Supporting Information

Development of Fluorescent Chalcone Library and its Application in the Discovery of a Mouse Embryonic Stem Cell Probe

Sung-Chan Lee,[1] Nam-Young Kang,[1] Sung-Jin Park,[1] Seong-Wook Yun,[1] Yogeswari Chandran,[1] Young-Tae Chang[1,2]

[1]Laboratory of Bioimaging Probe Development, Singapore Bioimaging Consortium, Agency for Science, Technology and Research (A*STAR), Biopolis, Singapore 138667
[2]Department of Chemistry & Medicinal Chemistry Program of Life Sciences Institute, National University of Singapore, Singapore 117543

List of Contents;

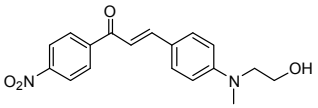
1. Material and Method

All reactions were performed in oven-dried glassware under a positive pressure of nitrogen. Unless otherwise noted, starting materials and solvents were purchased from Aldrich and Acros organics and used without further purification. Analytical TLC was carried out on Merck 60 F254 silica gel plate (0.25 mm layer thickness) and visualization was done with UV light. Column chromatography was performed on Merck 60 silica gel (230-400 mesh). NMR spectra were recorded on a Bruker Avance 300 NMR spectrometer. Chemical shifts are reported as δ in units of parts per million (ppm) and coupling constants are reported as a *J* value in Hertz (Hz). Mass of all the compounds was determined by LC-MS of Agilent Technologies with an electrospray ionization source. Spectroscopic measurements were performed on a fluorometer and UV/VIS instrument, Synergy 4 of bioteck company and Gemini XS fluorescence plate reader. The slit width was 1 nm for both excitation and emission. Relative quantum efficiencies were obtained by comparing the areas under the corrected emission spectrum. The following equation was used to calculate quantum yield

$$\Phi_x = \Phi_{st}(I_x/I_{st})(A_{st}/A_x)(\eta_x^2/\eta_{st}^2)$$

where Φst is the reported quantum yield of the standard, I is the integrated emission spectrum, A is the absorbance at the excitation wavelength, and η is the refractive index of the solvents used. The subscript x denotes unknown and st denotes standard. 5-Dimethylamino-1-naphthalenesulfonamide (DNSA) was used as standard.

General synthetic procedure of chalcone library



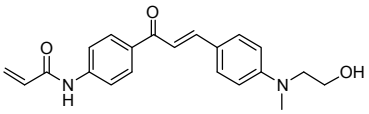
Synthesis of compound 1: To the solution of 4'-Nitroacetophenone (100mg, 0.6 mmole) and *N,N'* 2- 4-((2-hydroxyethyl)(methyl)amino)benzaldehyde (100mg, 0.55mmole) in EtOH, pyrrolidine 0. 1 mL was added and heat up with commercial Microwave Reactor(150MW) for 3 min. Resulted solution was cooled down and kept at the room temperature for 2 hrs to precipitate dark red solid. Solid was filtered and washed with EthylAcetate and Hexane (1:1) solution and dried as a dark red solid (100mg, 60%). Obtained solid was used for further reaction without any purification. ^{1}H-NMR (DMSO-d_6) δ 8.34 (m, 4H), 7.71 (m, 4H), 6.75 (d, J = 9, 2H), 4.75 (bt, 1H), 3.57 (bt, 2H), 3.49 (bt, 2H), 3.03 (s, 3H); ^{13}C-NMR ($CDCl_3$) δ 187.4, 151.6, 149.4, 146.9, 143.5, 131.4, 129.5, 128.3, 125.4, 123.7, 121.3, 115.3, 111.5, 58.2, 53.9, ESI-MS *m/z* (M+H) calc'd: 323.1, found 323.0.

Synthesis of compound 2: 2-ChloroTrityl Resin (200mg, 1 mmole/g) was pre-swelled in DCM. To the Resin solution compound 1 (100 mg) and 3 eq. of Pyridine were added and kept for overnight. The reaction mixture was filtered and washed with DMF, MeOH, DCM after 1hr MeOH capping procedure for extra resin chloride deactivation. Washed resin was dried with high vacuum dessicator and used for further reaction.

Synthesis of compound 5: Resin loaded compound 2 was treated with $SnCl_2$ hydrate 20 eq. in DMF solution. Reaction mixture was shaken overnight and washed with DMF, MeOH, DCM. After high vacuum drying, the resin was divided by 60 mg each in the 5 mL syringe. The reaction syringes were washed with DCM and pyridine 3 eq in DCM 1 mL was loaded in syringe and acidchlorides in 2mL loading was followed. After 30 min shaking, reaction syringes were washed out with DCM, MeOH, DCM each.

Cleavage protocol: 2 % TFA in DCM solution was loaded for cleavage. For 30 min, incubated with cleavage solution, aliquot was squeezed out and collected in 20 mL vial. 2.5% ammonia water in ACN was added for TFA neutralization and filter with silica end filled Tip. Dark brown solid was obtained after removing solvent and purity was analyzed by LC/MS instrument.LC/MS gradient condition was 5 % ACN to 100 % ACN in water with 4.3 x 50 mm C18 column. All HPLC solvent contains 0.1% formic acid for the LC/MS analysis.

Characteristics of CDg4 (CLA 7)



CLA 7 was synthesized according to the general synthetic procedure. ^{1}H-NMR (DMSO-d_6) δ 10.47 (s, 1H, NH), 8.12 (d, J = 8.7, 2H), 7.84 (d, J = 8.7, 2H,), 7.67 (d, J = 9.0, 2H), 7.63 (d, J = 2.1, 2H), 6.74 (d, J = 8.7, 2H), 6.48 (dd, J = 17.1, 9.9, 1H), 6.30 (dd, J = 16.8, 1.8, 1H), 5.82 (dd, J = 9.9, 1.8, 1H), 4.74 (s, 1H), 3.58 (t, J = 5.7, 2H), 3.48 (t, J = 5.7, 2H), 3.39 (s, 1H, OH), 3.01 (s, 3H); ^{13}C-NMR ($CDCl_3$) δ 187.09, 163.52, 151.08, 144.59, 142.85, 133.37, 131.60, 130.74, 129.46, 127.70, 121.74, 118.71, 115.70, 111.48, 58.16, 53.91, HRMS *m/z* (M+H) calc'd: 351.1703, found 351.1714.

Cell culture

mESC and MEF were kindly provided by Sai Kiang Lim (Institute of Medical Biology, Singapore). mESC was cultured in a culture dish coated with 0.1% gelatin using a high-glucose Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.1 mM non-essential amino acids, 0.1% ß–mercaptoethanol and 100 U/ml leukemia inhibitory factor (LIF, Chemicon). MEF was maintained in the same media as used for mESC without LIF and treated with mitomycin C (10 µg/ml) before used as feeder cell.

iPSC generation

MEF prepared from B6; CBATg(Pou5f1-EGFP)2Mnn/J mouse (Jackson Laboratory) were infected by pMX-Oct4, Sox2, Klf4 and c-Myc expressing retrovirus with 10 µg/ml polybrene (Sigma) in normal MEF culture medium for 1 day and the medium was replaced with iPS cell culture medium the next day (1 dpi). At 2 dpi, cells were harvested to seed 30,000 cells/well feeder cells at 10,000 cells/well of density in 12-well plates. The medium was replaced every day. At 15 dpi, Oct4-GFP positive colonies appeared and the fluorescence images taken by FITC filter (K. Takahashi, S. Yamanaka, *Cell* 2006, **126**, 663).

Neurosphere preparation and culture

E14.5 fetal mouse brains were trypsinized in 0.25% trypsin with 1mM EDTA solution (Invitrogen) for 30 mins at 37°C before neutralization with FBS. The tissues were triturated sequentially with a 10ml pipette followed by a 1ml blue tip and a 0.2 ml yellow tip attached to the 10ml pipette until the cell suspension flows through smoothly. The tissue suspension was washed 3 times with PBS by repeated resuspension and centrifugation and filtered through a 40 µm strainer. The obtained single cells were plated in a DMEM/F12 medium containing 10 ng/ml bFGF, 20 ng/ml EGF and B27 without vitamin A (Invitrogen) to grow forming spheres. All animal experiment procedures were performed in accordance with a protocol approved by the Institutional Animal Care and Use Committee.

Flow cytometry

MEFs and mESCs were cultured in 6 well plates for 24 h. Next day, 2µM of hit compounds including CDg4 was treated for 1hr. The cells were harvested by trypsin treatment, washed with PBS and resuspended in PBS. The fluorescence intensity of the cells was measured on Flow Cytometry (BDTM LSR II) with FITC filter (excitation at 488 nm, emission at 520 nm). The dot plot images for each cell type were overlaid using FlowJo7 (Three Star Inc.)

Glycogen quantification assay

Both mESC and mNSP were cultured as described above. Each 5 x 10^6 cells was homogenized in 1mL of dH_2O on ice. The homogenates was boiled for 5min to inactivate enzymes. The boiled samples were spanned at 13000 rpm for 5 min to remove insoluble material; the supernatant is ready for assay. It was added up to 50 µl of sample or buffer (blank) to test wells. The volume was adjusted to 50 µl with Hydrolysis Buffer. For unknown samples, we suggest testing several doses of your sample to ensure the readings are within the standard curve. Hydrolysis Enzyme Mix was added to Standard and samples and mixed well. It was incubated for 30 minutes at room temperature. To remove glucose background sample, it may do a glucose control without the addition of hydrolysis enzyme to determine the level of glucose background in your sample. The glucose background can then be subtracted from glycogen readings. Samples were incubated at room temperature for 30 minutes and protected from light. Color was measured as OD at 570 nm. Amount of glycogen is calculated by Glycogen assay kit (BioVision) as described

mESC differentiation and Immunohistochemistry

mES cells were stained with 2 µM of **CDg4** for 1 hr and were allowed to form embryoid bodies (EB) in the media without LIF. After 3 days, EB cells were transferred to 6well plate and allowed to grow in monolayer. Differentiated mESC were treated 2 µM of **CDg4** for 1 hr. After taking images, the cells were fixed with 4% paraformaldehyde for 10 minutes, washed with PBS and blocked with 2% bovine serum albumin for 30 minutes before incubation with a mouse monoclonal antibody against SSEA-1 (diluted 1:200, Millipore), smooth muscle actin (SMA) (1:250, Abcam), nestin (1:250, Milipore) or goat polyclonal antibody against SOX-17 (1:250, R & D System) overnight at 4°C. The antibody was visualized by Alexa Flour® 594-conjugated goat anti-mouse IgM (1:300, Invitrogen), Cy5-conjugated goat anti-mouse (1:300, Invitrogen), or Alexa Folur® 594-conjugated donkey anti-goat secondary antibody (1:300, Invitrogen).

Cytotoxicity test

To examine the effect of **CDg4** on cell growth, mESC cells were stained with 2 μM of **CDg4** from 1 hr to 24 hrs. The images were taken by 7X7 sites of images per well randomly using an ImageXpress. Total number of colonies was counted and compared with non-treated control. After 24 hrs, mESC cells were split. The next day cells were re-stained with 2 μM of **CDg4** for 1 hr and images were taken. Subsequently, 2nd splitting was carried out for a same procedure as the 1st splitting.

Triple colour staining of mESC

mESC were seeded on gelatine coated 12 well plates and allowed to grow. Next day, mESC colonies were stained by each of stem cell probes separately for single colour imaging. For **CDy1,** mESC was stained with 100 nM of dye for 1 hr and destained overnight before images were taken. For CDb8 and CDg4, the cells were incubated with 1 μM, 2 μM of the dyes respectively for 1 hr and images were taken. The images were taken using all three colour filters i.e. DAPI, FITC and TRITC to check whether each compound signal crosses over. For double colour imaging, the cells were stained with 100 nM of **CDy1** first then with **CDb8** or **CDg4. CDb8** and **CDg4**, cells were stained first with 1 μM of **CDb8** for 1 hr and washed three times with PBS and then 2 μM of **CDg4** was added for 1 hr staining. In order to perform tripe colour imaging, cells were stained in the order of **CDy1, CDb8** and **CDg4** as described above.

2. Supplemental figures

Figure S1. Spectroscopic property distribution of CLA library; a,b, Absorption and emission of CLA library in DMSO , c. Quantum yield distribution, d. cLogP distribution.

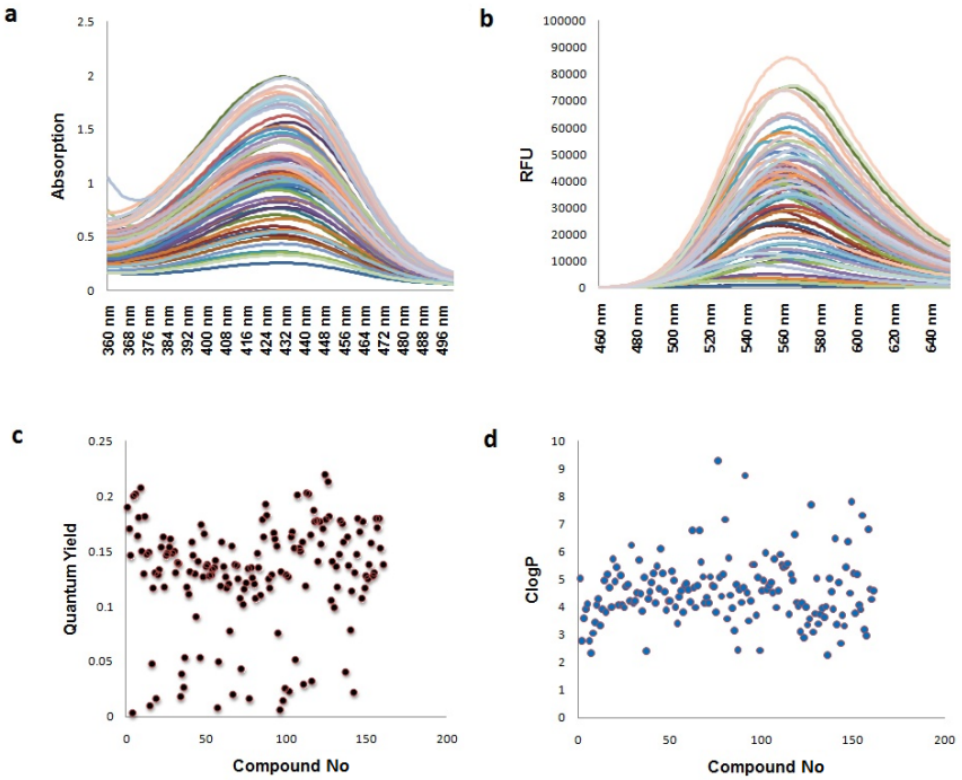


Figure S2. Structure vs quantum yield analysis; Structure and property relationships regarding to quantum yield was classified by two patterns. First, the compounds between 0.1 and 0.2 are not influenced by R group significantly, most of the aliphatic and electron donating substituted aromatic groups belong to this class. Second, interesting structural relationships are observed with the compounds which showed less than 0.1 (average; 0.04) quantum yield. Nitro substituted benzoyl group showed significantly low quantum yield with position dependency. p-Nitro was the most obvious position for the drastic change of quantum yield compared to others. Hetero-aromatic groups have also negative impact on quantum yield such as pyridyl, benzothiazolyl, pyrazine with involvement of electron withdrawing group.

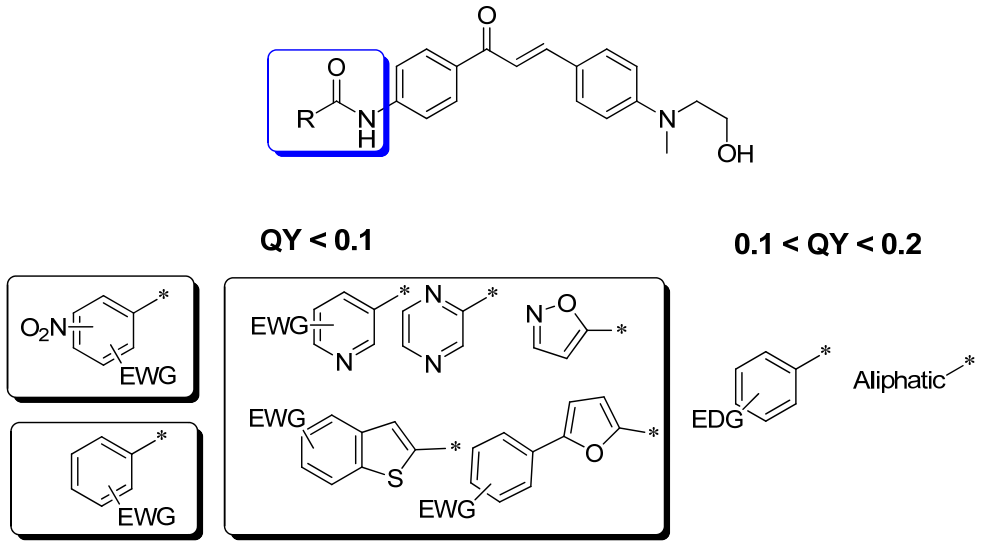


Figure S3. Differentiated mESC staining by CDg4. mESC was differentiated into three germ layer cell types by in vitro differentiated via embryonic body formation. None of cell types were stained by **CDg4**. The expressions of three germ layer markers Nestin (ectoderm), alpha-smooth muscle (SMA, mesoderm) and Sox17 (endoderm) were detected by immunofluorescencent staining. Scale bar: 100 μm

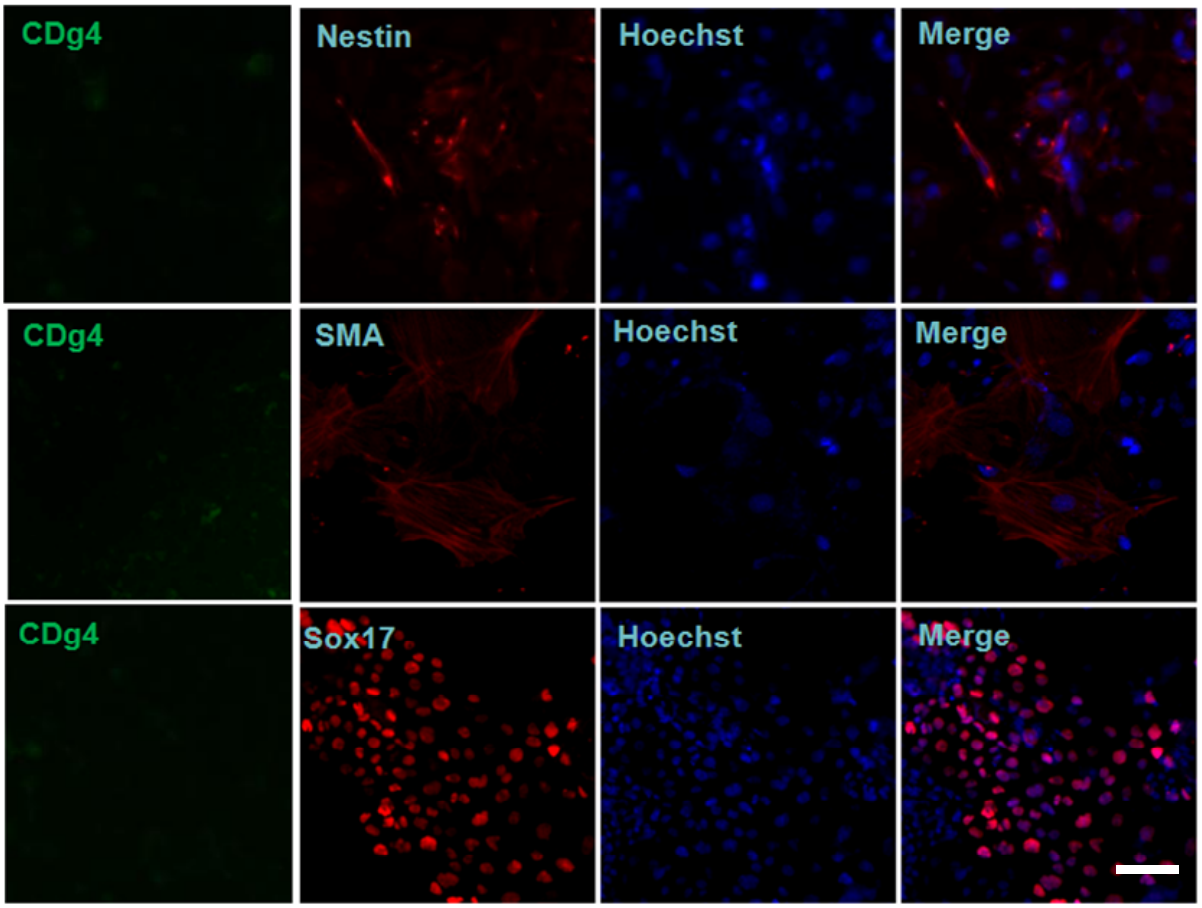


Figure S4. The effect of CDg4 to mESC properties. **a.** mESC staining with 2 μM of **CDg4** for 1 hr **b.** 1day after mESC staining of **CDg4 c.** mESC stained with **CDg4** with 2 μM for 1 day were sub-cultured and incubated overnight. Next day, cells were stained with 2 μM of **CDg4** for 1 hr and before images were taken **d.** 2nd sub culture mESC staining with 2 μM of **CDg4** for 1 hr. The Fluorescent images were taken after staining with **CDg4** without wash. Scale bar: 600 μm

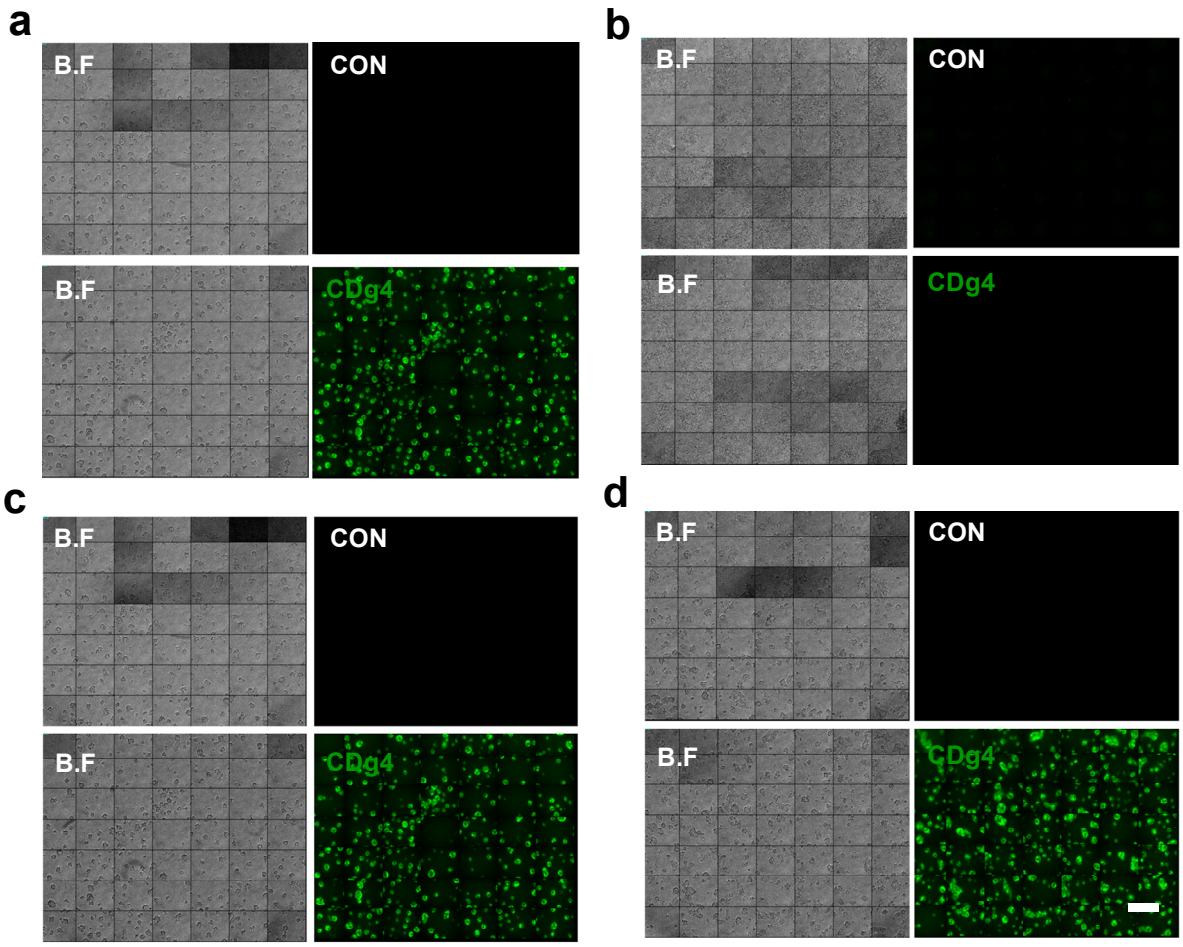
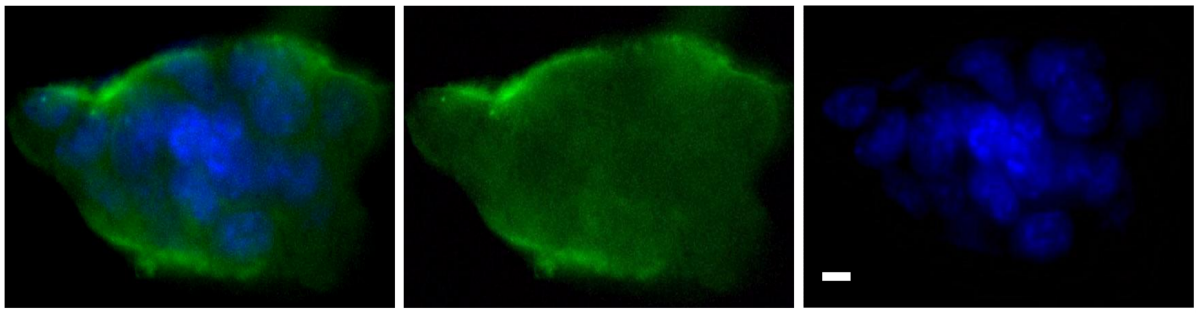


Figure S5. The fluorescent images of mESC colony stained by CDg4; Merged fluorescent images of **CDg4** and Hoechst 33342 (right), **CDg4** fluorescent image (middle) and Nucleus staining image with Hoechst 33342(left) 2 μM of **CDg4** is incubated for 1 hr. Scale bar: 10 μm. Images of stained cell colonies were taken by ×40 objective lenses.



7

Figure S6. Triple color imaging of mESC by stem cell probes: CDy1, CDb8 and CDg4 . a. Single color imaging of mESC. mESC cells were separately stained with **CDb8, CDg4** and **CDy1**. All images were taken using three filters; DAPI for **CDb8**, FITC for **CDg4** and TRITC for **CDy1. b.** Double color imaging of mESC. **CDg4** and **CDy**1(top); **CDy1** and **CDb8**(middle); **CDb8** and **CDg4**(bottom) **c.** Triple color imaging of mESC. mESC cells were stained with stem cell probes together : **CDy1, CDb8** and **CDg4**. Scale bar: 50 μm

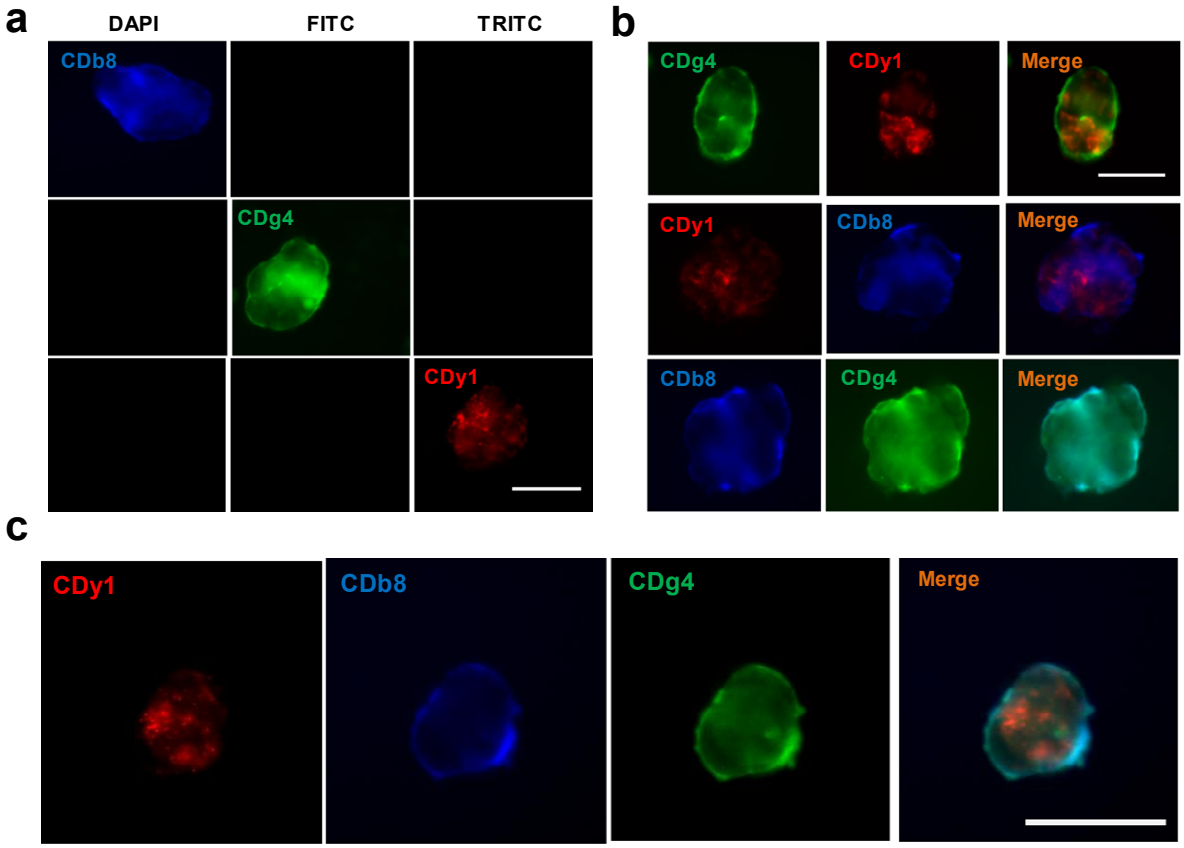


Figure S7. CDg4 staining of 2 different mESC cell lines. The other type of mESC, E14 was stained with 2 μM of **CDg4** for 1 hr. The same staining pattern was observed in both cell lines. Scale bar: 100 μm

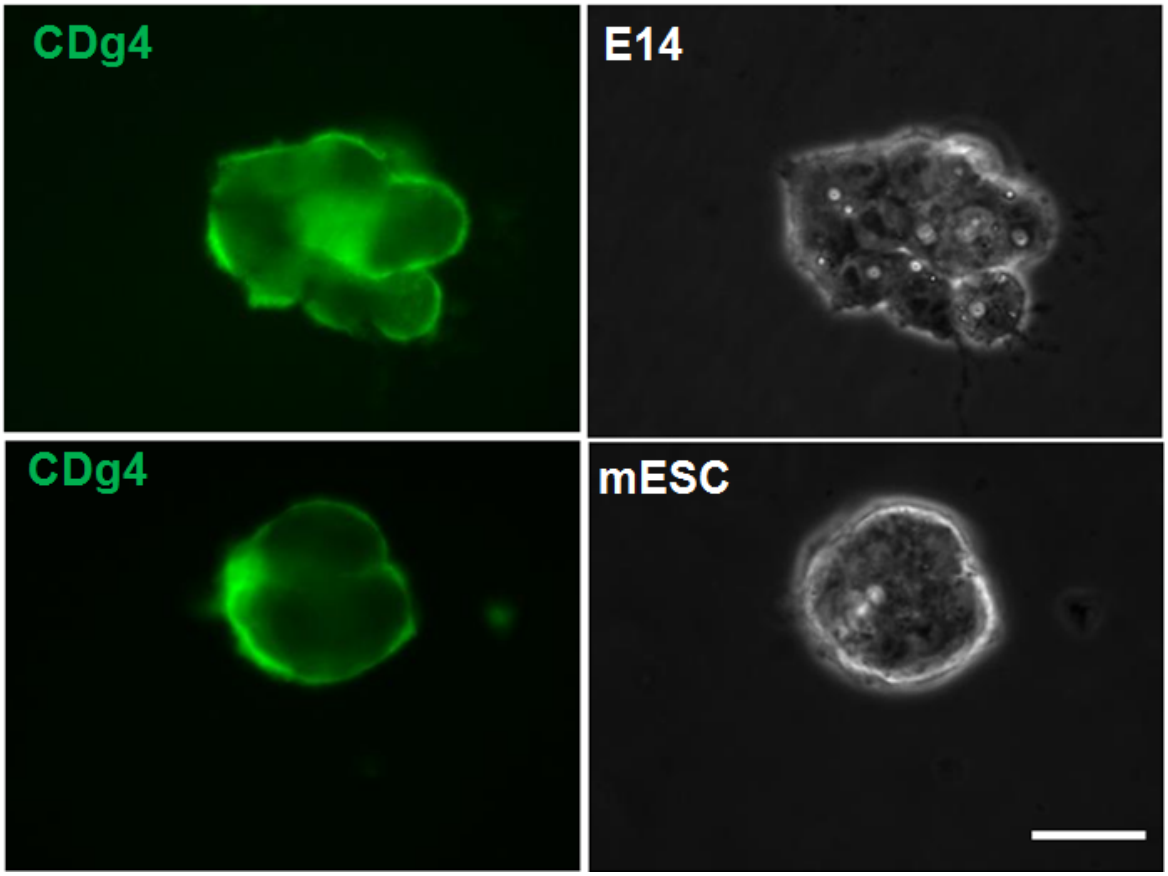


Figure S8. Selectivity of CDg4 for mouse induced pluripotent stem cell (iPSC). CDg4 staining of Oct4-GFP iPS at 15 dpi. The iPSC generated from Oct4-GFP transgenic mouse MEF were identified by GFP signal at 15 days post infection (dpi). Image of Oct4-GFP was taken (Oct4-GFP: FITC channel) and subsequtenly, the cells were stained with 2 μM of **CDg4** for 1 hr and image was taken (**CDg4**: FITC channel). B.F, Bright Field. Scale bar: 160 μm

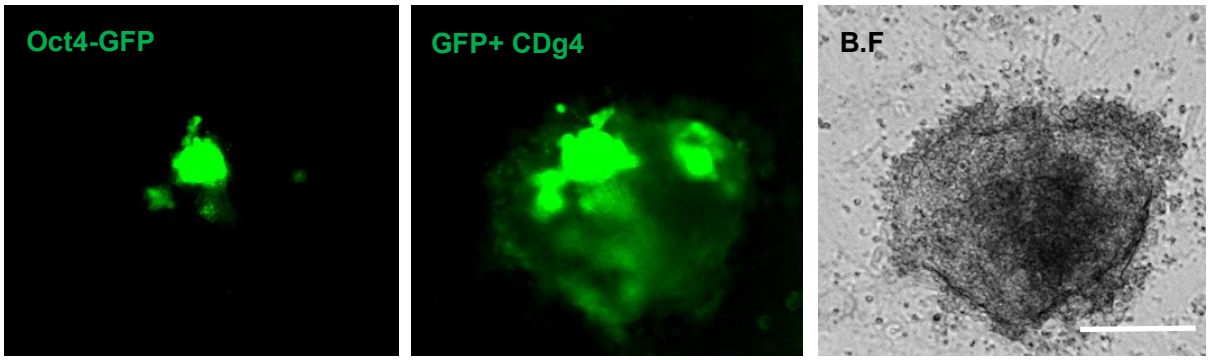
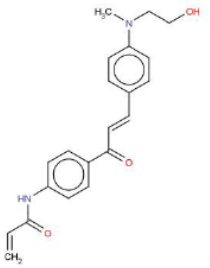


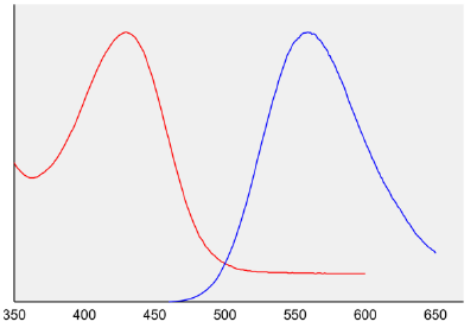
Figure S9. Optical properties and In vitro screening hitmap; Green colour refer to intensity increase, red colour is decrease by log2 unit.82 analytes and pH response were screened.

ChangLab Compound Report



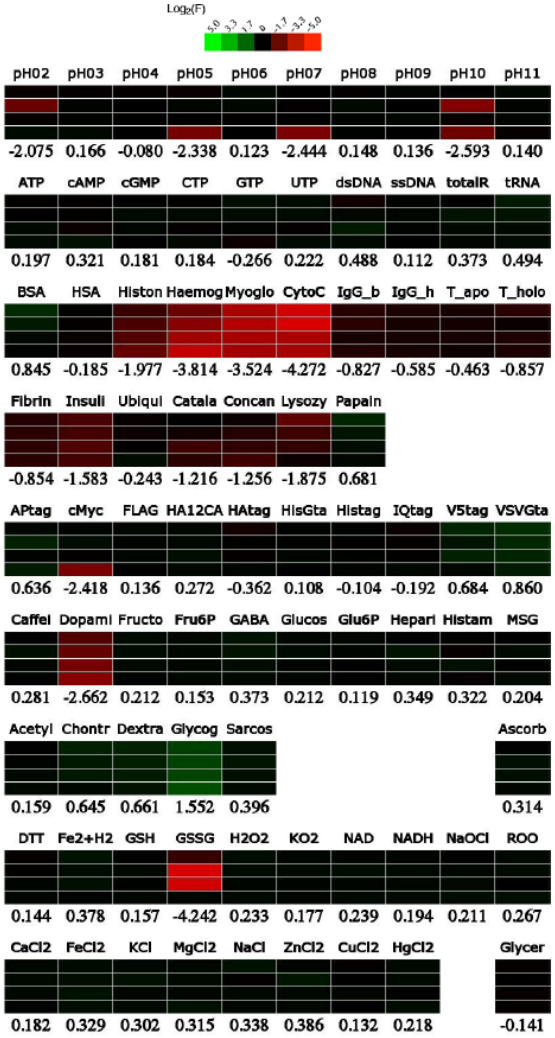
Chemical Information	
Universal Code	CLA7 (**CDg4**)
Plate Code	CLA1-A7
Mol. Weight	350.41
Exact Mass	350.1630
clogP	2.791800261
Permeability	24.3406
BBB	0.381651
tPSA	69.64
IUPAC Name	(*E*)-*N*-(4-(3-(4-((2-hydroxyethyl)(methyl)amino)phenyl)acryloyl)phenyl)acrylamide

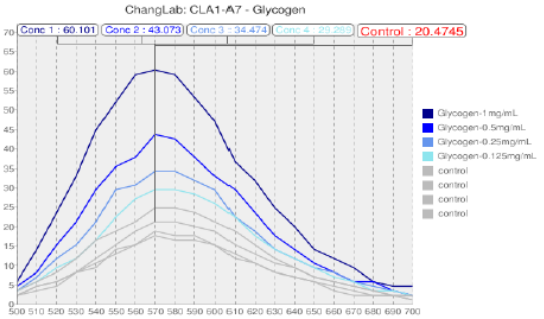
CLA1-A7



Spectral Information	
Abs(nm)	429
Excitation(nm)	430
C(abs)	50uM
EXT_Coeff	23600
Em(nm)	563
Max RFU	25709 (biotek)
C(em)	10uM
Quantum Yield	0.20
Remark	Biotek

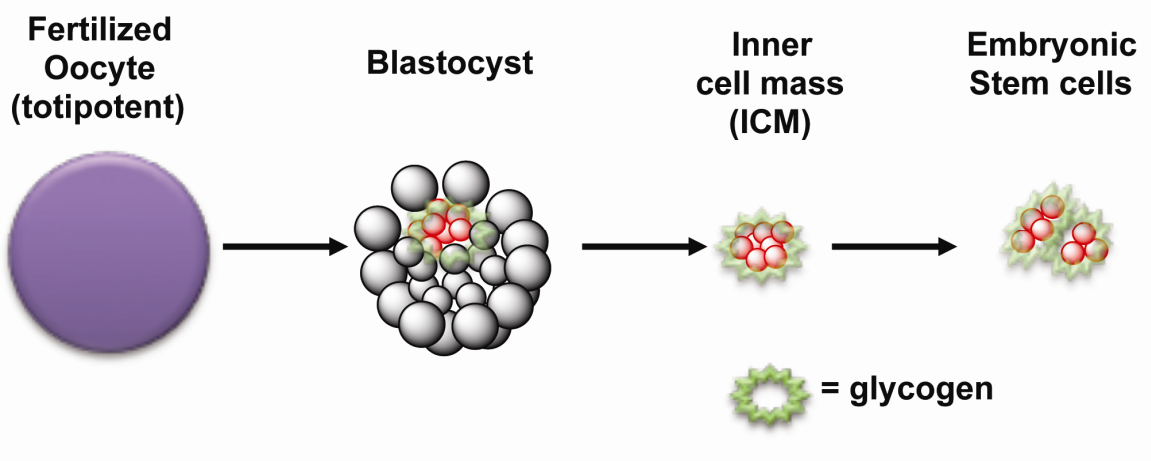
In Vitro Screen Heat Map (log2 scale)





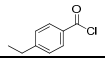
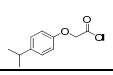
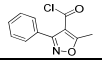
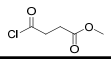
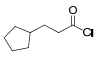
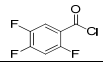
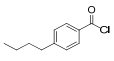
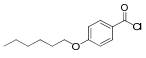
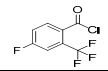
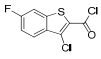
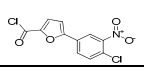
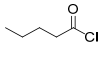
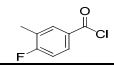
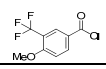
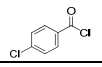
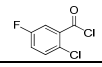
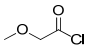
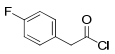
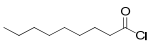
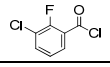
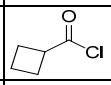
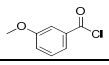
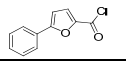
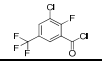
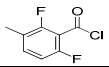
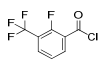
Probe concentration: 2μM, Ex: 435nm, Em: 580nm

Figure S10. Glycogen accumulation and distribution during embryogenesis.



3. Supplemental Tables

Table S1. Building Block for amide synthesis in Scheme 1; Compound code number is corresponding to building number.

ID	Structure	ID	Structure	ID	Structure	ID	Structure
C001		C028		C055		C080	
C002		C029		C057		C081	
C004		C031		C058		C082	
C005		C032		C059		C083	
C006		C033		C061		C084	
C007		C035		C062		C085	
C008		C036		C064		C086	
C010		C037		C065		C087	
C012		C038		C066		C088	
C014		C041		C068		C090	
C015		C042		C069		C091	
C016		C044		C070		C092	
C017		C045		C071		C094	
C018		C046		C072		C095	
C020		C047		C073		C096	
C022		C049		C074		C097	
C023		C050		C075		C098	
C024		C051		C076		C099	
C025		C052		C077		C100	
C026		C053		C078		C102	
C027		C054		C079		C103	

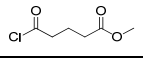
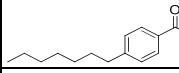
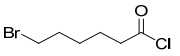
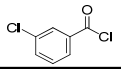
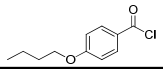
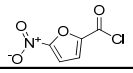
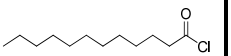
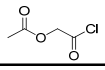
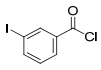
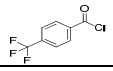
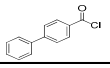
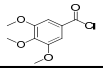
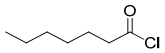
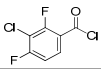
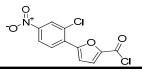
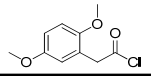
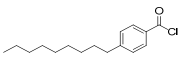
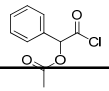
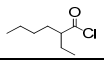
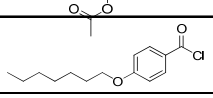
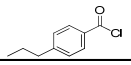
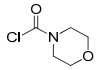
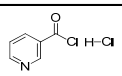
ID	Structure	ID	Structure	ID	Structure	ID	Structure
C104		C129		C158		C183	
C105		C130		C159		C185	
C106		C131		C161		C186	
C107		C132		C164		C188	
C108		C134		C165		C190	
C109		C136		C166		C192	
C110		C137		C167		C193	
C111		C138		C169		C194	
C112		C140		C170		C195	
C114		C142		C171		C197	
C115		C143		C172		C203	
C116		C144		C173		C409	
C117		C145		C174		C410	
C119		C146		C175			
C120		C147		C176			
C121		C148		C177			
C122		C150		C178			
C123		C152		C179			
C124		C153		C180			
C127		C155		C181			
C128		C156		C182			

Table S2. CLA library list with analysis data; extinction coefficient and quantum yield were measure with reference of Dansyl amide.

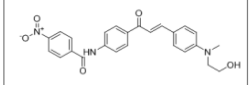
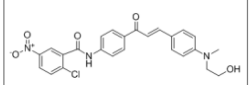
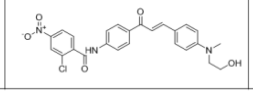
Code	Mass (calc.)	Mass (found)	Max_{Abs} (nm)	Max_{Em} (nm)	Ext. Coefficient	Quantum Yield
CLA-1	429.2	429.0	431	560	126061	0.19
CLA-2	411.2	411.0	428	555	25115	0.17
CLA-4	437.2	437.0	431	565	96339	0.14
CLA-5	446.2	446.0	433	551	75879	0.01
CLA-6	381.2	381.0	427	557	37091	0.20
CLA-7	351.2	351.0	429	563	23564	0.20
CLA-8	369.2	369.0	429	556	27442	0.16
CLA-10	353.2	353.0	427	554	28994	0.18
CLA-12	379.2	379.0	428	556	34036	0.20
CLA-14	451.2	450.9	431	565	37479	0.15
CLA-15	471.1	470.9	433	568	49115	0.13
CLA-16	458.2	458.0	431	593	127564	0.18
CLA-17	437.2	437.0	432	564	46545	0.14
CLA-18	545.1	544.8	431	567	56533	0.14
CLA-20	480.1	479.9	432	544	67152	0.01
CLA-22	487.2	486.9	432	563	40970	0.04
CLA-23	497.1	498.8	433	561	26376	0.11
CLA-24	437.2	436.9	432	564	40970	0.13
CLA-25	461.2	460.9	430	551	12412	0.01
CLA-26	503.1	504.1	431	564	50715	0.12
CLA-27	485.2	485.0	431	567	61430	0.13
CLA-28	431.2	431.0	430	561	54642	0.15
CLA-29	421.2	421.1	428	557	46303	0.16
CLA-31	455.2	454.9	431	562	32388	0.11
CLA-32	487.2	486.9	431	560	56970	0.14
CLA-33	433.2	433.0	430	563	53236	0.14
CLA-35	435.1	434.9	431	562	48485	0.15
CLA-36	433.2	433.0	429	557	39952	0.16
CLA-37	437.3	437.1	427	554	17648	0.14
CLA-38	431.2	431.0	431	562	52461	0.15
CLA-41	467.1	466.9	431	563	56533	0.13
CLA-42	415.2	415.0	430	562	59103	0.14
CLA-44	423.3	423.1	428	556	45382	0.13
CLA-45	466.1	465.9	432	550	58861	0.01
CLA-46	480.1	479.9	431	561	56824	0.03
CLA-47	491.1	490.9	431	555	27200	0.02
CLA-49	392.2	392.0	433	569	47806	0.05
CLA-50	521.1	520.9	431	563	50279	0.11
CLA-51	453.1	452.9	431	563	16970	0.11
CLA-52	465.2	464.9	430	562	59830	0.13
CLA-53	527.1	526.8	431	564	71176	0.15
CLA-54	431.2	431.0	430	559	74036	0.14
CLA-55	473.2	473.0	430	561	73212	0.13
CLA-57	551.1	550.7	432	568	78885	0.09
CLA-58	457.2	457.0	429	561	51103	0.14
CLA-59	509.1	508.9	430	539	42133	0.05
CLA-61	407.2	407.0	429	553	57697	0.17
CLA-62	471.1	470.9	432	564	62061	0.12
CLA-64	419.2	418.9	430	565	21091	0.16
CLA-65	419.2	419.0	431	565	60655	0.13
CLA-66	467.2	466.9	431	567	67006	0.13
CLA-68	453.1	452.9	431	566	69721	0.12
CLA-69	437.2	436.9	431	564	49455	0.12
CLA-70	448.2	447.9	431	565	62061	0.13
CLA-71	429.2	429.0	429	557	51248	0.13
CLA-72	445.2	445.0	429	561	61673	0.14

Code	Mass (calc.)	Mass (found)	Max_{Abs} (nm)	Max_{Em} (nm)	Ext. Coefficient	Quantum Yield
CLA-73	480.1	479.8	432	540	67830	0.01
CLA-74	487.2	486.9	431	560	57261	0.05
CLA-75	487.1	486.8	431	564	26521	0.11
CLA-76	407.2	407.0	429	556	55127	0.15
CLA-77	415.2	415.0	430	562	87273	0.13
CLA-78	451.3	451.0	427	555	61770	0.12
CLA-79	469.1	468.9	431	564	88097	0.11
CLA-80	482.2	482.0	431	561	84024	0.12
CLA-81	487.2	486.9	431	563	85915	0.07
CLA-82	501.3	501.0	429	561	89503	0.15
CLA-83	546.1	545.8	430	545	82667	0.02
CLA-84	499.2	498.9	431	563	88291	0.13
CLA-85	453.1	452.9	429	565	16000	0.13
CLA-86	445.2	445.0	430	562	66861	0.12
CLA-87	453.1	452.9	432	566	87418	0.10
CLA-88	521.1	520.8	433	563	92170	0.04
CLA-90	487.2	486.9	432	565	95952	0.10
CLA-91	433.2	433.0	432	566	92170	0.11
CLA-92	419.2	418.9	431	564	117285	0.12
CLA-94	541.3	541.2	429	563	56145	0.13
CLA-95	512.2	511.9	432	549	160242	0.01
CLA-96	433.2	433.0	431	562	119127	0.13
CLA-97	487.2	486.9	432	565	56679	0.12
CLA-98	485.3	485.0	428	562	115345	0.12
CLA-99	487.2	486.9	431	565	81067	0.10
CLA-100	495.2	495.0	430	563	83879	0.14
CLA-102	451.2	451.0	431	564	78255	0.13
CLA-103	491.1	490.9	432	570	38642	0.11
CLA-104	425.2	425.0	429	559	36945	0.17
CLA-105	435.1	434.9	431	565	52800	0.16
CLA-106	397.2	397.0	427	558	17115	0.19
CLA-107	429.2	429.0	429	562	55661	0.18
CLA-108	551.1	550.8	432	568	45042	0.12
CLA-109	471.1	470.9	432	568	49697	0.11
CLA-110	527.3	527.0	428	560	40679	0.12
CLA-111	415.2	415.0	430	561	53867	0.16
CLA-112	459.2	458.9	430	563	54594	0.16
CLA-114	467.1	466.9	431	563	54691	0.15
CLA-115	469.1	468.9	431	563	55758	0.07
CLA-116	546.1	545.9	432	544	139588	0.00
CLA-117	436.1	435.9	431	565	45091	0.13
CLA-119	512.2	511.9	431	547	60994	0.01
CLA-120	403.2	402.9	431	542	35782	0.02
CLA-121	469.1	468.9	431	565	62061	0.12
CLA-122	479.1	478.9	431	564	59782	0.12
CLA-123	537.2	536.9	432	559	59539	0.02
CLA-124	429.2	429.0	428	556	55564	0.16
CLA-127	449.2	448.9	428	557	33503	0.16
CLA-128	465.2	464.9	430	559	64048	0.15
CLA-129	507.1	506.8	432	564	34085	0.05
CLA-130	473.2	473.0	429	560	55321	0.20
CLA-131	487.2	486.9	431	563	51539	0.15
CLA-132	534.2	534.0	427	553	49309	0.15
CLA-134	477.2	477.0	439	582	129891	0.15
CLA-136	546.1	545.8	429	543	29139	0.02
CLA-137	469.1	469.0	425	563	18764	0.11
CLA-138	423.3	423.0	428	552	43539	0.20
CLA-140	443.2	443.0	428	560	41406	0.20

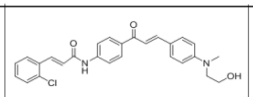
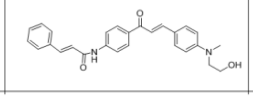
Code	Mass (calc.)	Mass (found)	Max_{Abs} (nm)	Max_{Em} (nm)	Ext. Coefficient	Quantum Yield
CLA-142	453.1	452.9	431	563	57164	0.16
CLA-143	487.2	486.9	431	545	44315	0.03
CLA-144	479.1	478.9	430	560	50473	0.18
CLA-145	471.3	471.0	429	562	44606	0.17
CLA-146	527.1	526.8	429	561	38158	0.17
CLA-147	479.1	478.8	430	562	52945	0.14
CLA-148	365.2	365.0	426	557	25309	0.17
CLA-150	453.1	452.9	431	563	52315	0.15
CLA-152	436.1	437.1	429	563	22109	0.17
CLA-153	393.2	394.2	426	553	26230	0.22
CLA-155	405.2	406.2	426	557	21333	0.17
CLA-156	404.2	404.0	428	556	38691	0.21
CLA-158	499.3	499.0	426	559	36461	0.18
CLA-159	436.1	436.0	429	555	45091	0.10
CLA-161	407.1	406.9	429	563	39321	0.14
CLA-164	469.2	468.9	431	565	58909	0.09
CLA-165	491.2	491.0	430	566	58327	0.13
CLA-166	455.2	454.9	433	565	58036	0.11
CLA-167	435.1	434.9	431	564	54933	0.14
CLA-169	379.2	379.0	428	557	43345	0.17
CLA-170	401.2	401.0	430	563	49261	0.17
CLA-171	410.2	410.0	425	537	70158	0.15
CLA-172	427.2	427.0	430	563	62448	0.04
CLA-173	459.2	459.0	429	558	72339	0.16
CLA-174	421.2	420.9	430	559	52121	0.13
CLA-175	535.1	534.8	430	567	71612	0.07
CLA-176	450.2	449.9	432	568	67539	0.11
CLA-177	509.0	508.7	429	548	22497	0.02
CLA-178	406.2	406.0	431	569	42764	0.13
CLA-179	405.2	405.0	431	565	81600	0.14
CLA-180	365.2	365.0	429	562	47127	0.18
CLA-181	485.2	484.9	432	565	78788	0.16
CLA-182	535.1	534.8	431	564	60364	0.10
CLA-183	473.1	474.9	428	562	57552	0.17
CLA-185	479.3	479.0	414	552	18327	0.11
CLA-186	527.1	526.8	431	569	50861	0.12
CLA-188	381.2	381.0	429	561	81988	0.12
CLA-190	409.2	409.0	428	561	66909	0.13
CLA-192	475.2	475.0	429	560	74036	0.15
CLA-193	473.2	473.0	432	565	52121	0.12
CLA-194	515.3	515.0	425	563	49358	0.13
CLA-195	391.2	391.0	430	566	56921	0.18
CLA-197	402.2	402.0	428	563	41600	0.17
CLA-203	463.3	463.0	423	557	32048	0.18
CLA-409	395.2	395.0	428	559	55467	0.15
CLA-410	405.2	405.0	428	562	56533	0.13

Table S3. Structure classification on quantum yield; collected structures' quantum yields are less than 0.1.

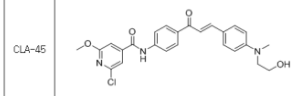
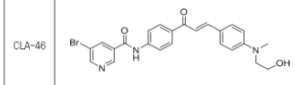
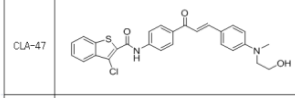
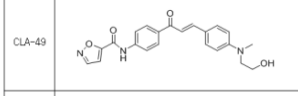
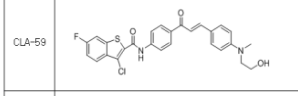
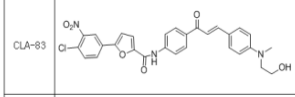
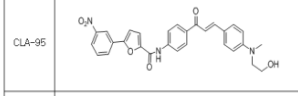
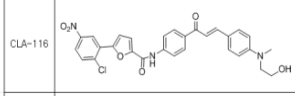
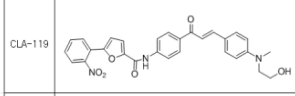
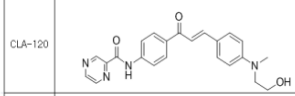
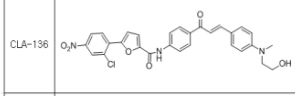
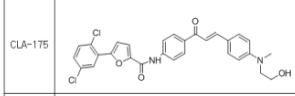
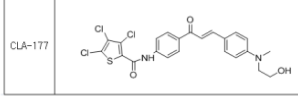
Nitro group

Code	Structure	Quantum Yield
CLA-5		0.003
CLA-20		0.010
CLA-73		0.008

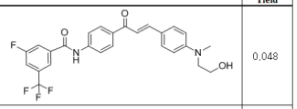
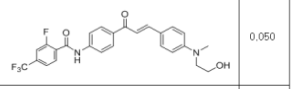
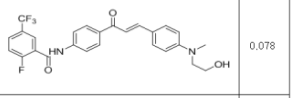
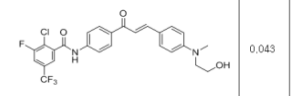
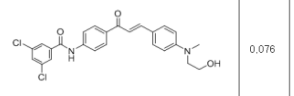
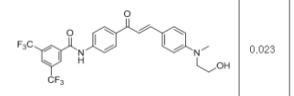
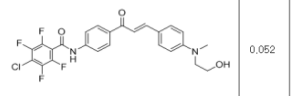
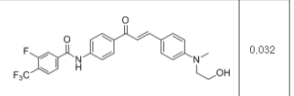
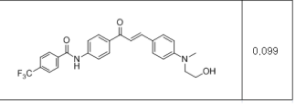
etc

Code	Structure	Quantum Yield
CLA-25		0.016
CLA-172		0.041

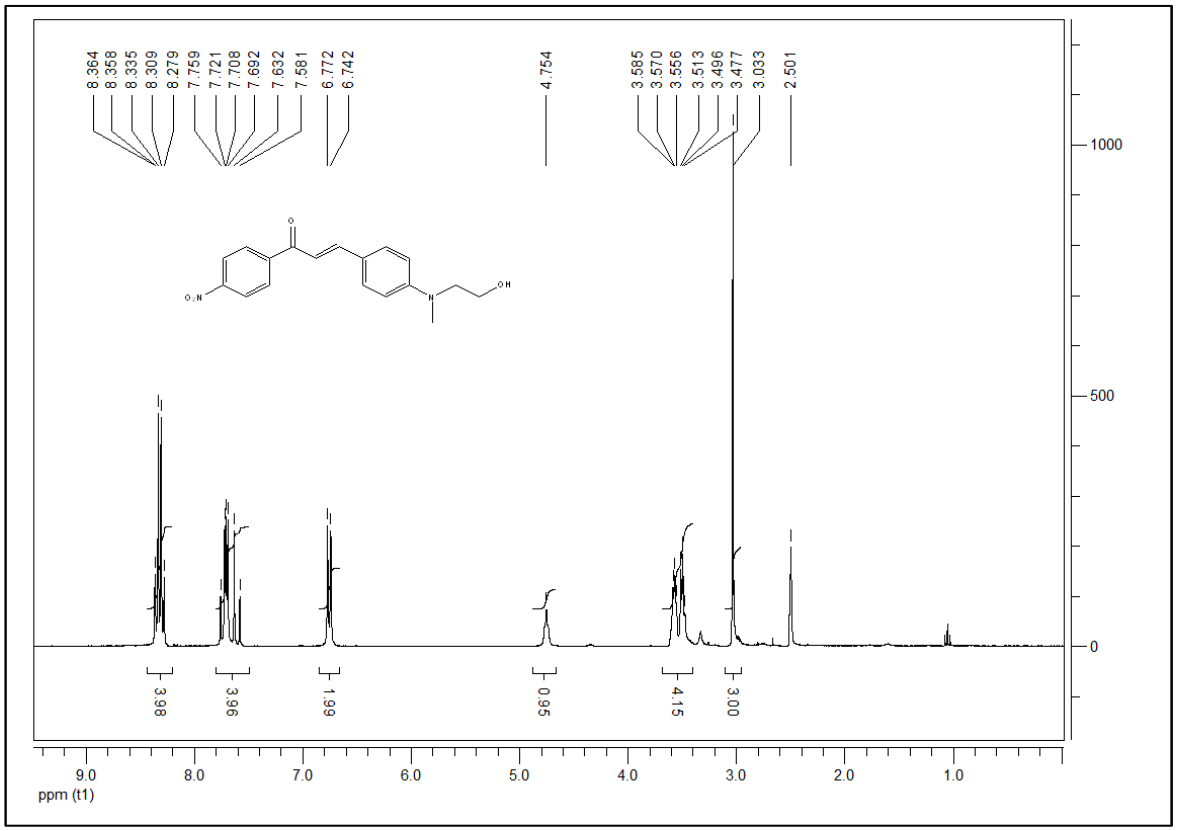
Hetero aromatic group

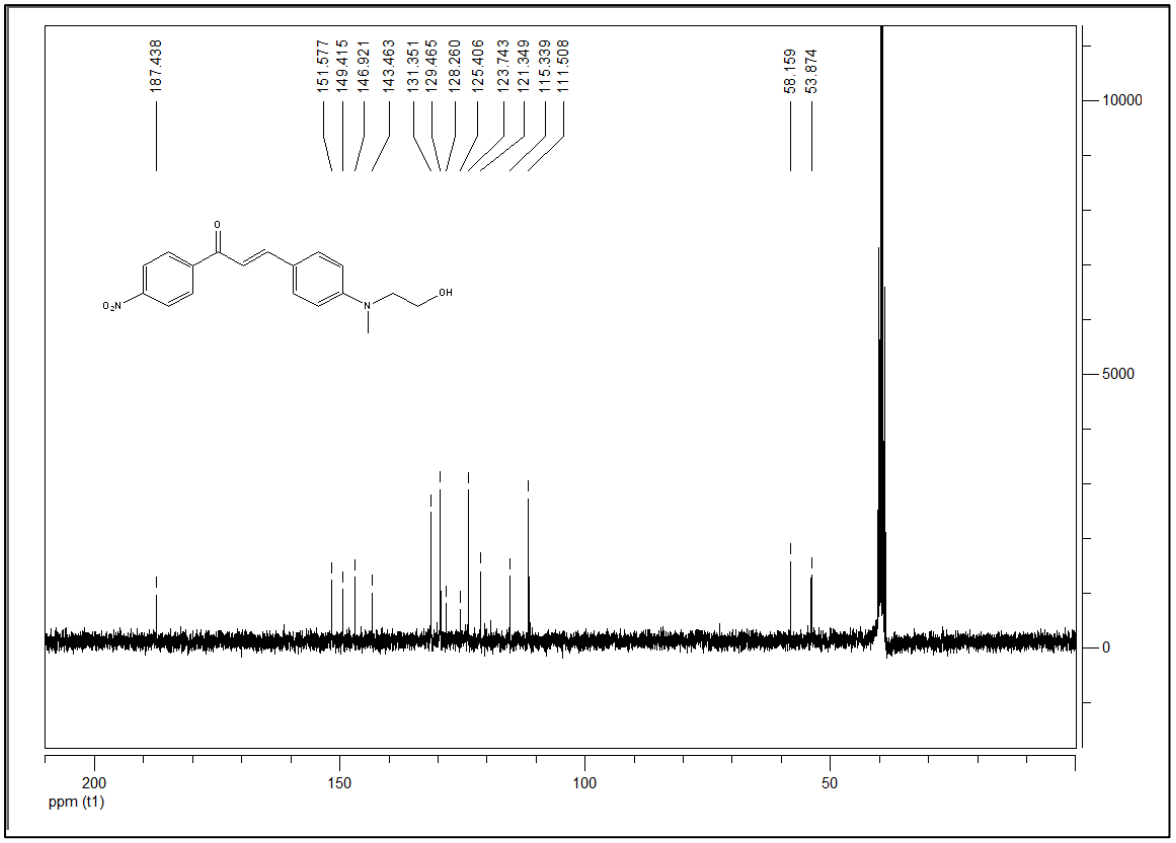
Code	Structure	Quantum Yield
CLA-45		0.018
CLA-46		0.039
CLA-47		0.027
CLA-49		0.054
CLA-59		0.054
CLA-83		0.020
CLA-95		0.016
CLA-116		0.006
CLA-119		0.015
CLA-120		0.026
CLA-136		0.029
CLA-175		0.079
CLA-177		0.022

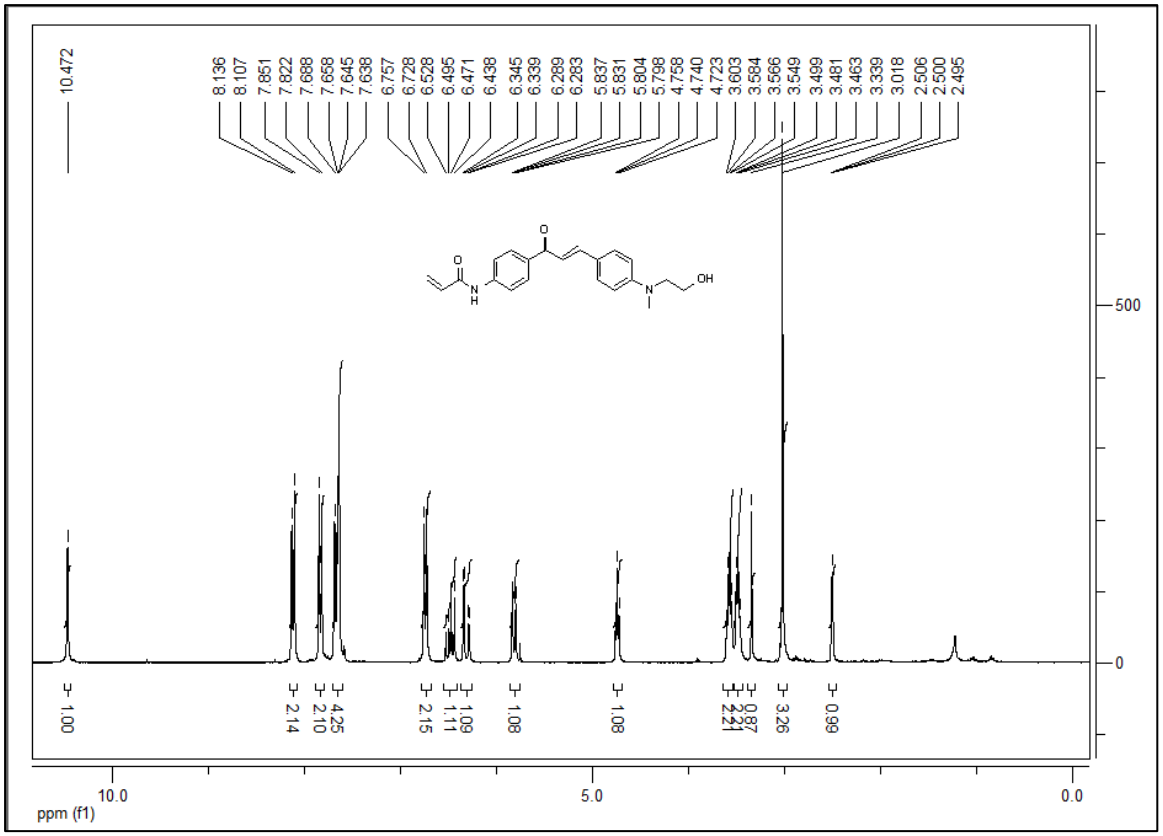
Electron withdrawing group

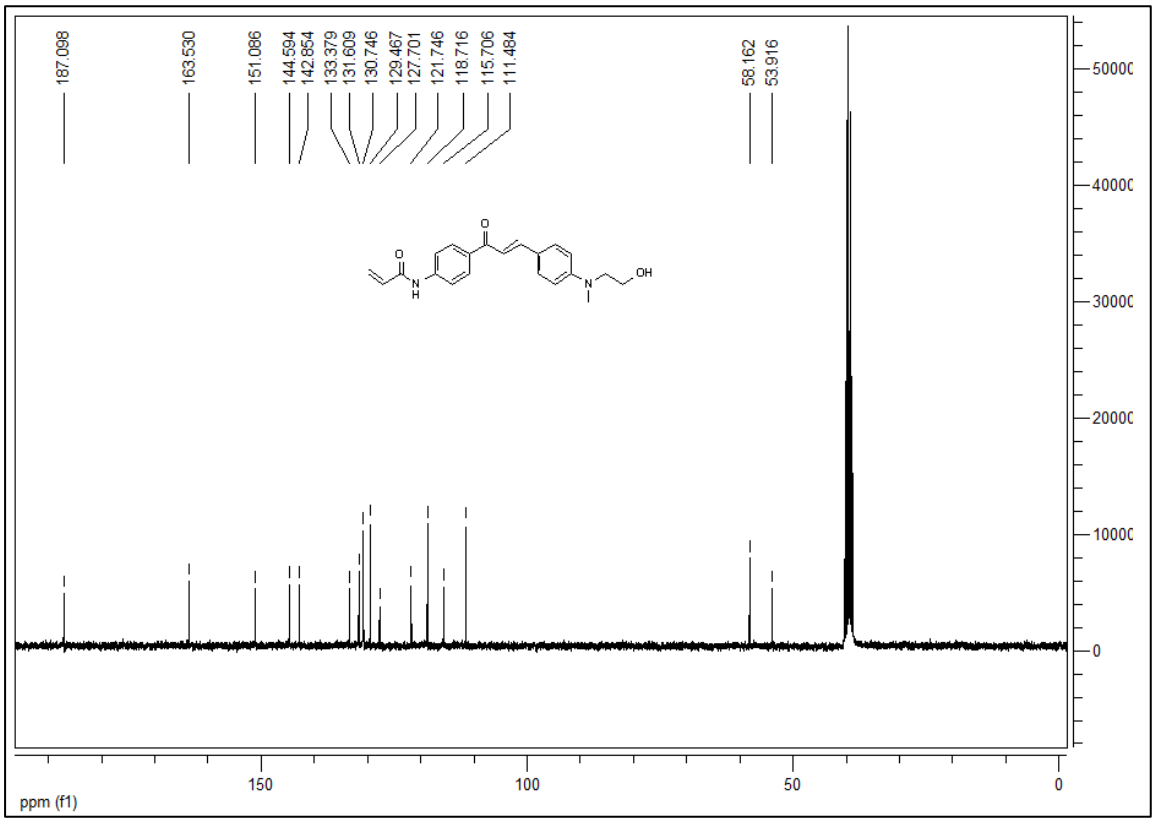
Code	Structure	Quantum Yield
CLA-22		0.048
CLA-74		0.050
CLA-81		0.078
CLA-88		0.043
CLA-115		0.076
CLA-123		0.023
CLA-129		0.052
CLA-143		0.032
CLA-164		0.099

4. 300MHz ^{1}H-NMR, ^{13}C-NMR data of intermediate, CLA7 and representative LC-MS spectrum of library compounds

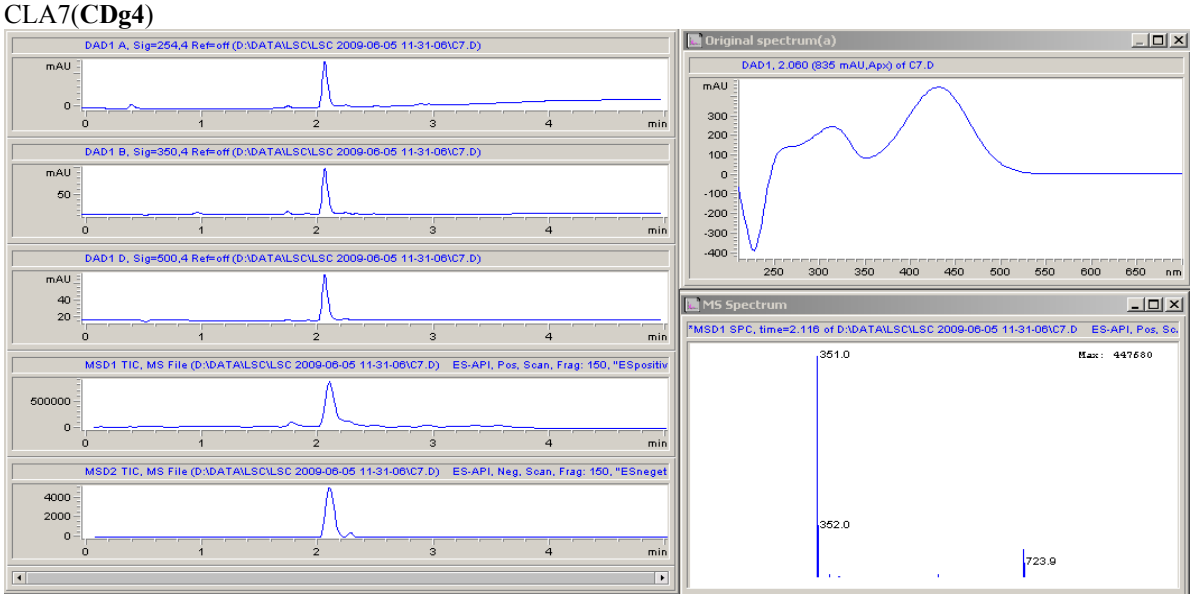




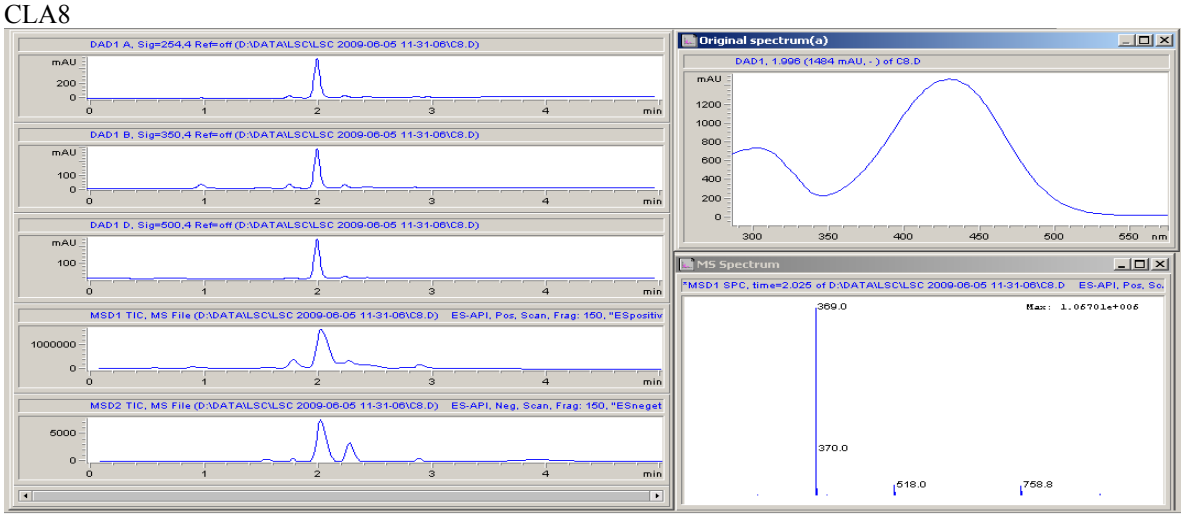

10.472
8.136
8.107
7.851
7.822
7.688
7.658
7.645
7.638
6.757
6.728
6.528
6.495
6.471
6.438
6.345
6.339
6.289
6.283
5.837
5.831
5.804
5.798
4.758
4.740
4.723
3.603
3.584
3.566
3.549
3.499
3.481
3.463
3.339
3.018
2.506
2.500
2.495
O
O
N
H
N
OH
500
0
1.00
2.14
2.10
4.25
2.15
1.11
1.09
1.08
1.08
2.21
0.87
3.26
0.90
10.0
5.0
0.0
ppm (f1)


187.098
163.530
151.086
144.594
142.854
133.379
131.609
130.746
129.467
127.701
121.746
118.716
115.706
111.484
58.162
53.916
O
O
N
H
N
OH
50000
40000
30000
20000
10000
0
150
100
50
0
ppm (f1)

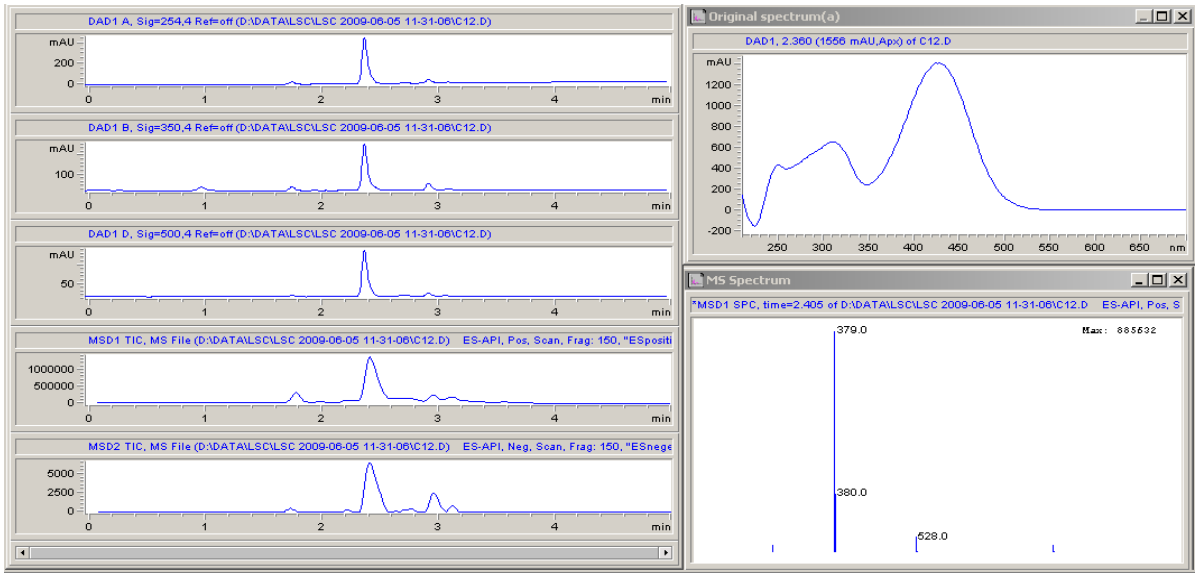
CLA7(**CDg4**)



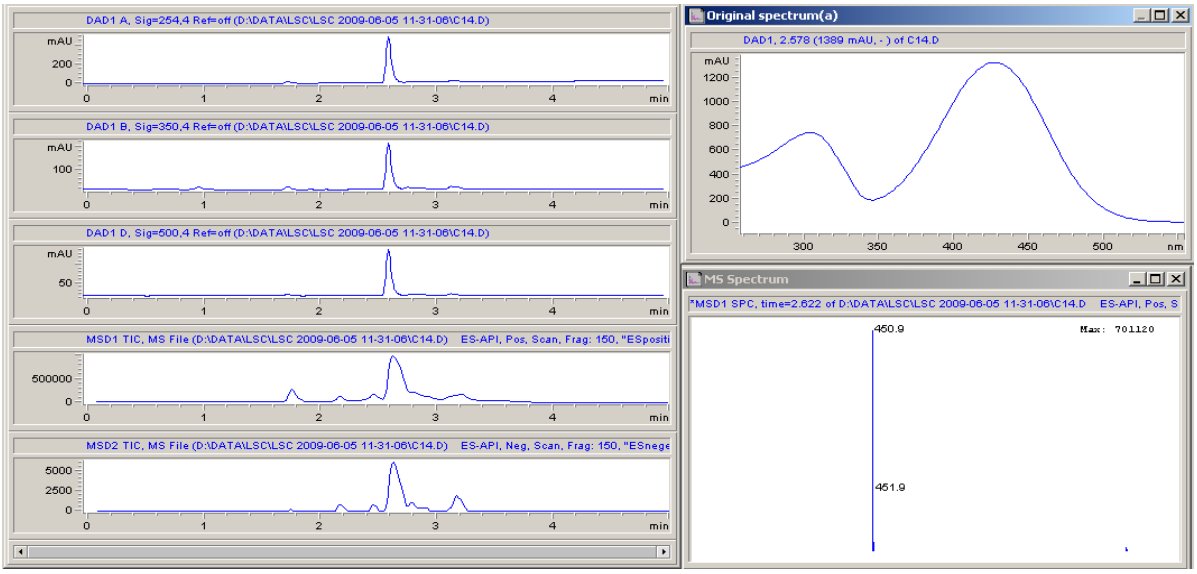
CLA8



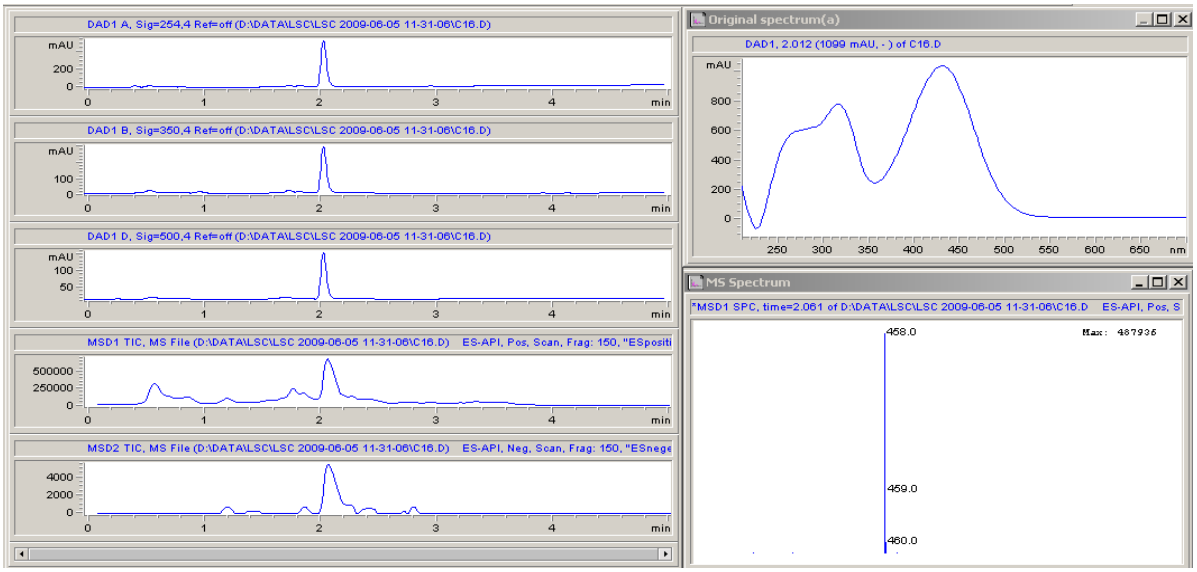
CLA12



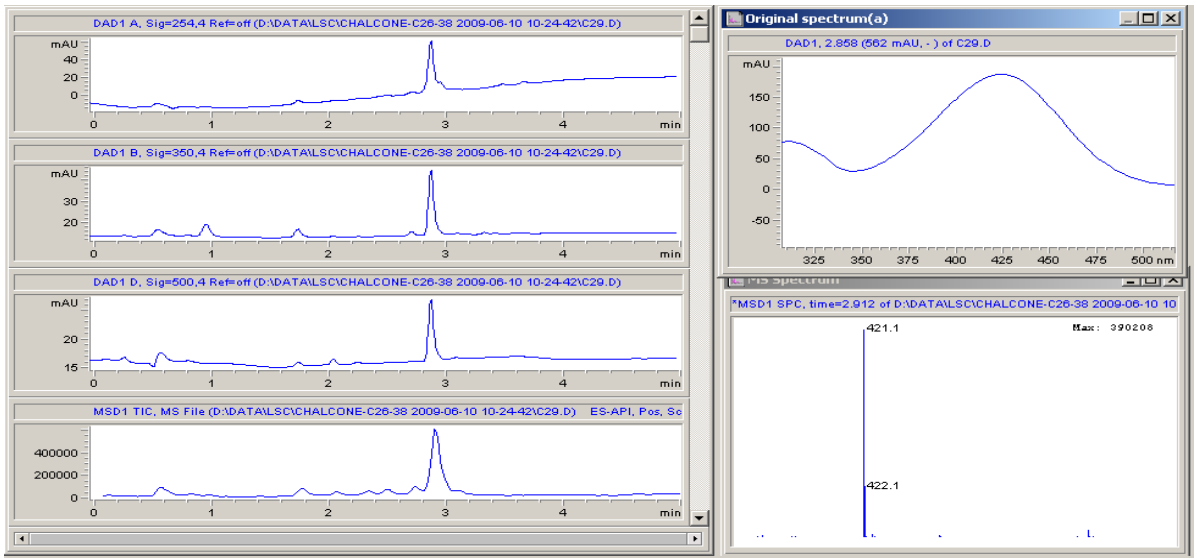
CLA14



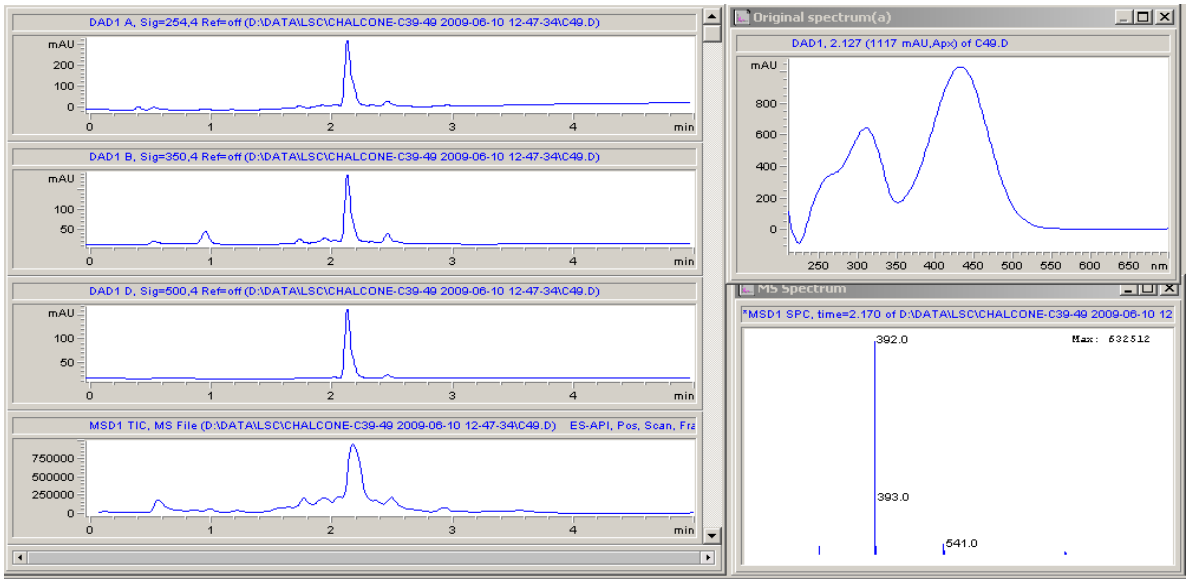
CLA16



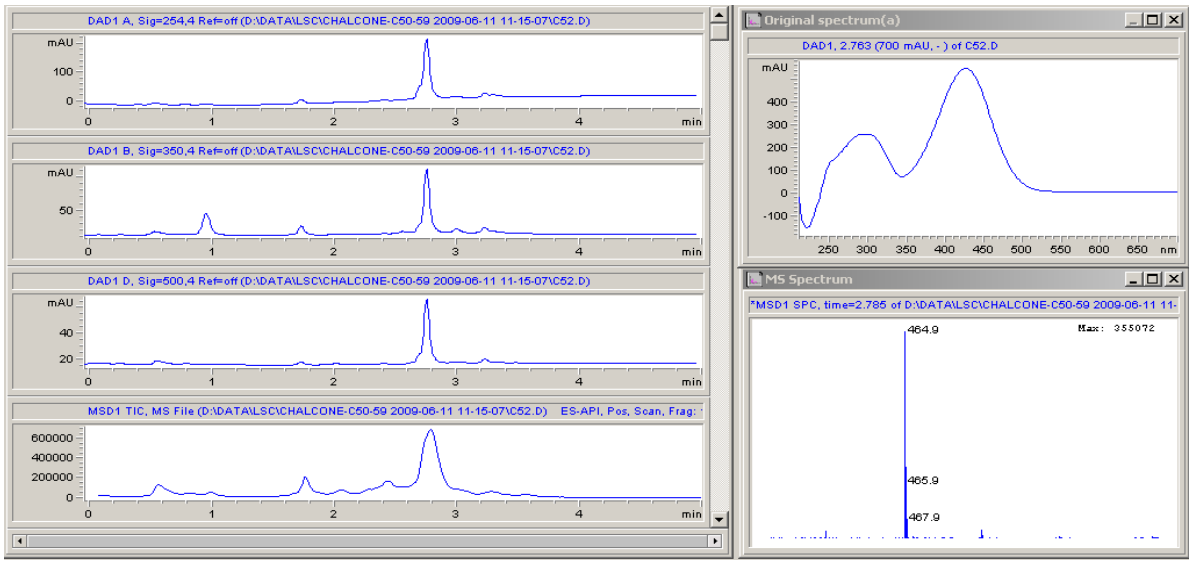
CLA29



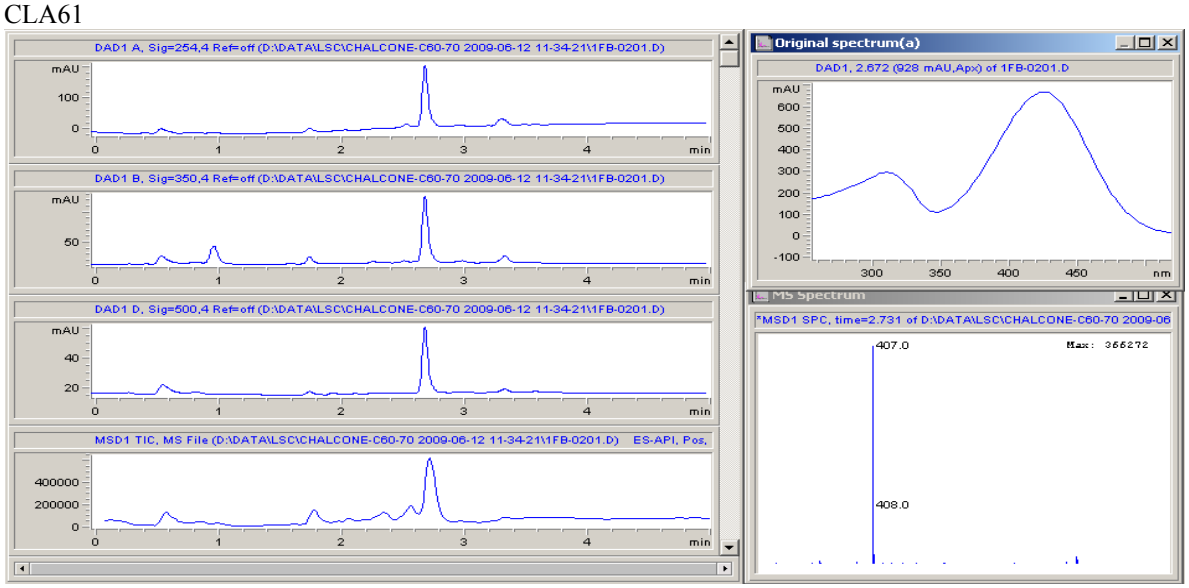
CLA49



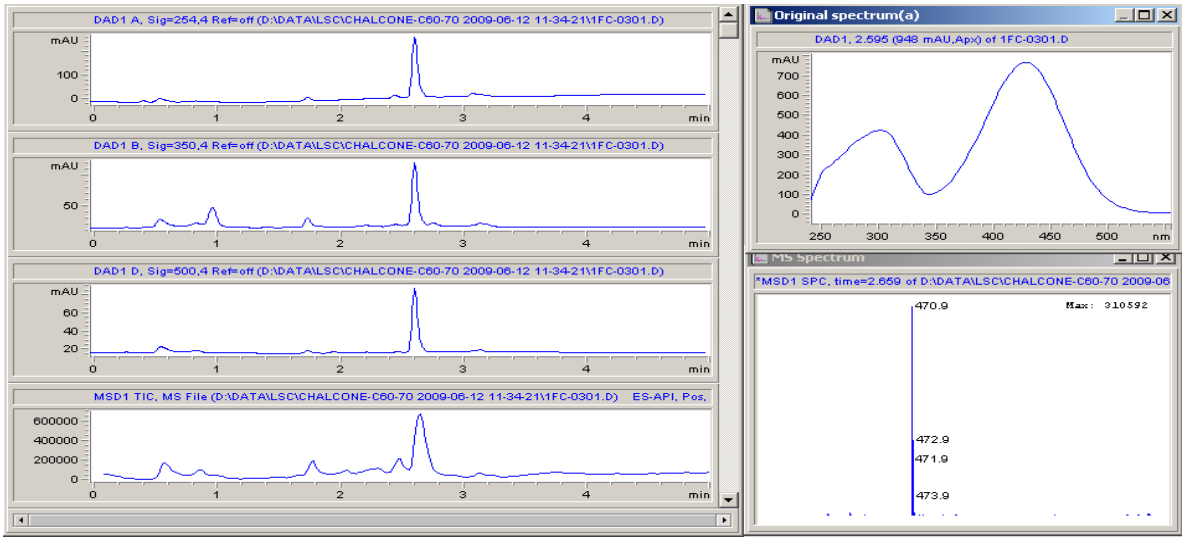
CLA52



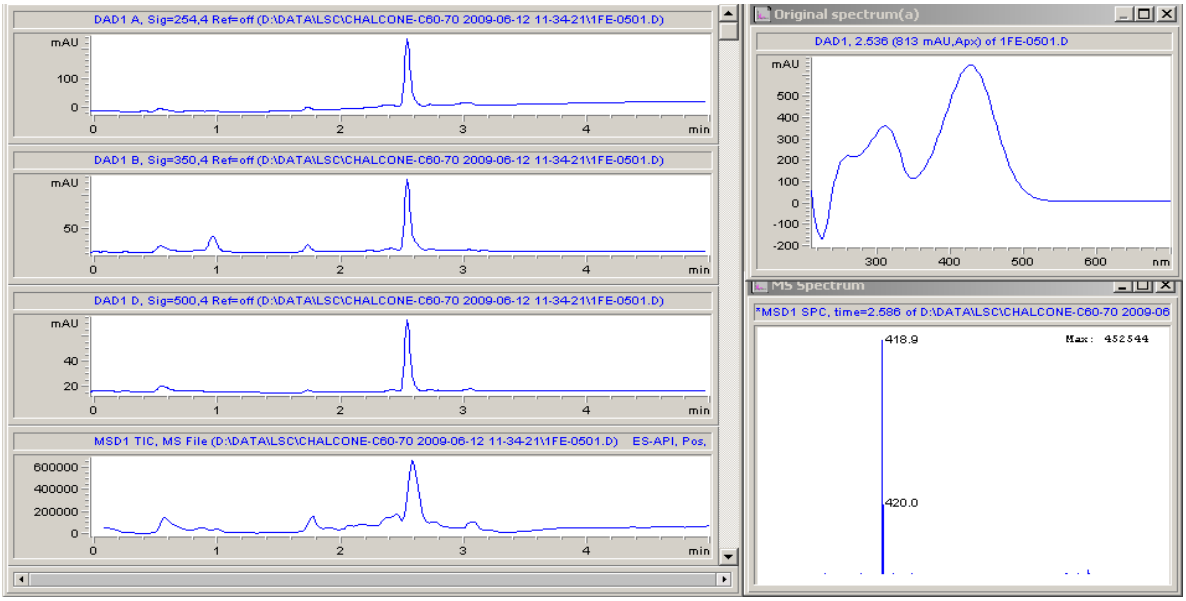
CLA61



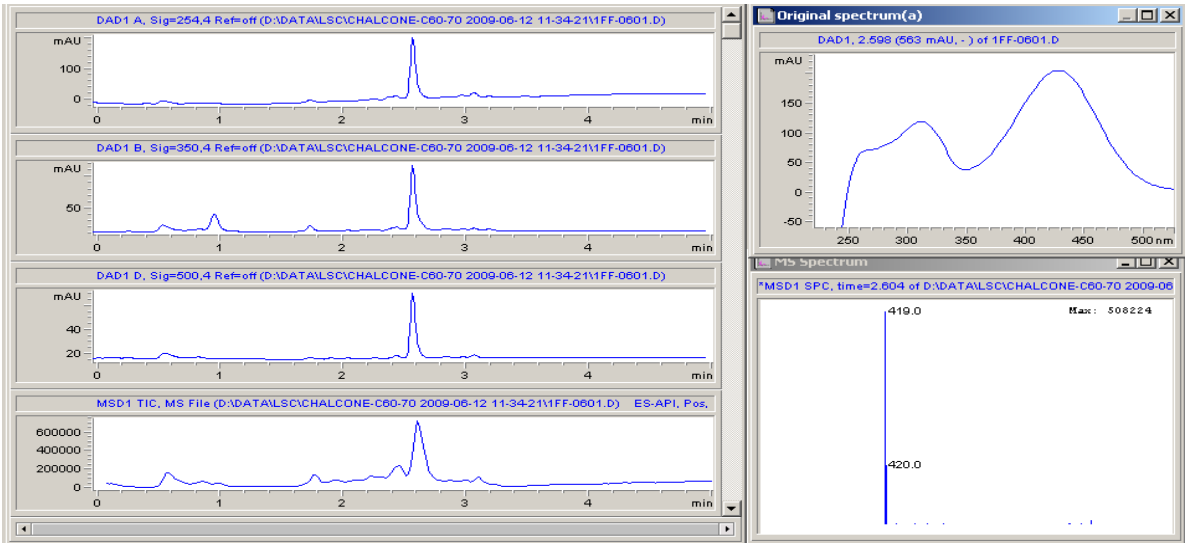
CLA62



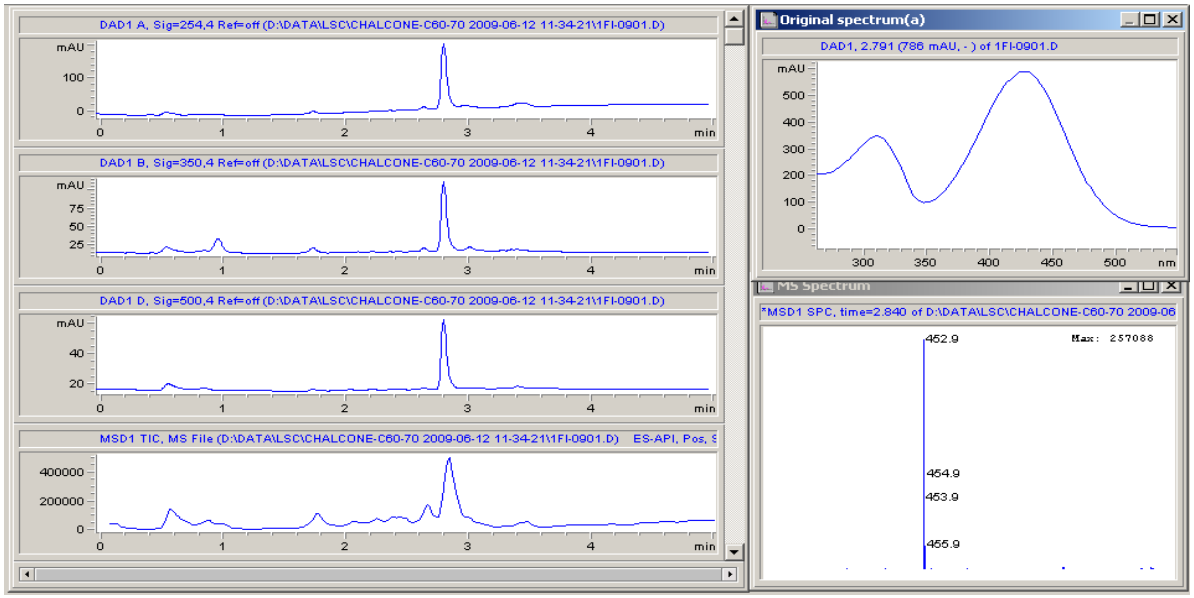
CLA64



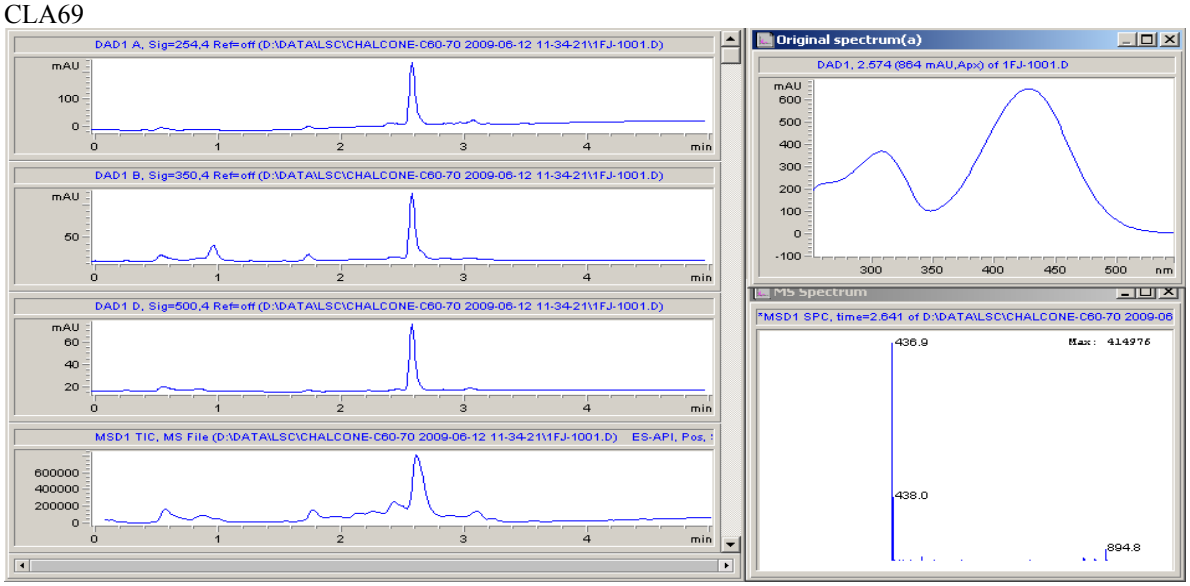
CLA65



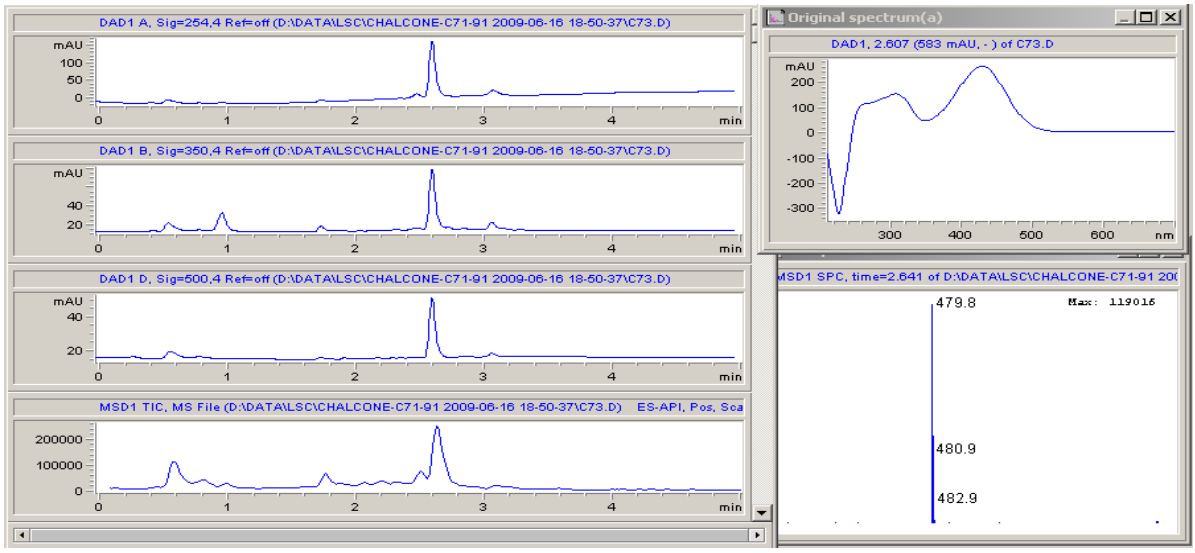
CLA68



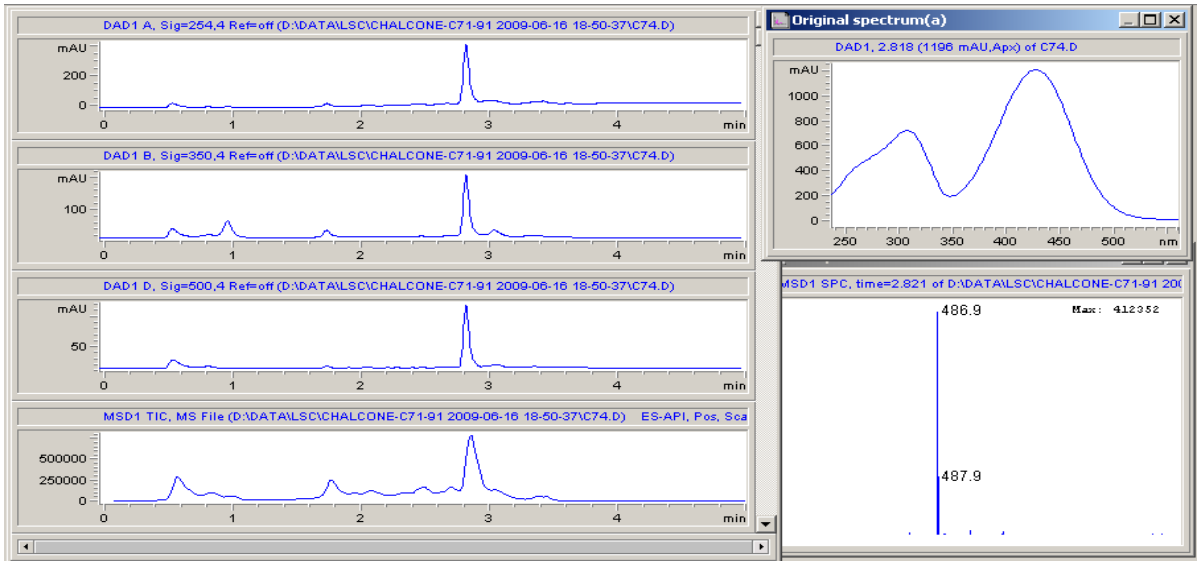
CLA69



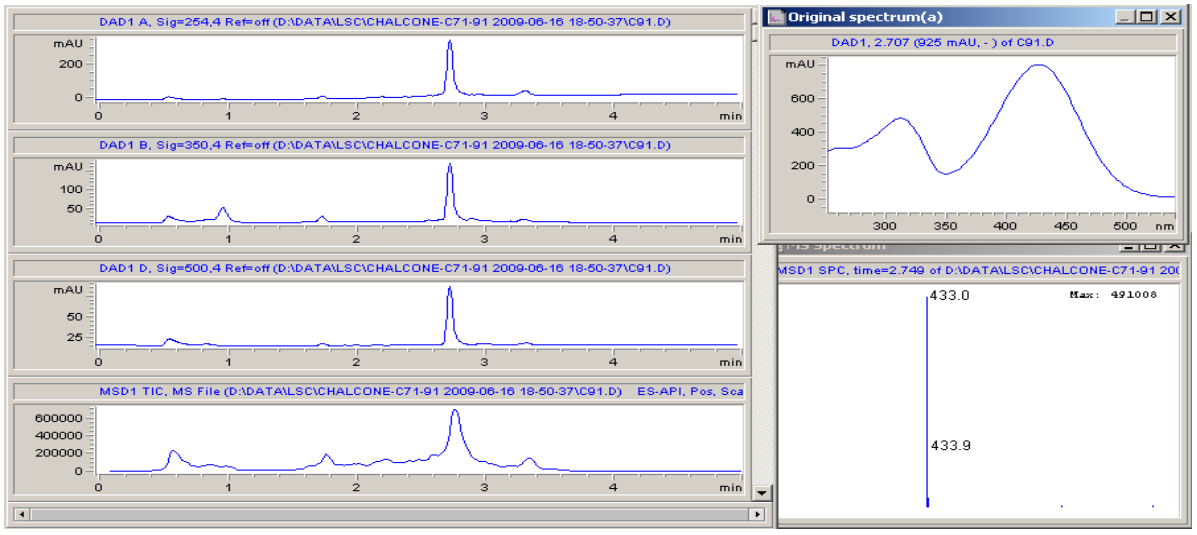
CLA73



CLA74



CLA91



CLA124