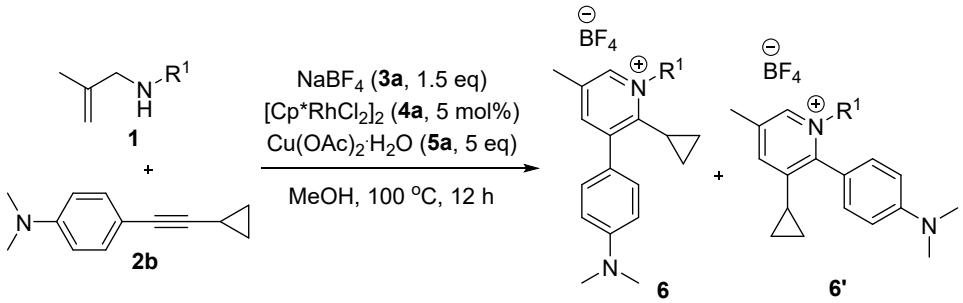
Table S1. Secondary allylamine and internal alkyne scope of the *N*-annulation process for representative pyridinium salt formation[a]



Entry	allylamine (**1**)	alkyne (**2**)	yield (**6**)[b]
1	R_1 = ph	R_2 = cyclopropyl, R_3 = *p*-$N(CH_3)_2C_6H_5$	**6b**+**6b'** (50%, 52:48)
2	R_1 = *p*-C_6H_5-ph	R_2 = cyclopentyl, R_3 = *p*-$N(CH_3)_2C_6H_5$	**6c** (6%)

[a]Unless otherwise noted, reactions were carried out with **1** (0.2 mmol), **2** (0.4 mmol), **3a** (5 mol %), **4a** (0.4 mmol), and **5a** (0.3 mmol) in 0.5 mL of MeOH at 100 °C. [b]All yields are isolated yields.

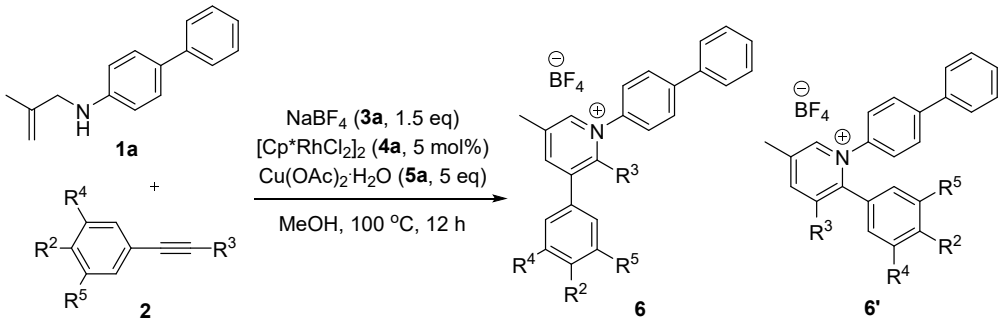
Table S2. Secondary Allylamine (**1**) Scope of the *N*-Annulation Process for Pyridinium Salt Formation[a]



entry	allylamine (**1**)	yield (%)[b]
1	**1c**, R_1 = Phenethyl	5 (**6d**), 3 (**6d'**)
2	**1d**, R_1 = *p*-$N(CH_3)_2$Ph	17 (**6e''**)[c]
3	**1a**, R_1 = *p*-C_6H_5-Ph	17 (**6f**), 11 (**6f'**)
4	**1e**, R_1 = *p*-*N*-methylpiperazine-Ph	6 (**6g**), 12 (**6g'**), 7 (**6g''**)[c]

[a]Unless otherwise noted, reactions were carried out with **1** (0.2 mmol), **2b** (0.4 mmol), **3a** (5 mol %), **4a** (0.4 mmol), and **5a** (0.3 mmol) in 0.5 mL of MeOH at 100 °C. [b]All yields are isolated yields. [c]cyclopropyl ring opened structure.

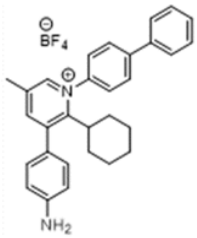
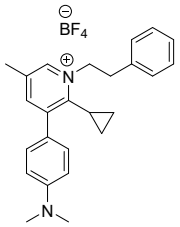
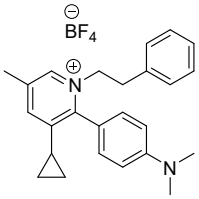
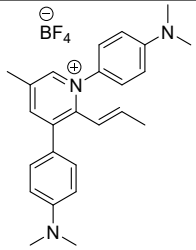
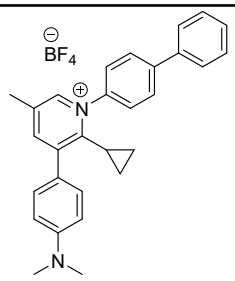
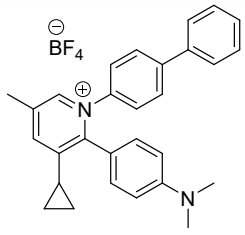
Table S3. Internal Alkyne (**2**) Scope of the *N*-Annulation Process for Pyridinium Salt Formation[a]

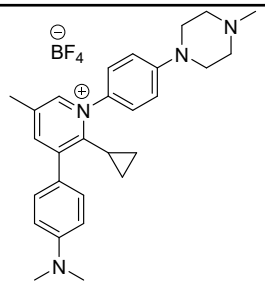
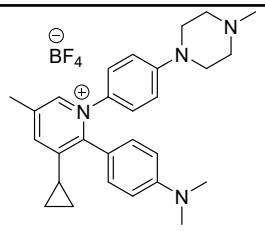
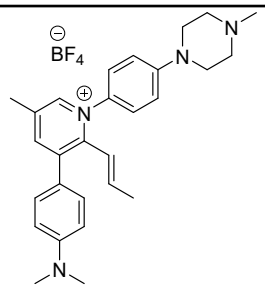
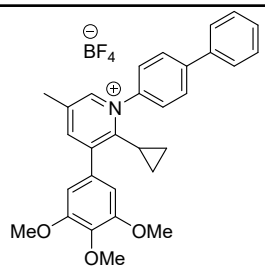
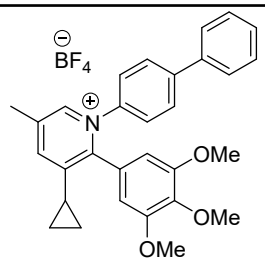
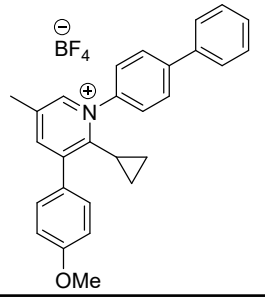


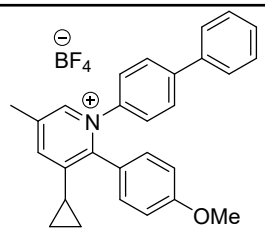
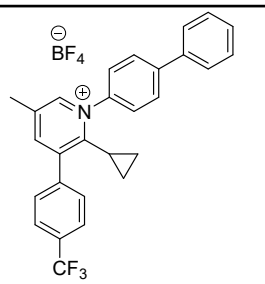
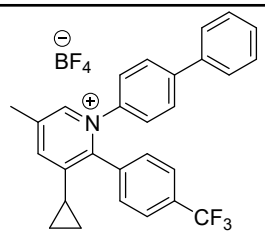
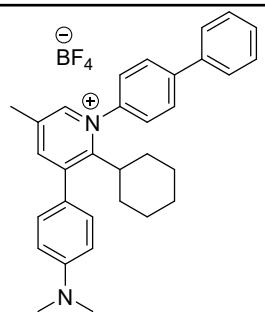
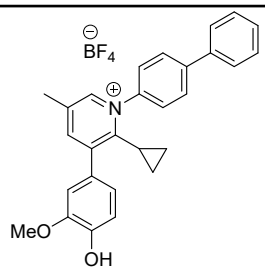
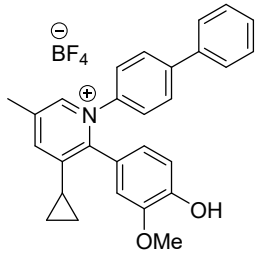
entry	alkyne (**2**)	yield (%)[b]
1	**2d**, R_2 = NH_2, R_3 = cyclohexyl, R_4 = H, R_5 = H	4 (**6a**)
2	**2e**, R_2 = OMe, R_3 = cyclopropyl, R_4 = OMe, R_5 = OMe	30 (**6h**), 12 (**6h'**)
3	**2f**, R_2 = OMe, R_3 = cyclopropyl, R_4 = H, R_5 = H	29 (**6i**), 9 (**6i'**)
4	**2g**, R_2 = CF_3, R_3 = cyclopropyl, R_4 = H, R_5 = H	46 (**6j**), 12 (**6j'**)
5	**2h**, R_2 = $N(CH_3)_2$, R_3 = cyclohexyl, R_4 = H, R_5 = H	12 (**6k**)
6	**2i**, R_2 = OH, R_3 = cyclohexyl, R_4 = OMe, R_5 = H	7 (**6l**), 5 (**6l'**)
7	**2j**, R_2 = CH_3, R_3 = cyclopropyl, R_4 = H, R_5 = H	46 (**6m**), 20 (**6m'**)

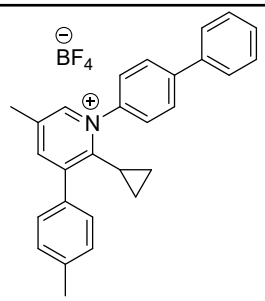
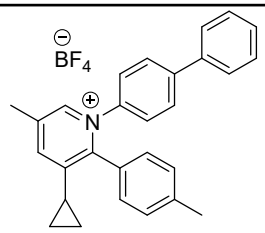
[a]Unless otherwise noted, reactions were carried out with **1a** (0.2 mmol), **2** (0.4 mmol), **3a** (5 mol %), **4a** (0.4 mmol), and **5a** (0.3 mmol) in 0.5 mL of MeOH at 100 °C. [b]All yields are isolated yields.

Table S4. ^{1}H NMR data and isolated yield of each pyridinium salt

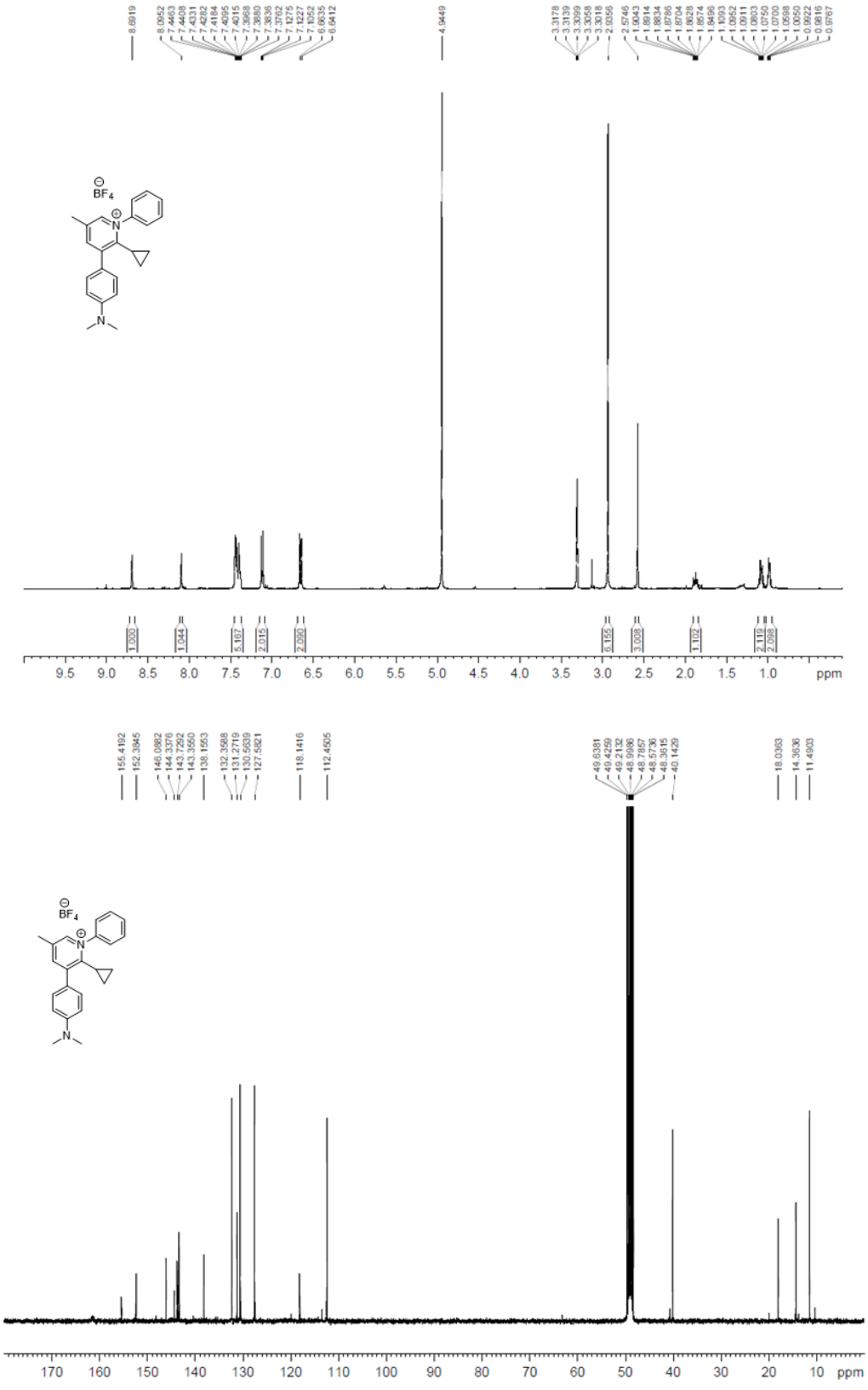
	structure	name	^{1}H NMR, MS	Yield (%)
1		**6a**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.68 (s, 1H), 8.26 (s, 1H), 7.69-7.37 (m, 9H), 6.92 (d, J = 8.12 Hz, 2H), 6.56 (d, J = 8 Hz, 2H), 2.65 (s, 3H), 1.82-1.68 (m, 5H), 1.50-1.41 (m, 2H), 1.20-1.09 (m, 4H); 419 $[M+H]^+$	4
2		**6d**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.75 (br-s, 1H), 7.49 (s, 1H), 7.21-7.20 (m, 3H), 7.04 (d, *J* = 8.48 Hz, 2H), 6.86-6.85 (m, 2H), 6.80 (d, *J* = 8.4 Hz, 2H), 4.65 (br-s, 2H), 3.09 (s, 6H), 3.08-3.03 (m, 2H), 2.45 (s, 3H), 1.64-1.57 (m, 1H), 0.99-0.96 (m, 2H), 0.78-0.75 (m, 2H); 357 $[M+H]^+$	5
3		**6d'**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.77 (br-s, 1H), 7.94 (s, 1H), 7.33-7.27 (m, 3H), 7.06 (d, J = 8.68 Hz, 2H), 6.98 (d, J = 6.48 Hz, 2H), 6.83 (d, J = 8.68 Hz, 2H), 5.23 (t, J = 5.72 Hz, 2H), 3.32 (br-s, 2H), 3.06 (s, 6H), 2.50 (s, 3H), 1.02-0.97 (m, 2H), 0.89-0.86 (m, 1H), 0.45-0.43 (m, 2H); 357 $[M+H]^+$	3
4		**6e''**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.47 (s, 1H), 8.33 (s, 1H), 7.04 (d, J = 8.28 Hz, 2H), 6.92 (d, J = 8.6 Hz, 2H), 6.58-6.53 (m, 4H), 6.51-6.49 (m, 1H), 6.11 (d, J = 15.76 Hz, 1H), 2.98 (s, 6H), 2.95 (s, 6H), 2.60 (s, 3H), 1.85 (d, J = 6.2 Hz,3H); 372 $[M+H]^+$	17
5		**6f**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.54 (s, 1H), 7.78 (s, 1H), 7.58 (d, J = 8.16 Hz, 2H), 7.55-7.52 (m, 2H), 7.45-7.37 (m, 5H), 7.06 (d, J = 8.8 Hz, 2H), 6.54 (d, J = 8.84 Hz, 2H), 2.93 (s, 6H), 2.60 (s, 3H), 1.88-1.82 (m, 1H), 1.10-1.05 (m, 2H), 0.94-0.90 (m, 2H); 405 $[M+H]^+$	17
6		**6f'**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.53 (s, 1H), 8.21 (s, 1H), 7.86 (d, J = 7.88 Hz, 2H), 7.70 (d, J = 7.96 Hz, 2H), 7.65 (d, J= 7.32 Hz, 2H), 7.53-7.42 (m, 5H), 7.13 (d, J = 7.88 Hz, 2H), 3.12 (s, 6H), 2.61 (s, 3H), 2.12-2.09 (m, 1H), 0.70-0.66 (m, 2H), 0.28-0.27 (m, 2H); 405 $[M+H]^+$	11

7		**6g**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.65 (br-s, 1H), 7.66 (s, 1H), 7.07 (br-s, 2H), 6.97 (d, J = 8.28 Hz, 2H), 6.80 (br-s, 2H), 6.56 (d, J = 8.28 Hz, 2H), 3.74-3.66 (m, 2H), 3.48-3.39 (m, 4H), 3.15-3.13 (m, 2H), 2.97 (s, 6H), 2.84 (s, 3H), 2.57 (s, 3H), 1.86-1.83 (m, 1H), 1.11-1.06 (m, 2H), 0.88-0.87 (m, 2H); 427 $[M+H]^+$	6
8		**6g'**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.58 (s, 1H), 8.13 (s, 1H), 7.43 (d, J = 7.88 Hz, 2H), 7.36 (d, J = 8.64 Hz, 2H), 7.08 (d, J = 7.88 Hz, 2H), 6.87 (d, J = 8.64 Hz, 2H), 3.93-3.90 (m, 2H), 3.64-3.62 (m, 2H), 3.57-3.50 (m, 2H), 3.21-3.14 (m, 2H), 3.08 (s, 6H), 2.91 (s, 3H), 2.59 (s, 3H), 2.04-2.01 (m, 1H), 0.76-0.71 (m, 2H), 0.21-0.19 (m, 2H); 427 $[M+H]^+$	12
9		**6g''**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.68 (br-s, 1H), 8.31 (s, 1H), 7.06 (br-s, 2H), 6.87 (d, J = 7.96 Hz, 2H), 6.80 (br-s, 2H), 6.56 (d, J = 7.88 Hz, 2H), 6.49-6.44 (m, 1H), 6.16 (d, J = 15.64 Hz, 1H), 3.81-3.76 (m, 2H), 3.49-3.45 (m, 4H), 3.12-3.08 (m, 2H), 2.99 (s, 6H), 2.85 (s, 3H), 2.60 (s, 3H), 1.88 (d, J = 6.12 Hz, 3H); 427 $[M+H]^+$	7
10		**6h**	^{1}H NMR 400 MHz, MeOD δ 8.82 (d, J = 0.76 Hz, 1H), 8.18 (d, J = 0.92 Hz, 1H), 7.72-7.70 (m, 2H), 7.60-7.52 (m, 4H), 7.47-7.37 (m, 3H), 6.73 (s, 2H), 3.73 (s, 6H), 3.70 (s, 3H), 2.62 (s, 3H), 1.91-1.87 (m, 1H), 1.16-1.11 (m, 2H), 1.07-1.03 (m, 2H); 452 $[M+H]^+$	30
11		**6h'**	^{1}H NMR 400 MHz, MeOD δ 8.88 (d, J = 1.16 Hz, 1H), 8.53 (d, J = 1.48 Hz, 1H), 8.01-7.97 (m, 2H), 7.85-7.82 (m, 2H), 7.77-7.74 (m, 2H), 7.54-7.43 (m, 3H), 6.96 (s, 2H), 3.92 (s, 6H), 3.85 (s, 3H), 2.63 (s, 3H), 2.36-2.29 (m, 1H), 0.70-0.65 (m, 2H), 0.34-0.29 (m, 2H); 452 $[M+H]^+$	12
12		**6i**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.56 (s, 1H), 7.82 (s, 1H), 7.56 (d, J = 7.64 Hz, 2H), 7.51 (d, J = 7.16 Hz, 2H), 7.45-7.36 (m, 5H), 7.20 (d, J = 8.52 Hz, 2H), 6.83 (d, J = 8.44 Hz, 2H), 3.77 (s, 3H), 2.61 (s, 3H), 1.76-1.72 (m, 1H), 1.09-1.04 (m, 2H), 0.94-0.90 (m, 2H); 392 $[M+H]^+$	29

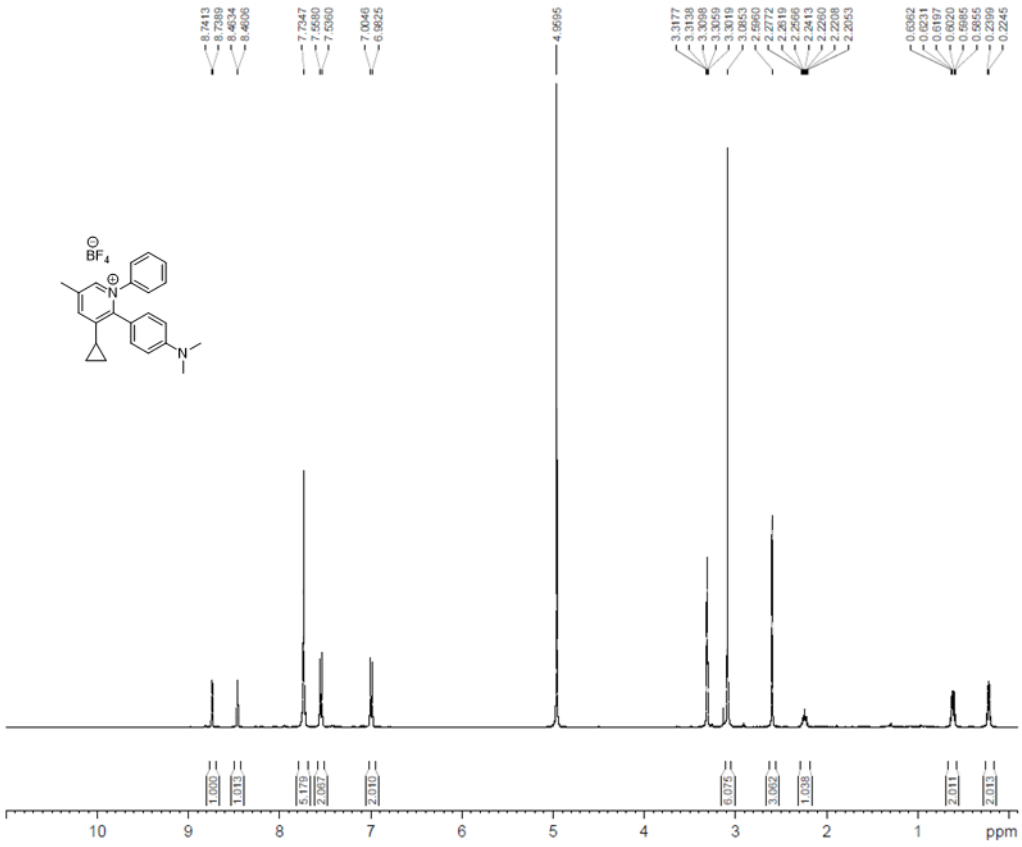
13		**6i'**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.63 (br-s, 1H), 8.19 (s, 1H), 7.85-7.76 (m, 4H), 7.65 (d, J = 7.36 Hz, 2H), 7.52-7.41 (m, 5H), 7.04 (d, J = 8.08 Hz, 2H), 3.88 (s, 3H), 2.61 (s, 3H), 2.11-2.10 (m, 1H), 0.66-0.64 (m, 2H), 0.28-0.27 (m, 2H); 392 $[M+H]^+$	9
14		**6j**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.59 (s, 1H), 7.88 (s, 1H), 7.63-7.53 (m, 6H), 7.49-7.38 (m, 7H), 2.63 (s, 3H), 1.63-1.56 (m, 1H), 1.07-1.05 (m, 2H), 0.95-0.94 (m, 2H); 430 $[M+H]^+$	46
15		**6j'**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.71 (br-s, 1H), 8.23 (s, 1H), 7.86-7.79 (m, 5H), 7.71-7.64 (m, 4H), 7.52-7.42 (m, 4H), 2.65 (s, 3H), 2.17 (br-s, 1H), 0.65-0.63 (m, 2H), 0.29 (br-s, 2H); 430 $[M+H]^+$	12
16		**6k**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.65 (br-s, 1H), 8.26 (s, 1H), 7.57-7.52 (m, 4H), 7.45-7.35 (m, 5H), 6.94 (d, J = 8.4 Hz, 2H), 6.54 (d, J = 8.28 Hz, 2H), 2.94 (s, 6H), 2.64 (s, 3H), 1.82-1.67 (m, 5H), 1.47-1.14 (m, 6H); 447 $[M+H]^+$	12
17		**6l**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.51 (s, 1H), 7.78 (s, 1H), 7.61-7.38 (m, 8H), 7.17 (br-s, 1H), 6.90-6.74 (m, 3H), 3.70 (s, 3H), 2.59 (s, 3H), 1.85-1.81 (m, 1H), 1.08-1.06 (m, 2H), 0.94-0.85 (m, 2H); 408 $[M+H]^+$	7
18		**6l'**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.53 (s, 1H), 8.21 (s, 1H), 7.85 (d, J = 7.84 Hz, 2H), 7.78 (d, J = 7.88 Hz, 2H), 7.65 (d, J = 7.28 Hz, 2H), 7.52-7.44 (m, 3H), 7.18 (s, 1H), 7.03 (d, J = 8.12 Hz, 1H), 6.91 (d, J = 7.84 Hz, 1H), 3.95 (s, 3H), 2.61 (s, 3H), 2.20-2.17 (m, 1H), 0.66-0.64 (m, 2H), 0.29-0.28 (m, 2H); 408 $[M+H]^+$	5

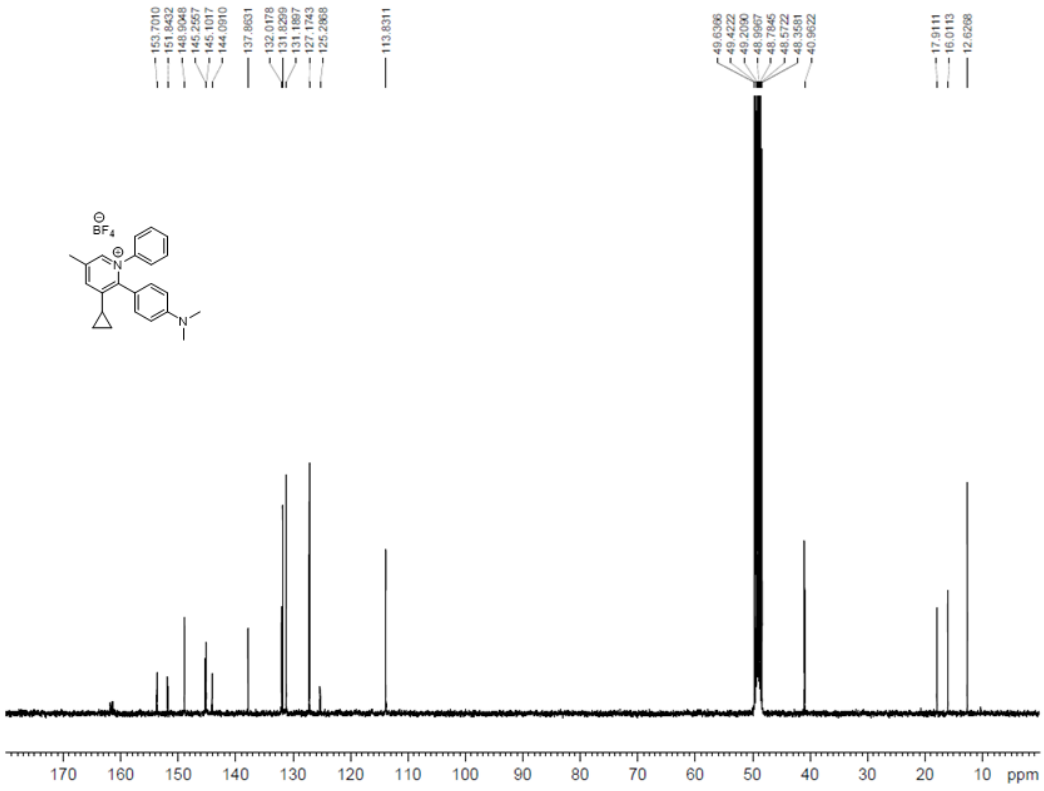
19		**6m**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.53 (br-s, 1H), 7.83 (s, 1H), 7.55-7.50 (m, 4H), 7.45-7.32 (m, 5H), 7.13-7.10 (m, 4H), 2.59 (s, 3H), 2.29 (s, 3H), 1.70 (br-s, 1H), 1.07-1.05 (m, 2H), 0.92 (br-s, 2H); LC/MS(ESI) m/z: 376 $[M+H]^+$	46
20		**6m'**	^{1}H NMR 400 MHz, $CDCl_3$ δ 8.68 (br-s, 1H), 8.18 (s, 1H), 7.86-7.75 (m, 4H), 7.65 (d, J = 7.36 Hz, 2H), 7.52-7.31 (m, 7H), 2.62 (s, 3H), 2.44 (s, 3H), 2.17-2.09 (m, 1H), 0.64-0.60 (m, 2H), 0.28-0.27 (m, 2H); LC/MS(ESI) m/z: 376 $[M+H]^+$	20

2-cyclopropyl-3-(4-(dimethylamino)phenyl)-5-methyl-1-phenylpyridin-1-ium tetrafluoroborate (**6b**)



3-cyclopropyl-2-(4-(dimethylamino)phenyl)-5-methyl-1-phenylpyridin-1-ium tetrafluoroborate (**6b'**)





1-([1,1'-biphenyl]-4-yl)-2-cyclopentyl-3-(4-(dimethylamino)phenyl)-5-methylpyridin-1-ium tetrafluoroborate (**6c**)

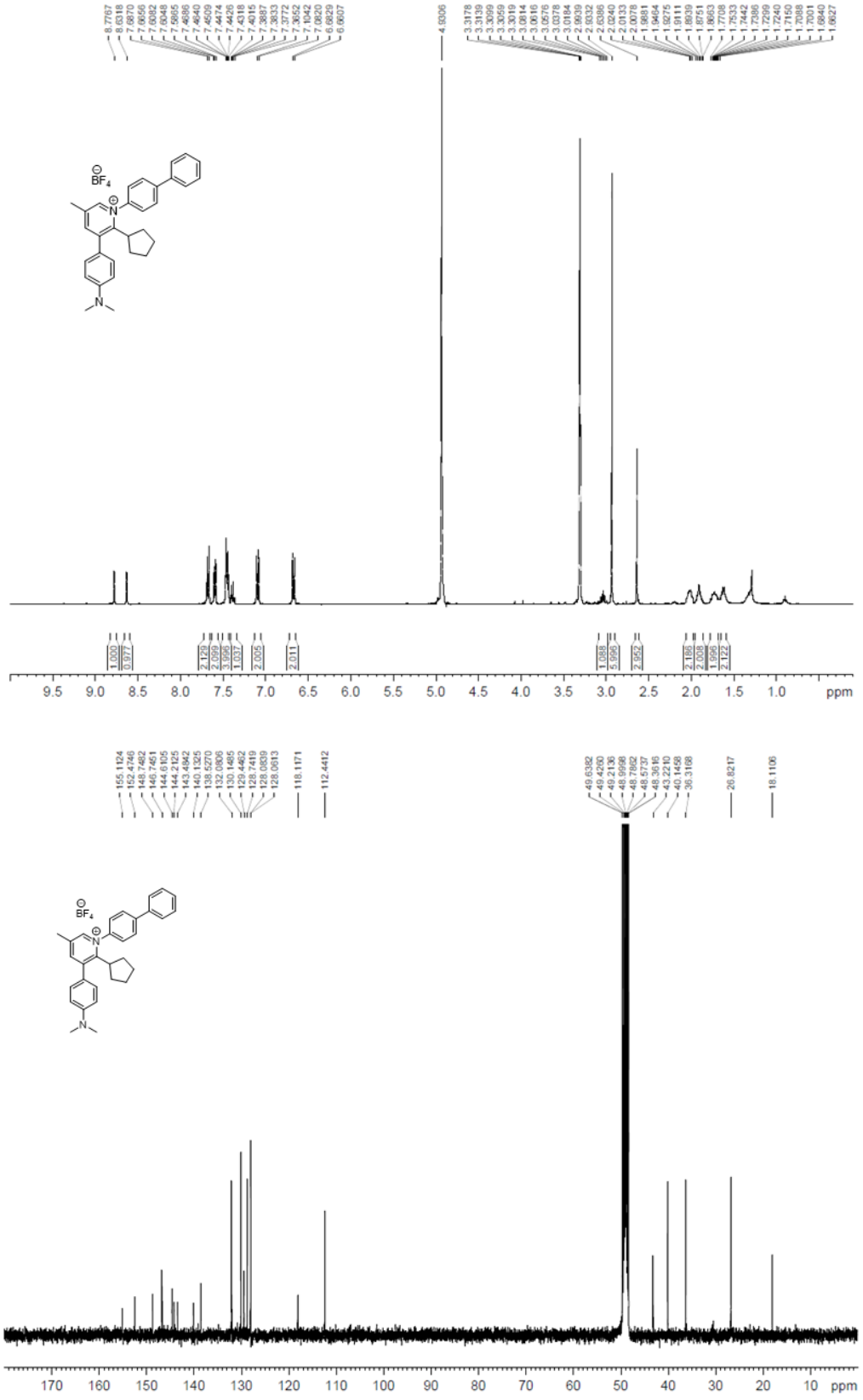


Figure S1. ^{1}H and ^{13}C NMR spectra of representative compounds (**6b**, **6b'** and **6c**)

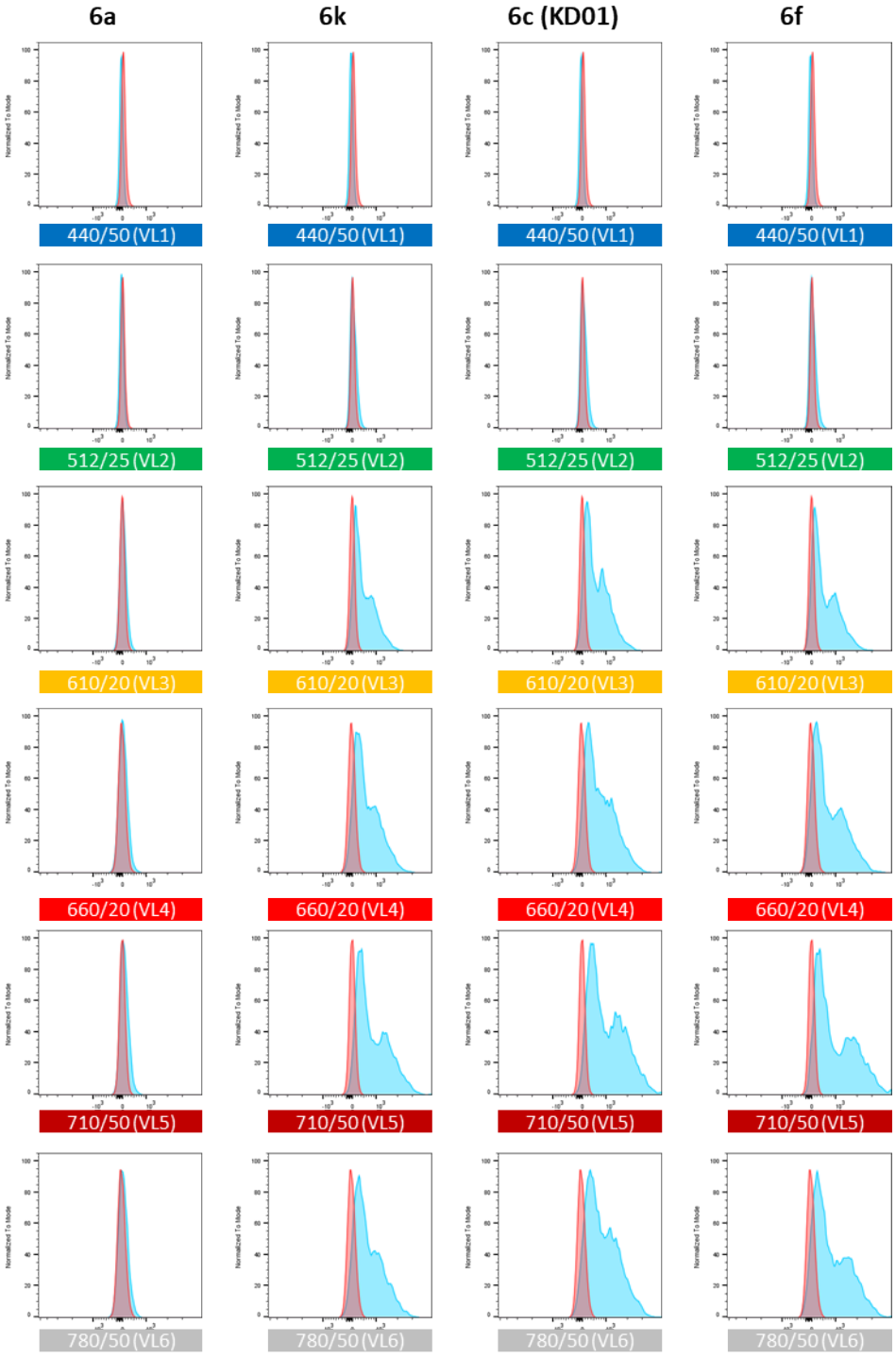


Figure S2. Flow cytometry profiles of primary mouse brain cells treated with lead compounds. Live cells were isolated from the brains of postnatal day 1 mice and incubated for 1 h with 10 µM of the indicated compounds (**6a**, **6k**, **6c/KD01**, and **6f**). Fluorescence signal from the stained cells was analyzed using a flow cytometer with a 405 nm excitation laser. The histograms show the fluorescence intensity detected in each of the six violet laser emission channels (VL1–VL6), which are defined by their central wavelength and bandpass filters.

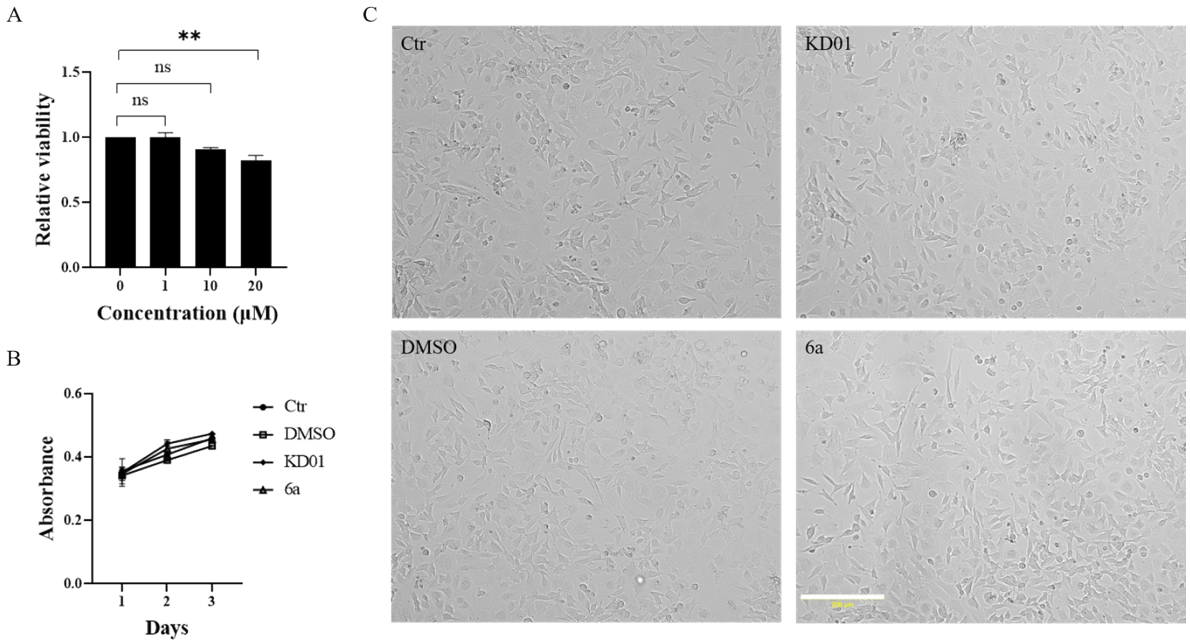


Figure S3 Biocompatibility of KD01 in SH-SY5Y cells. (A) Cell viability was assessed by the WST-1 assay after 1h treatment with **KD01** at the indicated concentrations. (B) Cell proliferation over three days was measured by WST-1 assay following a 1h treatment with DMSO (vehicle control), 10 μM **KD01**, or 10 μM **6a**. (C) Representative bright-field images of cells on day 3, showing normal morphology in all treatment groups. Scale bar, 200 μm. Data in (A) and (B) are presented as mean ± SD (n=3). **, $p < 0.01$ compared to the untreated control group; ns, not significant.

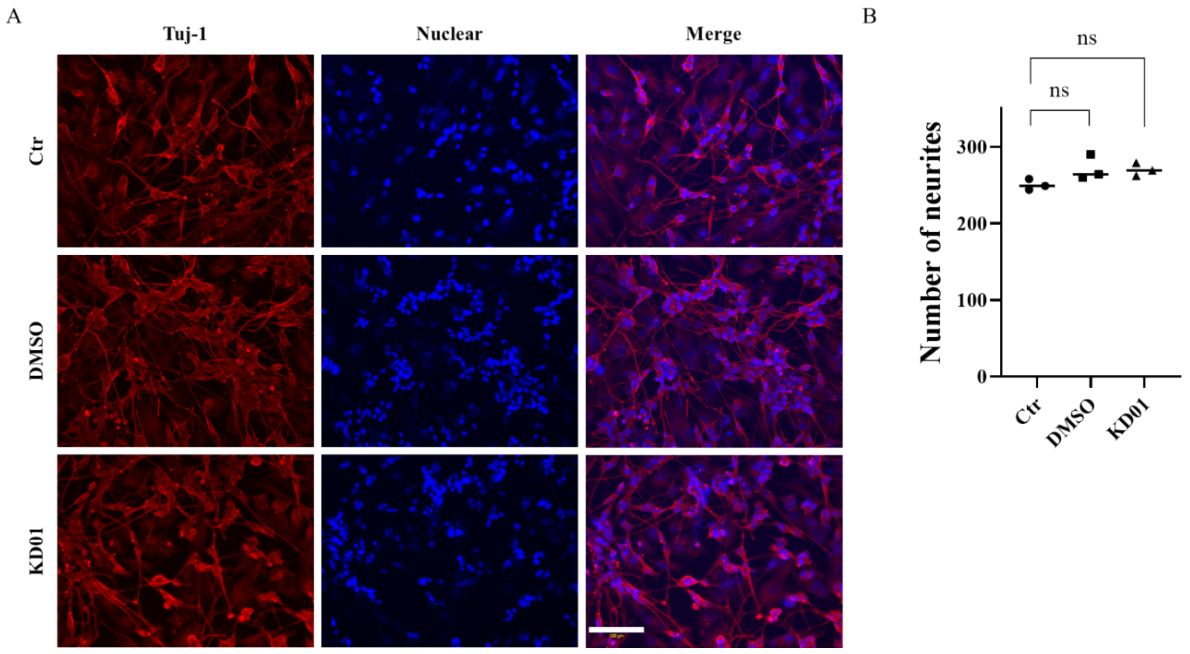
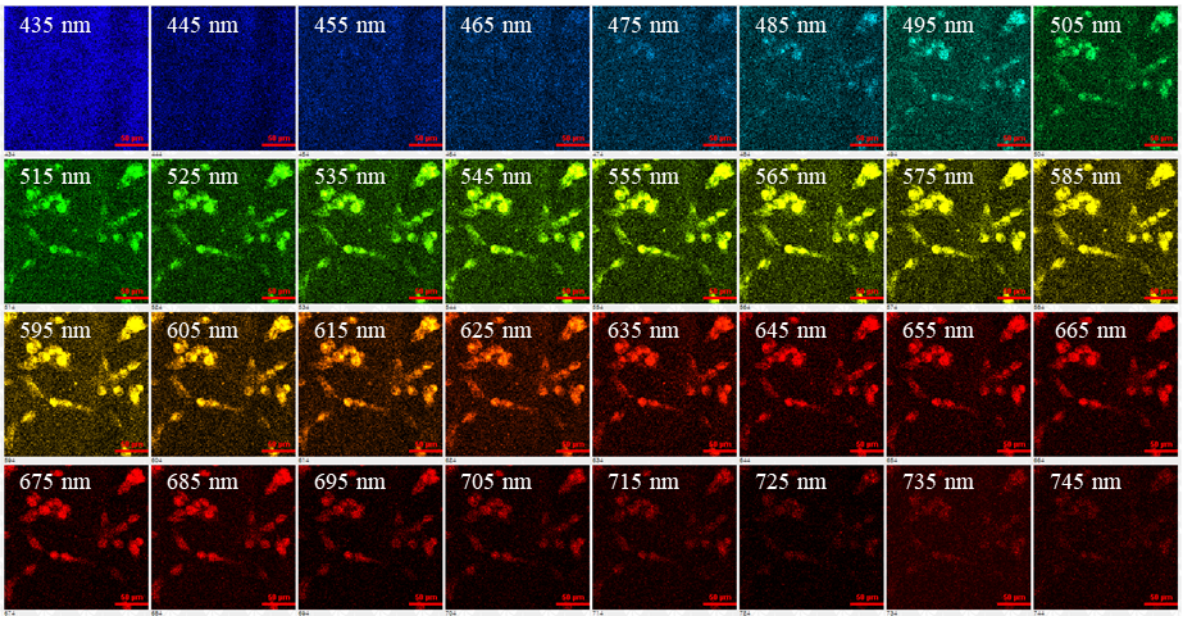
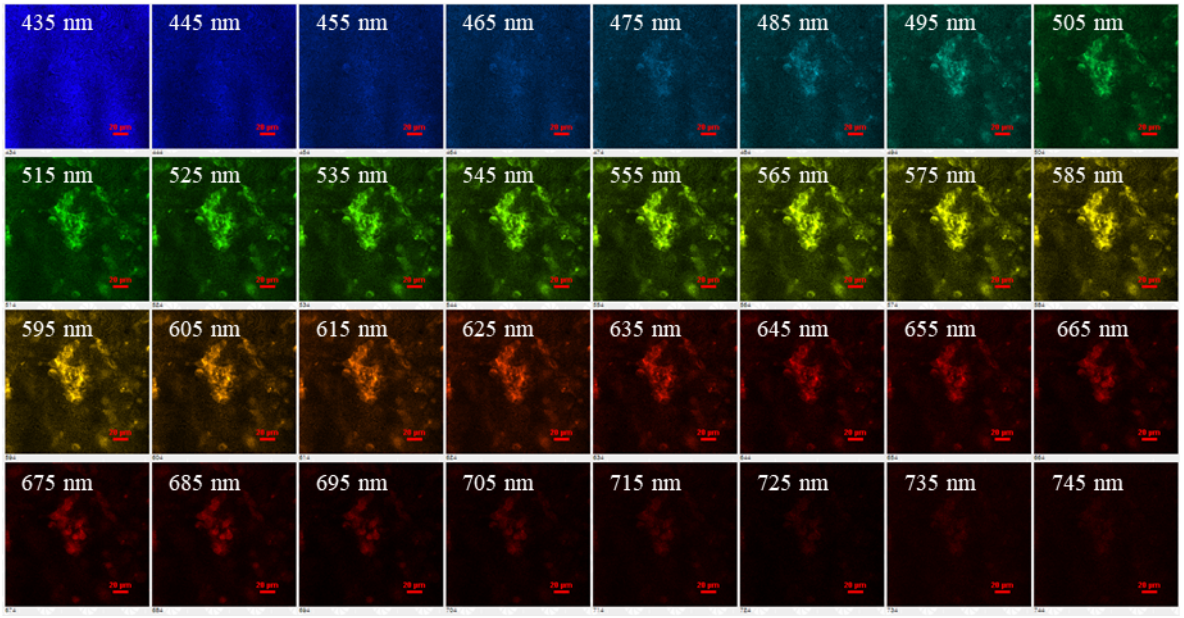


Figure S4. KD01 does not impair the neural differentiation potential of SH-SY5Y cells. (A) Representative confocal images and (B) quantification of neurite outgrowth from the differentiated SH-SY5Y cells. Cells were treated with 10 µM **KD01** or DMSO (vehicle control) for 1 h and then differentiated for 14 days. Cells were stained for neuronal markers Tuj1 (red) with DAPI (blue) as a nuclear counterstain. The results show that KD01-treated cells differentiated as effectively as the control group. Data in (B) are presented as the mean, with individual data points shown (n=3). Scale bar, 100 µm. ns, not significant.

A SH-SY5Y undifferentiation



B SH-SY5Y differentiation



C ReNcell differentiation

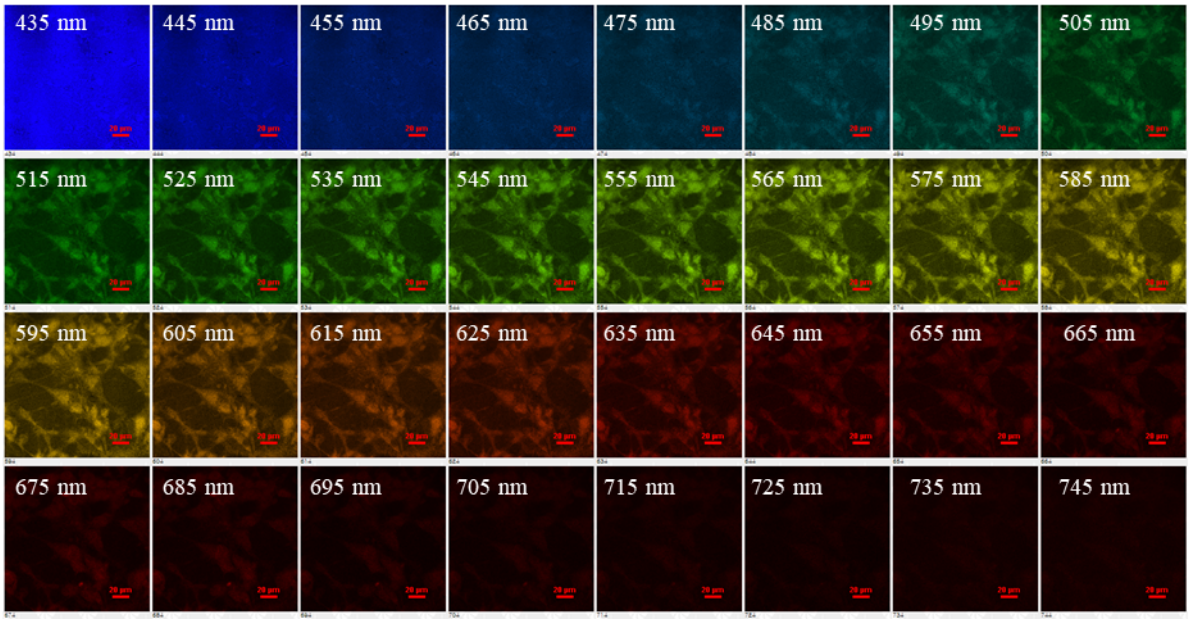


Figure S5. Spectral emission profiles of KD01 in various neural cell states. Representative spectral confocal images showing the fluorescence emission of KD01 in (A) undifferentiated SH-SY5Y cells, (B) differentiated SH-SY5Y cells, and (C) differentiated ReNcell VM cells. For spectral imaging, cells were excited at 405 nm, and emission was captured from 435 nm to 745 nm.

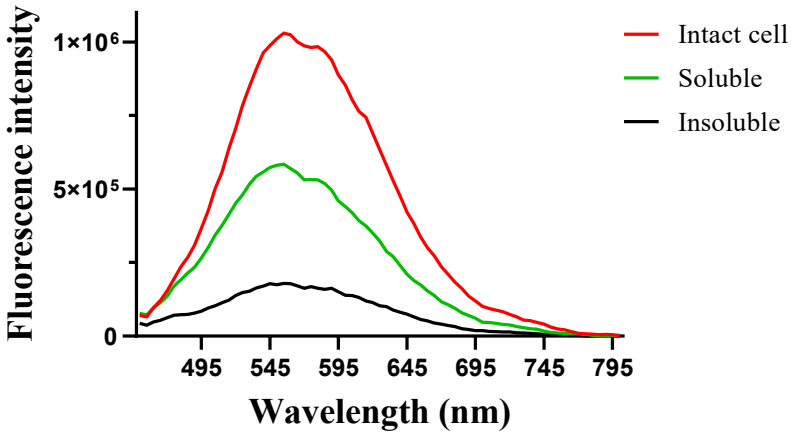


Figure S6. Fluorescence emission spectra of KD01 in different cellular fractions. For comparison, three parallel samples were prepared from an equivalent number of KD01-treated (100 μM, 1 h) SH-SY5Y cells. Sample 1 (Intact): The cell pellet was resuspended in PBS. Samples 2 & 3 (Fractions): An equivalent cell pellet was lysed with 1% Triton X-100 and separated by centrifugation into the soluble (supernatant) and insoluble (pellet) fractions. The fluorescence emission spectra of all three samples were recorded with excitation at 405 nm.