SUPPORTING INFORMATION

Bifunctional Coumarin Derivatives That Inhibit Transthyretin Amyloidogenesis and Serve as Fluorescent Transthyretin Folding Sensors

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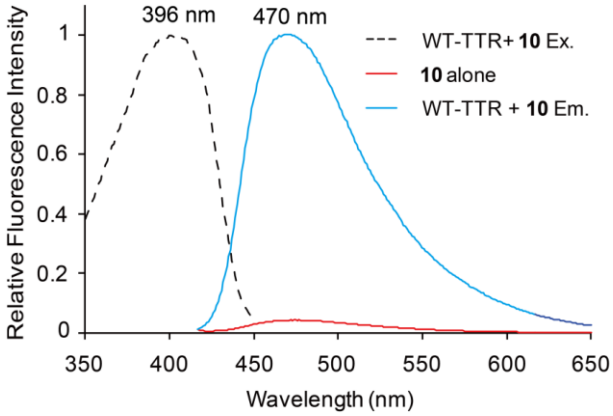
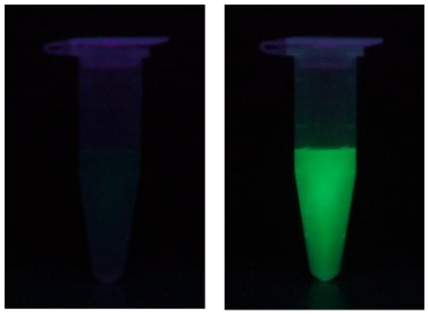
Figure S1. (A) Fluorescent spectra of **10** (7.2 μM) observed after 10 min of incubation with WT-TTR (3.6 μM, blue trace) and alone in phosphate buffer (red trace). Excitation spectrum is presented as a dotted black trace. Recombinant WT-TTR (3.6 μM, tetramer) was incubated with (B) **5** or (C) **10** (7.2 μM, respectively) and analyzed by illumination with a hand-held UV lamp.

A

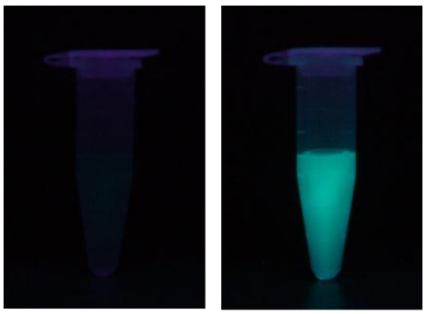


B



5 alone **5** + WT-TTR

C



10 alone **10** + WT-TTR

Figure S2. Fluorescence quenching of **5** in non-polar methylene chloride by addition of polar methanol.

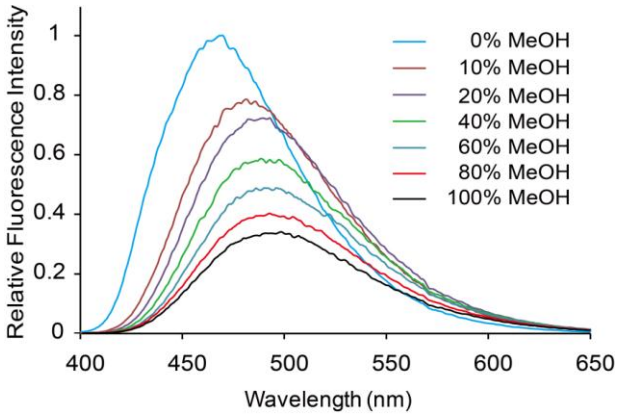
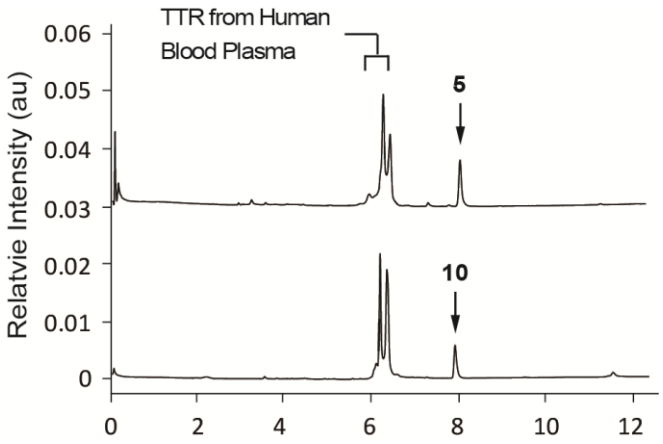


Figure S3. (A) C_{18}-RP-HPLC assessment of TTR binding stoichiometry of compounds **5** and **10** bound to human plasma TTR *ex vivo* after a 24 h incubation period (10.8 μM kinetic stabilizer concentration, maximum binding stoichiometry is 2 due to the two thyroxine binding pockets per TTR tetramer). (B) Fluorescence observed before and after 4 h of IPTG induction of WT-TTR expression in the presence of fluorogenic **5** (5 μM).

A



B

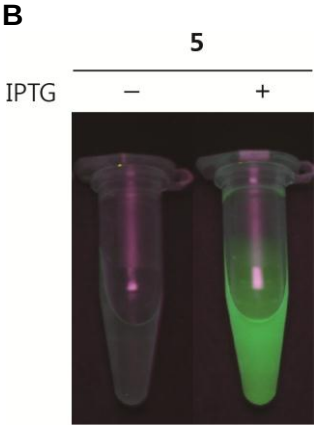
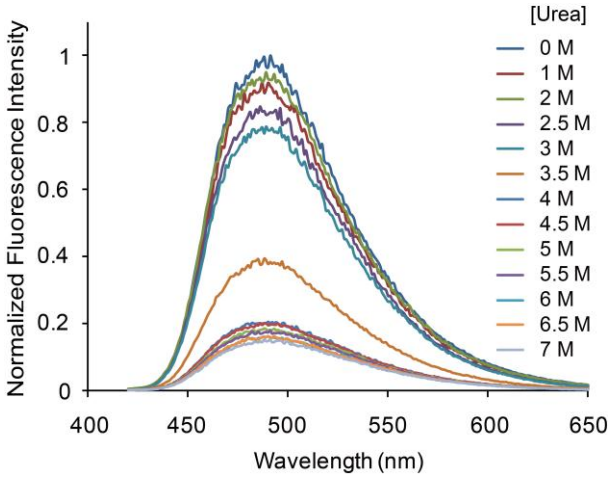


Figure S4. Urea concentration-dependent fluorescence spectra from (A) WT-TTR (3.6 μM) treated with **5** (7.2 μM) and (B) **5** (7.2 μM) without WT-TTR as a control.

A



B

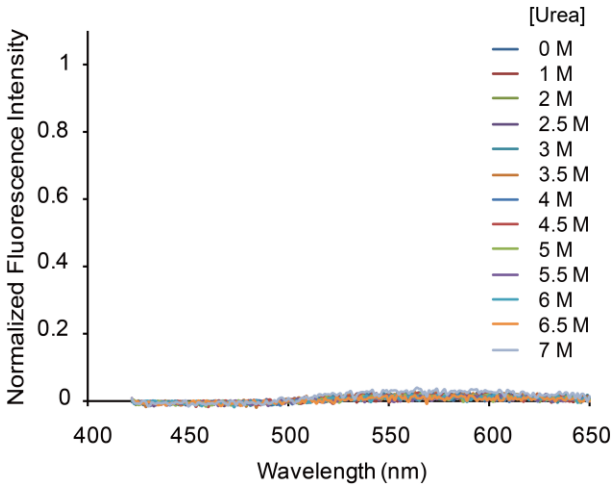


Table S1 Spectral properties of coumarin derivatives **1**-**10**.

Compound	λ_{ex}(nm)	λ_{em}(nm)	Relative fluorescence intensity[a]	Fold difference[b]
1	395	467	0.08	30
2	397	464	0.08	5
3	390	468	0.16	30
4	399	457	0.05	3
5	410	486	1.00	35
6	398	461	0.02	1
7	399	460	0.03	2
8	388	437	0.05	3
9	399	466	0.02	1
10	396	470	0.72	20

[a] Ratio of fluorescence intensity of each compound to that of **5** upon binding to WT-TTR in phosphate buffer

[b] Fold increase of fluorescence intensity upon binding to WT-TTR, compared to compound alone in phosphate buffer

Table S2. Data collection and refinement statistics for the crystal structure of TTR with **5**.

	WT-TTR $(5)_2$
Data Collection	
Beamline	SSRL 11-1
Wavelength (Å)	0.9797
Resolution (Å)	1.13 (1.13-1.19)[a]
Space group	$P2_12_12$
a, *b*, *c* (Å)	42.79, 85.65, 63.56
No. molecules in the a.u.	2
No. observations	534,387 (57,501)[a]
No. unique reflections	87,639 (11,620)
Completeness (%)	98.5 (90.7)
R_{sym} (%)[b]	2.1 (34.0)
R_{meas} (%)[c]	2.3 (37.9)
R_{pim} (%)[d]	0.9 (16.3)
Average I/σ	34.3 (4.3)
Redundancy	6.1 (4.9)
Refinement statistics	
Resolution (Å)	1.13-51.04
No. reflections (working set)	83,155 (5,012)
No. reflections (test set)	4,383 (246)
R_{cryst} (%)[e]	16.1 (31.2)
R_{free} (%)[f]	17.5 (32.9)
TTR/ligand/water atoms	1,989/48/251
Average B-values	
TTR	12.4
Ligand	18.1
Wilson B-value	10.2
Ramachandran plot	
Most favored (%)	99.6
Additionally allowed (%)	0.4
R.M.S deviations	
Bond lengths (Å)	0.15
Angles (°,)	1.72

[a] Numbers in parentheses throughout are for highest resolution sell of data.

[b] $R_{sym} = \sum_{hkl} \left| I - \langle I \rangle \right| \sum_{hkl} I$

[c] $R_{meas} = \sum_{hkl} [N/(N-1)]^{1/2} \sum i \left| Ii\,(hkl) - I(hkl) \right| / \sum_{hkl} \sum i\, Ii\,(hkl)$

[d] $R_{p.i.m} = \sum_{hkl} [1/(N-1)]^{1/2} \sum i \; | Ii\,(hkl) - I(hkl) | / \sum_{hkl} \sum i \; Ii\,(hkl)$

[e] $R_{cryst} = \sum_{hkl} \; | F_o - F_c | \sum_{hkl} F_o$

[f] R_{free} is the same as R_{cryst}, but for 5% of data excluded from refinement.

Acid-mediated Fibril Formation Assay with WT-TTR

WT-TTR was expressed and purified from an *E. coli* expression system as described previously.[1] Each coumarin derivative (1 μL of a 1.44 mM solution in DMSO, final concentration 14.4 μM, the minimum concentration required to occupy both T_4 binding sites) were added to 100 μL of a solution of WT-TTR homotetramer (0.4 mg/mL, 7.2 μM) in 10 mM phosphate, 100 mM KCl, and 1 mM EDTA (pH 7.0 phosphate buffer) in a 2 mL Eppendorf tube. The mixture was vortexed and preincubated at room temperature for 30 min. An acetate buffer solution (100 μL of 100 mM acetate, 100 mM KCl, 1 mM EDTA, pH 4.2) was added to lower the pH of the assay solution to 4.4. The tubes were incubated at 37 ^{o}C for 72 h without agitation. After the solution was vortexed to evenly distribute any precipitate, the turbidity of the solution at 600 nm was measured using a Thermo Genesis 10S UV-Vis spectrometer.

Binding Stoichiometry of 5 to TTR in Human Blood Plasma.

The plasma TTR binding selectivity assay has been previously described.[2] Briefly, to a 1 mL sample of human blood plasma in a 2 mL Eppendorf tube was added **5** (7.5 μL of a 1.44 mM solution in DMSO, final concentration: 10.8 μM) and then the plasma solution was incubated at 37 °C for 24 h on a rocker plate (30 rpm). A 1:1 (v/v) slurry of unfunctionalized sepharose resin in TSA buffer (10 mM Tris, 140 mM NaCl, pH 8.0) was added and the solution was incubated for 1 h at 4 °C on a rocker plate (18 rpm). The solution was then centrifuged and the supernatant was divided into two 400 μL aliquots, which were added to 200 μL of 1:1 (v:v) slurry of anti-TTR antibody conjugated sepharose resin in TSA. The solution was gently rocked (18 rpm) at 4 °C for 20 min, then centrifuged and the supernatant removed. The resin was washed three times by shaking for 1 min with 1 mL of TSA containing 0.05% saponin and then twice more with 1 mL of TSA. After centrifugation to remove the supernatant, 155 μL of triethylamine (100 mM, pH 11.5) was added to the sepharose resin to dissociate the TTR and bound test compound from the resin and the suspension was vortexed for 1 min. After centrifugation, the supernatant was analyzed by reverse phase HPLC on a Waters 600 E multi-solvent delivery system, using a Waters 486 tunable absorbance detector, a 717 autosampler, and a ThermoHypersil Keystone Betabasic-18 column (150 Å pore size, 3 μm particle size). The "A" mobile phase comprises 0.1%

TFA in 94.9% H_2O + 5% CH_3CN and the "B" mobile phase is made up of 0.1% TFA in 94.9% CH_3CN + 5% H_2O. Linear gradients were run from 100:0 A:B to 0:100 A:B for 9 min.

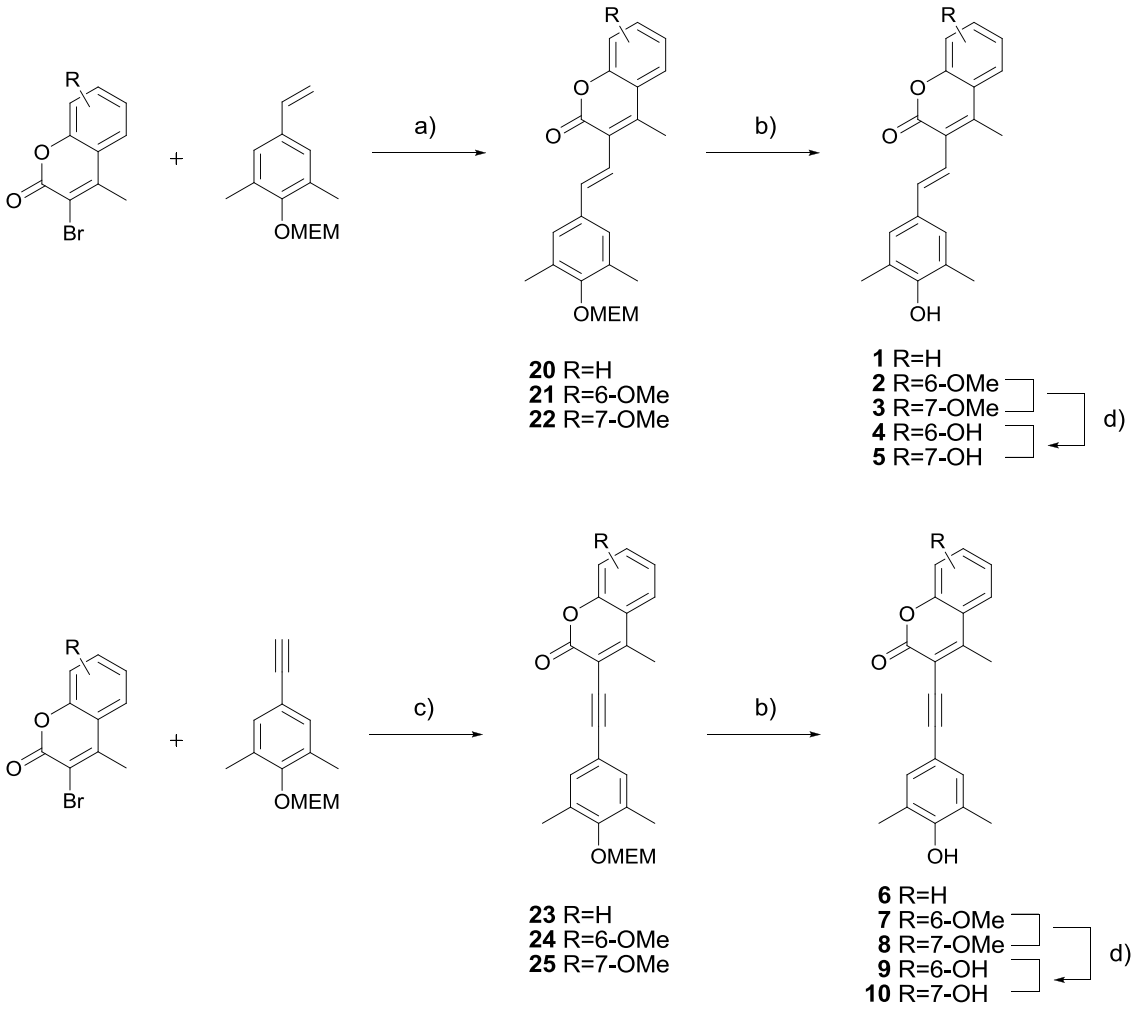
Fluorimetric Assay with WT-TTR and M-TTR. Each coumarin derivative (5 μL of a 1.44 mM solution in DMSO, final concentration: 7.2 μM) were added to 1 mL of a solution of WT-TTR (3.6 μM) or M-TTR (3.6 μM) in 10 mM phosphate, 100 mM KCl and 1 mM EDTA (pH 7.0 phosphate buffer). The samples were vortexed, and incubated for 10 min at 25 °C. The fluorescence changes were monitored using a Varian Cary Eclipse Fluorescence spectrophotometer at 20 °C in a 1 cm path length quartz cell. The excitation slit was set at 5 nm and the emission slit was set at 5 mm.

Urea-mediated TTR Dissociation Study. TTR (0.4 mg/mL) was incubated with increasing concentrations of urea in 10 mM sodium phosphate buffer, 100 mM KCl and 1 mM EDTA (pH 7.0). The mixtures were vortexed and incubated in the dark at 25 °C without agitation. Compound **5** was added to TTR-urea solution just before the fluorescence measurement.

Crystallization and Structure Determination of the WT-TTR–$(5)_2$. Wild type TTR protein was concentrated to 6 mg/mL in 10 mM sodium phosphate buffer and 100 mM KCl (pH 7.6) and co-crystallized at room temperature with a 5 molar excess of **5** using the vapor-diffusion sitting drop method. The crystals were grown from 1.395 M sodium citrate, 3.5% v/v glycerol at pH 5.5 and cryo-protected with 10% v/v glycerol. Data were collected at beam-lines 11-1 at the Stanford Synchrotron Radiation Lightsource (SSRL) at a wavelength of 0.9795 Å. Diffraction data were indexed, integrated, and scaled using XDS in space group $P2_12_12$ with two subunits observed per asymmetric unit. The structure was determined by molecular replacement using the model coordinates of 2FBR[3] in the program Phaser[4]. Ligand coordinates and restraints files were generated using the PRODRG server (http://davapc1.bioch.dundee.ac.uk/prodrg/). Ligands were assigned an occupancy of 0.5 to account for their position on the incident 2-fold symmetry axis (z- or C2). Further model building and refinement was completed using Coot[5] and Refmac5[4]. Hydrogens were added during refinement, and anisotropic *B*-values were calculated. Final

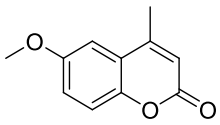
models were validated using the JCSG quality control server incorporating Molprobity[6], ADIT (http://rcsb-deposit.rutgers.edu/validate) WHATIF[7], Resolve[8], and Procheck[9]. Data collection and refinement statistics are displayed in Table S2.

Scheme S1. Synthesis of coumarin derivatives **1**-**10**

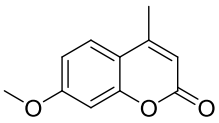


a) $Pd_2(dba)_3$, t-Bu Xphos, Et_3N, 70 °C; b) conc. HCl, MeOH:THF = 1:1; c) $PdCl_2(PPh_3)_2$, CuI, PPh_3, Et_3N, 1,4-dioxane; d) 1M BBr_3 in DCM, DCM

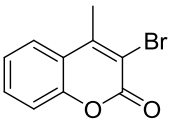
General synthetic method Unless otherwise indicated, all reactions were run under nitrogen gas. The progress of the reactions was monitored by TLC. All products were determined by ^{1}H, ^{13}C NMR and LC/MS. ^{1}H and ^{13}C spectra were recorded on a JEOL 400MHz spectrometer (JNM-AL400, ^{1}H=400MHz, ^{13}C=100MHz.). Chemical shifts are reported relative to internal $CDCl_3$ (Me_4Si, δ 0.0) and DMSO-d_6 (δ 2.50 for ^{1}H and 39.52 for ^{13}C). All mass spectrometry data were collected by Waters Fraction Lynx MS Autopurification System at the Korea Research Institute of Chemical Technology (KRICT). Final compound purities were determined by analytical reverse phase high performance liquid chromatography (RP-HPLC) performed on a Waters Alience 2695 separation module, using a Waters 2487 dual λ absorbance detector, equipped Alience 2695 autosampler and a Thermo Hypersil Keystone Betabasic-18 column (150 Å pore size, 3 μm particle size, mobile phase A= 0.1 % TFA in 94.9 % H_2O + 5 % MeCN, mobile phase B = 0.1 % TFA in 94.9 % MeCN + 5 % H_2O). Linear gradients were run from 100:1 to 0:100 A:B over 25 min.



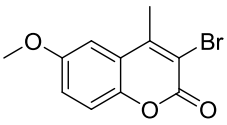
Representative Procedure for Methylation of Hydroxy Group (11). To a solution of 6-hydroxy-4-methyl-2H-chromen-2-one (1 g, 5.62 mmol), potassium carbonate (1.56 g, 11.24 mmol) in MeCN (13 mL) was added iodomethane (714 μL, 11.24 mmol) at 0 ^{o}C. The solution was refluxed for 6 h. The solution was evaporated then dissolved with EtOAc. The organic layer was washed with water and saturated brine. The solution was dried over $MgSO_4$ and concentrated. The residue was obtained and used without further purification in the next reaction to give **11** (0.9 g, 85 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (s, 3H), 3.87 (s, 3H), 6.31 (s, 1H), 7.02 (d, *J* = 2.80 Hz, 1H), 7.11 (dd, *J* = 9.02, 2.93 Hz, 1H), 7.28 (d, *J* = 5.24 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.61, 55.80, 107.66, 115.52, 118.01, 118.69, 120.46, 147.96, 152.03, 156.03, 161.05.



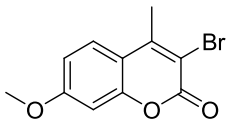
7-methoxy-4-methyl-2H-chromen-2-one (12). Compound **12** was prepared according to the representative procedure for methylation of hydroxyl group from 7-hydroxy-4-methyl-2H-chromen-2-one. The solution was refluxed for 16 h. The residue was obtained without further purification for the next reaction to give **12** (5.2 g, 96 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.40 (s, 3H), 3.88 (s, 3H), 6.14 (s, 1H), 6.82 (d, *J* = 2.44 Hz, 1H), 6.87 (dd, *J* = 8.72, 2.50 Hz, 1H), 7.50 (d, *J* = 8.78 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.58, 55.69, 100.86, 111.98, 112.31, 113.61, 125.56, 152.63, 155.39, 161.38, 162.74.



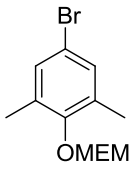
Representative Procedure for Bromination (13). To a solution of 4-methyl-2H-chromen-2-one (1 g, 6.24 mmol) in MeCN (25 mL) was added the solution of N-bromosuccinimide (1.46 g, 8.11mmol) in MeCN (15 mL) and then ammonium acetate (506 mg, 0.62mmol) at 0 ^{o}C. The solution was stirred for 48 h at r.t. The residue was subjected to column chromatography over silica gel (Hexane/EtOAc = 7:1) to give **13** (1.01 g, 68 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.65 (s, 3H), 7.32 - 7.39 (m, 2H), 7.55 - 7.61 (m, 1H), 7.68 (dd, *J* = 7.99, 1.04 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.41, 113.27, 117.09, 119.77, 124.86, 125.01, 132.00, 151.02, 151.93, 157.01.



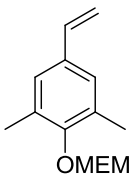
3-bromo-6-methoxy-4-methyl-2H-chromen-2-one (14). Compound **14** was prepared according to the representative procedure for bromination from compound **11**. The residue was subjected to column chromatography over silica gel (Hexane/EtOAc = 5:1) to give **14** (437 mg, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.62 (s, 3H), 3.88 (s, 3H), 7.07 (d, *J* = 2.80 Hz, 1H), 7.14 (dd, *J* = 9.02, 2.93 Hz, 1H), 7.29 (d, *J* = 9.02 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.56, 55.85, 108.08, 113.89, 117.98, 118.89, 120.23, 146.30, 150.61, 156.43, 157.11.



3-bromo-7-methoxy-4-methyl-2H-chromen-2-one (15). Compound **15** was prepared according to the representative procedure for bromination from compound **12**. The residue was recrystallized from hot EtOH to give **15** (0.98 g, 69 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.60 (s, 3H), 3.89 (s, 3H), 6.83 (d, *J* = 2.44 Hz, 1H), 6.90 (dd, *J* = 8.90, 2.56 Hz, 1H), 7.56 (d, *J* = 8.78 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.41, 55.80, 100.70, 109.72, 113.03, 113.43, 126.08, 151.21, 153.70, 157.45, 162.84.

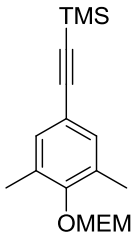


5-bromo-2-((2-methoxyethoxy)methoxy)-1,3-dimethylbenzene (16). To a solution of 4-bromo-2, 6-dimethylphenol (1.5 g, 7.4 mmol) and MEM-Cl (methoxyethoxymethyl chloride) (986 mg, 8.2 mmol) in THF (55 mL), sodium hydride (488 mg, 11.2 mmol) was slowly added in portion at 0 ^{o}C. The solution was allowed to reach room temperature, and stirred for 12 h. The solution was quenched with water. The solvent was evaporated and residue was dissolved with EtOAc. The organic layer was washed with water and saturated brine. The solution was dried over $MgSO_4$ and concentrated. The residue was subjected to column chromatography over silica gel (Hexane/EtOAc = 15:1) to give **16** (714 mg, 94 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.25 (s, 6H), 3.39 (s, 3H), 3.58 - 3.61 (m, 2H), 3.91 - 3.95 (m, 2H), 5.02 (s, 2H), 7.14 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.65, 59.06, 69.18, 71.75, 98.01, 116.75, 131.46, 133.31, 153.94.

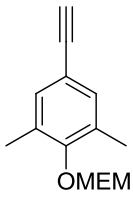


2-((2-methoxyethoxy)methoxy)-1,3-dimethyl-5-vinylbenzene (17). To a solution of compound **16** (700 mg, 2.4 mmol), bis(triphenylphosphine)-palladium(II) dichloride (169 mg,

0.5 mmol) in degassed 1, 4-dioxane (9 mL), tributyl(vinyl) tin (1.1 mL, 3.4 mmol) was added. The solution was stirred and refluxed for 10 h. The mixture was filtered through Celite pad. The filtrate was diluted with EtOAc. The organic layer was washed with 10% KI and saturated brine. The solution was dried over $MgSO_4$ and concentrated. The residue was subjected to column chromatography over silica gel (Hexane/EtOAc = 15:1) to give **17** (506 mg, 88 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.28 (s, 6H), 3.40 (s, 3H), 3.57 - 3.64 (m, 2H), 3.92 - 3.97 (m, 2H), 5.05 (s, 2H), 5.15 (dd, *J* = 10.85, 0.98 Hz, 1H), 5.63 (dd, *J* = 17.56, 0.98 Hz, 1H), 6.61 (dd, *J* = 17.56, 10.85 Hz, 1H), 7.07 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.83, 59.07, 69.09, 71.78, 97.93, 112.80, 126.74, 131.06, 133.53, 136.38, 154.50.

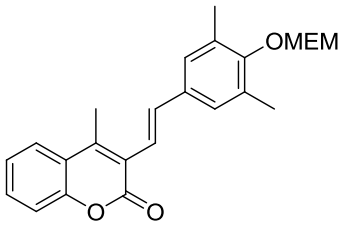


2((4-((2-methoxyethoxy)methoxy)-3,5-dimethylphenyl)ethynyl)trimethylsilane (18). To a solution of compound **16** (715 mg, 2.5 mmol), bis(triphenylphosphine)-palladium(II) dichloride (191 mg, 0.3 mmol), copper iodide (114 mg, 0.7 mmol) in Et_3N (4 mL), trimethylsilylacetylene (0.7 mL, 4.9 mmol) was added and stirred at 80 ^{o}C for 12 h. The mixture was filtered over Celite. The filtrate was diluted with EtOAc. The solution was washed with water, 5 % citric acid and saturated brine. The solution was dried over $MgSO_4$ and concentrated. The residue was subjected to column chromatography over silica gel (Hexane/EtOAc = 100:1 to 35:1) to give **18** (714 mg, 94 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.23 (s, 9H), 2.24 (s, 6H), 3.40 (s, 3H) , 3.56 - 3.62 (m, 2H), 3.90 - 3.94 (m, 2H), 5.04 (s, 2H), 7.15 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -0.09, 16.61, 59.06, 69.17, 71.76, 92.94, 97.98, 105.07, 118.75, 131.18, 132.58, 155.27.



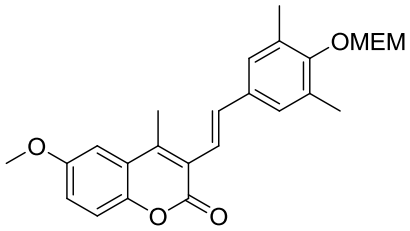
5-ethynyl-2-((2-methoxyethoxy)methoxy)-1,3-dimethylbenzene (19). To a solution of compound **18** (1 g, 3.23 mmol) in MeOH (4.6 mL), potassium carbonate (449 mg, 3.23 mmol) was added at 0 ^{o}C. The mixture was allowed to reach room temperature and stirred for 13 h. The solution was diluted with EtOAc and washed with water and saturated brine. The solution was dried over $MgSO_4$ and concentrated. The residue was subjected to column chromatography over silica gel (Hexane/EtOAc = 9:1) to give **19** (765 mg, 100 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.26 (s, 6H), 2.98 (s, 1H), 3.40 (s, 3H), 3.58 - 3.62 (m, 2H), 3.91 - 3.95 (m, 2H), 5.05 (s, 2H), 7.17 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.64, 59.06, 69.17, 71.75, 76.08, 83.54, 97.97, 117.69, 131.33, 132.72, 155.48.

General Procedure for Heck Reaction. To a solution of substituted coumarin (1 equiv.), vinylbenzene **17** (1.2 equiv.), tris(dibenzylideneacetone)dipalladium(0) (0.2 equiv.), 2-di-tert-butylphosphino-2',4',6'-triisopropylbiphenyl (0.257 equiv.) in Et_3N were stirred under N_2 at 70 ^{o}C for 10 h. The mixture was filtered over Celite. The filtrate was diluted with EtOAc and washed with 5 % citric acid, saturated NH_4Cl and brine. The solution was dried over $MgSO_4$ and concentrated. The compound was purified by column chromatography (Hexanes/EtOAc) or RP-HPLC. All compounds were characterized by ^{1}H, ^{13}C-NMR, and mass spectrometry.

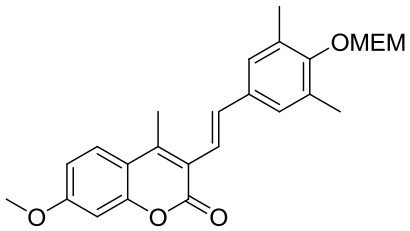


***(E)*-3-(4-((2-methoxyethoxy)methoxy)-3,5-dimethylstyryl)-4-methyl-2H-chromen-2-one (20).** Compound **20** was prepared according to the general procedure for Heck reaction from **13** and **17**. The residue was subjected to column chromatography over silica gel (Hexane/EtOAc =

3:1) to give **20** (159 mg, 96 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.34 (s, 6H), 2.60 (s, 3H), 3.41 (s, 3H), 3.59 - 3.65 (m, 2H), 3.92 - 3.98 (m, 2H), 5.08 (s, 2H), 7.06 (d, *J* = 16.22 Hz, 1H), 7.22 (s, 2H), 7.28 - 7.35 (m, 2H), 7.46 - 7.53 (m, 2H), 7.67 - 7.72 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.39, 16.89, 59.08, 69.13, 71.77, 97.97, 116.71, 119.88, 120.89, 122.51, 124.24, 124.89, 127.44, 130.87, 131.31, 133.32, 136.38, 145.50, 151.96, 155.05, 160.14; LC/MS (ES+) m/z 395.16 $[M+H]^+$.



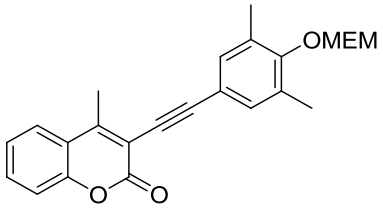
***(E)*-6-methoxy-3-(4-((2-methoxyethoxy)methoxy)-3,5-dimethylstyryl)-4-methyl-2H-chromen-2-one (21).** Compound **21** was prepared according to the general procedure for Heck reaction from **14** and **17**. The residue was subjected to column chromatography over silica gel (Hexane/EtOAc = 2:1) to give **21** (116 mg, 74 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.32 (s, 6H), 2.58 (s, 3H), 3.42 (s, 3H), 3.60 - 3.66 (m, 2H), 3.88 (s, 3H), 3.94 - 4.01 (m, 2H), 5.08 (s, 2H), 7.03 - 7.13 (m, 3H), 7.22 (s, 2H), 7.27 (d, *J* =8.78 Hz, 2H), 7.50 (d, *J* = 16.10 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.51, 16.88, 55.79, 59.08, 69.13, 71.77, 97.97, 108.05, 117.56, 117.82, 120.00, 121.38, 122.81, 127.45, 131.31, 133.32, 136.46, 145.13, 146.41, 155.05, 156.03, 160.27; LC/MS (ES+) m/z 425.09 $[M+H]^+$.



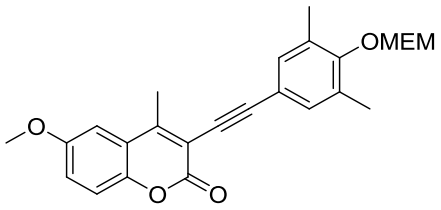
***(E)*-7-methoxy-3-(4-((2-methoxyethoxy)methoxy)-3,5-dimethylstyryl)-4-methyl-2H-chromen-2-one (22).** Compound **22** was prepared according to the general procedure for Heck

reaction from **15** and **17**. The residue was subjected to column chromatography over silica gel (Hexane/EtOAc = 2:1) to give **22** (92 mg, 58 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.25 (s, 6H), 2.57 (s, 3H), 3.26 (s, 3H), 3.48 - 3.53 (m, 2H), 3.81 - 3.89 (m, 2H), 3.86 (s, 3H), 5.02 (s, 2H), 6.95 - 6.99 (m, 2H), 7.11 (d, *J* = 16.22 Hz, 1H), 7.29 (s, 2H), 7.49 (d, *J* = 16.22 Hz, 1H), 7.80 (d, *J* = 9.27 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.18, 16.59, 55.92, 58.16, 68.55, 71.24, 97.51, 100.32, 112.49, 113.91, 117.99, 120.30, 127.08, 127.14, 131.01, 133.15, 134.00, 147.51, 153.22, 154.66, 159.38, 162.08; LC/MS (ES+) m/z 425.03 $[M+H]^+$.

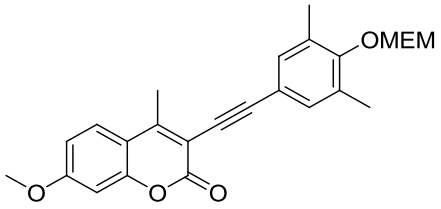
General Procedure for Sonogashira Coupling Reaction. To a solution of substituted coumarin (1 equiv.), ethynylbenzene **19** (2 equiv.), bis(triphenylphosphine)palladium(II) dichloride (0.02 equiv.), copper iodide (0.1 equiv.), triphenyl phosphine (0.2 equiv.) was added Et_3N (1.9 equiv.) in degassed 1,4-dioxane (0.1 M). The solution was stirred under N_2 at 60 ^{o}C for 10 h. The mixture was filtered over Celite. The filtrate was diluted with EtOAc and washed with water and saturated brine. The organic layer was dried over $MgSO_4$ and concentrated. The compound was purified by column chromatography (Hexanes/EtOAc) or RP-HPLC. All compounds were characterized by ^{1}H, ^{13}C-NMR, and mass spectrometry.



3-((4-((2-methoxyethoxy)methoxy)-3,5-dimethylphenyl)ethynyl)-4-methyl-2H-chromen-2-one (23). Compound **23** was prepared according to the general procedure for Sonogashira reaction from **13** and **19**. The residue was subjected to column chromatography over silica gel (Hexane/EtOAc = 3:1) to give a mixture (35 mg) of **23** and ethynylbenzene homocoupling compound. The mixture was used without further purification in the next reaction.

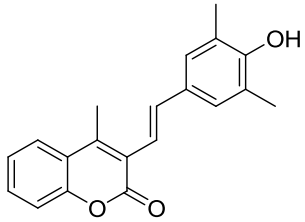


6-methoxy-3-((4-((2-methoxyethoxy)methoxy)-3,5-dimethylphenyl)ethynyl)-4-methyl-2*H*-chromen-2-one (24). Compound **24** was prepared according to the general procedure for Sonogashira reaction from **14** and **19**. The residue was subjected to column chromatography over silica gel (Hexane/EtOAc = 2:1) to give **24** (59 mg, 38 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29 (s, 6H), 2.67 (s, 3H), 3.41 (s, 3H), 3.58 - 3.63 (m, 2H), 3.88 (s, 3H), 3.92 - 3.97 (m, 2H), 5.08 (s, 2H), 7.05 (s, 1H), 7.10 (d, *J* = 9.02 Hz, 1H), 7.27 - 7.29 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.64, 17.34, 55.80, 59.05, 69.17, 71.72, 82.27, 97.98, 99.69, 107.77, 111.84, 117.91, 118.14, 118.93, 120.28, 131.42, 132.44, 146.63, 152.67, 155.71, 156.22, 159.42; LC/MS (ES+) m/z 423.00 $[M+H]^+$.

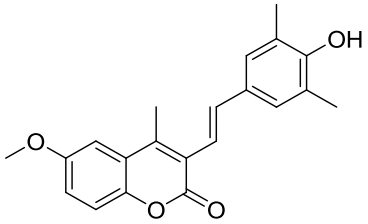


7-methoxy-3-((4-((2-methoxyethoxy)methoxy)-3,5-dimethylphenyl)-ethynyl)-4-methyl-2H-chromen-2-one (25). Compound **25** was prepared according to the general procedure for Sonogashira reaction from **15** and **19**. The residue was subjected to column chromatography over silica gel (Hexane/EtOAc = 2:1) to give **25** (72 mg, 46 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.28 (s, 6H), 2.65 (s, 3H), 3.41 (s, 3H), 3.58 - 3.63 (m, 2H), 3.88 (s, 3H), 3.93 - 3.97 (m, 2H), 5.08 (s, 2H), 6.82 (d, *J* = 2.44 Hz, 1 H), 6.89 (dd, *J* = 8.90, 2.56 Hz, 1H), 7.38 (br, 1H), 7.55 (d, *J*=8.90 Hz, 1H), 7.72 - 7.78 (br, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.66, 17.25, 55.77, 59.08, 69.19, 71.75, 82.24, 98.00, 100.74, 108.32, 112.84, 113.51, 118.43, 126.09, 131.38, 132.34, 153.48, 154.09, 155.50, 159.85, 162.90; LC/MS (ES+) m/z 423.00 $[M+H]^+$.

General Procedure for MEM Deprotection of MEM protected compound. To a solution of MEM protected compound (1 equiv.) in mixed solution of MeOH (methanol, 0.12 M) and THF (0.12 M) was slowly added conc. HCl (35~37 %, 4 equiv.). The solution was stirred for 11 h. The mixture was diluted with EtOAc and washed with water, saturated $NaHCO_3$ and brine. The solution was dried over $MgSO_4$ and concentrated. The compound was purified by column chromatography (Hexanes/EtOAc) or RP-HPLC. All compounds were characterized by ^1H, ^{13}C-NMR, and mass spectrometry.

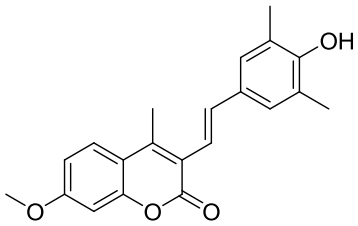


***(E)*-3-(4-hydroxy-3,5-dimethylstyryl)-4-methyl-2H-chromen-2-one (1).** Compound **1** was prepared according to the general procedure for MEM deprotection reaction from compound **20**. The residue was subjected to column chromatography over silica gel (Hexane/EtOAc = 4:1) to give **1** (68 mg, 87 %). ^1H NMR (400 MHz, DMSO-d_6) δ 2.20 (s, 6H), 2.60 (s, 3H), 7.04 (d, J = 16.2 Hz, 1H), 7.22 (s, 2H), 7.35 - 7.41 (m, 2H), 7.48 (d, J = 16.22 Hz, 1H), 7.55 - 7.61 (m, 1H), 7.88 (dd J = 8.29, 1.34 Hz, 1H), 8.51 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.16, 16.59, 116.15, 117.77, 120.56, 121.52, 124.55, 124.57, 125.72, 127.20, 128.26, 131.14, 136.01, 145.84, 151.31, 154.08, 159.20; LC/MS (ES+) m/z 307.22 $[M+H]^+$; HRMS (ES+) m/z calcd for $C_{20}H_{18}O_3$ (M^+) 306.1256, found 306.1263; Purity: 95 %.



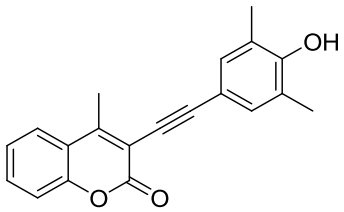
***(E)*-3-(4-hydroxy-3,5-dimethylstyryl)-6-methoxy-4-methyl-2H-chromen-2-one (2).**

Compound **2** was prepared according to the general procedure for MEM deprotection reaction from compound **21**. The residue was used without further purification in the next reaction. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.28 (s, 6H), 2.58 (s, 3H), 3.88 (s, 3H), 4.74 (s, 1H), 7.02 (d, *J* = 16.34 Hz, 1H), 7.07 (q, *J* = 1.00 Hz, 1H), 7.09 - 7.11 (m, 1H), 7.20 (s, 2H), 7.27 (d, *J* = 9.02 Hz, 1H), 7.48 (d, *J*=16.10 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.29, 16.57, 55.75, 108.32, 117.17, 117.89, 118.38, 121.07, 121.72, 124.57, 127.19, 128.29, 136.06, 145.63, 145.67, 154.07, 155.81, 159.29; LC/MS (ES+) m/z 337.21 $[M+H]^+$; Purity: 97 %.

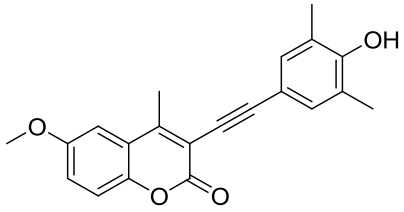


***(E)*-3-(4-hydroxy-3,5-dimethylstyryl)-7-methoxy-4-methyl-2H-chromen-2-one (3).**

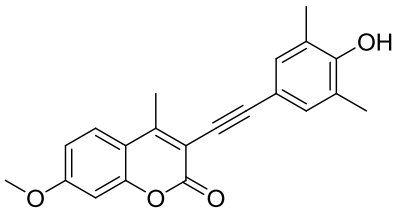
Compound **3** was prepared according to the general procedure for MEM deprotection reaction from compound **22**. The residue was subjected to column chromatography over silica gel (Hexane/EtOAc = 3:1) to give **3** (26 mg, 28 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.19 (s, 6H), 2.56 (s, 3H), 3.86 (s, 3H), 6.93 - 7.03 (m, 3H), 7.19 (s, 2H), 7.44 (d, *J* = 16.22 Hz, 1H), 7.79 (d, *J* = 9.63 Hz, 1H), 8.46 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.10, 16.58, 55.88, 100.31, 112.40, 114.02, 117.89, 118.39, 124.53, 126.90, 126.98, 128.49, 134.83, 146.39, 153.05, 153.83, 159.47, 161.85; LC/MS (ES+) m/z 337.21 $[M+H]^+$; Purity: 98 %.



3-((4-hydroxy-3,5-dimethylphenyl)ethynyl)-4-methyl-2H-chromen-2-one (6). Compound **6** was prepared according to the general procedure for MEM deprotection reaction from compound **23**. The residue was subjected to reverse phase C-18 column chromatography (mobile phase A= 0.1 % TFA in H_2O, mobile phase B = 0.1 % TFA in MeCN). Linear gradients were run from 60 %: 40% to 45%: 55% (A:B) to give **6** (22 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.19 (s, 6H), 2.68 (s, 3H), 7.18 (s, 2H), 7.39 - 7.45 (m, 2H), 7.62-7.66 (m, 1H), 7.83 - 7.88 (m, 1H), 8.86 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.31, 17.22, 81.65, 99.67, 110.46, 112.13, 116.53, 119.46, 124.88, 125.00, 125.94, 131.72, 132.34, 151.65, 153.48, 154.91, 158.44; LC/MS (ES+) m/z 305.27 $[M+H]^+$; HRMS (ES+) m/z calcd for $C_{20}H_{16}O_3$ (M^+) 304.1099, found 304.1105; Purity: 97 %.



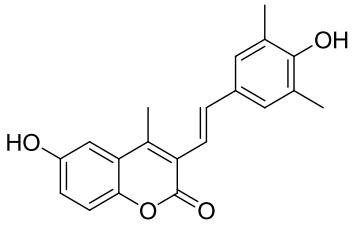
***(E)*-3-(4-hydroxy-3,5-dimethylstyryl)-7-methoxy-4-methyl-2H-chromen-2-one (7).** Compound **7** was prepared according to the general procedure for MEM deprotection reaction from compound **24**. The residue was subjected to column chromatography over silica gel (Hexane/EtOAc = 2:1) to give **7** (107 mg, 99 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.18 (s, 6H), 2.67 (s, 3H), 3.86 (s, 3H), 7.18 (s, 2H), 7.21 - 7.26 (m, 2H), 7.36 (d, J = 8.78 Hz, 1H), 8.86 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.30, 17.35, 55.84, 81.80, 99.83, 108.36, 110.70, 112.16, 117.60, 119.57, 119.95, 124.99, 131.72, 146.01, 153.26, 154.90, 155.98, 158.54; LC/MS (ES+) m/z 335.19 $[M+H]^+$; Purity: 95 %.



3-((4-hydroxy-3,5-dimethylphenyl)ethynyl)-7-methoxy-4-methyl-2H-chromen-2-one (8).

Compound **8** was prepared according to the general procedure for MEM deprotection reaction from compound **25**. The residue was subjected to column chromatography over silica gel (Hexane/EtOAc = 2:1) to give **8** (83 mg, 89 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.17 (s, 6H), 2.63 (s, 3H), 3.87 (s, 3H), 6.98 - 7.03 (m, 2H), 7.15 (s, 2H), 7.74 - 7.78 (m, 1H), 8.79 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.33, 17.22, 56.05, 81.69, 98.61, 100.73, 107.13, 112.42, 112.79, 113.01, 124.94, 127.15, 131.57, 153.56, 153.91, 154.68, 158.93, 162.77; LC/MS (ES+) m/z 335.19 $[M+H]^+$; Purity: 97 %.

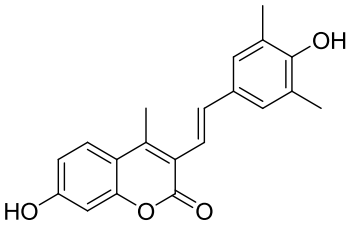
General Procedure for Demethylation. Compound (1 equiv.) in DCM was added 1 M BBr_3 (3 equiv) in DCM at -78 ^{o}C. After 10 min the solution was allowed to reach room temperature. The solution was stirred for 1 h and diluted with EtOAc. The organic layer was washed with 5% citric acid and saturated brine. The solution was dried over $MgSO_4$ and concentrated. The compound was purified by column chromatography (Hexanes/EtOAc) or RP-HPLC. All compounds were characterized by ^{1}H, ^{13}C-NMR, and mass spectrometry.



***(E)*-6-hydroxy-3-(4-hydroxy-3,5-dimethylstyryl)-4-methyl-2H-chromen-2-one (4).**

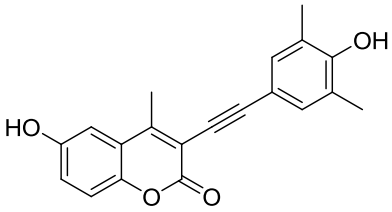
Compound **4** was prepared according to the general procedure for demethylation from compound **2 (**20 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.19 (s, 6H), 2.53 (s, 3H), 6.99 - 7.05 (m, 2H), 7.13 (d, J = 2.68 Hz, 1H), 7.19 - 7.25 (m, 3H), 7.46 (d, J = 16.22 Hz, 1H), 8.50 (s, 1H), 9.70 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.18, 16.54, 109.77, 116.97, 117.93, 119.03, 121.10, 121.51,

124.52, 127.10, 128.24, 135.88, 144.62, 145.45, 153.89, 153.98, 159.41; LC/MS (ES+) m/z 323.26 $[M+H]^+$; Purity: 96 %.



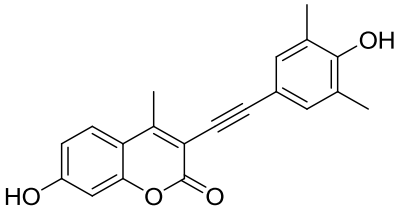
***(E)*-7-hydroxy-3-(4-hydroxy-3,5-dimethylstyryl)-4-methyl-2H-chromen-2-one (5).**

Compound **5** was prepared according to the general procedure for demethylation from compound **3** (36%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.19 (s, 6H), 2.54 (s, 3H), 6.71 (d, J = 1.95 Hz, 1H), 6.82 (dd, J = 8.72, 2.01 Hz, 1H), 6.99 (d, J = 16.22 Hz, 1H), 7.18 (s, 2H), 7.42 (d, J = 16.22 Hz, 1H), 7.71 (d, J = 8.90 Hz, 1H), 8.44 (s, 1H), 10.52 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.11, 16.58, 101.83, 112.94, 113.21, 117.57, 118.03, 124.53, 126.91, 127.12, 128.57, 134.43, 146.70, 153.15, 153.73, 159.60, 160.65; LC/MS (ES+) m/z 323.19 $[M+H]^+$; HRMS (ES+) m/z calcd for $C_{20}H_{18}O_4$ (M^+) 322.1205, found 322.1199; Purity: 96 %.



6-hydroxy-3-((4-hydroxy-3,5-dimethylphenyl)ethynyl)-4-methyl-2H-chromen-2-one (9).

Compound **9** was prepared according to the general procedure for demethylation from compound **7** (70 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (s, 6H), 2.37 (s, 3H), 7.06 (s, 1H), 7.07 - 7.16 (m, 1H), 7.28 (d, J = 8.78 Hz, 1H), 7.31 (s, 2H), 8.75 (s, 1H), 9.80 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.41, 16.59, 110.05, 117.37, 119.76, 120.40, 121.90, 123.46, 124.29, 127.71, 128.97, 130.94, 145.35, 148.46, 154.11, 154.82, 158.94; LC/MS (ES^+) m/z 321.31 $[M+H]^+$; Purity: 96 %.



7-hydroxy-3-((4-hydroxy-3,5-dimethylphenyl)ethynyl)-4-methyl-2H-chromen-2-one (10).

Compound **10** was prepared according to the general procedure for demethylation from compound **8** (39 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.18 (s, 6H), 2.61 (s, 3H), 6.74 (d, J = 2.44 Hz, 1H), 6.85 (dd, J = 8.78, 2.32 Hz, 1H), 7.15 (s, 2H), 7.69 (d, J = 8.78 Hz, 1H), 8.80 (s, 1H), 10.75 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.33, 17.16, 81.81, 98.20, 102.24, 106.20, 111.94, 112.54, 113.58, 124.94, 127.43, 131.53, 153.67, 154.17, 154.60, 159.10, 161.74; LC/MS (ES+) m/z 321.24 $[M+H]^+$; HRMS (ES+) m/z calcd for $C_{20}H_{16}O_4$ (M^+) 320.1049, found 320.1041; Purity: 98 %.

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