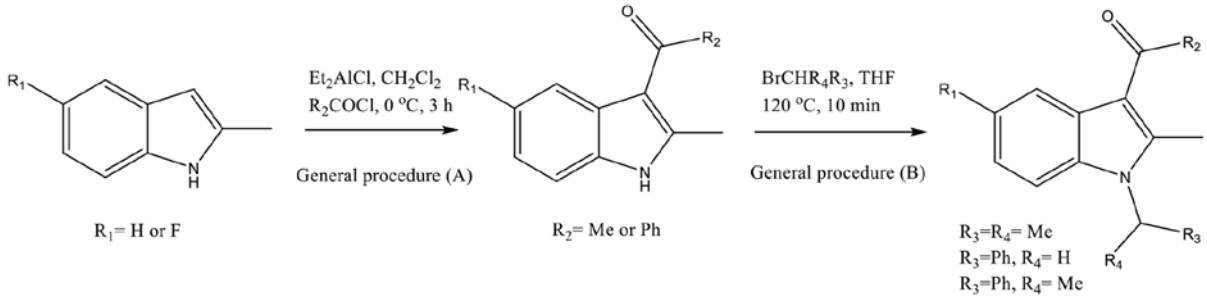
Below are the synthetic schemes of the derivative compounds described in text.

General Synthetic Scheme

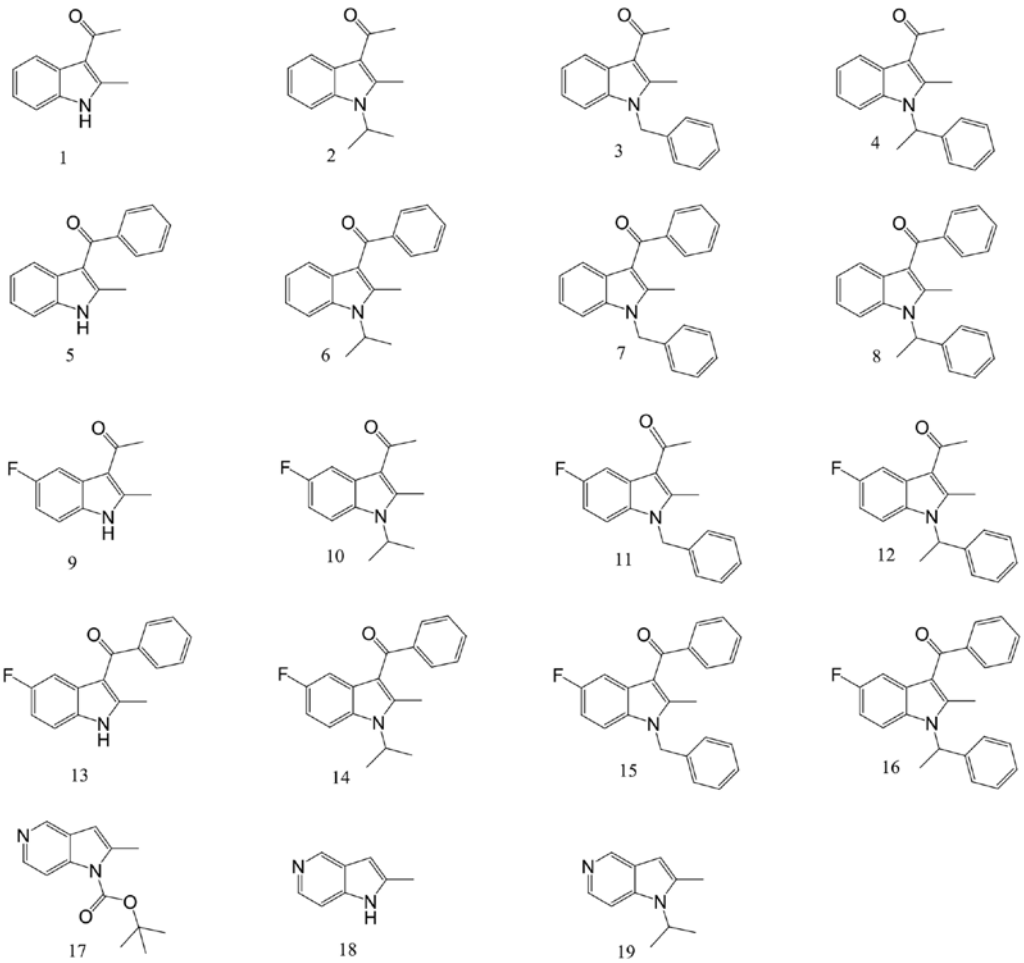
Scheme 1



General procedure (A): Acylation of indoles at the 3-position (1). To a CH_2Cl_2 solution (5 ml) of 2-methyl-1*H*-indole (1.0 mmol) was added 1.4 ml (1.5 equiv.) of Et_2AlCl (1.07 mol/l in hexane) dropwise at 0˚C . The mixture was stirred at 0˚C for 30 min. To this solution was added dropwise CH_2Cl_2 solution (4 ml) of acyl chloride (1.5 equiv.) at 0˚C. The resulting solution was stirred at 0˚C for 3 h, and pH 7 aqueous buffer (10 ml) was added to quench the reaction and extracted by ethyl acetate. After the usual workup, the crude product was purified by flash column chromatography to provide the 3-acetylindole.

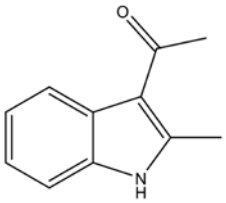
General procedure (B): N-alkylation of 3-acylindoles. To a solution of 3-acylindole (1.0 mmol) in dimethylformamide (10 ml) was added NaH (5 equiv.) and alkyl halide (5 equiv.). The reaction mixture was heated at 120˚C for 10 min with stirring in microwave reactor. After the reaction it was quenched with water and extracted by ethyl acetate. After usual workup, the crude product was purified by flash column chromatography to provide the N-alkylindole.

Chemical structures of synthetic compounds



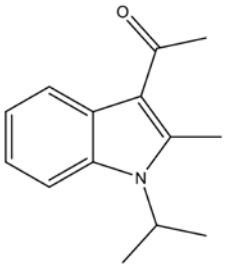
Characterization of synthetic compounds

1-(2-Methyl-1*H*-indol-3-yl)-ethanone



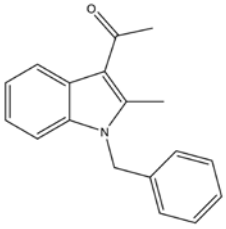
Synthesized according to the general procedure (A) with 2-methyl-1*H*-indole and acetyl chloride to give the titled compound (yield 74%) as light yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.50 (brs, 1H), 8.06 (d, J=8.2, 1H), 7.33 (d, J=7.5, 1H), 7.21-7.25 (m, 2H), 2.88 (s, 3H), 2.66 (s, 3H); LCMS m/z:196 ($M+Na^+$), 174 ($M+H^+$).

1-[2-Methyl-1-(1-methylethyl)-1*H*-indol-3-yl]-ethanone



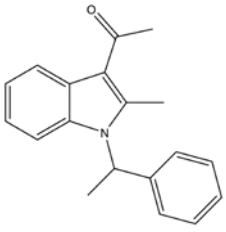
Synthesized according to the general procedure (B) with 1-(2-Methyl-1*H*-indol-3-yl)-ethanone and 2-bromopropane at 120˚C for 10 min to give titled compound (yield 52%) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.97 (d, J=7.5, 1H), 7.54 (d, J=8.0, 1H), 7.18-7.25 (m, 2H), 4.82 (m, 1H), 2.80 (s, 3H), 2.69 (s, 3H), 1.65 (s, 3H), 1.63 (s, 3H); LCMS m/z:453 ($2M+Na^+$), 238 ($M+Na^+$), 216 ($M+H^+$).

1-[2-methyl-1-(phenylmethyl)-1*H*-indol-3-yl]-ethanone



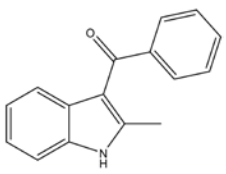
Synthesized according to the general procedure (B) with 1-(2-Methyl-1*H*-indol-3-yl)-ethanone and benzyl bromide at R.T. for 1 h to give titled compound (yield 87%) as light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 (d, J=7.6, 1H), 7.18-7.32 (m, 6H), 6.98 (d, J=6.4, 2H), 5.37 (s, 2H), 2.73 (s, 3H), 2.69 (s, 3H); LCMS m/z:549 ($2M+Na^+$), 286 ($M+Na^+$), 264 ($M+H^+$).

1-[2-methyl-1-(1-phenylethyl)-1*H*-indol-3-yl]-ethanone



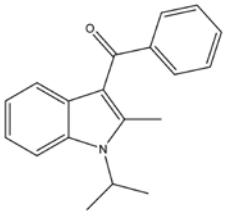
Synthesized according to the general procedure (B) with 1-(2-Methyl-1*H*-indol-3-yl)-ethanone and 1-bromoethylbenzene at room temperature for 1 h to give titled compound (yield 87%) as light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, J=8.4, 1H), 7.15-7.21 (m, 3H), 7.23-7.35 (m, 3H), 7.04 (d, J=4.0, 2H), 5.91 (q, J=7.2, 1H), 2.77 (s, 3H), 2.70 (s, 3H), 1.97 (d, J=7.2, 3H); LCMS m/z:278($M+H^+$).

(2-Methyl-1*H*-indol-3-yl)-phenyl-methanone



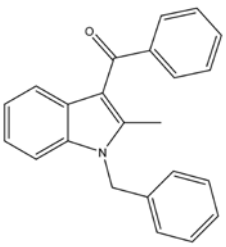
Synthesized according to the general procedure (A) with 2-methyl-1*H*-indole and benzoyl chloride to give the titled compound (yield 79%) as light yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.50 (brs, 1H), 8.48 (brs, 1H), 7.75 (d, J=7.2, 2H), 7.54 (t, J=7.2, 1H), 7.45 (t, J=7.2, 1H), 7.38 (d, J=7.2, 1H), 7.32 (d, J=7.2, 1H), 7.16 (t, J=7.2, 1H), 7.07 (t, J=7.2, 1H), 2.55 (s, 3H); LCMS m/z:493 ($2M+ Na^+$), 258 ($M+Na^+$), 236 ($M+H^+$).

1-[2-Methyl-1-(1-methylethyl)-1*H*-indol-3-yl]-phenyl-methanone



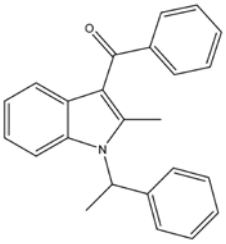
Synthesized according to the general procedure (B) with (2-methyl-1*H*-indol-3-yl)-phenyl-methanone and 2-bromopropane at 120˚C for 40 min to give titled compound (yield 27%) as light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (dd, J=8.4, 1.6, 2H), 7.54 (d, J=8.0, 2H), 7.44 (t, J=8.4, 2H), 7.28 (d, J=7.6, 1H), 7.17 (t, J=7.0, 1H), 7.02 (t, J=7.0, 1H), 4.82 (m, 1H), 2.62 (s, 3H), 1.72 (s, 3H), 1.69 (s, 3H); LCMS m/z:577 ($2M+Na^+$), 278 ($M+H^+$).

1-[2-methyl-1-(phenylmethyl)-1*H*-indol-3-yl]-phenyl-methanone



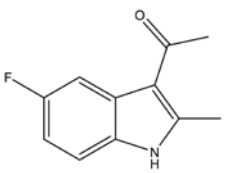
Synthesized according to the general procedure (B) with (2-methyl-1*H*-indol-3-yl)-phenyl-methanone and benzyl bromide at room temperature for 1 h to give titled compound (yield 82%) as light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (d, J=7.6, 2H), 7.56 (t, J=6.4, 1H), 7.48 (t, J=7.6, 2H), 7.25-7.37 (m, 5H), 7.02-7.08 (m, 3H), 5.41 (s, 2H), 2.56 (s, 3H); LCMS m/z:673 ($2M+Na^+$), 651 ($2M+H^+$), 326 ($M+H^+$).

1-[2-methyl-1-(1-phenylethyl)-1*H*-indol-3-yl]-phenyl-methanone



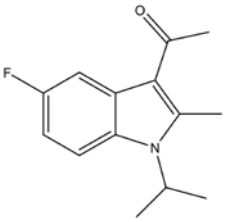
Synthesized according to the general procedure (B) with (2-methyl-1*H*-indol-3-yl)-phenyl-methanone and 1-bromoethylbenzene at 120˚C for 2 h to give titled compound (yield 91%) as light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (d, J=7.6, 2H), 7.55 (t, J=6.4, 1H), 7.48 (t, J=7.6, 2H), 7.20-7.37 (m, 6H), 6.95-7.06 (m, 3H), 5.92 (q, 1H), 2.70 (s, 3H), 2.02 (d, J=7.0, 3H); LCMS m/z:701 ($2M+Na^+$), 340 ($M+H^+$).

1-(5-Fluoro-2-methyl-1*H*-indol-3-yl)-ethanone



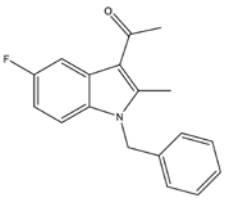
Synthesized according to the general procedure (A) with 5-fluoro-2-methyl-1*H*-indole and acetyl chloride to give the titled compound (yield 73%) as light yellow solid. ^{1}H NMR (400 MHz, DMSO-d6): δ 11.93 (brs, 1H), 7.73 (d, J=8.0, 1H), 7.36 (dd, J=8.0, 5.2, 1H), 7.00 (t, J=5.6, 1H), 2.69 (s, 3H), 2.50 (s, 3H); LCMS m/z:214 ($M+Na^+$), 192 ($M+H^+$).

1-[5-Fluoro-2-methyl-1-(1-methylethyl)-1*H*-indol-3-yl]-ethanone



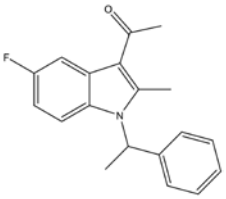
Synthesized according to the general procedure (B) with 1-(5-Fluoro-2-methyl-1*H*-indol-3-yl)-ethanone and 2-bromopropane at 120˚C for 20 min to give titled compound (yield 14%) as light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (dd, J=10.2, 2.5, 1H), 7.45 (dd, J=9.2, 4.6, 1H), 6.95 (t, J=9.2, 1H), 4.82 (m, 1H), 2.80 (s, 3H), 2.64 (s, 3H), 1.64 (d, J=7.6, 6H); LCMS m/z:234 ($M+H^+$).

1-[5-Fluoro-2-methyl-1-(phenylmethyl)-1*H*-indol-3-yl]-ethanone



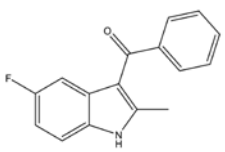
Synthesized according to the general procedure (B) with 1-(5-Fluoro-2-methyl-1*H*-indol-3-yl)-ethanone and benzyl bromide at room temperature for 1 h to give titled compound (yield 84%) as light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (d, J=7.6, 1H), 7.20-7.31 (m, 3H), 7.15 (d, J=4.5, 1H), 6.87-6.95 (m, 3H), 5.33 (s, 2H), 2.72 (s, 3H), 2.64 (s, 3H); LCMS m/z:585 ($2M+Na^+$), 304 ($M+Na^+$), 282 ($M+H^+$).

1-[5-Fluoro-2-methyl-1-(1-phenylethyl)-1*H*-indol-3-yl]-ethanone



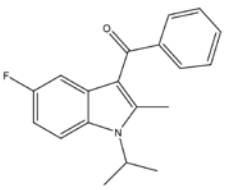
Synthesized according to the general procedure (B) with 1-(5-Fluoro-2-methyl-1*H*-indol-3-yl)-ethanone and 1-bromoethylbenzene at 120˚C for 20 min to give titled compound (yield 4%) as light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (dd, J=10.4, 2.8, 1H), 7.25-7.39 (m, 3H), 7.14 (d, J=8.0, 2H), 6.92 (dd, J=7.4, 4.5, 1H), 6.76 (t, J=7.4, 1H), 5.88 (q, J=7.2, 1H), 2.81 (s, 3H), 2.67 (s, 3H), 1.96 (d, J=7.2, 3H); LCMS m/z:296 ($M+H^+$).

(5-Fluoro-2-methyl-1*H*-indol-3-yl)-phenyl-methanone



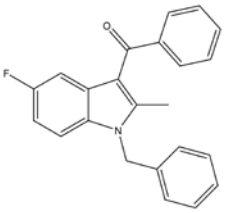
Synthesized according to the general procedure (A) with 5-fluoro-2-methyl-1*H*-indole and benzoyl chloride to give the titled compound (yield 75%) as light yellow solid; ^{1}H NMR (400 MHz, DMSO-d6): δ 12.08 (brs, 1H), 7.57-7.62 (m, 3H), 7.50-7.53 (m, 2H), 7.38 (dd, J=9.2, 5.2, 1H), 6.95-7.03 (m, 2H), 2.34 (s, 3H); LCMS m/z:529 (2M+ Na^+), 276 (M+Na^+), 254 (M+H^+).

1-[5-Fluoro-2-methyl-1-(1-methylethyl)-1*H*-indol-3-yl]-phenyl-methanone



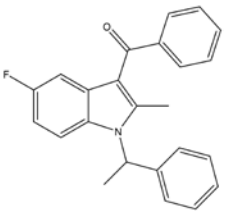
Synthesized according to the general procedure (B) with (5-fluoro-2-methyl-1*H*-indol-3-yl)-phenyl -methanone and 2-bromopropane at 120˚C for 20 min to give titled compound (yield 17%) as light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (d, J=6.8, 2H), 7.40-7.56 (m, 4H), 6.88-6.96 (m, 2H), 4.78 (m, 1H), 2.58 (s, 3H), 1.67 (d, J=7.6, 6H); LCMS m/z:613 (2M+Na^+), 296 (M+H^+).

1-[5-Fluoro-2-methyl-1-(phenylmethyl)-1*H*-indol-3-yl]-phenyl-methanone



Synthesized according to the general procedure (B) with (5-Fluoro-2-methyl-1*H*-indol-3-yl)-phenyl-methanone and benzyl bromide at room temperature for 1 h to give titled compound (yield 84%) as light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (dd, J=8.4, 1.6, 2H), 7.57 (t, J=8.4, 1H), 7.47 (m, 2H), 7.25-7.33 (m, 3H), 7.17 (m, 1H), 7.00-7.05 (m, 3H), 6.90 (m, 1H), 5.38 (s, 2H), 2.53 (s, 3H); LCMS m/z:709 (2M+Na^+), 344 (M+H^+).

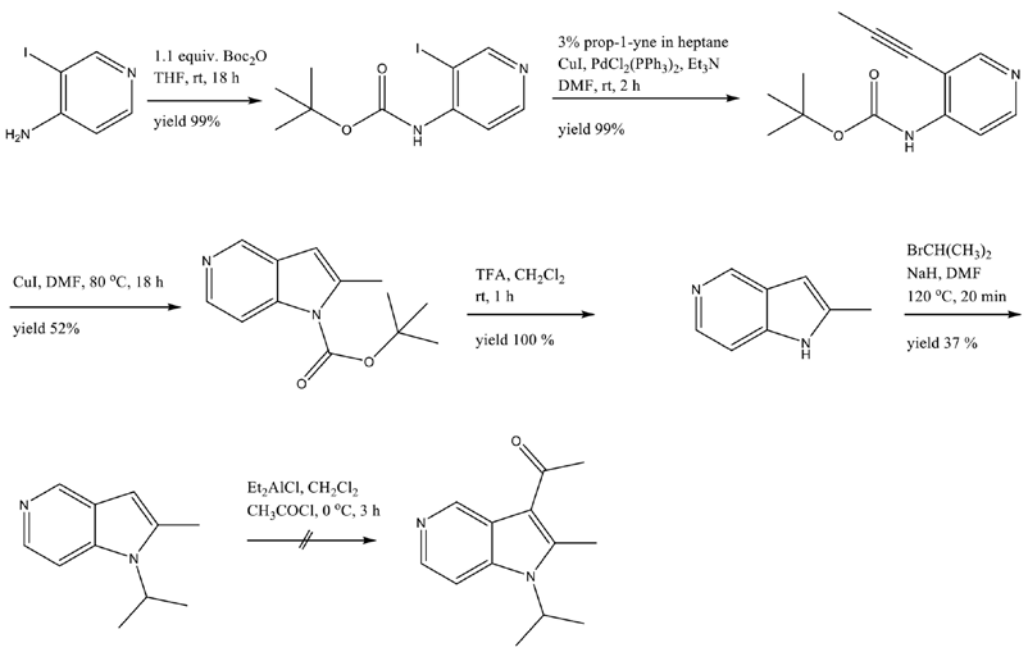
1-[5-Fluoro-2-methyl-1-(1-phenylethyl)-1*H*-indol-3-yl]-phenyl-methanone



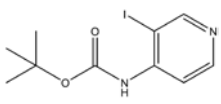
Synthesized according to the general procedure (B) with (5-Fluoro-2-methyl-1*H*-indol-3-yl)-phenyl-methanone and 1-bromoethylbenzene at 120˚C for 20 min to give titled compound (yield 35%) as light yellow oil. ^{1}H NMR (400 MHz, CD_3OD): δ 7.77 (d, J=6.8, 2H), 7.45-7.60 (m, 3H), 7.20-7.40 (m, 5H), 6.88-6.96 (m, 2H), 6.72 (t, J=7.4, 1H), 5.87 (q, J=6.4, 1H), 2.60 (s, 3H), 2.00 (d, J=6.4, 3H); LCMS m/z:737 (2M+Na^+), 358 (M+H^+).

Synthetic scheme of 1*H*-pyrrolo[3,2-c]pyridine derivatives

Scheme 2

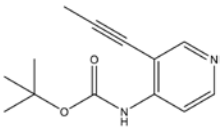


_N_-(3-Iodo-4-pyridinyl)-1,1-dimethylethyl ester carbamic acid



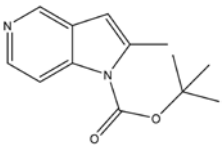
Di-*tert*-butyl dicarbonate (1.26 ml, 5.5 mmol) was added to a stirred suspension of 3-iodo-4-aminopyridine (1.1 g, 5.0 mmol) in tetrahydrofurane (5 ml) at 0°C . The solution was stirred at room temperature for 18 h. After usual workup, the crude product was purified by flash column chromatography to provide the titled compound (1.59 g, 71%) as an off-white solid. Pyridin-4-yl-carbamic acid tert-butyl ester, yield (39.62 g, 99%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.64 (1H, s), 8.23 (d J=6.0, 1H), 8.00 (d, J=6.0, 1H), 7.04 (br s, 1H), 1.43 (9H, s); LCMS m/z:321 ($M+H^+$)

_N_-[3-(1-Propyn-1-yl)-4-pyridinyl]-1,1-dimethylethyl carbamic acid



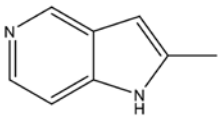
3% Propyne in hexane (12 ml, 4.6 mmol) was added dropwise to the mixture of *N*-(3-iodo-4-pyridinyl-1,1-dimethylethyl ester carbamic acid (500 mg, 1.56 mmol), triethylamine (1.2 ml, 8.56 mmol) and copper (I) iodide (30 mg, 0.16 mmol) and palladium bistriphenylphosphine dichloride (55 mg, 0.078 mmol) in DMF (1.4 ml) at -78°C. The mixture was stirred at R.T. for 2 h. After usual workup, the crude product was purified by flash column chromatography to provide the titled compound (358 mg, 99%) as light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (brs, 1H), 8.28 (d, J=7.5, 1H), 7.98 (d, J=7.2, 1H), 7.28 (brs, 1H), 2.08 (s, 3H) 1.46 (s, 9H); LCMS m/z:233 ($M+H^+$).

2-Methyl-1*H*-pyrrolo[3,2-c]pyridine-1-carboxylic acid 1,1-dimethylethyl ester



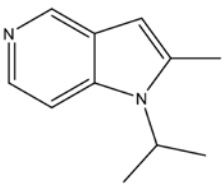
To the solution of *N*-[3-(1-propyn-1-yl)-4-pyridinyl]-1,1-dimethylethyl carbamic acid (360 mg, 1.55 mmol) in *N,N*-Dimethylformamide (6.6 ml) was added copper (I) iodide (15 mg, 0.08 mmol). The reaction mixture was stirred at 80°C for 18 h. After usual workup, the crude product was purified by flash column chromatography to provide the titled compound (189 mg, 52%) as light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.73 (s, 1H), 8.36 (d, J=7.5, 1H), 7.90 (d, J=7.5, 1H), 6.36 (s, 1H), 2.60 (s, 3H) 1.67 (s, 9H); LCMS m/z:233 ($M+H^+$).

2-Methyl-1*H*-Pyrrolo[3,2-c]pyridine



To the solution of 2-Methyl-1*H*-pyrrolo[3,2-c]pyridine-1-carboxylic acid 1,1-dimethylethyl ester (50 mg, 0.22 mmol) in dichloromethane (1 ml) was added trifluoroacetic acid (1 ml). The reaction mixture was stirred at rt for 18 h and then the solvent was remover under reduced pressure to provide the titled compound (28.4 mg, 100%) as light yellow oil. ^{1}H NMR (400 MHz, CD_3OD): δ 8.89 (s, 1H), 8.21 (d, J=6.0, 1H), 7.78 (d, J=6.0, 1H), 6.69 (s, 1H), 2.54 (s, 3H); LCMS m/z:133 ($M+H^+$).

2-Methyl-1-(1-methylethyl)-1*H*-Pyrrolo[3,2-c]pyridine



Synthesized according to the general procedure (B) with2-Methyl-1*H*-Pyrrolo[3,2-c]pyridine and 2-bromopropane at 120°C for 20 min to give titled compound (yield 37%) as light yellow solid. ^{1}H NMR (400 MHz, CD_3OD): δ 8.60 (s, 1H), 8.04 (d, J=6.0, 1H), 7.53 (d, J=6.4, 1H), 6.35 (s, 1H), 4.75 (m, 1H), 247 (s, 3H), 1.60 (d, J=7.6, 6H); LCMS m/z:175 ($M+H^+$).

Reference

1. Okauchi T, Itonaga M, Minami T, Owa T, Kitoh K and Yoshino H: A general method for acylation of indoles at the 3-position with acyl chlorides in the presence of dialkylaluminum chloride. Org Lett 2: 1485-1487, 2000.

Figure S1. Effect of NSI-1 on Notch family receptors. Using Gal4DBD fused ICD of human Notch1, Notch2 and Notch3, the effect of NSI-1 on their transactivation activity was analyzed via luciferase assay. Notch-ICDΔ and Gal4DBD fusion proteins derived from Notch1, Notch2 and Notch3 were constructed (shown above). Preparation of these constructs was described in the Materials and methods section in the main text. Numbers indicate amino acid positions in full-length proteins. These were expressed in the SH-SY5Y cell line, treated with DMSO, 10 μM DAPT or 15 μM NSI-1 for 24 h and then analyzed using luciferase activity. NSI-1 significantly reduced luciferase activities in all constructs, indicating that NSI-1 functions on all Notch family receptors. ICD, intracellular domain; NSI-1, Notch signaling inhibitor-1; Gal4DBD, Gal4DNA-binding domain; N, N-termini, C, C-termini. **P<0.01, *P<0.05, one-way ANOVA with Tukey's post hoc test.

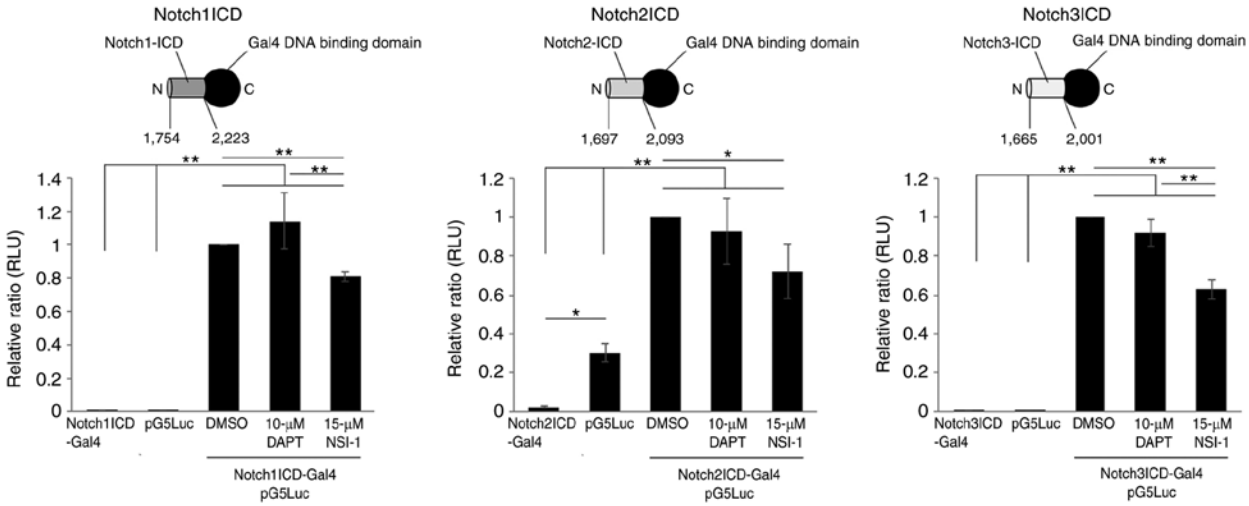


Figure S2. Analysis of the mRNA expression of Firefly luciferase produced from the pG5Luc plasmid by RT-qPCR analysis. To confirm the expression of the Firefly luciferase gene from the pG5Luc plasmid, cells transfected pG5Luc and other plasmids were subjected to RT-qPCR analysis. Plasmids encoding proteins indicated at the bottom with pG5Luc, which were used in Fig. 3D and Fig. S1, were transfected in SH-SY5Y cells and total RNA was isolated 24 h later using TRIzol and subjected to RT-qPCR analysis. The mRNA expression of Firefly luciferase was calculated using GAPDH as an internal control. The results from cells transfected with the Gal4DBD fusion protein-coding plasmid and pG5Luc in each graph was set to 1.0. N=3, mean ± SD. **P<0.01, one-way ANOVA with Tukey's post hoc test. N1, Notch1; N2, Notch2; N3, Notch3; ICD, intracellular domain; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

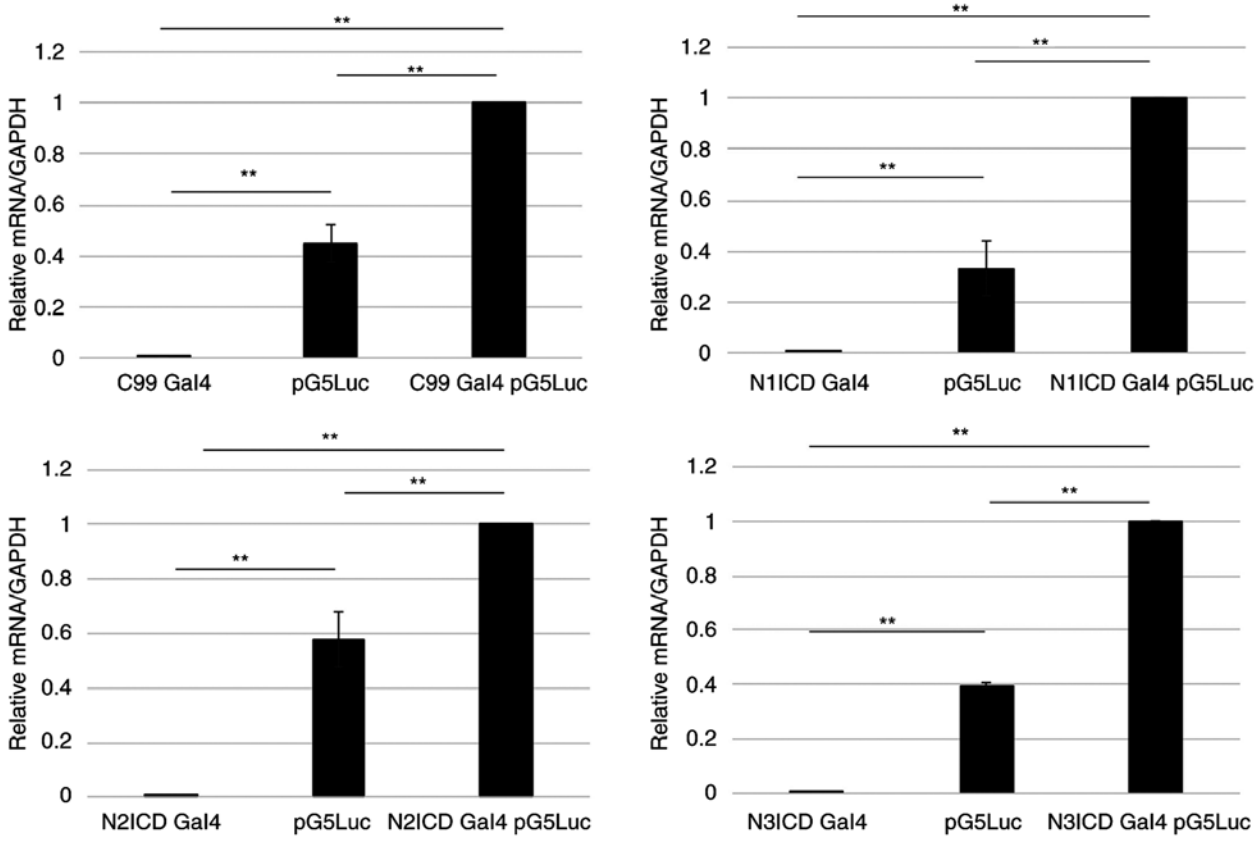


Figure S3. Confirmation of transfection efficacy of plasmids encoding Gal4DBD fusion proteins analyzed by immunostaining. To confirm the transfection efficacy of plasmids used in Fig. 3D and Fig. S1, cells transfected with those plasmids were subjected to immunostaining using an anti-Gal4DBD antibody and DAPI stain. Plasmids encoding proteins, indicated on the left, which were used in Fig. 3D and Fig. S1, were transfected in SH-SY5Y cells and fixed for immunostaining 24 h later. Images of DAPI staining (left), anti-Gal4DBD antibody staining (center) and Merge images (right) are shown. Scale bar, 10 μm. Gal4DBD, Gal4DNA binding domain; ICD, intracellular domain.

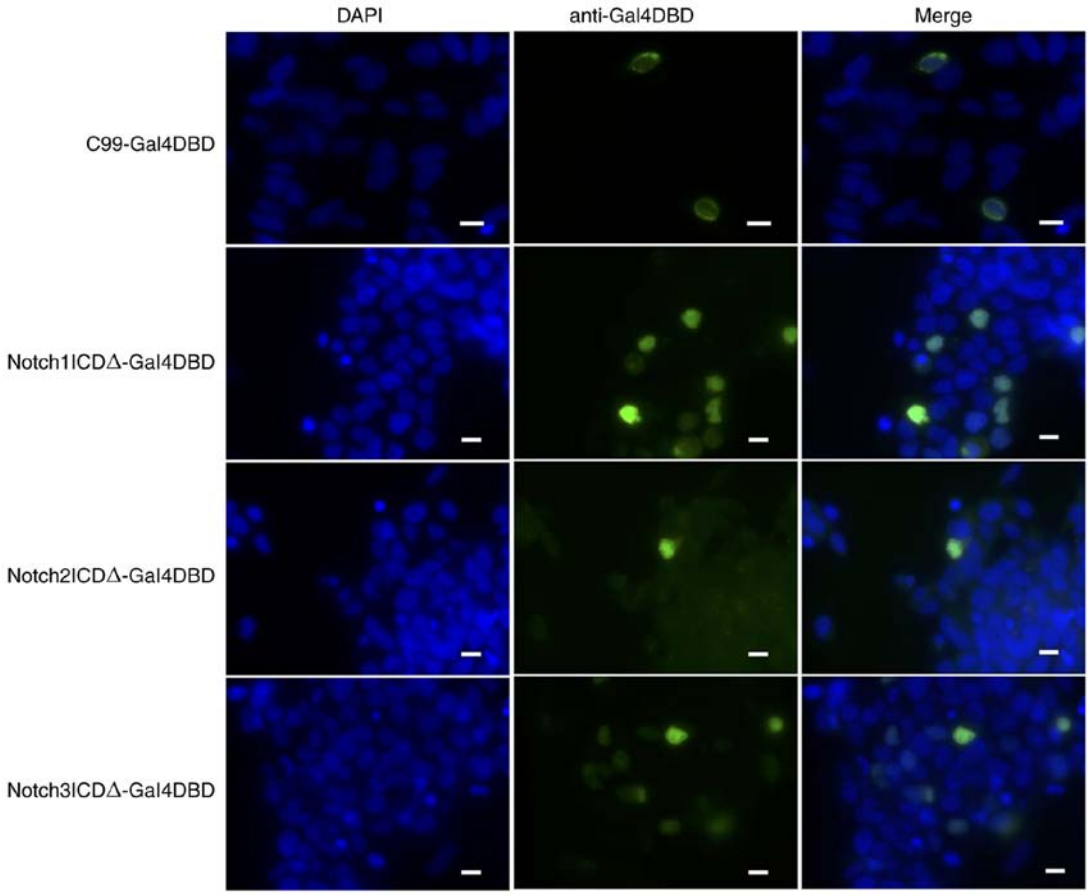


Figure S4. Results of the LDH assay for cells treated with DMSO, DAPT or NSI-1. The viability of cells treated with DMSO, DAPT or NSI-1 was analyzed using an LDH assay. Cell lines shown at top were treated with DMSO, 10 μM DAPT and 15 μM NSI-1 for the indicated durations and medium was collected for the LDH assay. The y-axis indicates the value of OD450 following subtraction of the values of BG (no cells). The x-axis indicates incubation time (h) following addition of reagents (N=3, mean ± SD; **P<0.01, *P<0.05, one-way ANOVA with Tukey's post hoc test.). Significantly higher values were observed in NSI-1-treated cells following DMSO treatment at certain points. However, there was no reverse correlation with the results of the MTT assay shown in Fig. 5A. These results indicate that NSI-1 and DAPT suppressed cell proliferation rather than inducing cell death. NSI-1, Notch signaling inhibitor-1; DAPT, N-[N-(3,5-difluorophenacetyl)-l-alanyl]-S-phenylglycine t-butyl ester; LDH, lactate dehydrogenase; MCF7, Michigan Cancer Foundation-7.

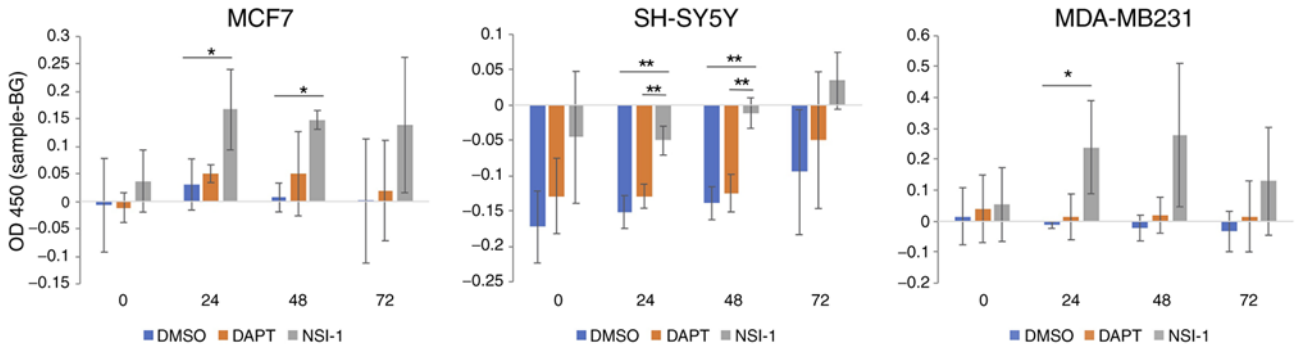


Figure S5. NSI-1 has no additive effect of on DAPT treatment in the production of NICD. The additive effect of NSI-1 on the suppression of NICD production by DAPT treatment was analyzed by immunoblotting. NotchΔEΔGal4 was expressed in SH-SY5Y cells, which were treated 24 h later with DMSO (-), 1 μM DAPT and 1 μM NSI-1, as indicated, for 24 h. The cells were harvested and used for immunoblotting using anti-activated Notch1 (upper panel) and anti-α-tubulin (lower panel) antibodies. The graph shows quantification of protein band intensities. Arrows indicate specific bands detected (N=3, mean ± SD; **P<0.01, one-way ANOVA with Tukey's post hoc test.). Treatment with 1 μM DAPT significantly suppressed the production of NICD compared with that following DMSO treatment, whereas 1 μM NSI-1 had no effect. When cells were treated with both reagents simultaneously, the production of NICD was suppressed at the same level as that following treatment with of 1 μM DAPT only, indicating that NSI-1 had no additive effect on the suppression of NICD production by DAPT treatment. NICD, Notch intracellular domain; NSI-1, Notch signaling inhibitor-1; DAPT, N-[N-(3,5-difluorophenacetyl)-l-alanyl]-S-phenylglycine t-butyl ester; n.s., not significant.

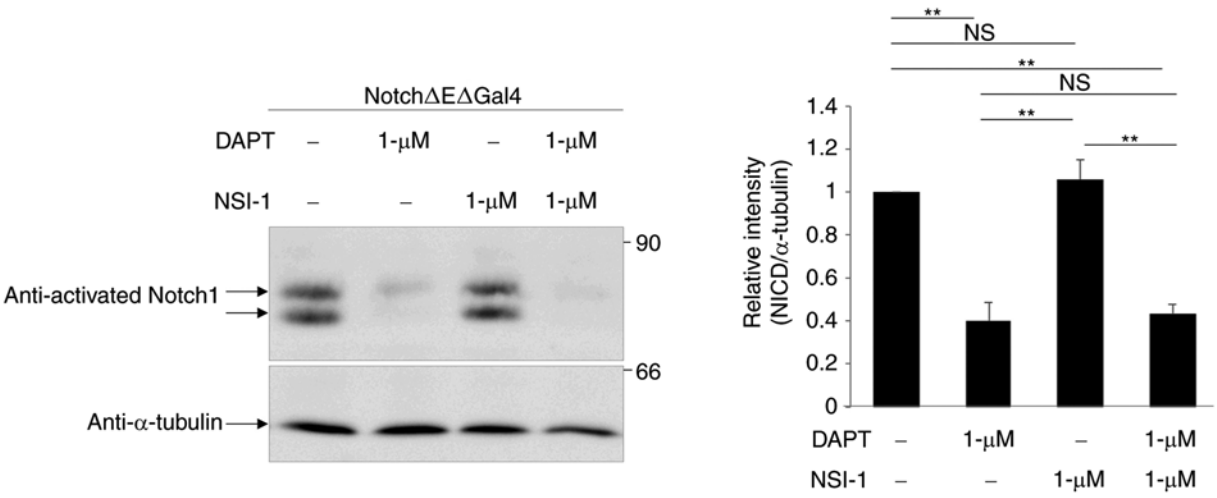


Figure S6. Results of RT-qPCR analysis of the expression of *HES1* in Notch1 OE cells and their parental cell lines. Gene expression of *HES1* upon the overexpression of human Notch1 was analyzed by RT-qPCR analysis using MCF7, SH-SY5Y and MDA-MB-231 cell lines. A plasmid encoding human Notch1 was transfected and total RNA was isolated using TRIzol reagent 24 h later. RT-qPCR analysis was performed, the expression values were normalized by GAPDH and the value in control cells was set to 1.0 (N=3, mean ± SD; $^{***}P<0.005$, $^{*}P<0.05$ with Student's t-test). Notch1 OE significantly increased the gene expression of *HES1* compared with that in parental cells in all cell lines. These results indicate that the overexpression of Notch1 is sufficient to facilitate Notch signaling activity. RT-qPCR, reverse transcription-quantitative PCR; hairy and enhancer of split-1; OE, overexpression; MCF7, Michigan Cancer Foundation-7.

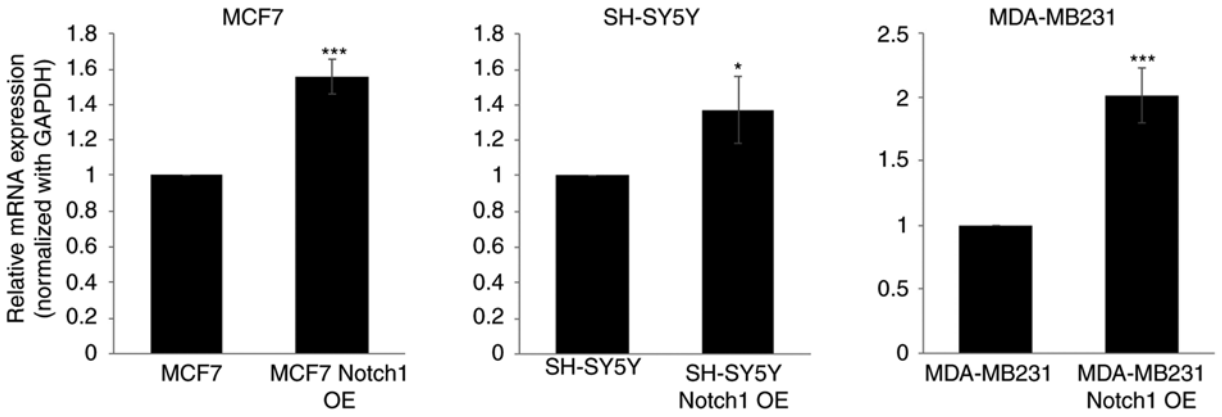


Figure S7. Expression of Notch family receptors in cell lines examined. Expression of endogenous Notch2 and Notch3 and Notch1 was analyzed by immunoblotting using specific antibodies. Cell lysates were prepared from cell lines using RIPA buffer and immunoblotting was performed as described in the Materials and methods section in the main text using specific antibodies against Notch1, Notch2, Notch3 and a-tubulin indicated on the left side of the blots. Numbers on the right of the blots indicate protein standard (kDa). The SH-SY5Y cell line expressed Notch2 and Notch3 at the highest level and expressed Notch1 at a lower level than levels in the other cell lines. NTM, Notch transmembrane/intracellular region.