Electronic Supplementary Material (ESI) for ChemComm.

SUPPLEMENTARY INFORMATION

A highly sensitive fluorescent probe that quantifies transthyretin in human plasma as an early diagnostic tool for Alzheimer's disease

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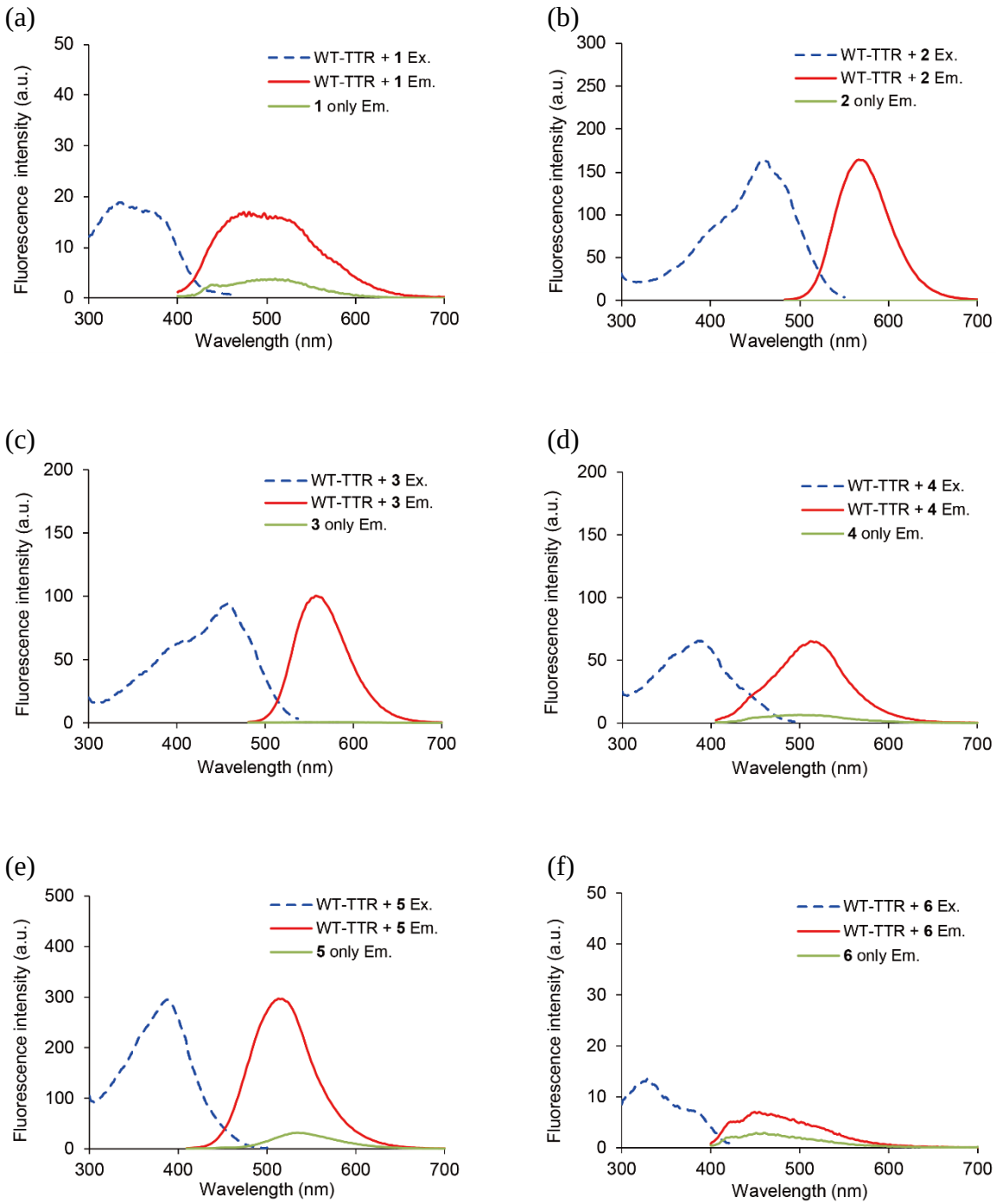
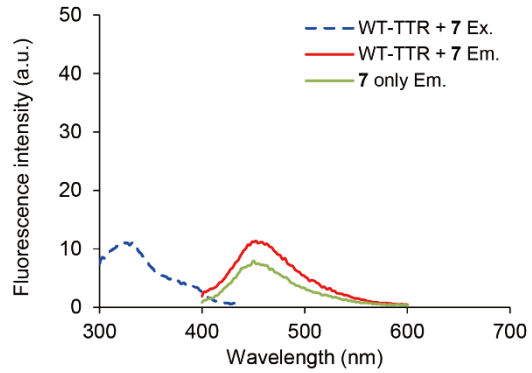
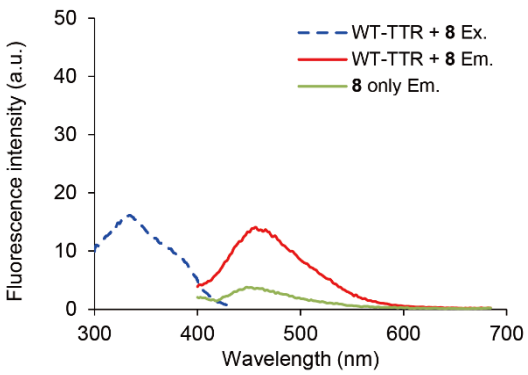
Figure S1. Fluorescent spectra of probes **1-10** (4 μM) observed after 1 h incubation with WT-TTR (2 μM, red trace) and alone in PBS (green trace). λ_{ex} and λ_{em} values are presented in Table 1.



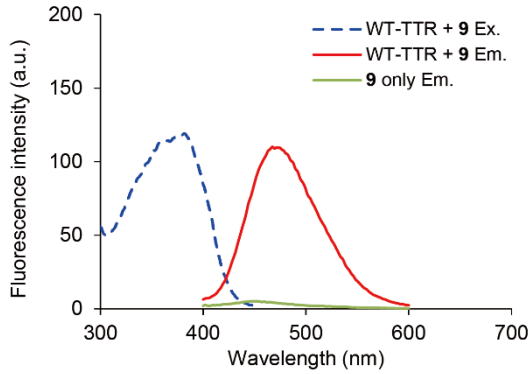
(g)


WT-TTR + 7 Ex.
WT-TTR + 7 Em.
7 only Em.
Fluorescence intensity (a.u.)
Wavelength (nm)
0
10
20
30
40
50
300
400
500
600
700

(h)


WT-TTR + 8 Ex.
WT-TTR + 8 Em.
8 only Em.
Fluorescence intensity (a.u.)
Wavelength (nm)
0
10
20
30
40
50
300
400
500
600
700

(i)


WT-TTR + 9 Ex.
WT-TTR + 9 Em.
9 only Em.
Fluorescence intensity (a.u.)
Wavelength (nm)
0
50
100
150
200
300
400
500
600
700

(j)

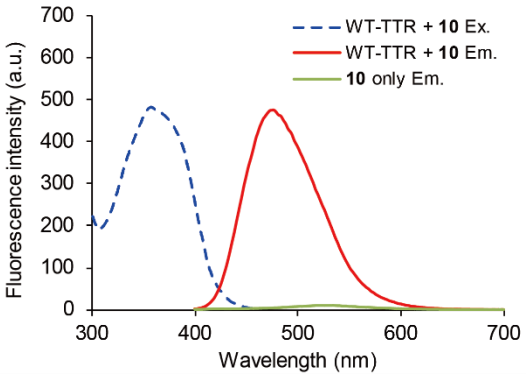

WT-TTR + 10 Ex.
WT-TTR + 10 Em.
10 only Em.
Fluorescence intensity (a.u.)
Wavelength (nm)
0
100
200
300
400
500
600
700
300
400
500
600
700

Figure S2 (a) Fluorescence quenching of probe **10** (10 μM) in less-polar aprotic acetonitrile by addition of polar protic water. λ_{ex} = 357 nm. (b) Fluorescence quenching of probe **10** (10 μM) in less-polar aprotic methylene chloride by addition of polar protic methanol. λ_{ex} = 357 nm. (c) The fluorescence spectra of probe **10** (4 μM) in the presence of different TTR concentrations (0.005 – 0.1 μM) in PBS

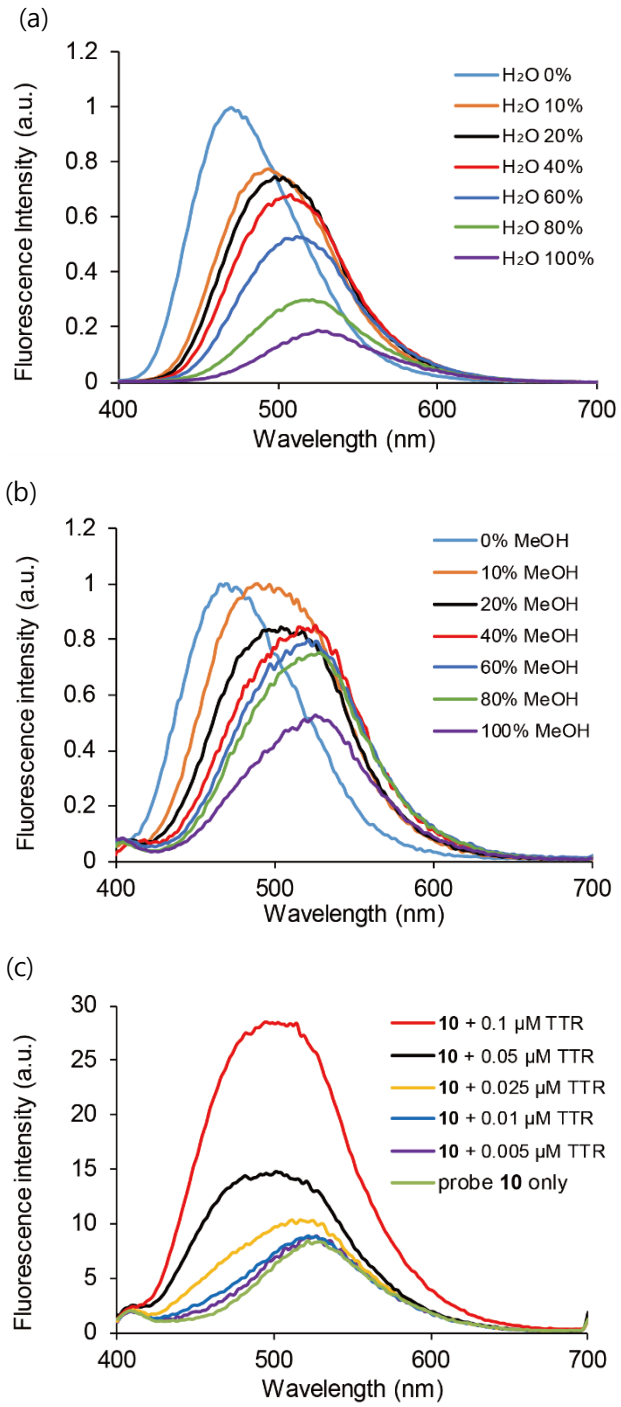
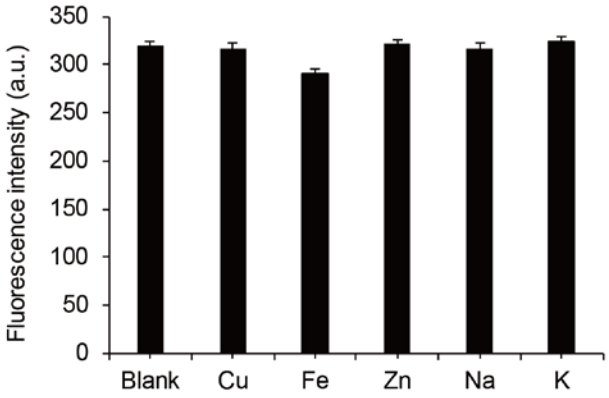


Figure S3 (a) Fluorescence intensity of probe **10** (4 μM) upon addition of WT-TTR (1 μM in 10 mM phosphate buffer) in the presence of various metal ions (20 μM) (b) Fluorescence intensity of probe **10** (4 μM) upon addition of WT-TTR (1 μM in 10 mM PBS) with different pH value (from 5 to 8). λ_{ex} = 357 nm

(a)



(b)

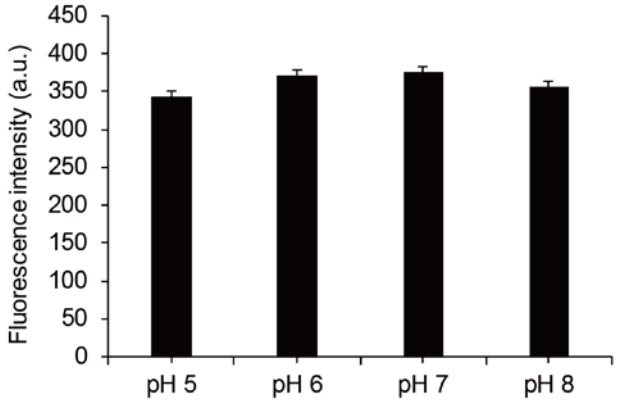
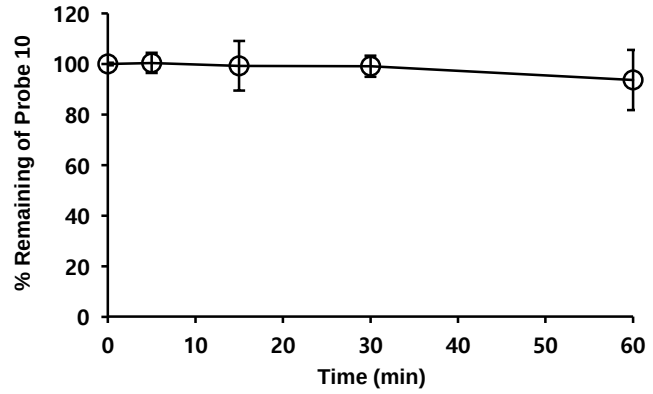


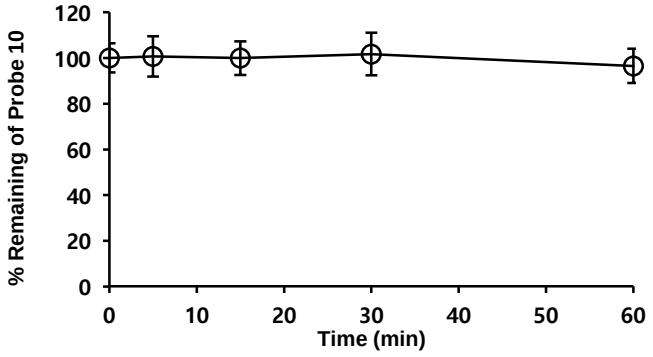
Figure S4 Temporal profiles for the remaining of probe 10 in (a) the rat plasma and (b) the human plasma. Each data point represents the mean ± SD (n =3).

(a)



time (min)	% Remaining	SD	$t_{1/2}$ (min)
0	100.0	0.5	
5	100.4	4.0	
15	99.3	9.8	
30	99.1	4.2	624.75
60	93.6	11.9	

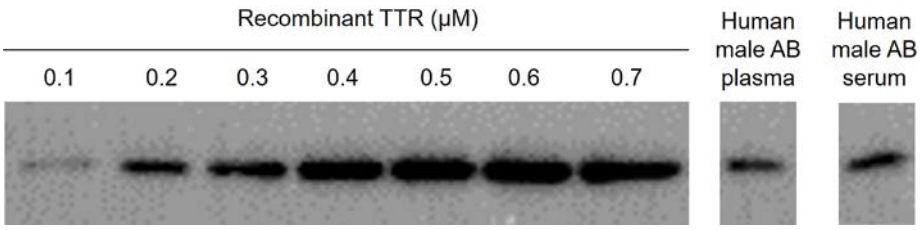
(b)



time (min)	% Remaining	SD	$t_{1/2}$ (min)
0	100.0	6.4	
5	100.7	8.8	
15	100.0	7.4	
30	101.7	9.3	1213.499
60	96.5	7.5	

Figure S5 (a) Densitometry analysis of western-blot assay for recombinant TTR, human plasma and human serum (b) the linear relationship between densitometry intensity and TTR concentrations.

(a)



(b)

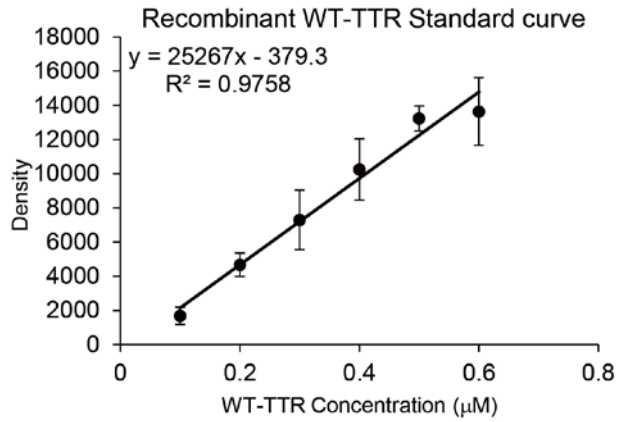
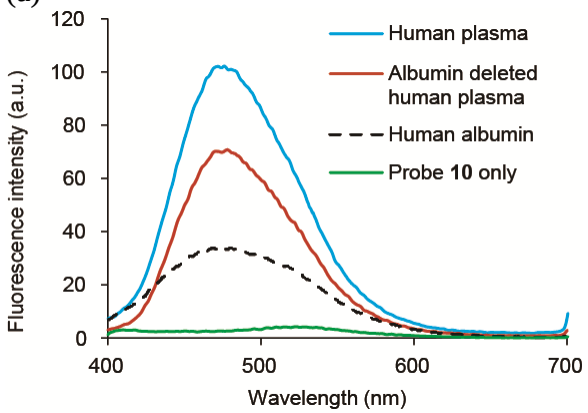


Figure S6 (a) Fluorescence spectra of probe **10** in the presence of 18-fold diluted human male AB plasma and 18-fold diluted albumin (0.63 mM). (b) Fluorescence spectra of probe **10** in the presence of 18-fold diluted human male AB clotted serum and 18-fold diluted albumin.

(a)



(b)

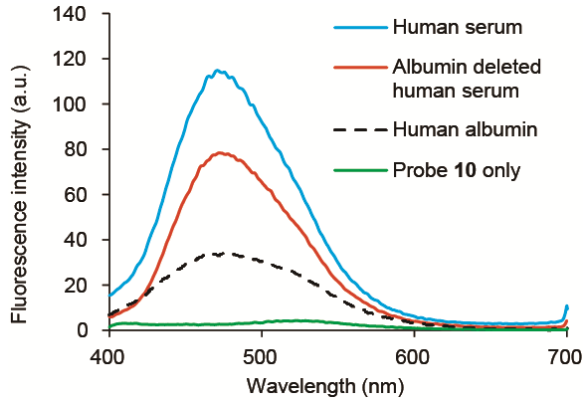
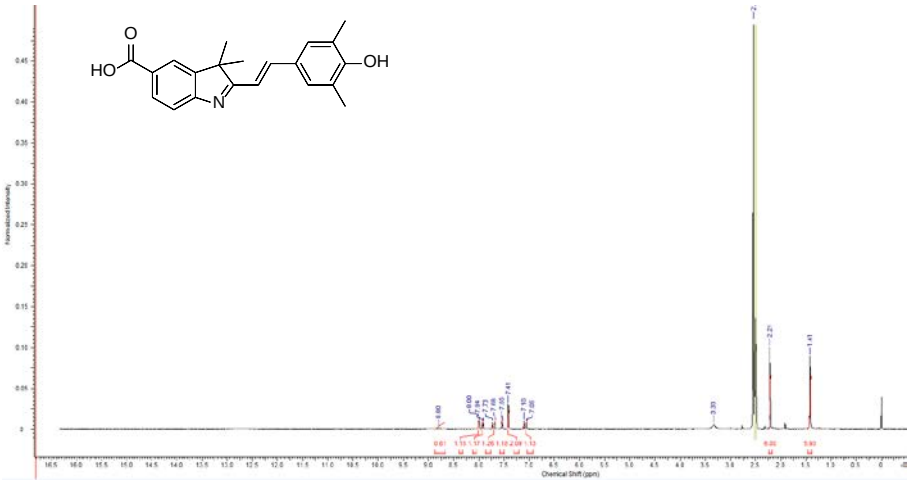
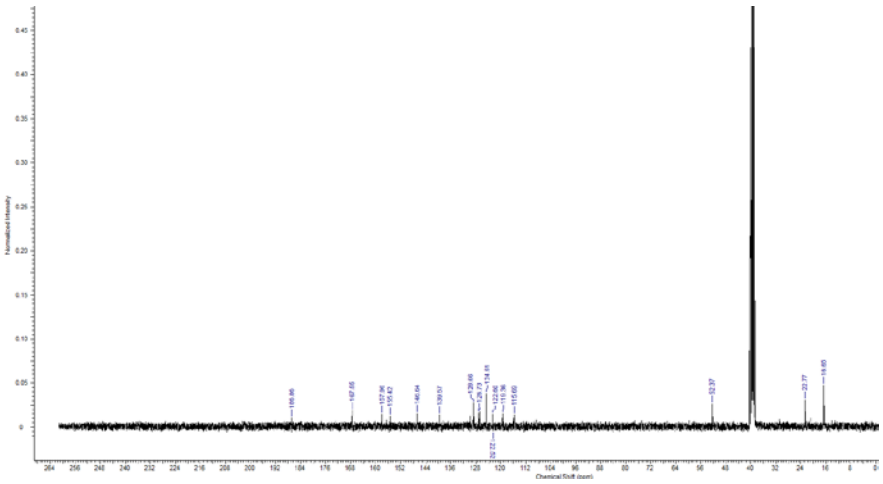
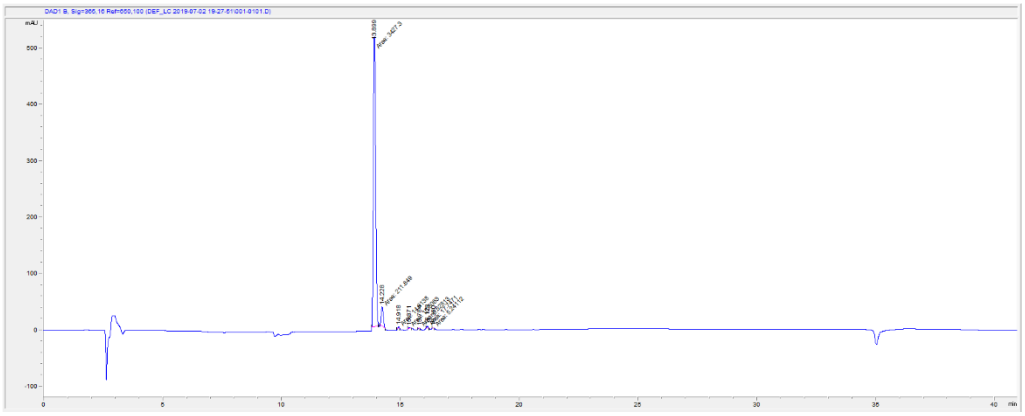


Figure S7 ^{1}H, ^{13}C NMR spectra, and HPLC trace of compound **1**

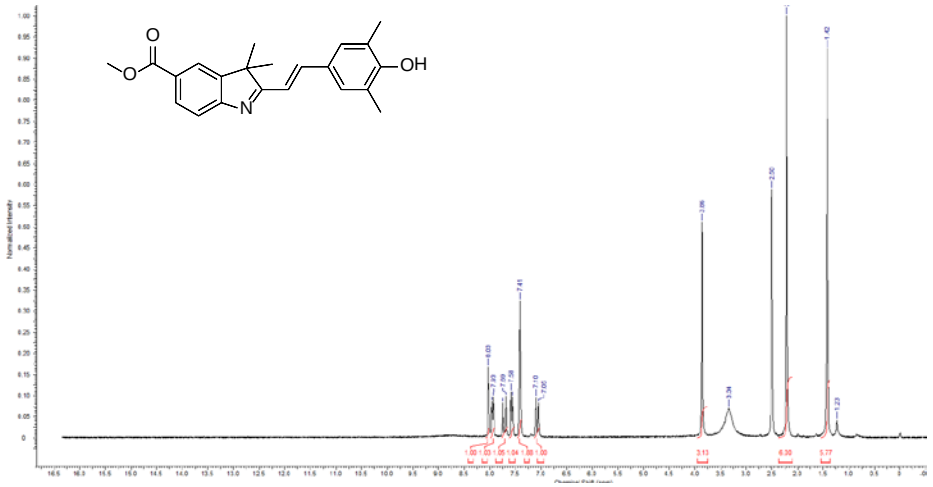


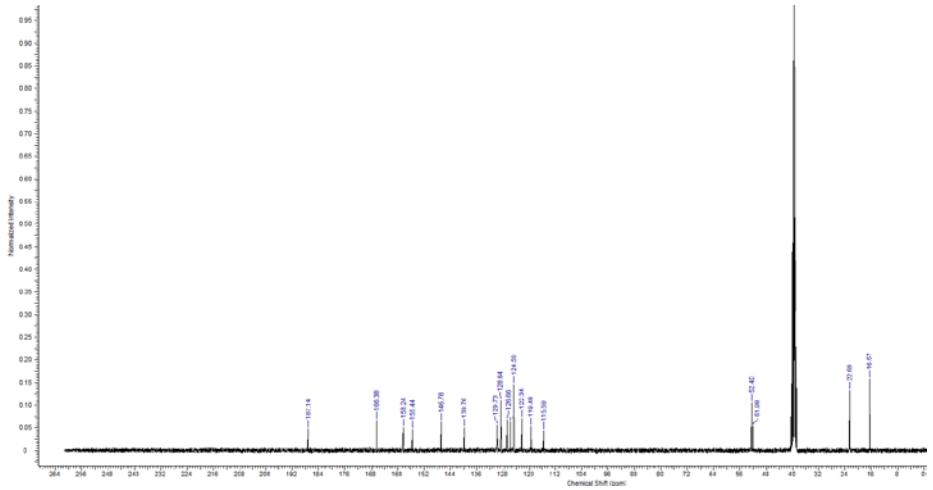


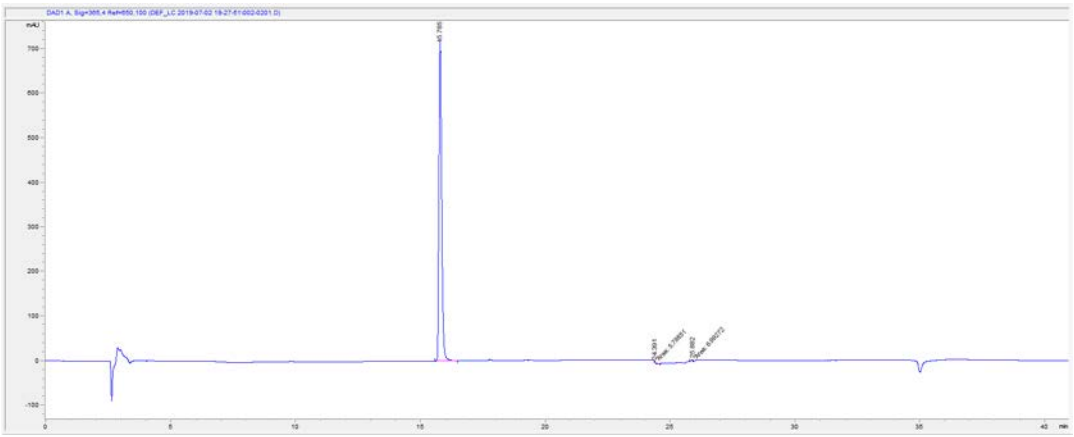


#	Time	Area	Height	Width	Area%	Symmetry
1	13.899	3427.3	515.5	0.1108	92.653	0.801
2	14.228	211.8	34.7	0.1019	5.727	0.859
3	14.918	14.6	3.5	0.0688	0.395	1.118
4	15.371	15.8	2.7	0.0978	0.427	0.643
5	15.784	6.5	1.8	0.0604	0.176	1.353
6	16.128	17.7	3.3	0.0887	0.480	0.668
7	16.38	5.2	1.3	0.0689	0.142	0.981

Figure S8 ^{1}H, ^{13}C NMR spectra, and HPLC trace of compound **2**

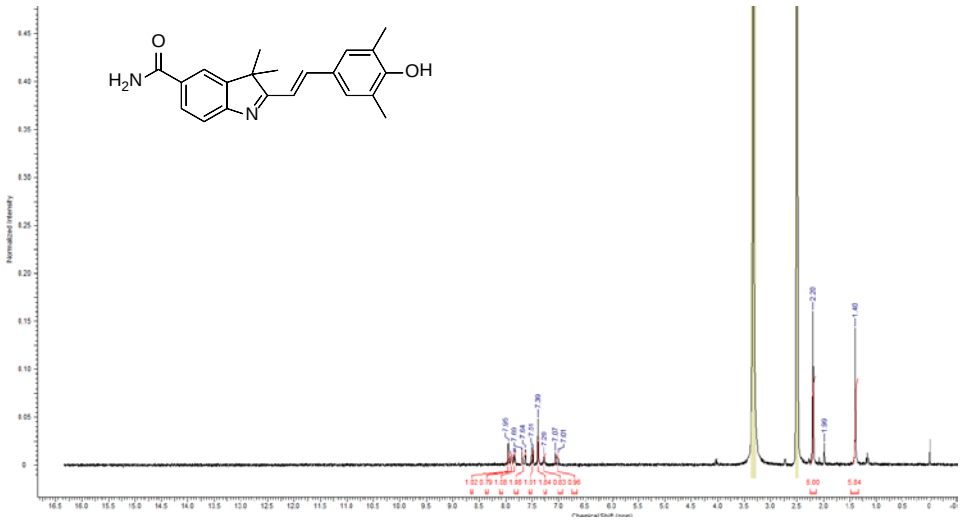


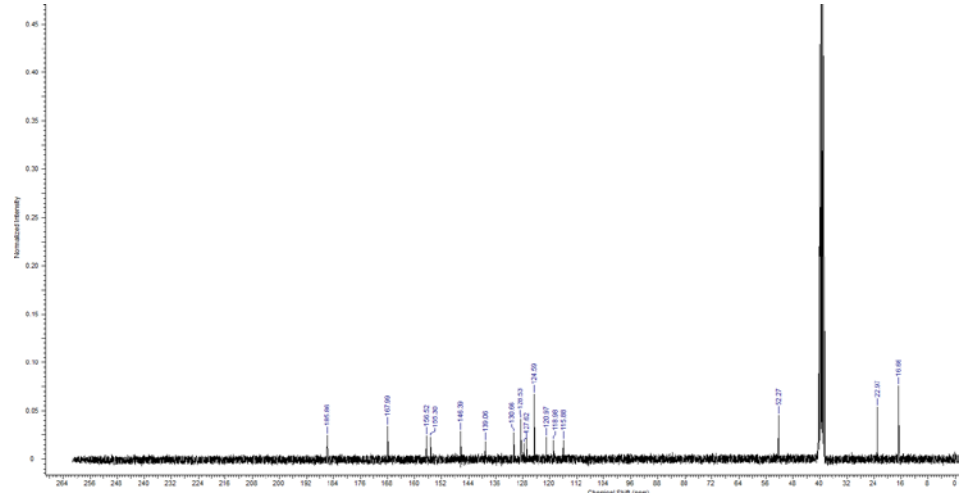


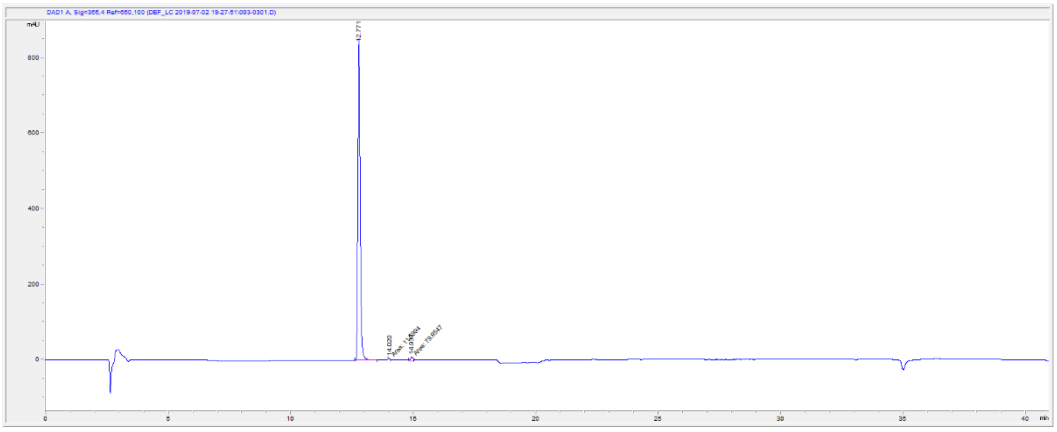


#	Time	Area	Height	Width	Area%	Symmetry
1	15.785	5498.1	722	0.1168	99.768	0.657
2	24.391	5.8	2.1	0.0459	0.105	0
3	25.882	7	1.6	0.0719	0.127	1.526

Figure S9 ^{1}H, ^{13}C NMR spectra, and HPLC trace of compound **3**

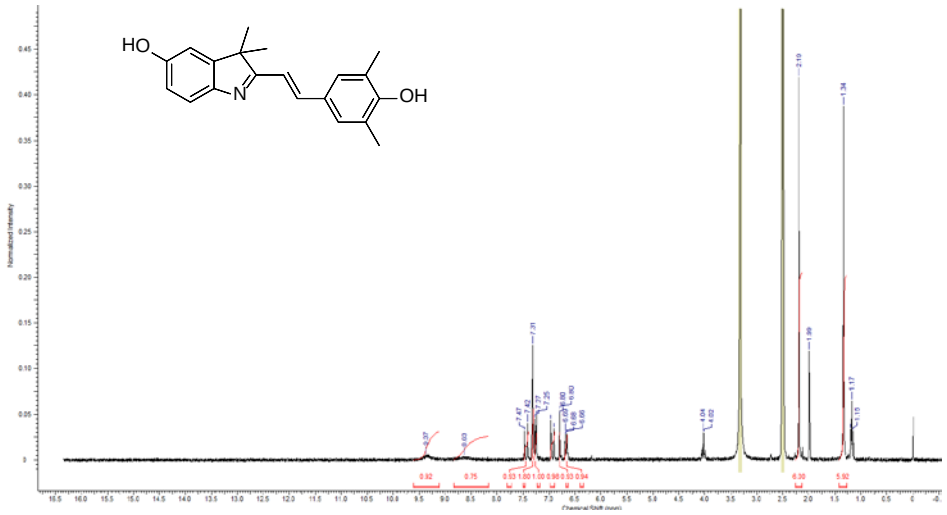


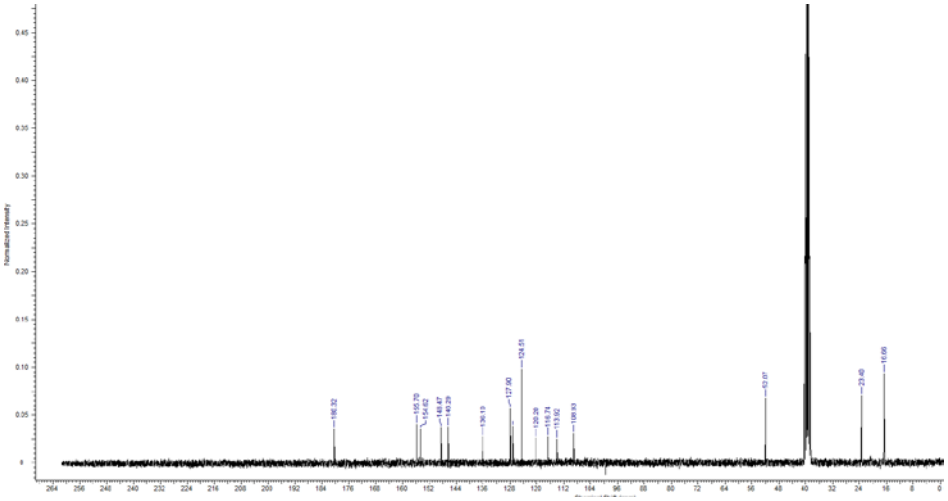


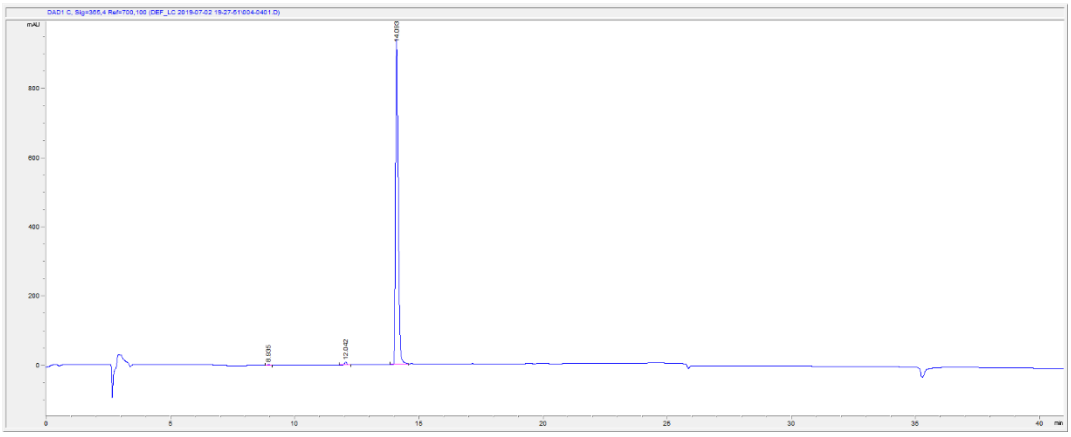


#	Time	Area	Height	Width	Area%	Symmetry
1	12.771	5789.7	852.7	0.1052	98.443	0.702
2	14.02	11.6	2.8	0.0695	0.197	0.382
3	14.937	80	10.4	0.128	1.359	1.062

Figure S10 ^{1}H, ^{13}C NMR spectra, and HPLC trace of compound **4**

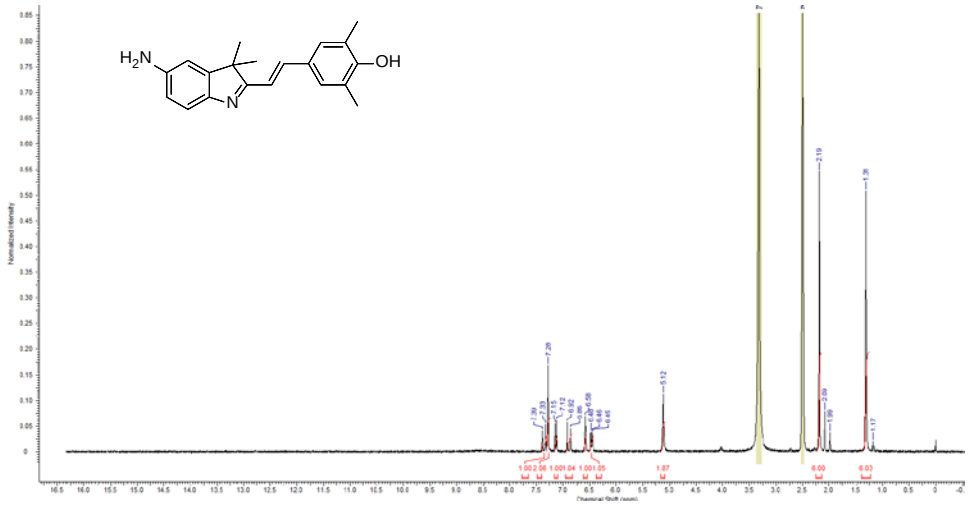


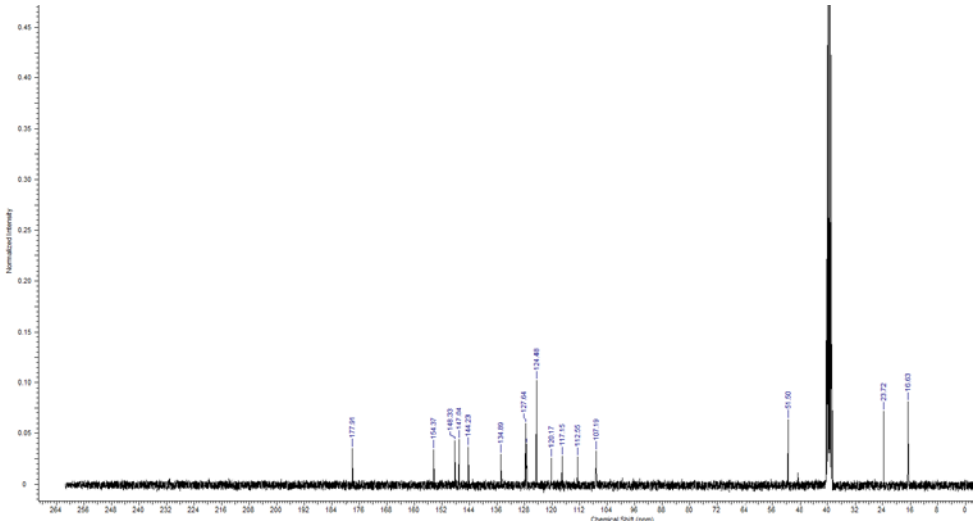


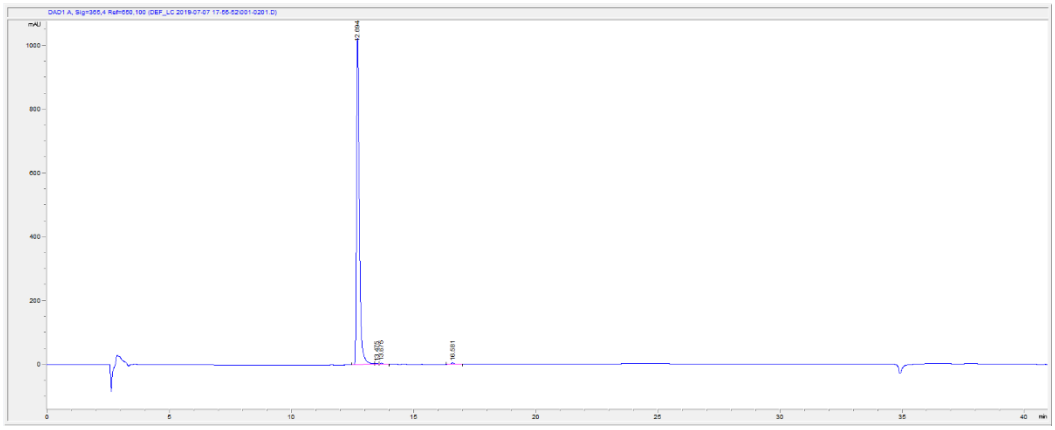


#	Time	Area	Height	Width	Area%	Symmetry
1	8.935	13.2	2.2	0.0927	0.183	0.912
2	12.042	50.7	7.9	0.1007	0.703	0.912
3	14.093	7147.7	941.8	0.1165	99.114	0.645

Figure S11 ^{1}H, ^{13}C NMR spectra, and HPLC trace of compound **5**

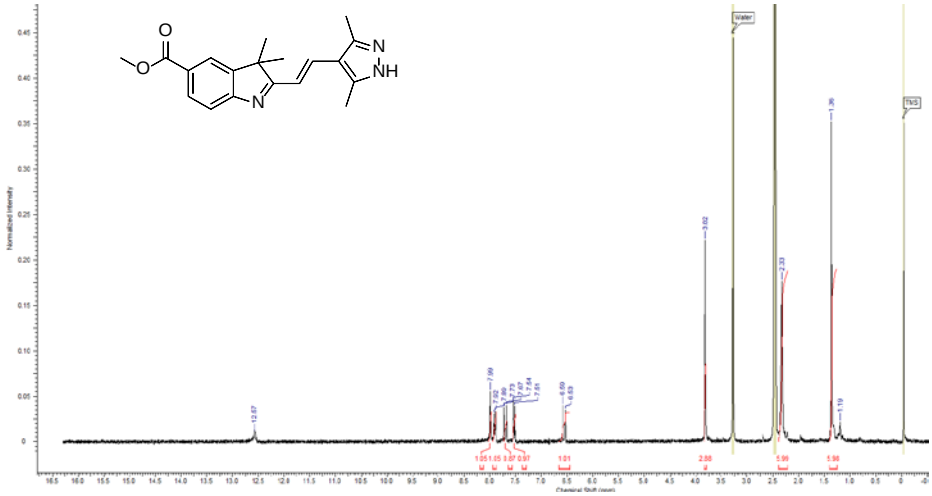


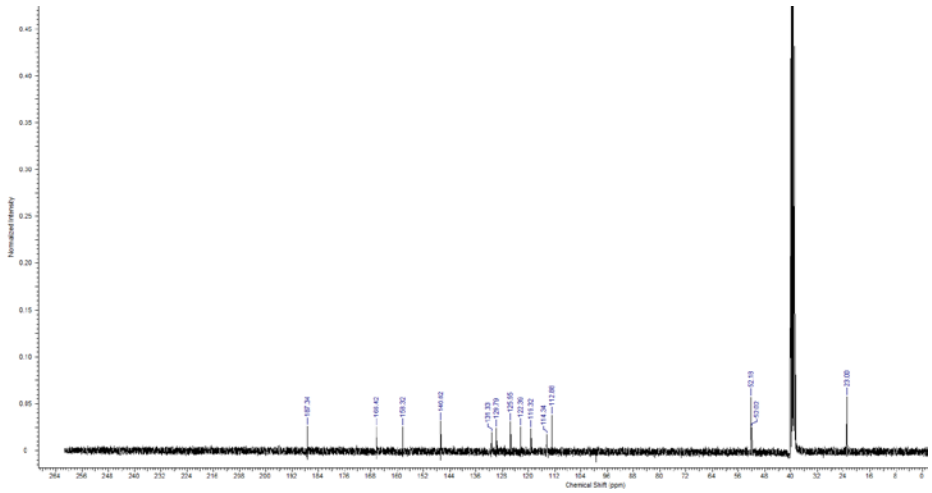


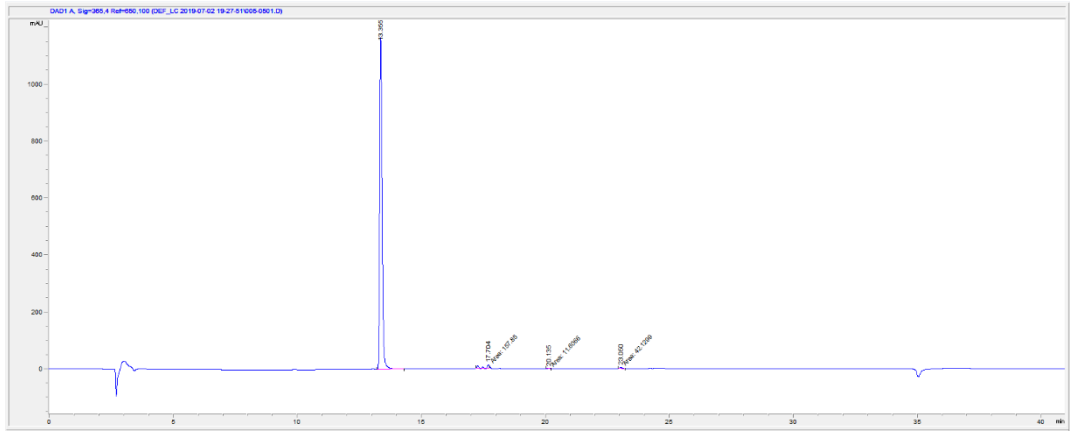


#	Time	Area	Height	Width	Area%	Symmetry
1	12.694	8332.9	1023.2	0.123	98.736	0.546
2	13.475	26.5	2.9	0.1339	0.314	0.843
3	13.675	40	4.4	0.1304	0.474	0.643
4	16.581	40.1	4.8	0.1281	0.475	0.669

Figure S12 ^{1}H, ^{13}C NMR spectra, and HPLC trace of compound **6**

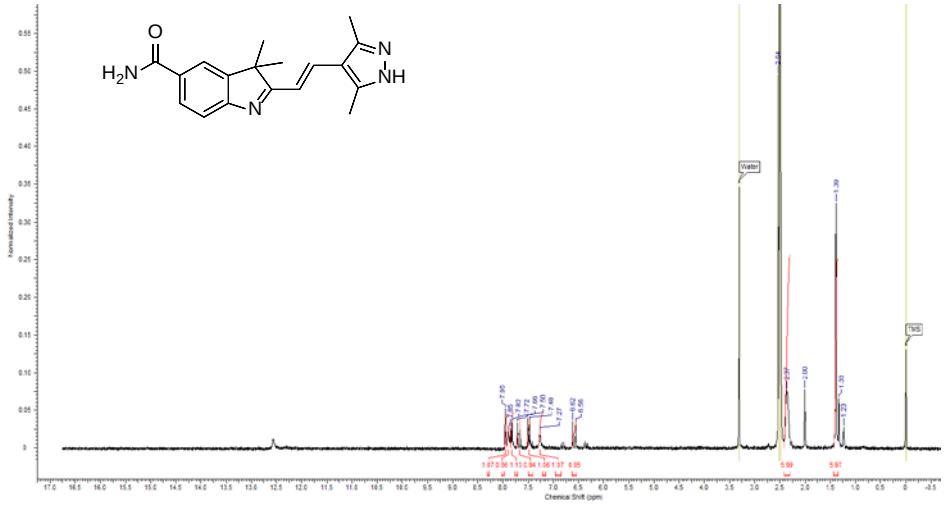


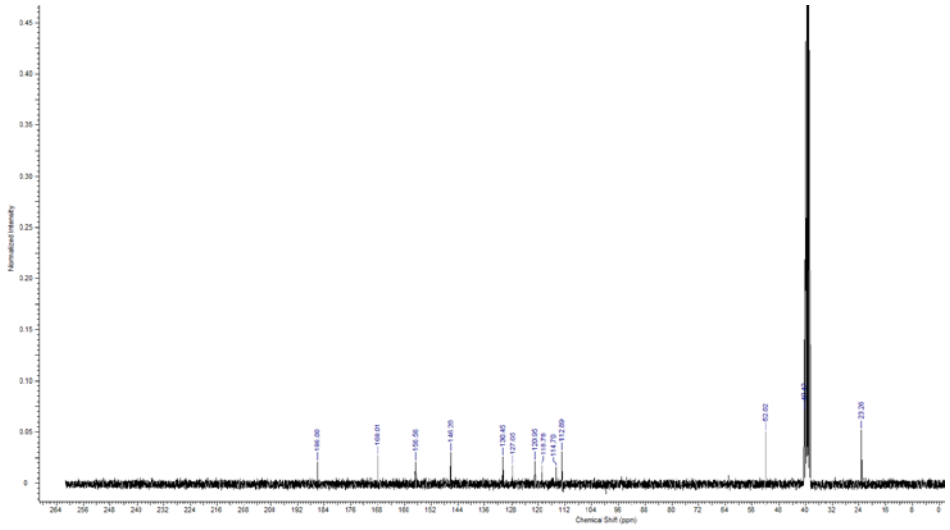


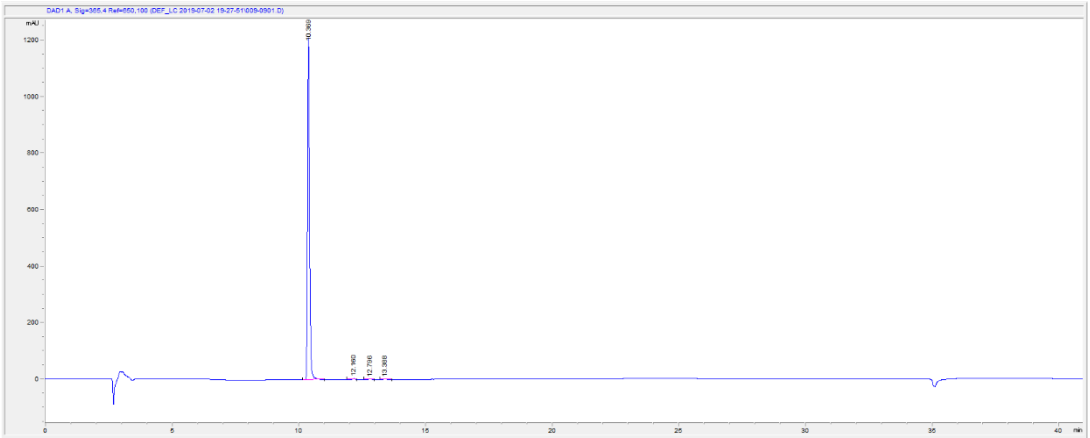


#	Time	Area	Height	Width	Area%	Symmetry
1	13.355	8116.4	1165.9	0.1072	97.459	0.685
2	17.704	157.8	13.2	0.1993	1.895	5.063
3	20.135	11.6	1.7	0.1147	0.139	0.96
4	23.05	42.1	4	0.1756	0.506	0.294

Figure S13 ^{1}H, ^{13}C NMR spectra, and HPLC trace of compound **7**

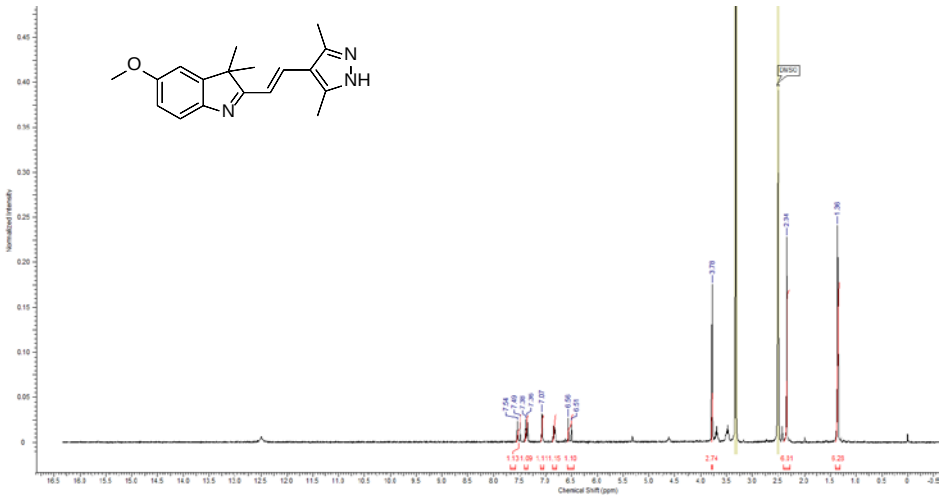


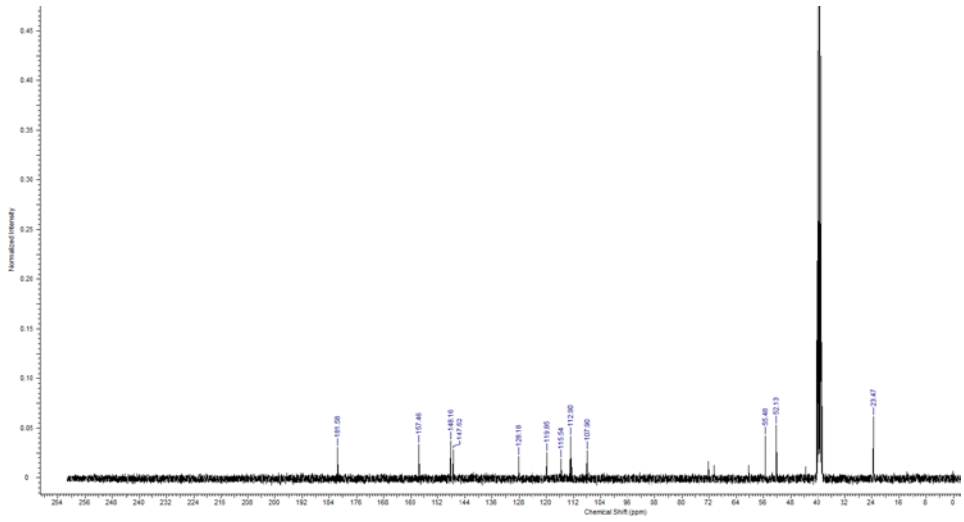


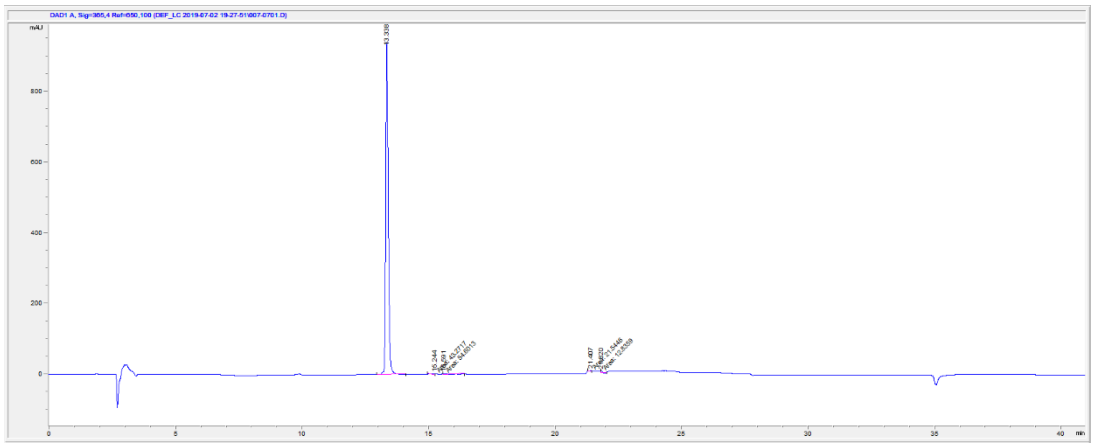


#	Time	Area	Height	Width	Area%	Symmetry
1	10.369	7058.3	1211.6	0.09	99.466	0.777
2	12.16	17.5	2.9	0.0949	0.247	1.063
3	12.796	11.4	1.6	0.1115	0.161	0.774
4	13.388	9	1.2	0.1137	0.127	0.765

Figure S14 ^{1}H, ^{13}C NMR spectra, and HPLC trace of compound **8**

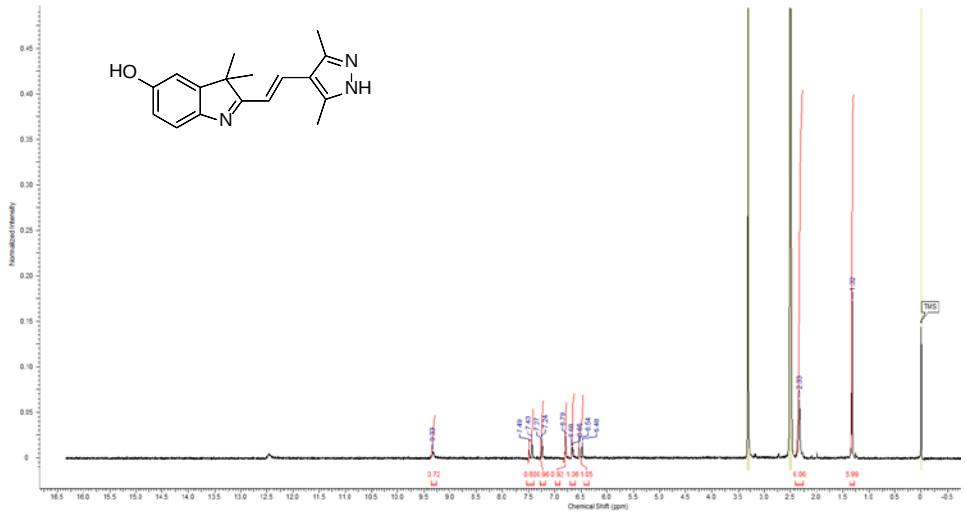


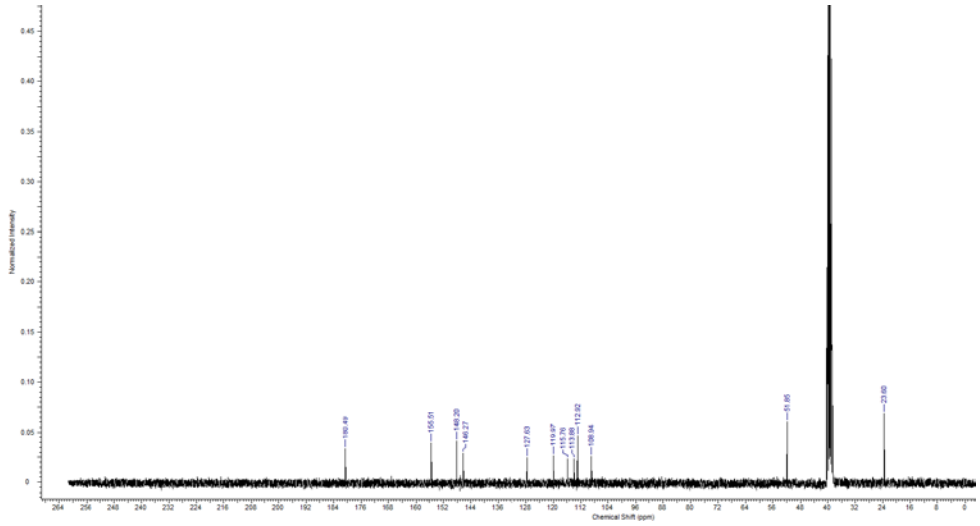


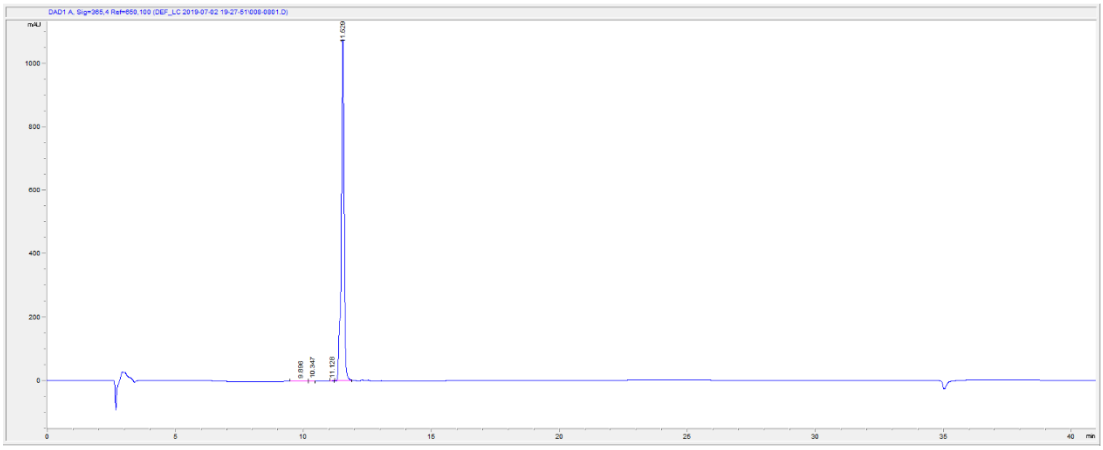


#	Time	Area	Height	Width	Area%	Symmetry
1	13.338	6660	939.8	0.1087	98.053	0.7
2	15.244	43.3	5.1	0.1426	0.637	0.385
3	15.591	54.6	3.3	0.2739	0.804	0.145
4	21.407	21.5	2.9	0.1251	0.317	1.265
5	21.82	12.8	3.6	0.0415	0.189	0

Figure S15 ^{1}H, ^{13}C NMR spectra, and HPLC trace of compound **9**

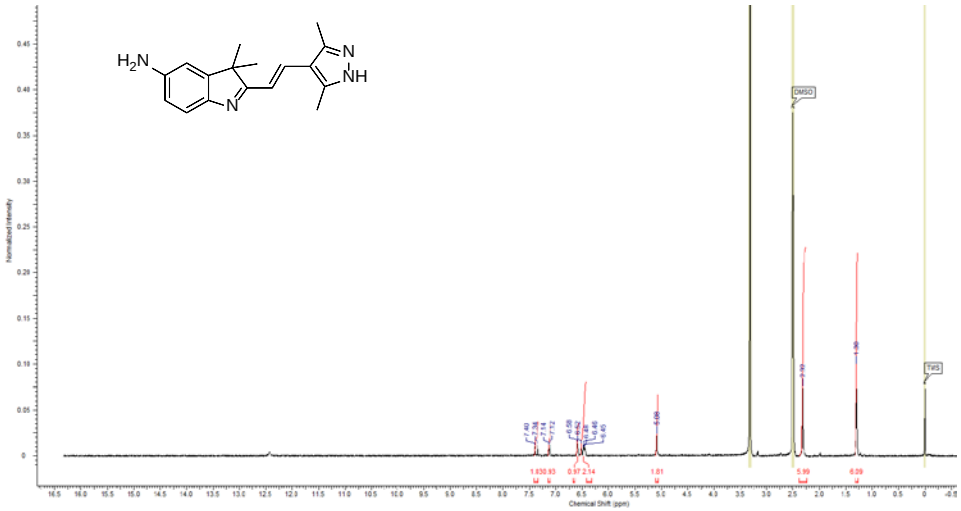


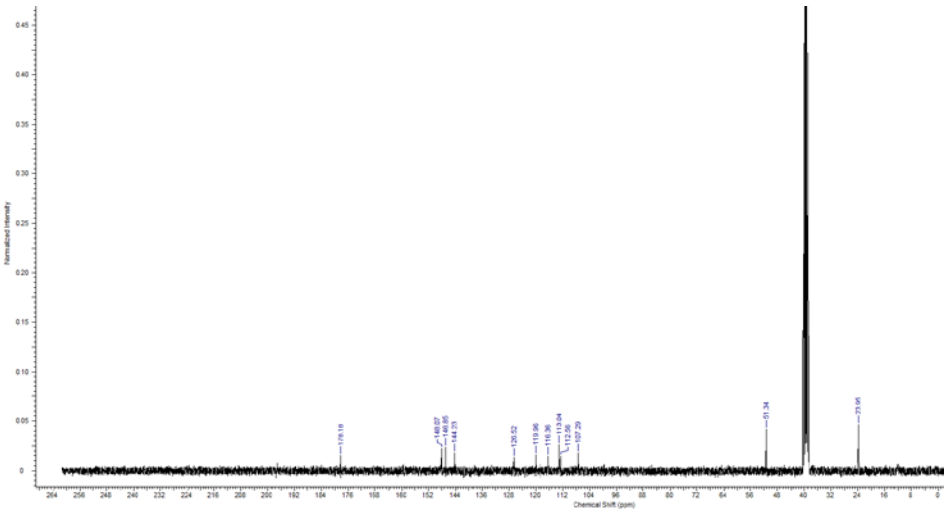


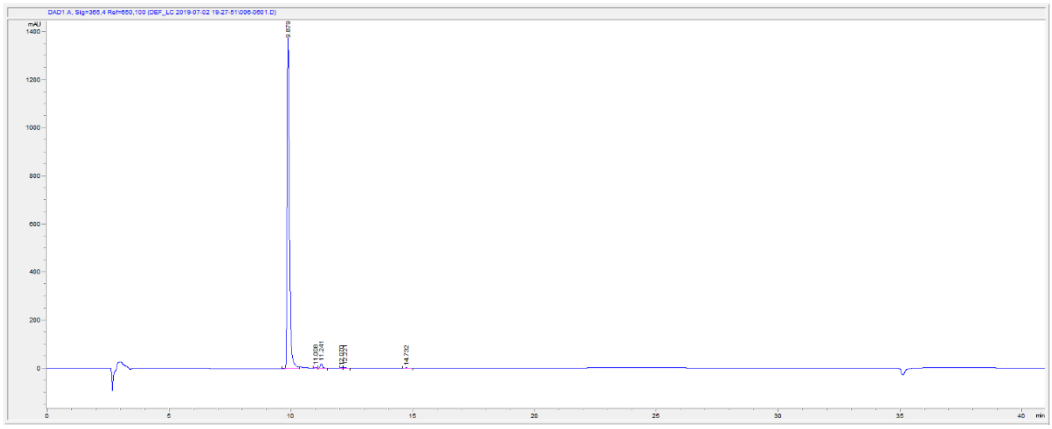


#	Time	Area	Height	Width	Area%	Symmetry
1	9.896	16	1.4	0.1628	0.194	1.441
2	10.347	9	1.4	0.0975	0.109	0.83
3	11.128	5.2	1.1	0.0769	0.063	1.137
4	11.529	8217.2	1077.2	0.113	99.634	1.103

Figure S16 ^{1}H, ^{13}C NMR spectra, and HPLC trace of compound **10**



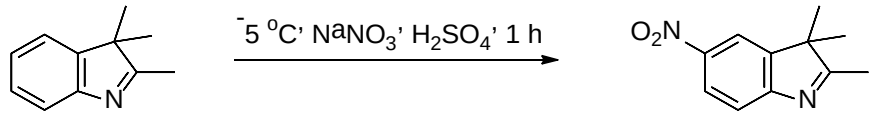




#	Time	Area	Height	Width	Area%	Symmetry
1	9.879	9013.6	1373.8	0.0985	97.875	0.641
2	11.008	15.4	2.8	0.0868	0.167	0.816
3	11.241	118.7	19.1	0.0944	1.289	0.769
4	12.07	16.7	2.7	0.0935	0.181	1.115
5	12.221	26.3	4.4	0.0934	0.286	0.866
6	14.732	18.6	2.8	0.1025	0.202	0.816

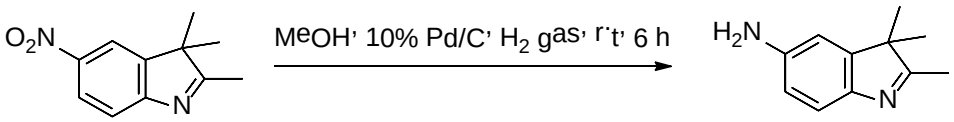
General Procedure

All reactions were run under an atmosphere of argon gas except indicated experiments. Anhydrous solvent was used in a large case of reactions. Solvent was dried according to standard procedures. Several distilled solvents were used after the degasification by bubbling an inert argon gas. The progress of the reaction was monitored by TLC. ^{1}H, ^{13}C NMR spectra of all products were recorded on a BRUKER 300 MHz spectrometer (Fourier 300, ^{1}H = 300 MHz, ^{13}C = 75 MHz). Chemical shifts of spectra were referenced in Me_4Si, $CDCl_3$, and DMSO as the internal standard. All mass spectrometry data were collected by Waters Fraction Lynx MS Autopurification System at Daegu-Gyeongbuk Medical Innovation Foundation. The purity of final compound was determined by analytical (reverse phase high performance liquid chromatography) RP-HPLC. Analytical RP-HPLC was performed on Agilent 1100 series, using Thermo Hypersil Keystone Betabasic-18 column (150 Å pore size, 3 μm particle size; mobile phase A, 0.1% TFA in 95% H_2O + 5% CH_3CN; mobile phase B, 0.1% TFA in 95% CH_3CN + 5% H_2O; all percentages are v/v). Linear gradients were run from 100:0 to 0:100 A:B over 30 min. The spectroscopic study was performed utilizing a Genesis 10S UV Vis Spectrometer and a Perkin Elmer LS55.



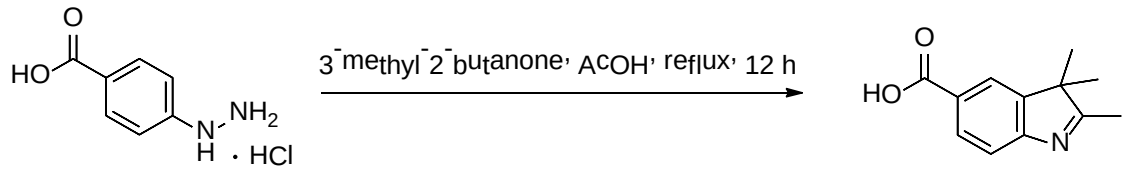
2,3,3-Trimethyl-5-nitro-3H-indole[1]

2,3,3-Trimethylindolenine (1.0 mL, 6.3 mmol) was stirred in ice water bath for 5 min. $NaNO_3$ (0.56 g, 6.5 mmol) was added in one portion. H_2SO_4 (16.0 mL, 300 mmol) was added dropwise over 10 min. During the addition of H_2SO_4, the color of reaction mixture was changed from yellow to red. The resulting solution was stirred for 1 h at -5 °C. The solution was neutralized slowly with solid NaOH, while maintaining a reaction temperature of -5 °C. This crude product was dissolved in EtOAc and, washed with H_2O (3 × 20 mL). The organic layer was isolated and dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield 2,3,3-trimethyl-5-nitro-3H-indole (1.2 g, 93%) as a dark red solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.41 (d, J = 2.23 Hz, 1H), 8.23 (dd, J = 8.47, 2.24 Hz, 1H), 7.65 (d, J = 8.57 Hz, 1H), 2.30 (s, 3H), 1.34 (s, 6H).



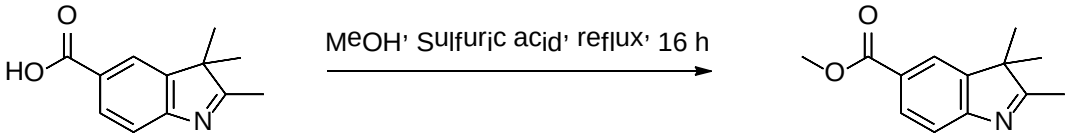
2,3,3-Trimethyl-3H-indol-5-amine[2]

To a solution of 2,3,3-trimethyl-5-nitro-3H-indole (500 mg, 2.45 mmol) in MeOH (16 mL) was added 10% Pd/C (53 mg, 0.049 mmol) and, stirred for 6 h at room temperature. The reaction mixture filtered through celite and washed with EtOAc. Combined filtrate was evaporated by rotary evaporator. The residue was subjected to chromatography over silica gel (50% EtOAc in Hexane) to give 2,3,3-trimethyl-3H-indol-5-amine (272 mg, 64%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.05 (d, J = 8.10 Hz, 1H), 6.56 (d, J = 1.68 Hz, 1H), 6.42 (dd, J = 8.20, 1.68 Hz, 1H), 4.95 (s, 2H), 2.09 (s, 3H), 1.16 (s, 6H).



2,3,3-Trimethyl-3H-indole-5-carboxylic acid[3]

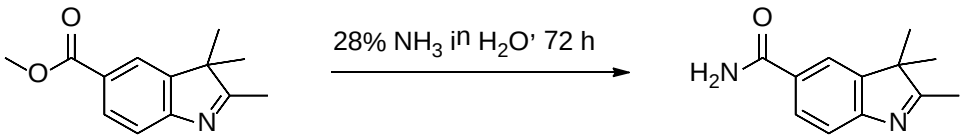
4-Hydrazinobenzoic acid hydrochloride (1.0 g, 5.3 mmol) and 3-methyl-2-butanone (0.90 mL, 9.0 mmol) were dissolved in acetic acid (10 mL). The solution was refluxed for 12 h and then allowed to cool to room temperature. The solvent was removed under reduced pressure. The resulting residue was diluted with DCM and washed with sat. $NaHCO_3$. The organic extracts were dried over Na_2SO_4, filtered, and evaporated to afford 2,3,3-trimethyl-3H-indole-5-carboxylic acid as a brown solid (870 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13-8.16 (m, 1H), 8.06 (d, J = 3 Hz, 1H), 7.66 (d, J = 6 Hz, 1H), 2.37 (s, 3H), 1.37 (s, 6H).



Methyl 2,3,3-trimethyl-3H-indole-5-carboxylate[4]

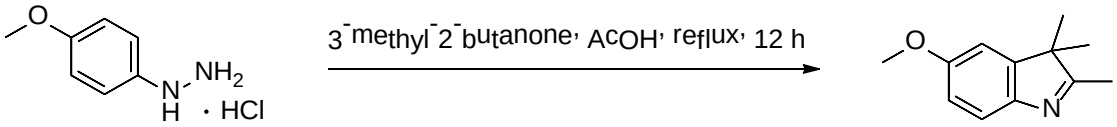
2,3,3-Trimethyl-3H-indole-5-carboxylic acid (500 mg, 2.46 mmol) in MeOH (7.9 mL) and sulfuric acid (820 μL, 15.4 mmol) was refluxed for 16 h. The mixture allowed to cool to room temperature and $NaHCO_3$ was added until gas evolution cease. The reaction mixture was extracted with EtOAc, washed with brine and dried over Na_2SO_4. The crude material was purified by column chromatography (silica gel, 33% EtOAc in Hexane) to give methyl 2,3,3-

trimethyl-3H-indole-5-carboxylate (448 mg, 84%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.02 (d, J = 1.12 Hz, 1H), 7.94 (dd, J = 8.01, 1.68 Hz, 1H), 7.54 (d, J = 8.01 Hz, 1H), 3.85 (s, 3H), 2.26 (s, 3H), 1.29 (s, 6H).



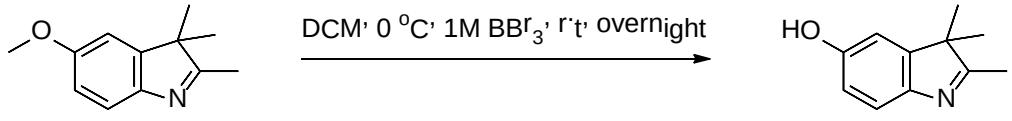
2,3,3-Trimethyl-3H-indole-5-carboxamide[5]

Methyl 2,3,3-trimethyl-3H-indole-5-carboxylate (200 mg, 0.92 mmol) was dissolved in 28% aqueous ammonia (2.6 mL), and the solution was stirred at 50 ℃ for 48 h. The solvent was removed under reduced pressure. The residue was dissolved in water and extracted with DCM. The organic extracts were dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by column chromatography (silica gel, 80% EtOAc in Hexane) to give 2,3,3-trimethyl-3H-indole-5-carboxamide (136 mg, 73%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.94 (s, 1H), 7.90 (s, 1H), 7.54 (d, J = 9 Hz, 1H), 7.46 (d, J = 6 Hz, 1H), 7.28 (s, 1H), 2.24 (s, 3H), 1.27 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.61, 168.12, 156.09, 145.85, 130.89, 127.48, 121.17, 118.74, 53.39, 22.48, 15.34.



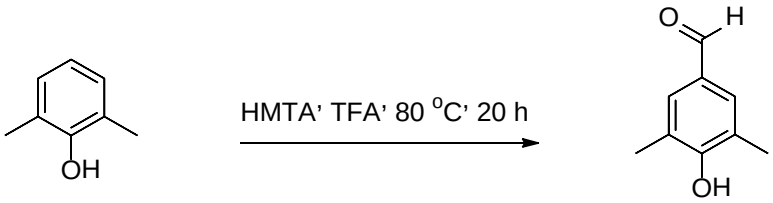
5-Methoxy-2,3,3-trimethyl-3H-indole[6]

4-Methoxyphenylhydrazine hydrochloride (500 mg, 2.90 mmol) and 3-methyl-2-butanone (0.29 mL, 2.9 mmol) were dissolved in acetic acid (5 mL). The solution was refluxed for 12 h and then allowed to cool to room temperature. The solvent was removed under reduced pressure. The resulting residue was diluted with DCM and was washed with sat. $NaHCO_3$. The organic extracts were dried over Na_2SO_4, filtered, and evaporated to afford 5-methoxy-2,3,3-trimethyl-3H-indole (359 mg, 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, J = 6 Hz, 1H), 6.83 (s, 1H), 6.81 (d, J = 3 Hz, 1H), 3.83 (s, 3H), 2.24 (s, 3H), 1.29 (s, 6H).



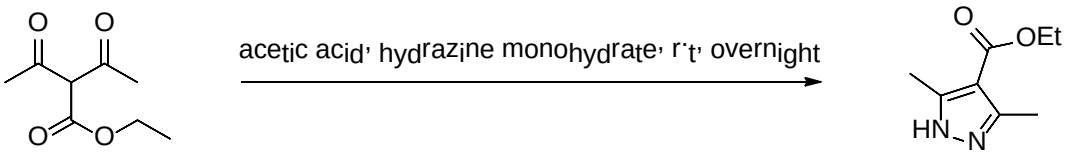
2,3,3-Trimethyl-3H-indol-5-ol[7]

To a solution of 5-methoxy-2,3,3-trimethyl-3H-indole (300 mg, 1.59 mmol) in DCM at 0 ℃ was added 1 M BBr_3 in DCM (3.1 mL, 3.1 mmol). The solution was allowed to room temperature and stirred overnight. To the resulting residue was added sat. $NaHCO_3$ and the solution was then diluted with water and a small amount of MeOH. The solution was extracted with DCM, dried over Na_2SO_4, filtered, and evaporated. The crude material was purified by column chromatography (silica gel, 5% MeOH in DCM) to give 2,3,3-trimethyl-3H-indol-5-ol (190 mg, 68%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.24 (s, 1H), 7.18 (d, J = 8.20 Hz, 1H), 6.76 (d, J = 2.24 Hz, 1 H), 6.63 (dd, J = 8.15, 2.28 Hz, 1 H), 2.13 (s, 3H), 1.18 (s, 6H).



4-Hydroxy-3,5-dimethylbenzaldehyde[8]

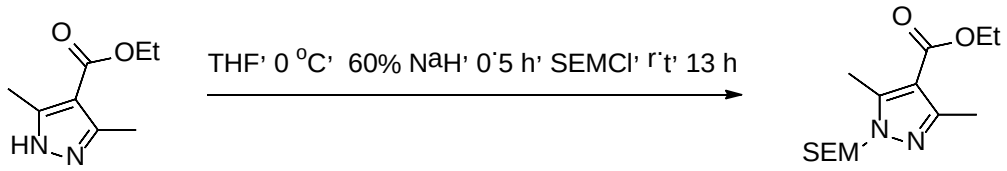
2,6-Dimethylphenol (12.2 g, 100 mmol) and HMTA (hexamethylenetetramine, 14 g, 100 mmol) were dissolved in TFA (Trifluoroacetic acid, 143 mL). The mixture was stirred and heated to 80 ℃ for 20 h. The reaction was quenched by addition of water (120 mL) and the resulting mixture was stirred for 1 h. The mixture was neutralized by solid K_2CO_3 to pH ~7 and extracted with diethyl ether. The combined organic extracts were washed with brine, and dried with $MgSO_4$. The solution was filtered and concentrated. The residue was subjected to chromatography over silica gel (33% EtOAc in Hexane) to give 4-hydroxy-3,5-dimethylbenzaldehyde (9.6 g, 67 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.82 (s, 1H), 7.55 (s, 2H), 5.25 (s, 1H), 2.32 (s, 6H).



Ethyl 3,5-dimethyl-1H-pyrazole-4-carboxylate[9]

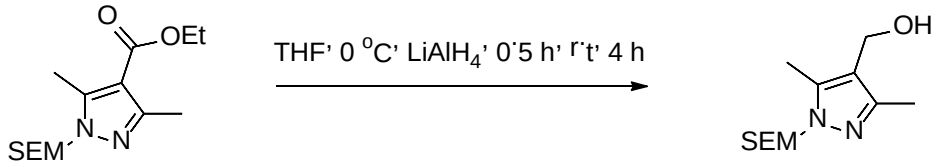
To a solution of ethyl diacetoacetate (8.97 g, 50 mmol) in AcOH (100 mL) was added hydrazine monohydrate (2.72 mL, 55.0 mmol) and the mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure. The residue was diluted with water, neutralized with sat. $NaHCO_3$. Then mixture was filtered, washed with water and then

dried to give ethyl 3,5-dimethyl-1H-pyrazole-4-carboxylate (7.3 g, 88%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.63 (br, s, 1H), 4.18 (q, J = 7.1 Hz, 2H), 2.33 (s, 6H), 1.27 (t, J = 7.1, 3H).



Ethyl 3,5-dimethyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazole-4-carboxylate

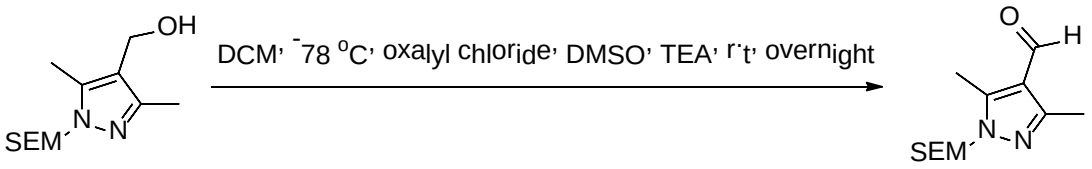
To a solution of ethyl 3,5-dimethyl-1H-pyrazole-4-carboxylate (7.00 g, 41.6 mmol) in 84 mL of THF was added slowly NaH (60% in Oil, 2.50 g, 62.4 mmol) at 0 °C under argon atmosphere. After the mixture was allowed to stir for 30 min, SEMCl (8.50 mL, 45.8 mmol) was added slowly. The reaction solution allowed to warm to room temperature and stirred for 13 h. The solution was quenched with water and the solvent was evaporated under reduced pressure. The residue was dissolved with EtOAc, washed with brine, and dried with $MgSO_4$. The solution was filtered and concentrated. The residue was subjected to column chromatography over silica gel (10% EtOAc in Hexane) to give ethyl 3,5-dimethyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazole-4-carboxylate (9.7 g, 78 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.36 (s, 2H), 4.30 (q, J = 7.08 Hz, 2H), 3.56 (t, J = 8.10 Hz, 2H), 2.57 (s, 3H), 2.42 (s, 3H), 1.36 (t, J = 7.12 Hz, 3H) 0.89 (t, J=8.20 Hz, 2H), -0.02 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.98, 151.85, 146.50, 112.37, 79.13, 67.97, 61.14, 19.22, 15.84, 15.69, 12.41, 0.00.



(3,5-Dimethyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazol-4-yl)methanol

To a solution of ethyl 3,5-dimethyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazole-4-carboxylate (5.00 g, 16.7 mmol) in THF (100 mL) was added $LiAlH_4$ (953 mg, 25.1 mmol) portionwise at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, then 4 h at room temperature. The reaction mixture was cooled in an ice bath, and carefully quenched with H_2O and extracted with EtOAc. The organic phase was combined, dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The mixture was purified by column chromatography over silica gel (50% EtOAc in Hexane) to give (3,5-dimethyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazol-4-yl)methanol (4.0 g, 89 %). ^{1}H NMR (300 MHz,

$CDCl_3$) δ 5.32 (s, 2H), 4.49 (s, 2H), 3.56 (t, *J* = 8.10 Hz, 2H), 2.32 (s, 3H), 2.26 (s, 3H), 0.89 (t, *J* = 8.40 Hz, 2H), 0.00 (s, 9H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.69, 139.92, 118.82, 78.96, 67.60, 56.29, 19.23, 13.11, 10.61, 0.00.

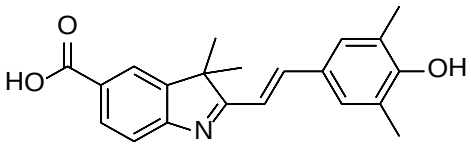


3,5-Dimethyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazole-4-carbaldehyde

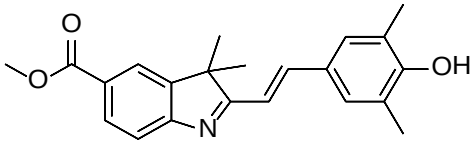
To a solution of oxalyl chloride (1.54 mL, 17.2 mmol) in DCM (133 mL) at -78 °C was added a solution of DMSO (2.66 mL, 37.4 mmol) in DCM (25 mL) under argon atmosphere. After 30 min, a solution of (3,5-dimethyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazol-4-yl)methanol (4.00 g, 15.6 mmol) in DCM (33 mL) was added. The mixture was stirred for 30 min and Et_3N (11 mL, 78 mmol) was added. The mixture was warmed to room temperature and then stirred overnight. Then, the mixture was extracted with DCM and the organic layer was washed with water. The organic layer was dried over Na_2SO_4 and the solvents were removed. The residue was subjected to column chromatography over silica gel (16% EtOAc in to give 3,5-dimethyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazole-4-carbaldehyde (2.3 g, 62 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.00 (s, 9H), 0.92 (t, *J* = 8.4 Hz, 2H), 2.47 (s, 3H), 2.60 (s, 3H), 3.61 (t, *J* = 8.3 Hz, 2H), 5.39 (s, 2H), 9.98 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.57, 152.05, 147.05, 120.49, 79.00, 68.29, 19.23, 14.05, 11.25, 0.00.

General Procedure for syntheses of 1-5 and SEM protected 6-10

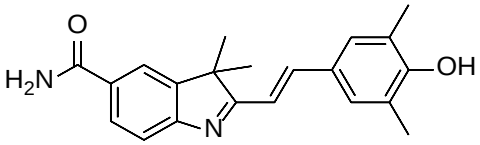
Concentrated hydrochloric acid (1.4 mL per 1.0 mmol of each compound) was added dropwise into compound **1-10**. The mixture was stirred at room temperature for 30 min. Then excess reagent was removed by rotary evaporation under reduced pressure to give HCl salt. The HCl salt was then dissolved in anhydrous EtOH and then substituted aldehyde was added. The mixture was allowed to reflux for 4 h. Cooled to room temperature and extracted with DCM, washed with water, brine, dried over Na_2SO_4, and concentrated. The crude material was purified by column chromatography (silica gel, 16% EtOAc in Hexane) to give **1-5** and SEM protected **6-10**.



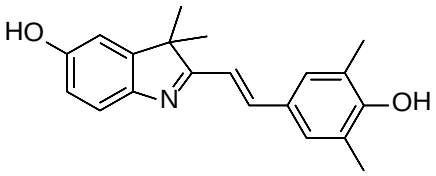
(*E*)-2-(4-Hydroxy-3,5-dimethylstyryl)-3,3-dimethyl-3H-indole-5-carboxylic acid (1). Yield: 17%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.80 (s, 1H), 8.00 (s, 1H), 7.94 (d, J = 9 Hz, 1H), 7.73 (d, J = 15 Hz, 1H), 7.55 (d, J = 6 Hz, 1H), 7.41 (s, 1H), 7.10 (d, J = 15 Hz, 1H), 2.21 (s, 3H), 1.41 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 186.66, 167.55, 157.96, 155.42, 146.64, 139.57, 128.66, 127.01, 126.73, 124.61, 119.36, 115.69, 52.37, 22.77, 16.65; HRMS (ESI) (m/z): calcd for $C_{21}H_{21}NO_3$ $[M]^+$ 335.1521, found 335.1518; Purity: 93%.



(*E*)-Methyl 2-(4-hydroxy-3,5-dimethylstyryl)-3,3-dimethyl-3H-indole-5-carboxylate (2). Yield: 41%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.76 (br. s., 1H), 8.03 (s, 1H), 7.95 (d, J = 8.20 Hz, 1H), 7.72 (d, J = 16.39 Hz, 1H), 7.57 (d, J = 8.01 Hz, 1H), 7.41 (s, 2 H), 7.08 (d, J = 16.11 Hz, 1H), 3.86 (s, 3H), 2.21 (s, 6H), 1.42 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 187.14, 166.38, 158.24, 155.44, 146.78, 139.76, 129.73, 128.64, 126.66, 125.77, 124.59, 122.34, 119.48, 115.59, 52.40, 51.98, 22.69, 16.57; HRMS (ESI) (m/z): calcd for $C_{22}H_{23}NO_3$ $[M]^+$ 349.1678, found 349.1675; Purity: 100%

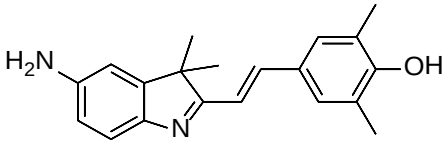


(*E*)-2-(4-Hydroxy-3,5-dimethylstyryl)-3,3-dimethyl-3H-indole-5-carboxamide (3). Yield: 15%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.91 (s, 1H), 7.84 (d, J = 9.4 Hz, 1H), 7.66 (d, J = 16 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.39 (s, 2H), 7.28 (s, 1H), 7.04 (d, J = 16.5 Hz, 1H), 2.20 (s, 6H), 1.40 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 185.56, 167.99, 156.52, 155.30, 146.39, 139.06, 130.66, 128.53, 127.62, 126.78, 124.59, 120.97, 118.98, 115.88, 52.27, 22.97, 16.66; HRMS (ESI) (m/z): calcd for $C_{21}H_{22}N_2O_2$ $[M]^+$ 334.1681, found 334.1679; Purity: 98%.



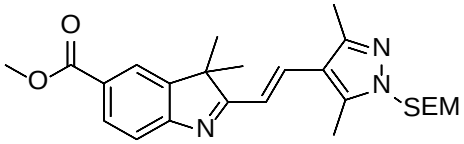
(*E*)-2-(4-Hydroxy-3,5-dimethylstyryl)-3,3-dimethyl-3H-indol-5-ol (4).

Yield: 18%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.37 (br. s., 1H), 8.63 (br, s, 1H), 7.44 (d, *J* = 16.5 Hz, 1H), 7.31 (s, 2H), 7.27 (d, *J* = 8.4 Hz, 1H), 6.93 (d, *J* = 16.4 Hz, 1H), 6.80 (s, 1H), 6.67 (dd, *J* = 8.1, 2.3 Hz , 1H), 2.19 (s, 6H), 1.34 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 180.32, 155.70, 154.62, 148.47, 146.29, 136.10, 127.90, 127.21, 124.51, 120.20, 116.74, 113.92, 108.93, 52.07, 23.40, 16.66; HRMS (ESI) (m/z): calcd for $C_{20}H_{21}NO_2$ $[M]^+$ 307.1572, found 307.1568; Purity: 99%.



(*E*)-4-(2-(5-Amino-3,3-dimethyl-3H-indol-2-yl)vinyl)-2,6-dimethylphenol (5).

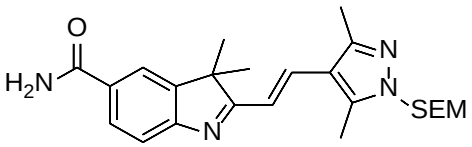
Yield: 18%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.51 (br. s., 1H), 7.36 (d, *J* = 16.7 Hz, 1H), 7.28 (s, 2H), 7.14 (d, *J* = 8.1 Hz, 1H), 6.89 (d, *J* = 16.2 Hz, 1H), 6.58 (d, *J* = 1.7 Hz, 1H), 6.47 (dd, *J* = 8.2, 1.9 Hz, 1H), 5.12 (s, 2H), 2.19 (s, 6H), 1.31 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 177.91, 154.37, 148.33, 147.04, 144.23, 134.89, 127.64, 127.42, 124.48, 120.17, 117.15, 112.55, 107.19, 51.50, 23.72, 16.63; HRMS (ESI) (m/z): calcd for $C_{20}H_{22}N_2O$ $[M+H]^+$ 307.1805, found 307.1798; Purity: 99%.



(*E*)-Methyl2-(2-(3,5-dimethyl-1((2(trimethylsilyl)ethoxy)methyl)-1H-pyrazol-4-yl)vinyl) -3,3-dimethyl-3H-indole-5-carboxylate.

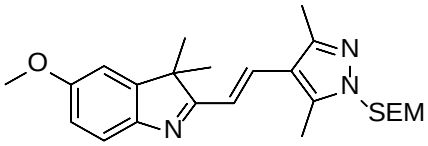
Yield: 48%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.04 (s, 1H), 7.96 (dd, *J* = 8.1, 1.6, 1H), 7.73 (d, *J* = 16.4 Hz, 1H), 7.58 (d, *J* = 8.1 Hz, 1H), 6.68 (d, *J* = 16.4 Hz, 1H), 5.38, (s, 2H), 3.86 (s, 3H), 3.54 (t, *J* = 8.2 Hz, 2H), 2.45 (s, 3H), 2.37 (s, 3H), 1.41 (s, 6H), 0.84 (t, *J* = 7.9 Hz 2H), -0.04(s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 188.49, 167.73, 159.51, 148.02, 147.99, 142.18,

132.02, 131.12, 127.10, 123.76, 120.82, 117.02, 116.24, 78.26, 66.76, 53.62, 53.36, 24.21, 18.48, 15.18, 11.10, 0.00.



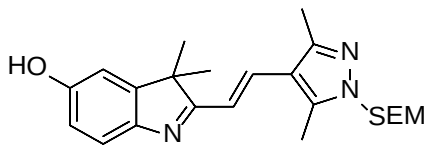
(*E*)-2-(2-(3,5-Dimethyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazol-4-yl)vinyl)-3,3-dimethyl-3H-indole-5-carboxamide.

Yield: 77%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.97 (s, 1H), 7.90 (s, 1H), 7.86 (d, J = 7.9 Hz, 1H), 7.68 (d, J = 16.7 Hz, 1H), 7.51 (d, J = 8.1 Hz, 1H), 7.28 (s, 1H), 6.66 (d, J = 16.4 Hz, 1H), 5.38, (s, 2H), 3.53 (t, J = 7.9 Hz, 2H), 2.44 (s, 3H), 2.36 (s, 3H), 1.40 (s, 6H), 0.84 (t, J = 7.9 Hz 2H), -0.04 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 187.13, 169.29, 157.73, 147.84, 147.57, 141.90, 131.97, 131.26, 128.97, 122.29, 120.24, 117.36, 116.24, 78.26, 66.73, 53.43, 24.46, 18.47, 15.17, 11.08, 0.00.



(*E*)-2-(2-(3,5-Dimethyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazol-4-yl)vinyl)-5-methoxy-3,3-dimethyl-3H-indole.

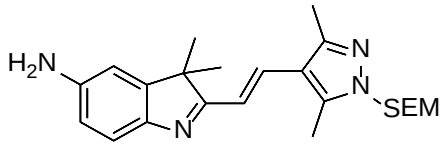
Yield: 96%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.50 (d, J = 16.5 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 7.07 (s, 1H), 6.84 (dd, J = 8.4, 2.5 Hz 1H), 6.60 (d, J = 16.5 Hz, 1H), 5.36, (s, 2H), 3.78 (s, 3H), 3.53 (t, J = 8 Hz, 2H), 2.42 (s, 3H), 2.33 (s, 3H), 1.36 (s, 6H), 0.84 (t, J = 8 Hz, 2H), -0.04 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 182.73, 158.93, 149.57, 148.74, 147.46, 141.11, 128.92, 121.34, 118.21, 116.34, 114.01, 109.23, 78.21, 66.68, 56.82, 53.53, 24.70, 18.47, 15.12, 11.04, 0.03.



(*E*)-2-(2-(3,5-dimethyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazol-4-yl)vinyl)-3,3-dimethyl-3H-indol-5-ol.

Yield: 28%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.38 (br. s., 1H), 7.45 (d, J = 16.86 Hz, 1H), 7.27 (d, J = 8.38 Hz, 1 H), 6.81 (d, J = 2.51 Hz, 1H), 6.68 (dd, J = 8.66, 2.70 Hz, 1H), 6.58 (d,

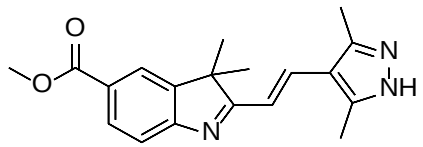
J=15.90 Hz, 1H), 5.36 (s, 2H), 3.52 (t, *J* = 7.92 Hz, 2H), 2.41 (s, 3H), 2.33 (s, 3H), 1.33 (s, 6H), 0.83 (t, *J* = 7.9 Hz, 2H), -0.04 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 185.33, 156.62, 148.83, 147.19, 120.83, 114.77, 110.50, 109.23, 78.21, 66.68, 56.82, 53.53, 24.70, 18.47, 15.12, 11.04, 0.03.



(*E*)-2-(2-(3,5-dimethyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazol-4-yl)vinyl)-3,3-dimethyl-3H-indol-5-amine.

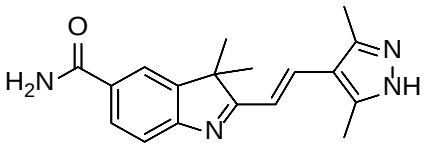
Yield: 56%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.36 (d, *J* = 16.6 Hz, 1H), 7.14 (d, *J* = 8 Hz, 1H), 6.51 (m, 3H), 5.35 (s, 2H), 5.11 (s, 2H), 3.52 (t, *J* = 7.9 Hz, 2H), 2.40 (s, 3H), 2.32 (s, 3H), 1.30 (s, 6H), 0.83 (t, *J* = 7.9 Hz, 2H), -0.04 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 179.27, 149.48, 148.34, 147.19, 145.45, 140.55, 127.23, 121.45, 119.01, 116.51, 113.91, 108.58, 78.19, 66.64, 52.71, 25.18, 18.47, 15.10, 11.02, 0.00.

General Procedure for SEM deprotection (6-10). The SEM protected compound (1 equiv.) was diluted with 4 N HCl in dioxane (10 equiv.) and heated to 60 ºC for 3 h. The reaction mixture was neutralized with sat. $NaHCO_3$. The solution was diluted with EtOAc, washed with brine, dried over Na_2SO_4 and concentrated. The crude material was purified by column chromatography (silica gel, Hexane/EtOAc).

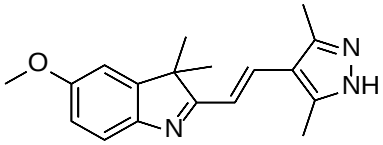


(*E*)-Methyl-2-(2-(3,5-dimethyl-1H-pyrazol-4-yl)vinyl)-3,3-dimethyl-3H-indole-5-carboxylate (6).

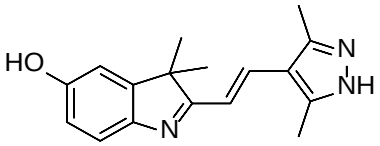
Yield: 48%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.60 (br. s., 1H), 8.03 (s, 1H), 7.95 (d, *J* = 8.1 Hz, 1H), 7.74 (d, *J* = 16.3 Hz, 1H), 7.56 (d, *J* = 8.1 Hz, 1H), 6.60 (d, *J* = 16.3 Hz, 1H), 3.86 (s, 3H), 2.37 (s, 6H), 1.40 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 187.34, 166.42, 158.32, 146.62, 131.33, 129.79, 125.55, 122.39, 119.32, 114.34, 112.88, 52.18, 52.02, 23.00; HRMS (ESI) (m/z): calcd for $C_{19}H_{21}N_3O_2$ $[M]^+$ 323.1634, found 323.1630; Purity: 97%.



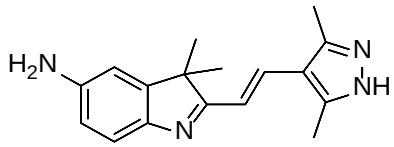
(*E*)-2-(2-(3,5-dimethyl-1H-pyrazol-4-yl)vinyl)-3,3-dimethyl-3H-indole-5-carboxamide (7). Yield: 28%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.57 (br. s., 1H), 7.95 (s, 1H), 7.89 (s, 1H), 7.84 (d, *J* = 9 Hz, 1H), 7.69 (d, *J* = 16.5 Hz, 1H), 7.49 (d, *J* = 8.2 Hz, 1H), 7.27 (s, 1H), 6.59 (d, *J* = 16.3 Hz, 1H), 2.37 (s, 6H), 1.39 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 186.00, 168.01, 156.56, 146.20, 130.45, 127.65, 120.95, 118.78, 114.70, 112.89, 52.02, 40.42, 23.26; HRMS (ESI) (m/z): calcd for $C_{18}H_{20}N_4O$ $[M]^+$ 308.1637, found 308.1634; Purity: 99%.



(*E*)-2-(2-(3,5-dimethyl-1H-pyrazol-4-yl)vinyl)-5-methoxy-3,3-dimethyl-3H-indole (8). Yield: 96%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.49 (br. s., 1H), 7.51 (d, *J* = 16.5 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 7.07 (d, *J* = 2.2 Hz, 1H), 6.83 (dd, *J* = 8.2, 2.4 Hz, 1H), 6.53 (d, *J* = 16.5 Hz, 1H) 3.78 (s, 3H), 2.34 (s, 6H), 1.36 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 181.58, 157.46, 148.16, 147.52, 128.18, 119.85, 115.54, 112.90, 112.62, 107.90, 55.48, 52.13, 43.63, 23.47; HRMS (ESI) (m/z): calcd for $C_{18}H_{21}N_3O$ $[M]^+$ 295.1685, found 295.1685; Purity: 98%.



(*E*)-2-(2-(3,5-dimethyl-1H-pyrazol-4-yl)vinyl)-3,3-dimethyl-3H-indol-5-ol (9). Yield: 28%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.45 (br. s., 1H), 9.33 (br. s., 1H), 7.46 (d, *J* = 16.5 Hz, 1H), 7.25 (d, *J* = 8.1 Hz, 1H), 6.79 (s, 1H), 6.67 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.51 (d, *J* = 16.6 Hz, 1H), 2.33 (s, 6H), 1.32 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 180.49, 155.51, 148.20, 146.27, 127.63, 119.97, 115.76, 113.88, 112.92, 108.94, 51.85, 23.60; HRMS (ESI) (m/z): calcd for $C_{17}H_{19}N_3O$ $[M]^+$ 281.1528, found 281.1524; Purity: 100%.



(*E*)-2-(2-(3,5-dimethyl-1H-pyrazol-4-yl)vinyl)-3,3-dimethyl-3H-indol-5-amine (10).

Yield: 56%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.44 (br. s., 1H) 7.37 (d, J = 16.5 Hz, 1H), 7.13 (d, J = 8.2 Hz, 1H), 6.59 (d, J = 2.2 Hz, 1H), 6.47 (m, 2H), 5.09 (s, 2H), 2.32 (s, 6H), 1.29 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 178.18, 148.07, 146.85, 144.23, 126.52, 119.96, 116.36, 113.04, 112.56, 107.29, 51.34, 23.95; HRMS (ESI) (m/z): calcd for $C_{17}H_{20}N_4$ $[M+H]^+$ 281.1761, found 281.1758; Purity: 98%

Fluorescence assay with WT-TTR and M-TTR.

Each indole derivative (1.5 μL of a 0.4 mM solution in DMSO, final concentration: 4 μM) was added to 150 μL of a solution of WT-TTR (2 μM) or M-TTR (8 μM) in PBS (10 mM phosphate, 100 mM KCl and 1 mM EDTA, pH 7.0). The samples were vortexed, and incubated for 1 h at room temperature. The fluorescence changes were monitored using a Perkin Elmer LS55 Fluorescence Detector in a 1 cm path length quartz cell. The excitation slit was set at 10 nm and the emission slit was set at 5 nm.

Probe 10 titration assay

Each compound (1.5 μL, 0.1–0.6 mM solution in DMSO) were added to 150 μL of a solution of WT-TTR (2 μM) in PBS. The samples were vortexed, and incubated for 1 h at room temperature. The fluorescence changes were monitored using a Perkin Elmer LS55 Fluorescence Detector in a 1 cm path length quartz cell. The excitation slit was set at 10 nm and the emission slit was set at 5 nm.

WT-TTR titration assay

Probe **10** (1.5 μL, 0.4 mM solution in DMSO) were added to 150 μL of a solution of WT-TTR (0.1–1 μM) in PBS. The samples were vortexed, and incubated for 1 h at room temperature. The fluorescence changes were monitored using a Perkin Elmer LS55 Fluorescence Detector in a 1 cm path length quartz cell. The excitation slit was set at 10 nm and the emission slit was set at 5 nm.

Determination of the limit of detection (LOD)

The minimum detectable value was calculated by using $3\sigma/k$ rule where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity and WT-TTR concentration.

Western blot

Samples (human male AB plasma, human male A serum, sigmaaldrich) were centrifuged to 4 ℃ for 10 min at 13000 RPM and diluted at 1:18 in Tris-Glycine-SDS running buffer (25 mM Tris base, 192 mM Glycine, 0.1% SDS, 1 mM DTT, pH 8.3). 20 μL samples were loaded in Novex™Wedgewell™ 16% tris-glycine mini gel (12 well, Invitrogen) to separate proteins after 10 min boiling, and transfer them to PVDF (polyvinylidene fluoride) membrane (Immobilon®-P). Membrane was blocked with 5% skim milk in TBST (24.7 mM tris, 137 mM NaCl, 2.7 mM KCl, 0.05% Tween20), treated with rabbit anti-human TTR antibody (Invitrogen, 1:2000) to 4 ℃ for 18 h, and reacted with Goat anti-rabbit IgG(H+L) antibody (Invitrogen, 1:2000) for 1 h at room temperature. Proteins are visualized with ECL solution (SuperSignal™) and analyzed using an image analyzer (Chemi doc, Vilber)

Plasma Stability of probe 10

The stability of probe **10** in human plasma (Sigma-aldrich Inc. St. Louis, MO, USA) was determined. The stock solution of probe **10** was prepared to 10 mg/mL in DMSO prior to the start of the experiment. The stock solution was diluted with methanol to a concentration of 200 μg/mL. The diluted probe **10** solution (5 μL) was added to 495 μL of freshly obtained rat plasma or human plasma and 50 μL of sample was dispensed (0 min). After 5, 15, 30, and 60 min incubation in a shaking water bath (37℃, 140 rpm), the reaction was terminated by adding 200 μL of ice-cold acetonitrile solution containing 3.33 ng/mL carbamazepine, and mixing on a vortexer. The solution was clarified by centrifugation at 10,000 × g for 3 min at 4 ^{o}C, and the clear supernatants were collected and transferred to liquid chromatography vials. The samples were analyzed by LC-MS/MS for quantification of probe **10**.

Fluorescence assay with human plasma and serum.

Prior to Fluorescent measurement, plasma and serum samples were diluted with Tris-Glycine buffer (25 mM Tris, 25 mM NaCl, pH 7.5), and albumin was removed using Pierce™

Albumin Depletion Kit (Thermo scientific), following the manufacturer's instructions.

Probe **10** (1.5 μL of a 0.4 mM solution in DMSO, final concentration: 4 μM) was added to 150 μL of a diluted solution of human plasma or serum sample. The samples were vortexed, and incubated for 1 h at room temperature. The fluorescence changes were monitored using a Perkin Elmer LS55 Fluorescence Detector in a 1 cm path length quartz cell. The excitation slit was set at 10 nm and the emission slit was set at 5 nm.

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