Supplementary Materials

for

A rationally engineered specific near-infrared fluorogenic substrate of human pancreatic lipase for functional imaging and inhibitor screening

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[1]These authors made equal contributions to this work.

Contents

Recombinant expression and purification of hPL

The human pancreatic cDNA was cloned into the pTT5 vector with the N-terminal mouse IgG κ chain signal peptide and C-terminal His10-tag. Freestyle 293-F cells (Invitrogen) were cultured in SMM 293T-II medium (Sino Biological Inc.) at 37 °C, 130 rpm under 5% CO_2. The pTT5 containing human pancreatic lipase cDNA plasmid was pre-mixed with PEI MAX 40K (Polysciences) for 30 min before transfection. Transfection was started by adding plasmid-PEI MAX 40K mixture when the cell density reached 2.0×10^6 cells/mL. After 72 h, the transfected cells were centrifuged at 1000 rpm for 10 min and the conditioned medium was collected. The conditioned medium was loaded on the Ni Sepharose excel resin (GE Healthcare). The resin was washed with wash buffer 1 containing 25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$, 2 mM imidazole. The protein was eluted with elute buffer 1 containing 25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$, 200 mM imidazole. The eluted protein was diluted 10 times with a buffer (25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM $CaCl_2$) and incubated with Ni-NTA agarose (Qiagen) for further purification. The agarose was washed with wash buffer 2 containing 25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM $CaCl_2$, 20 mM imidazole. Then the protein was eluted with elute buffer 2 containing 25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM $CaCl_2$, 300 mM imidazole. The human pancreatic lipase-containing fractions were concentrated using a 10 kDa molecular weight cut-off (MWCO) concentrator (Millipore). The concentrated sample was purified on a Superdex 200 10/300 GL column (GE Healthcare) equilibrated in protein storage buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$).

Cytotoxicity assays

The determination of cell viability was done by the CCK-8 assay. AR42J cells were inoculated at a density of 1×10^4 in 96-well plates and cultured in Dulbecco's Modified Eagle medium: nutrient mixture F-12 (DMEM/F-12) containing 10% fetal bovine serum (FBS) and maintained in a 5% CO_2 incubator at 37°C for 48 h. The cells were then incubated with different concentrations of **7-STCFC** for 12 hours, followed by washing with PBS. Finally, the CCK-8 assay was used to assess cytotoxicity. Medium containing 10% CCK-8 was added and incubated for a further 3 hours. The absorbance was measured at 450 nm.

Fluorescence imaging of rat and monkey pancreas slices

Monkey and mouse pancreatic tissues were cut into 150 μm using a Microtome Cryostat (Leica, Wetzlar, Germany) and placed in cell culture dishes. These slices were divided into three groups. Slices in blank groups without adding **7-STCFC** and inhibitor, Slices in experimental groups were only incubated with **7-STCFC** (20 μM) for 30 min, and slices in negative control groups were treated with orlistat (25 μM) for 15 min, and then incubated with **7-STCFC** (20 μM) for 30 min. Finally, slices were observed under confocal microscope (Leica SP8, Wetzlar, Germany). λ_{ex} = 552 nm, λ_{em} =650 − 690 nm.

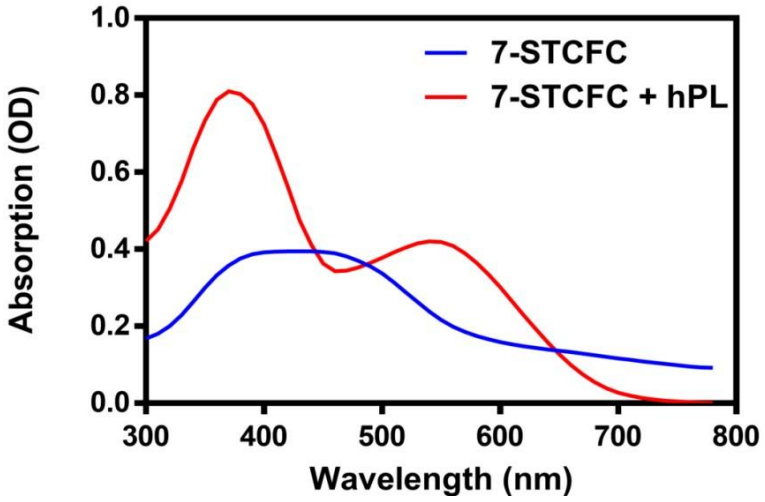


Fig. S1. The change in absorption spectrum of **7-STCFC** (50 μM) in presence of hPL (10 μg/mL).

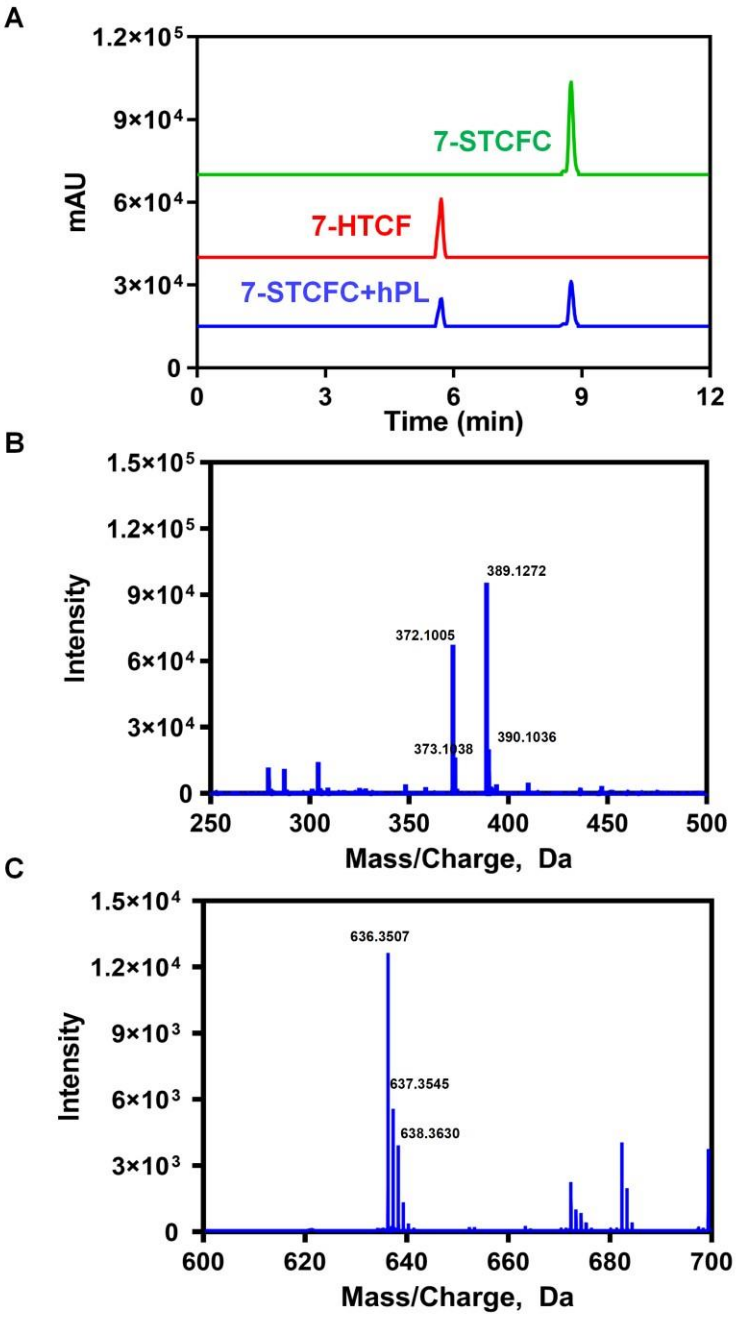


Fig. S2. (A) Representative LC-UV chromatograms of **7-STCFC** incubation samples at 37 °C, UV detector was set at 468 nm. Mass spectra of **7-STCFC** with the quasi-molecular ion peak m/z = 636.3507, monitored under negative mode (C), and its hydrolytic product **7-HTCF** with the quasi-molecular ion peak m/z = 372.1005 (B) monitored under positive mode.

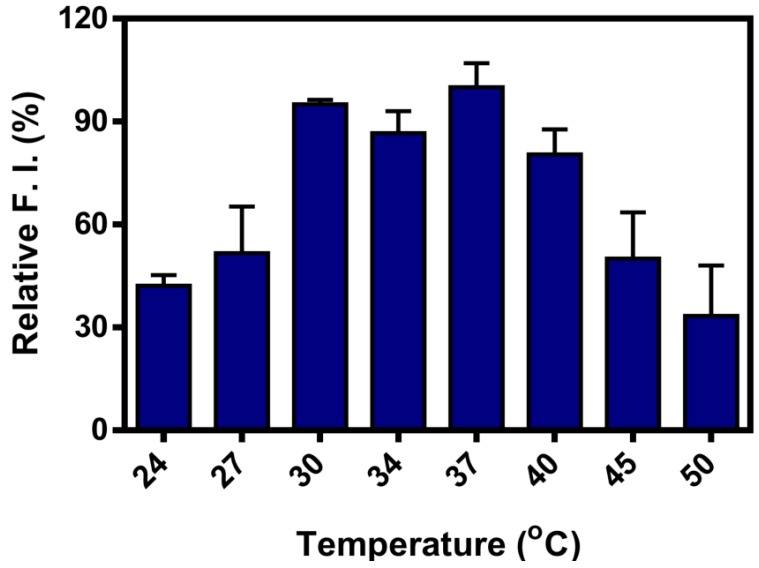


Fig. S3. The effect of temperature on the fluorescence response of **7-STCFC** to hPL

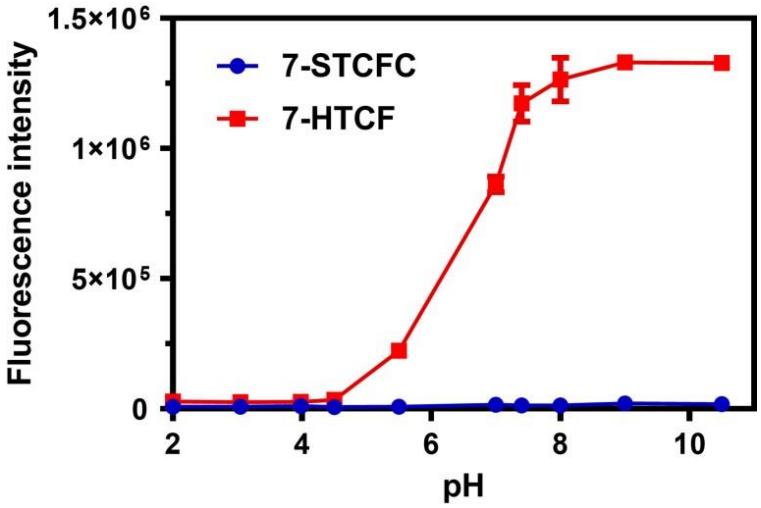


Fig. S4. The effects of pH values on the fluorescence intensity of **7-STCFC** and its metabolite **7-HTCF** (5 μM).

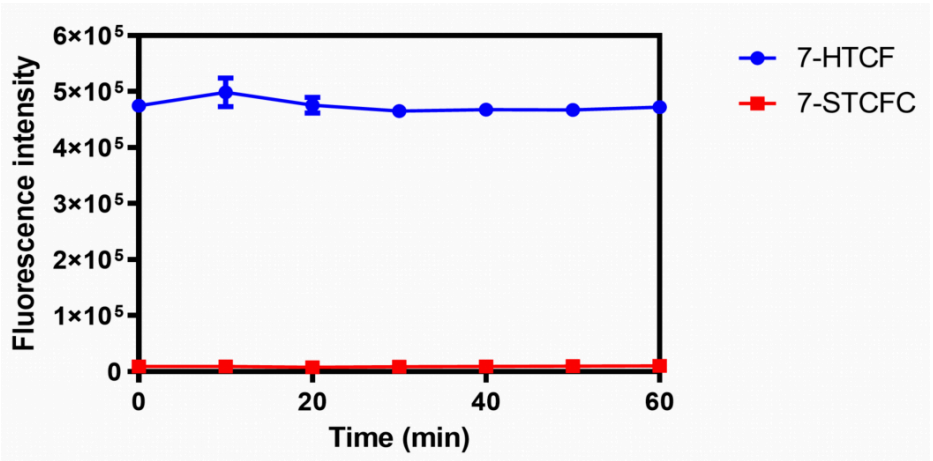


Fig. S5. The photostability of **7-STCFC** (5 μM) and **7-HTCF** (5 μM), following continuous illumination at 570 nm for different time.

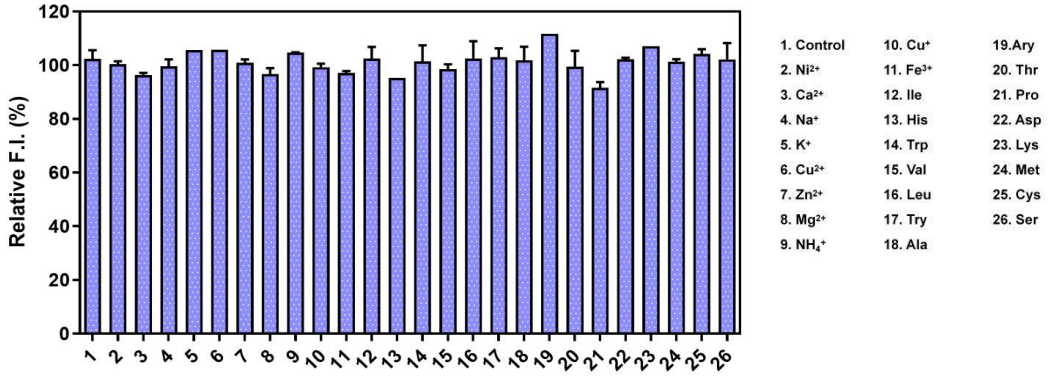


Fig. S6. Fluorescence responses of **7-STCFC** (5 μM) to various analytes in aqueous solution. Relative fluorescence intensity (%) = Fluorescence intensity of adding analytes / Fluorescence intensity of control.

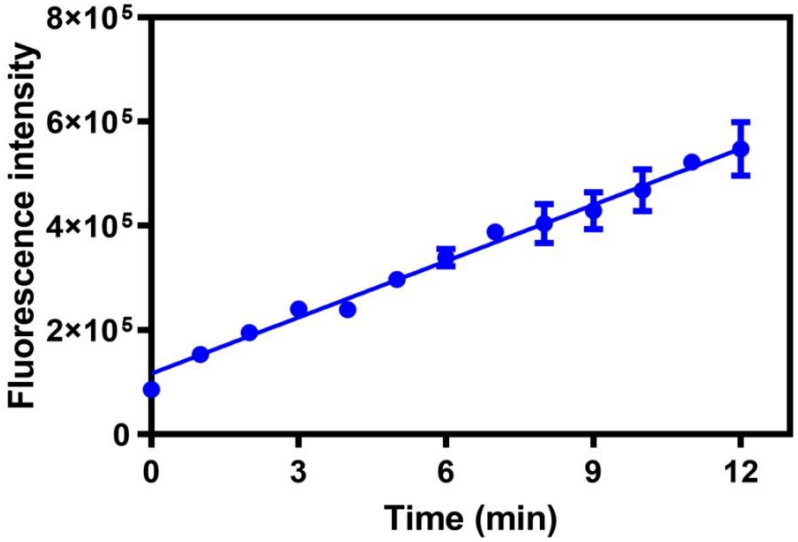


Fig. S7. The Linear in fluorescence intensity of **7-STCFC** (5 μM) over time upon addition of hPL (1 μg/mL) in buffer at 37 °C.

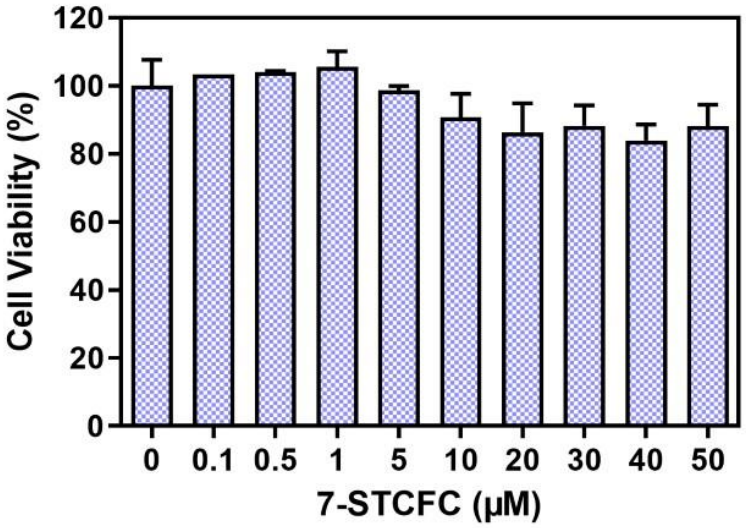


Fig. S8. The cytotoxicity of **7-STCFC** in AR42J

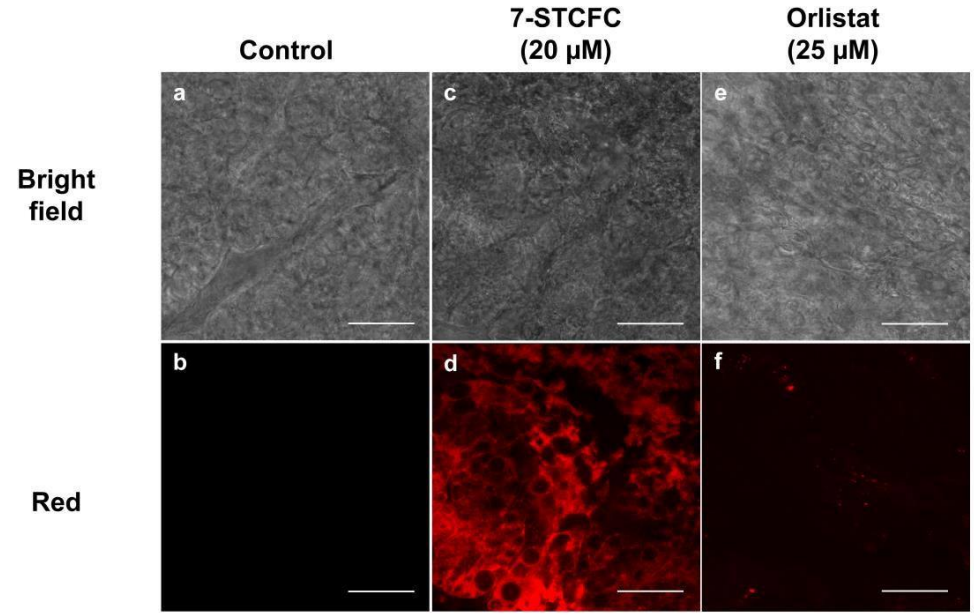


Fig. S9. Confocal images of rat pancreas slices. Rat pancreas slices were incubated with PBS only for 30 min (a-b); (c-d) Rat pancreas slices were incubated with **7-STCFC** (20 μM) for 30 min; (e-f) Rat pancreas slices were pre-treated with orlistat for 30 min and then incubated with **7-SCTCF** for 30 min. λ_{ex} = 550 nm, λ_{em} = 650− 690 nm. Scale bars = 25 μm.

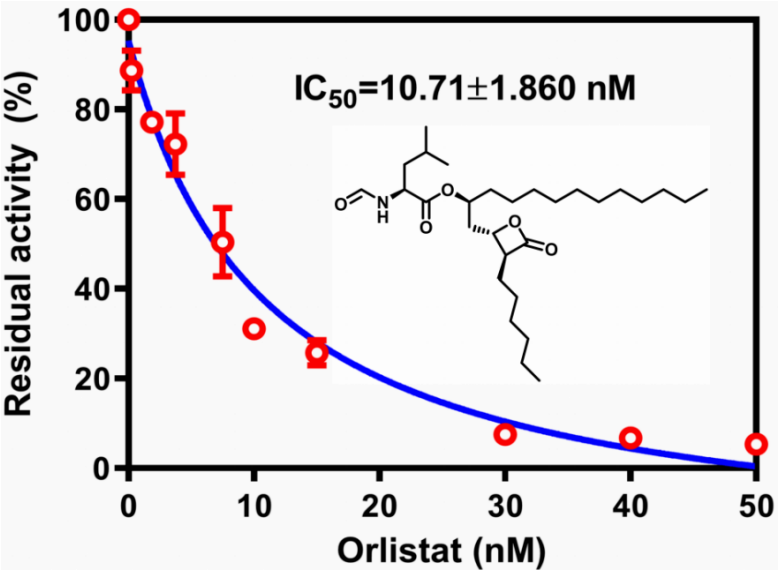


Fig. S10. Dose-inhibition curves of orlistat against hPL catalysed **7-STCFC** hydrolysis. All data were expressed as mean ± SD.

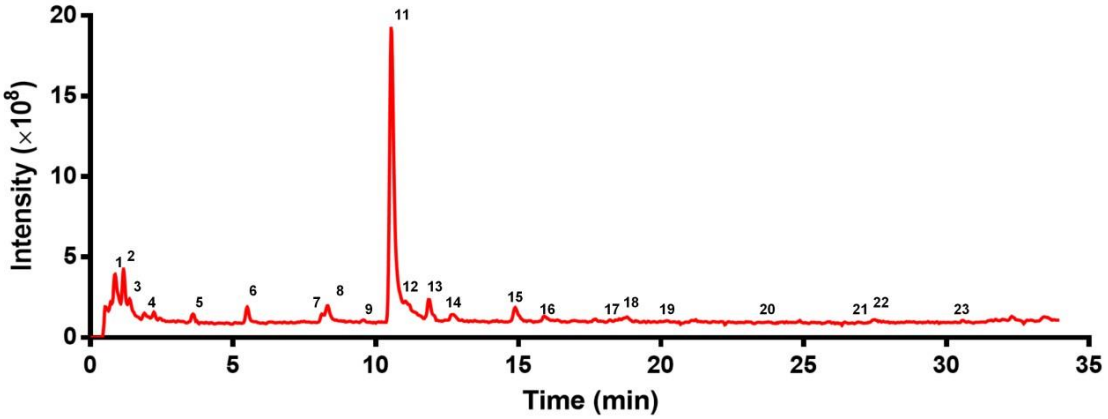


Fig. S11. Total ion chromatograms of pu-er tea extract in positive ion mode.

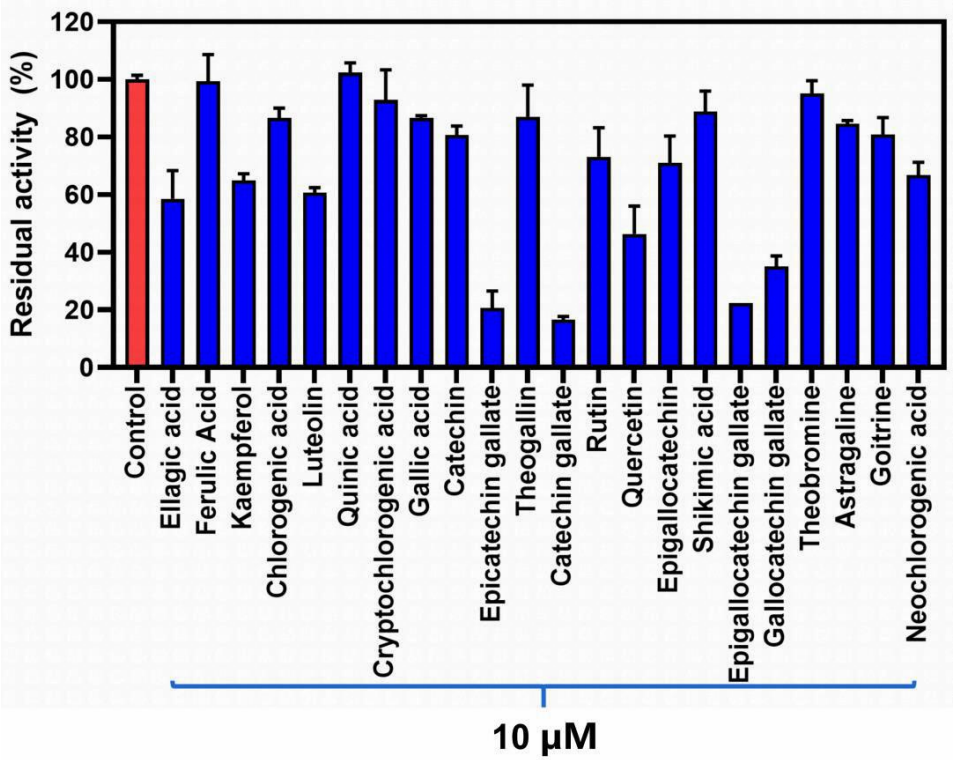


Fig. S12. Inhibitory effects of constituents in the pu-er tea against hPL-catalyzed **7-STCFC** hydrolysis. Data were shown as mean ± SD.

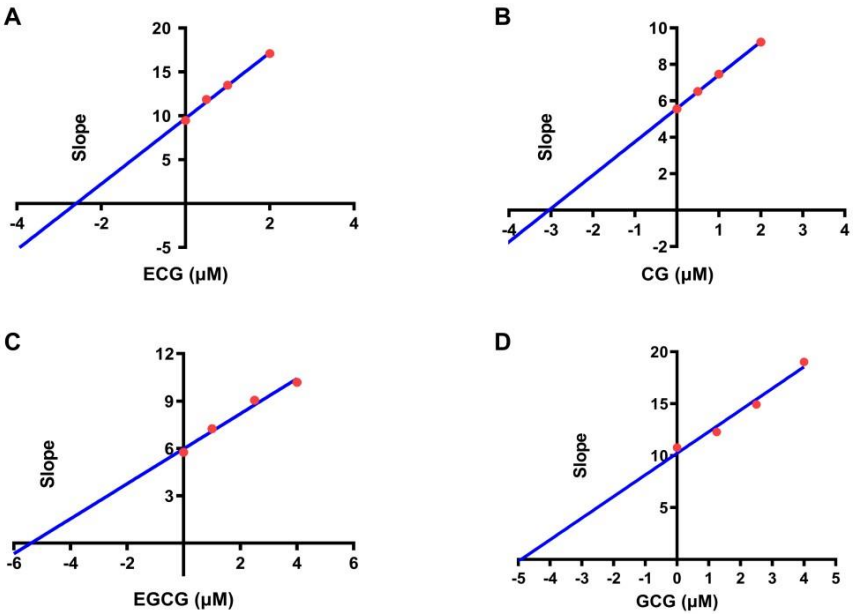


Fig. S13. The second plot of slopes from the Lineweaver–Burk plots.

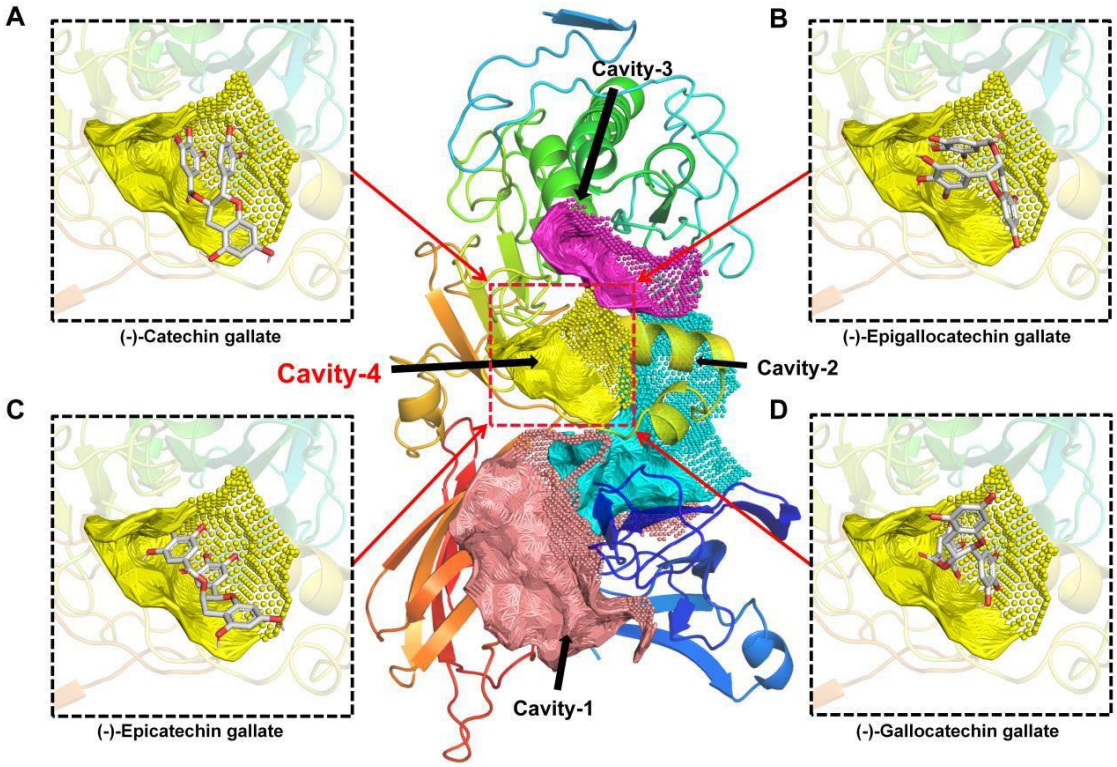


Fig. S14. An equilibrium stereo detailed view of hPL docked with (A) (-)-Catechin gallate; (B) (-)-Epigallocatechin gallate; (C) (-)-Epicatechin gallate; (D) (-)-Gallocatechin gallate in protein binding cavities-4.

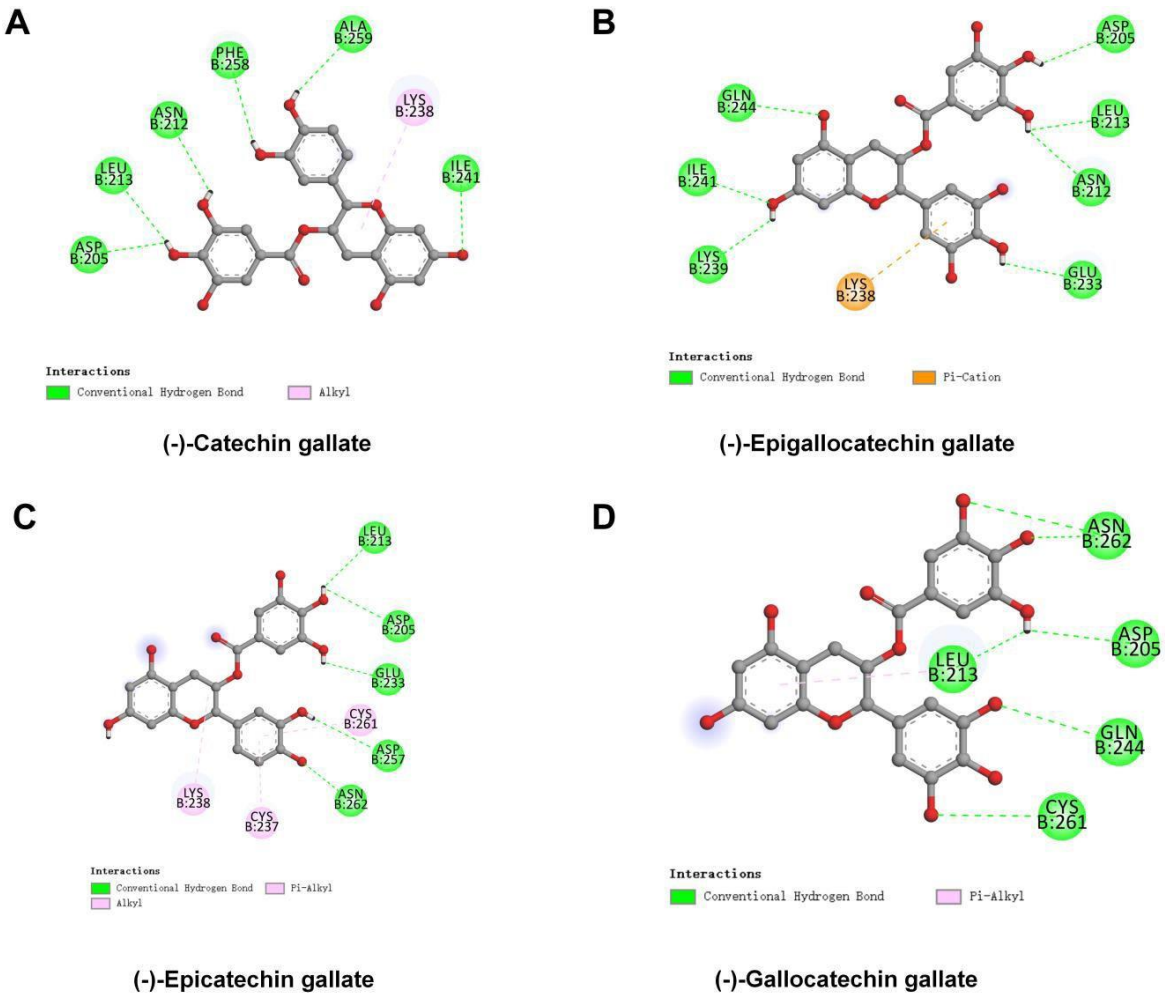


Fig. S15. 2D representation of the interactions between **(A)** (-)-Catechin gallate **(B)** (-)-Epigallocatechin gallate **(C)** (-)-Epicatechin gallate **(D)** (-)-Gallocatechin gallate and the amino acid residues of hPL

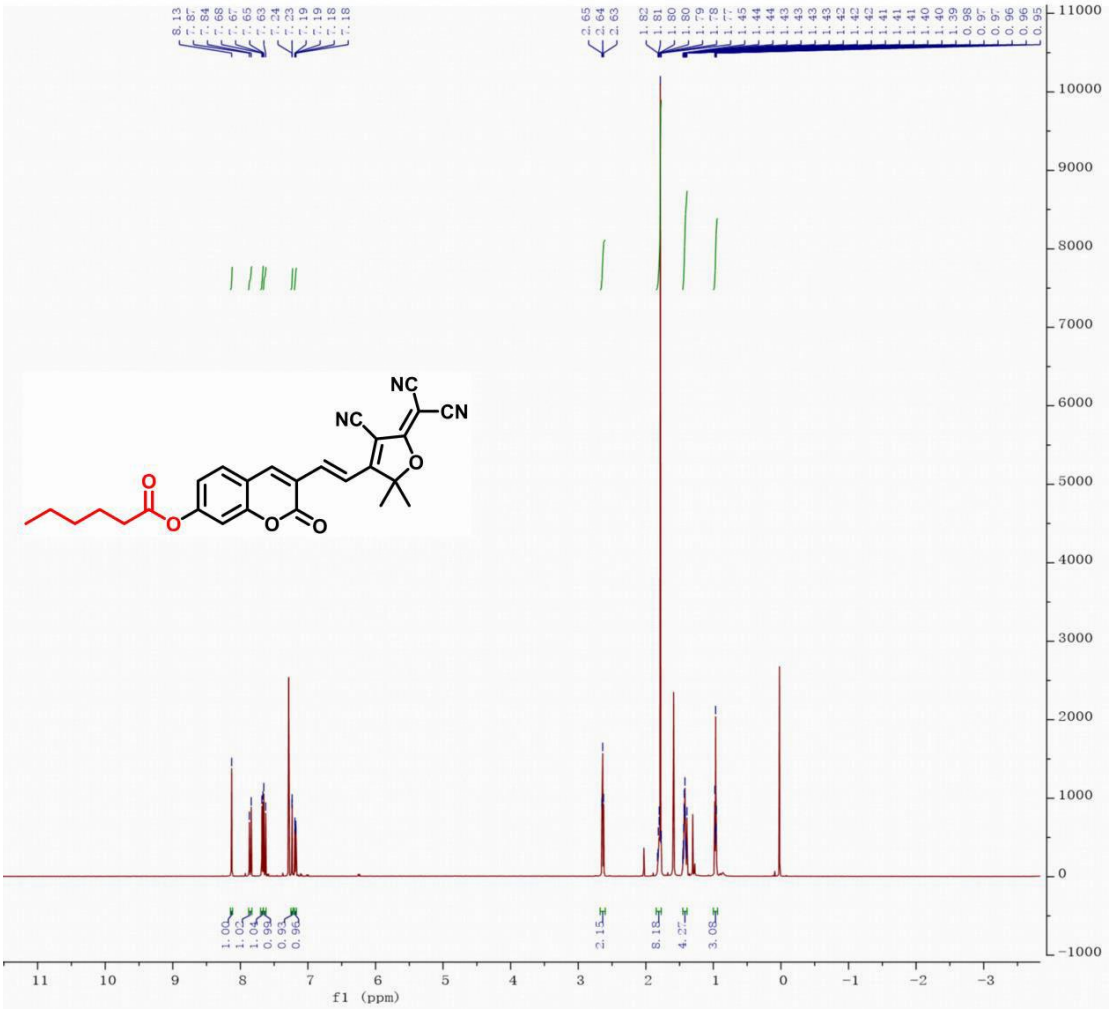


Fig. S16. ^{1}H NMR spectra of compound **L-6C**.

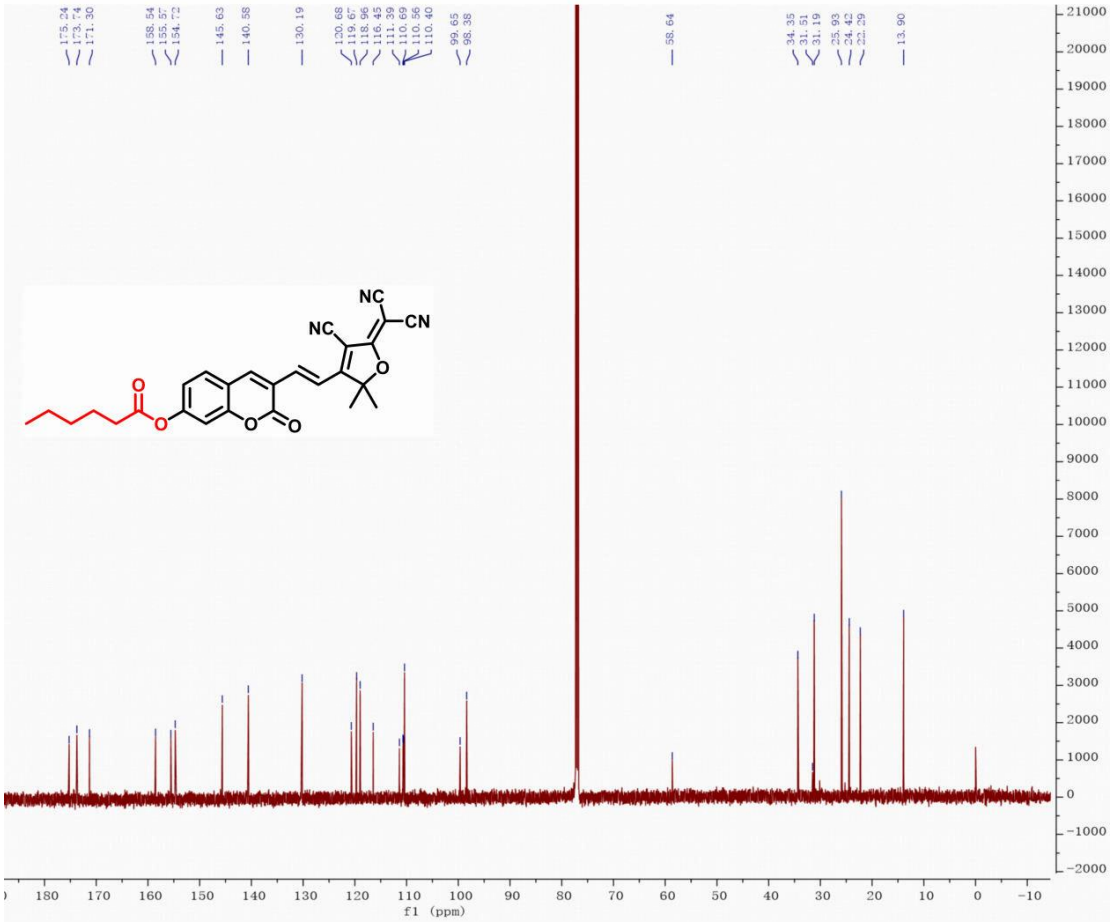


Fig. S17. [1]C NMR spectra of compound **L-6C**.

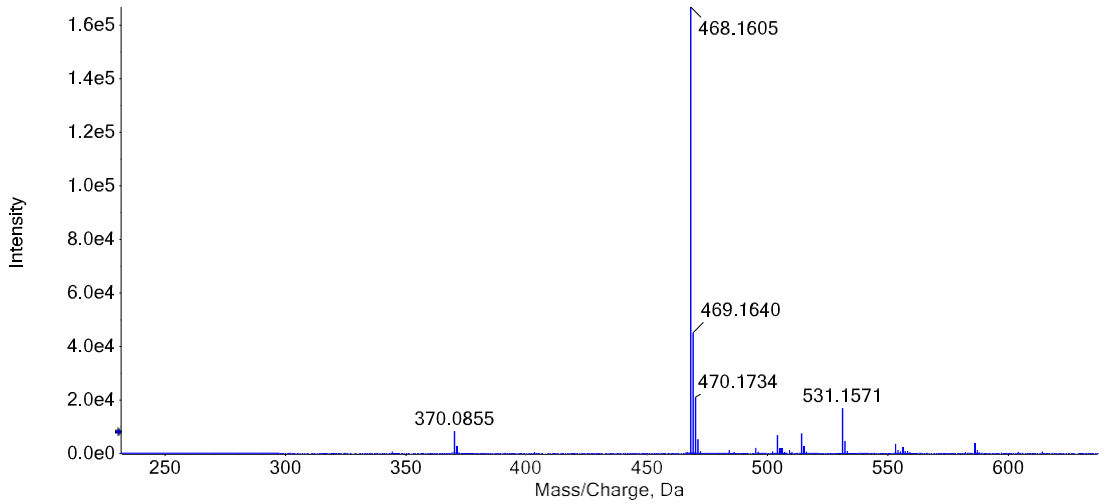


Fig. S18. HRMS spectrum of compound **L-6C**.

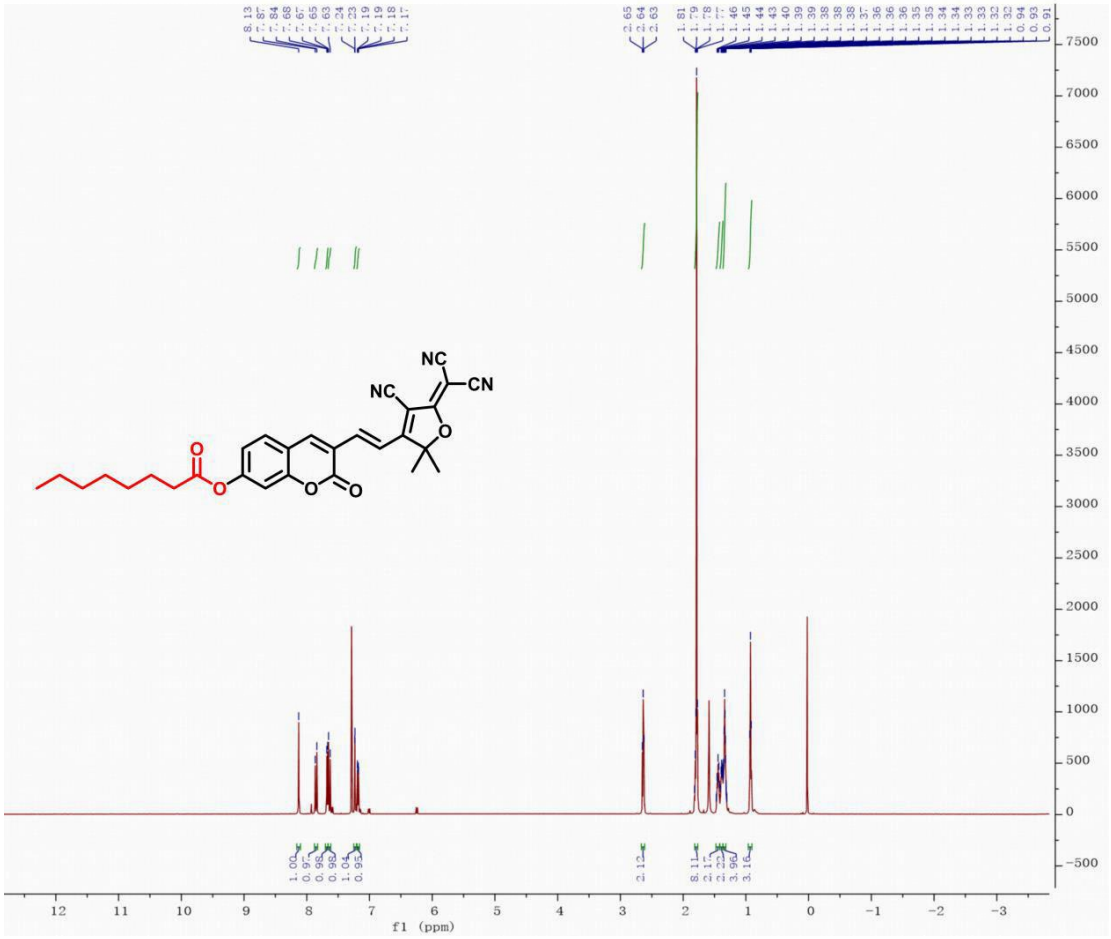


Fig. S19. ^{1}H NMR spectra of compound **L-8C.**

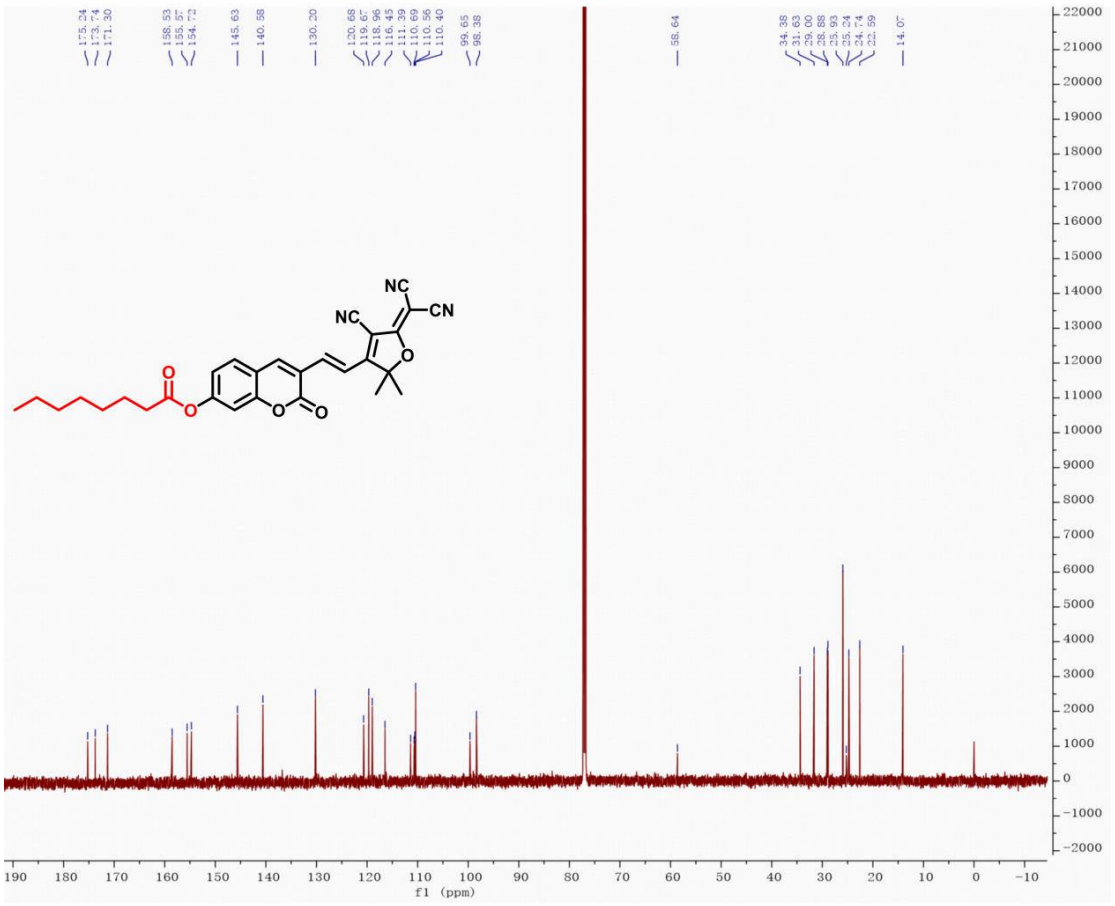


Fig. S20. ^{1}C NMR spectra of compound **L-8C.**

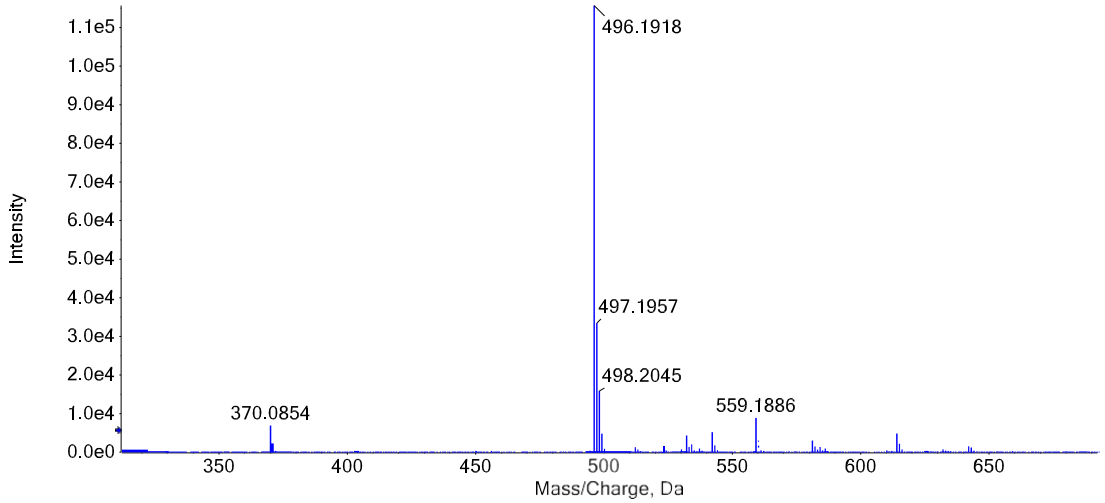


Fig. S21. HRMS spectrum of compound **L-8C.**

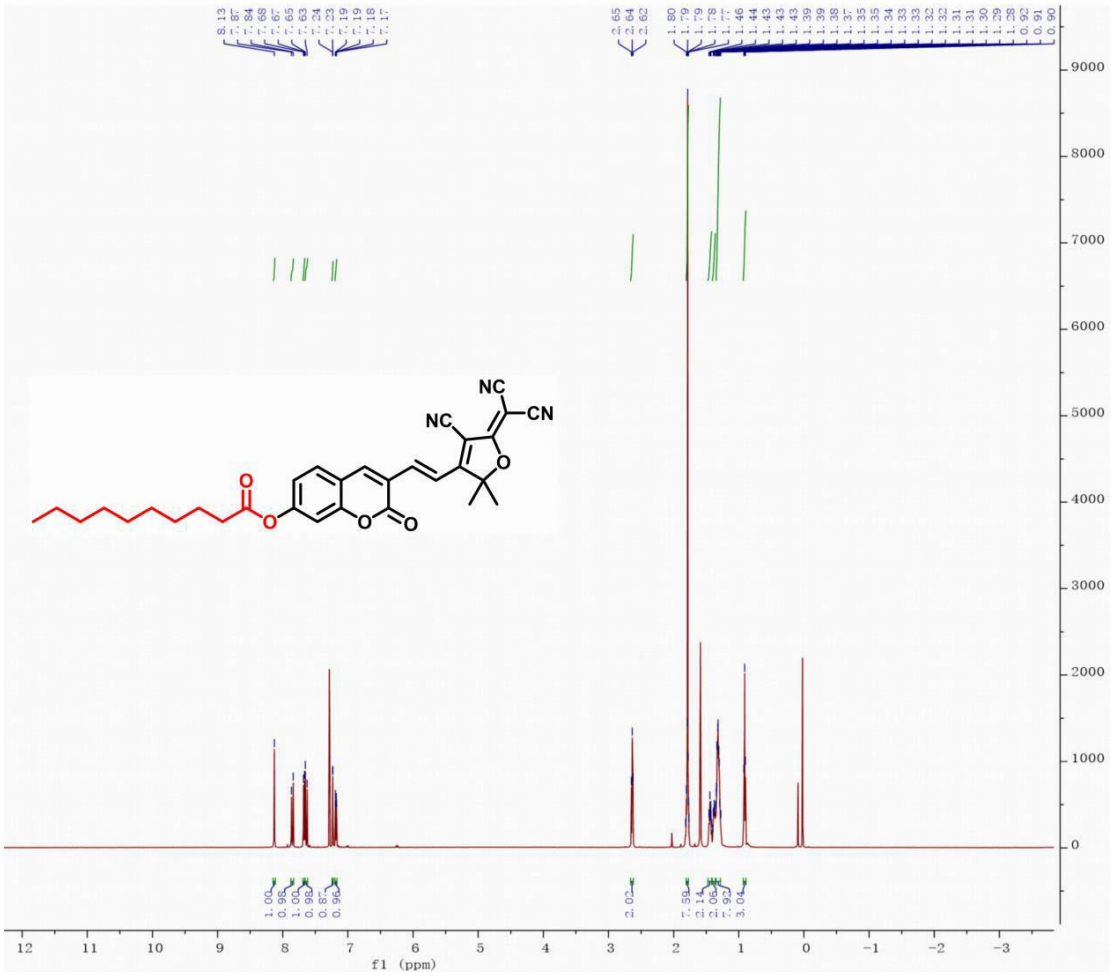


Fig. S22. ^{1}H NMR spectra of compound **L-10C.**

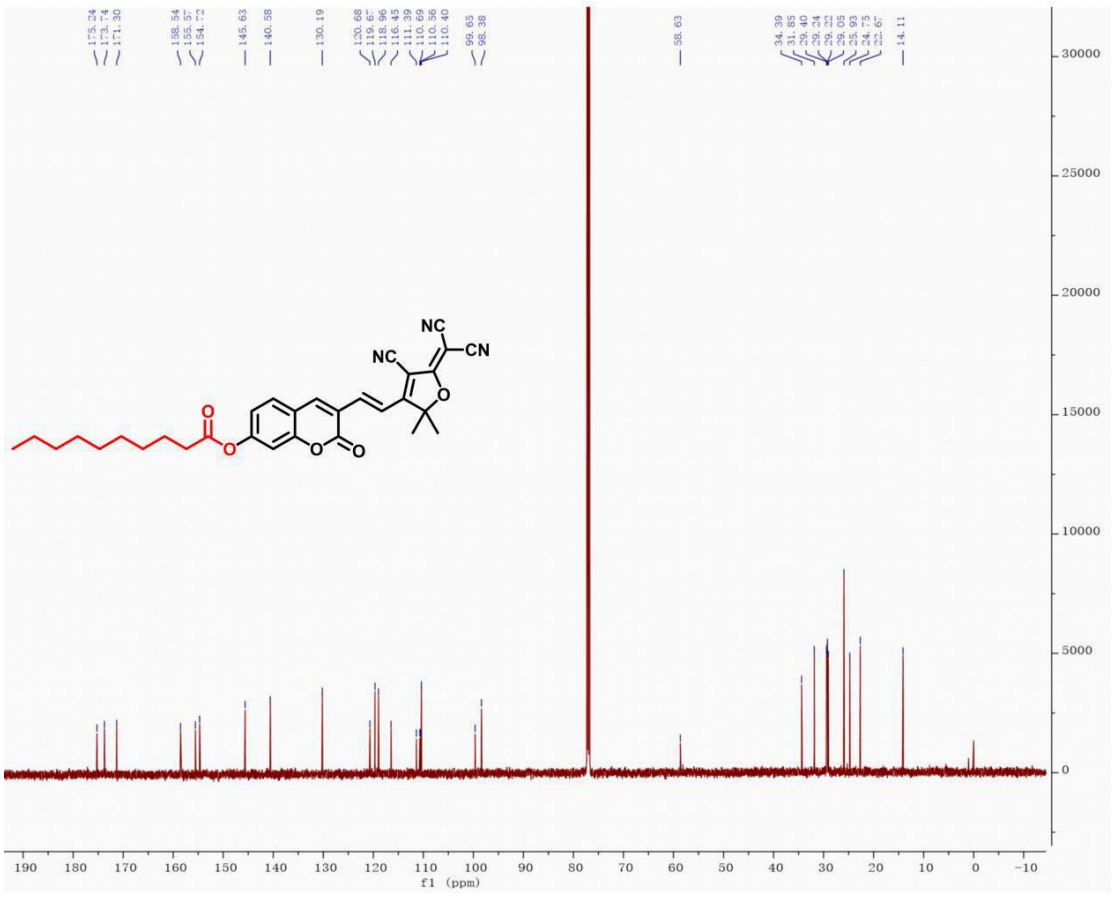


Fig. S23. ^{1}C NMR spectra of compound **L-10C.**

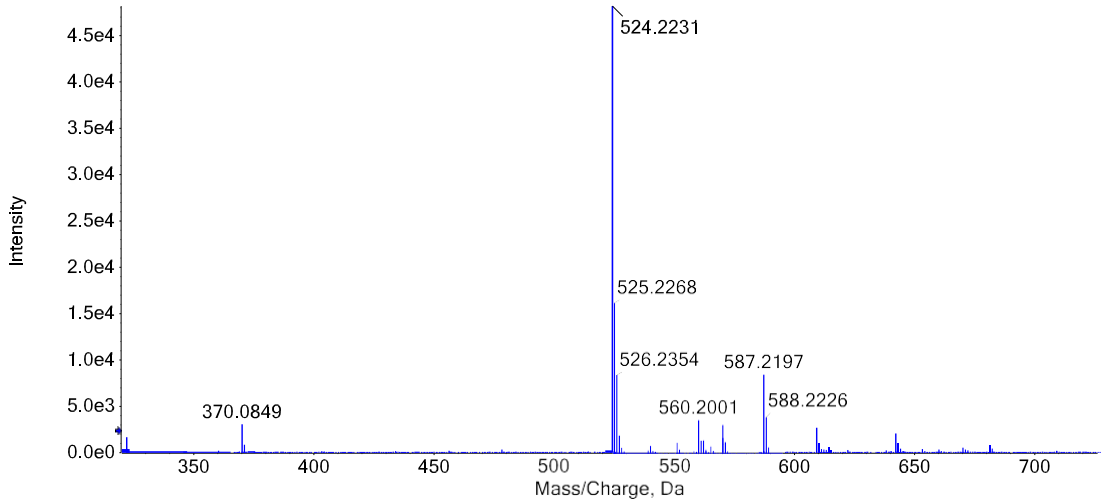


Fig. S24. HRMS spectrum of compound **L-10C.**

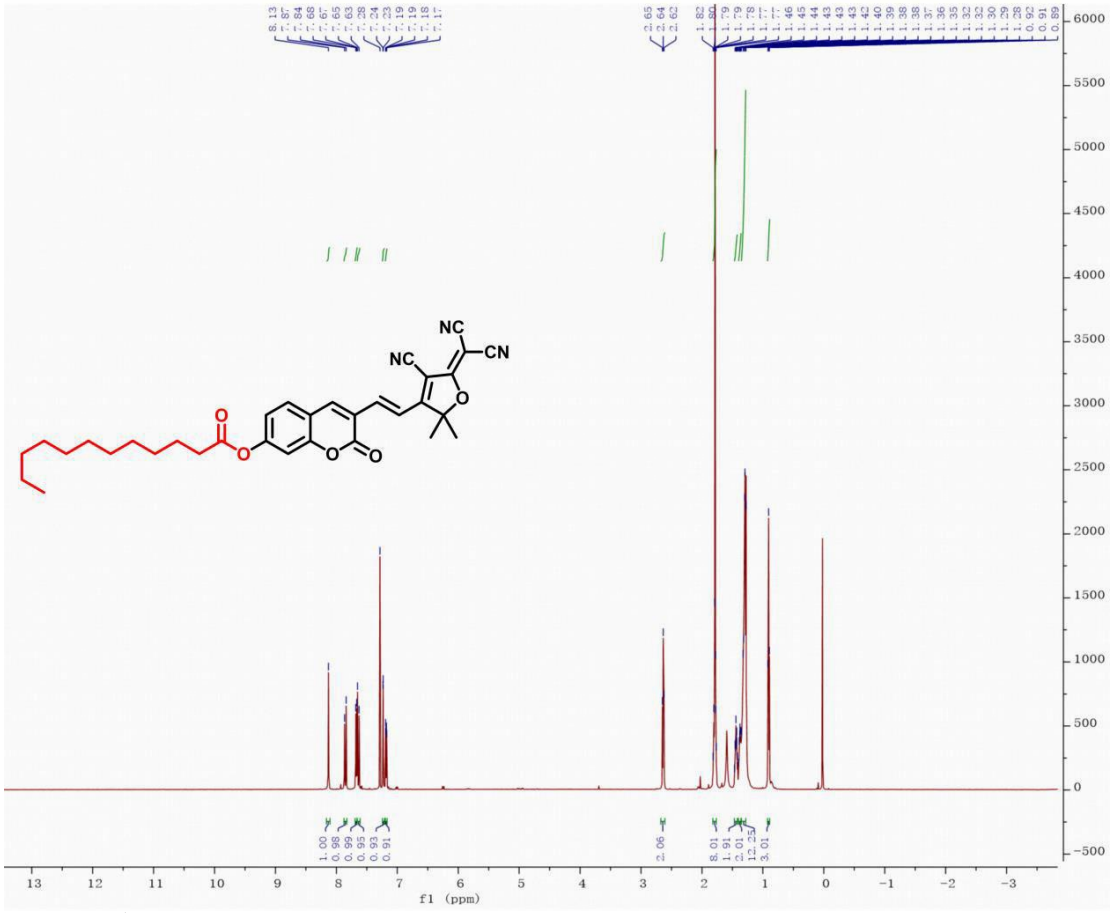


Fig. S25. ^{1}H NMR spectra of compound **L-12C.**

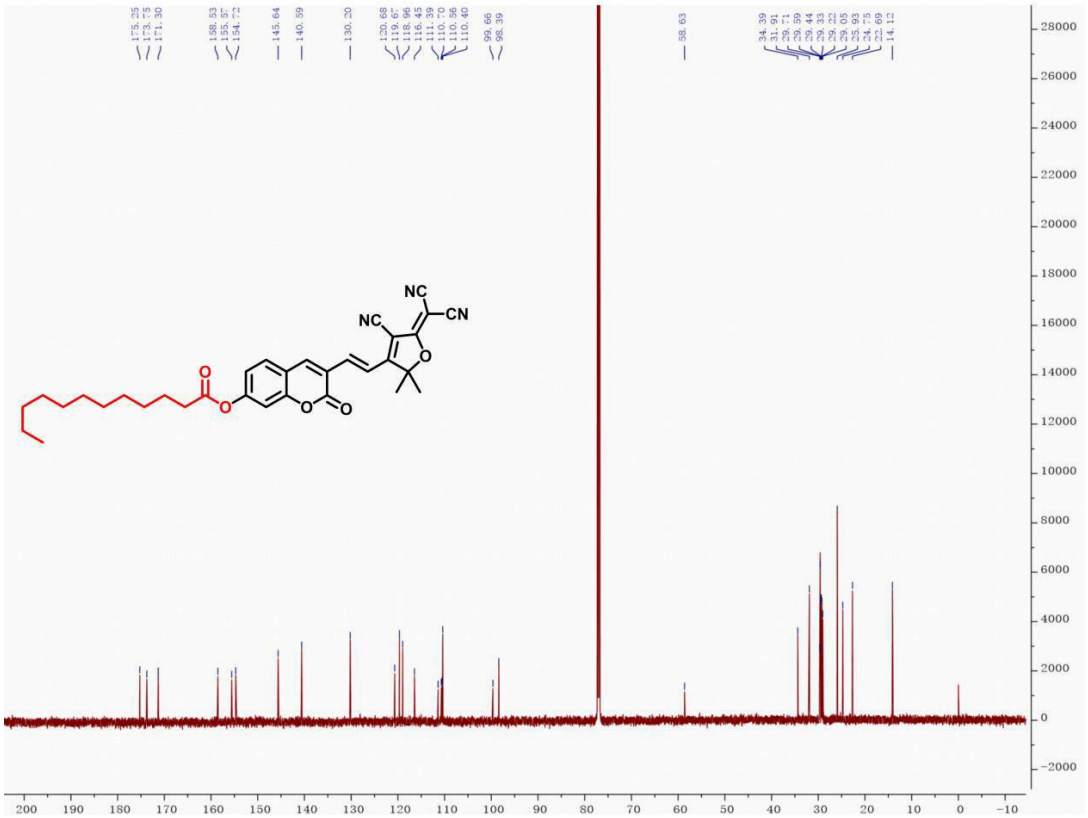


Fig. S26. [1]C NMR spectra of compound **L-12C.**

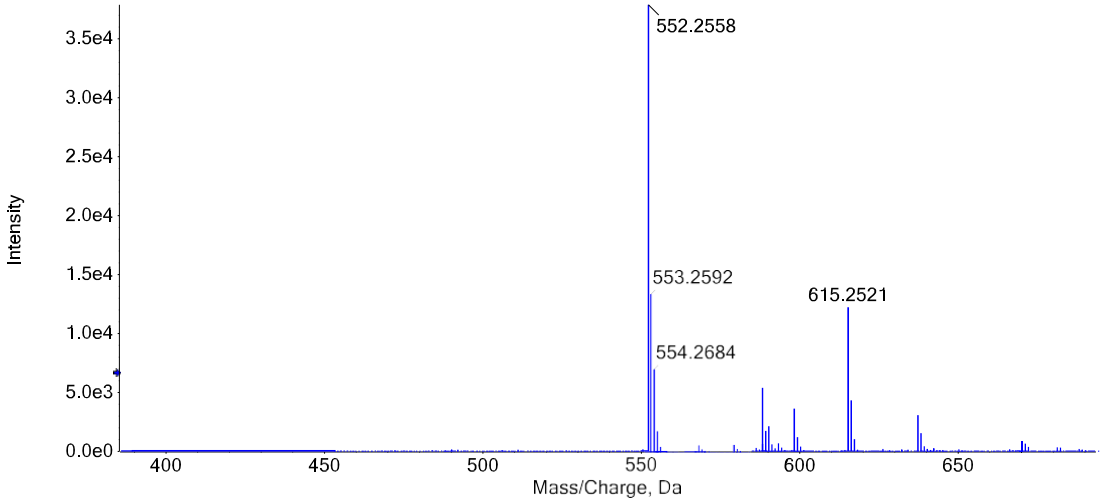


Fig. S27. HRMS spectrum of compound **L-12C**.

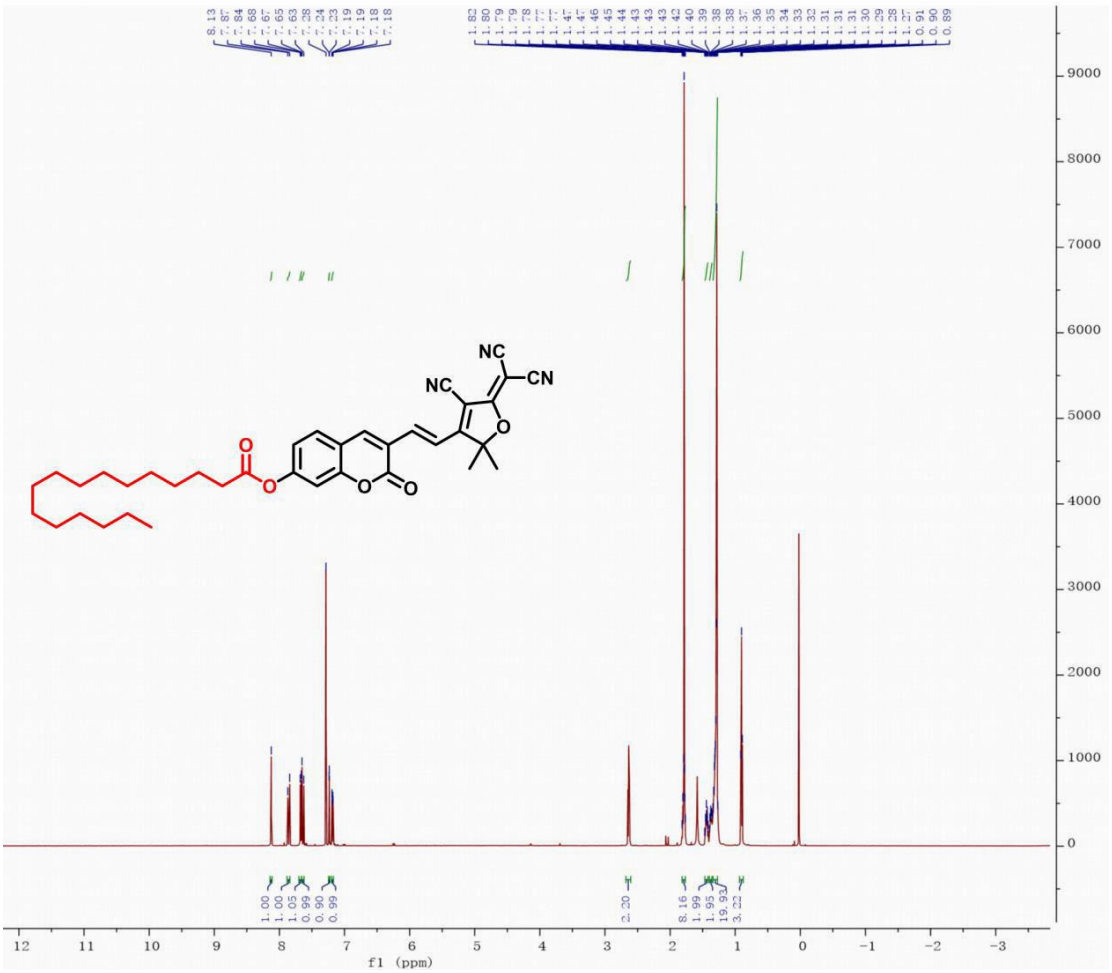


Fig. S28. [1]H NMR spectra of compound **L-16C.**

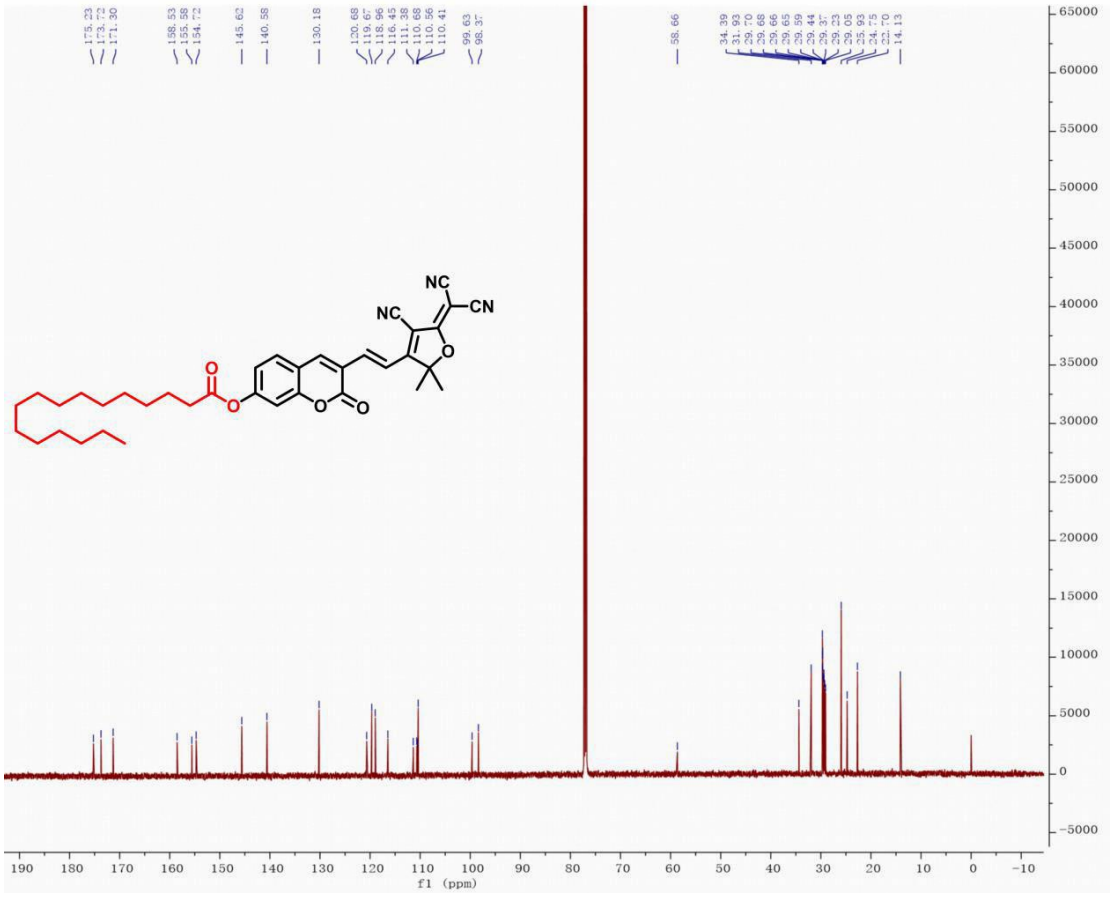


Fig. S29. ^{1}C NMR spectra of compound **L-16C.**

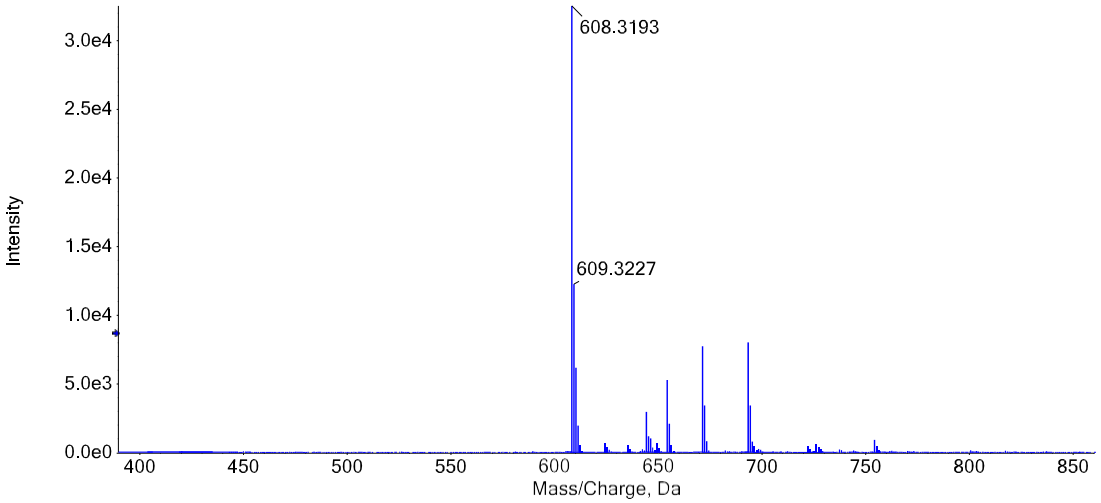


Fig. S30. HRMS spectrum of compound **L-16C**.

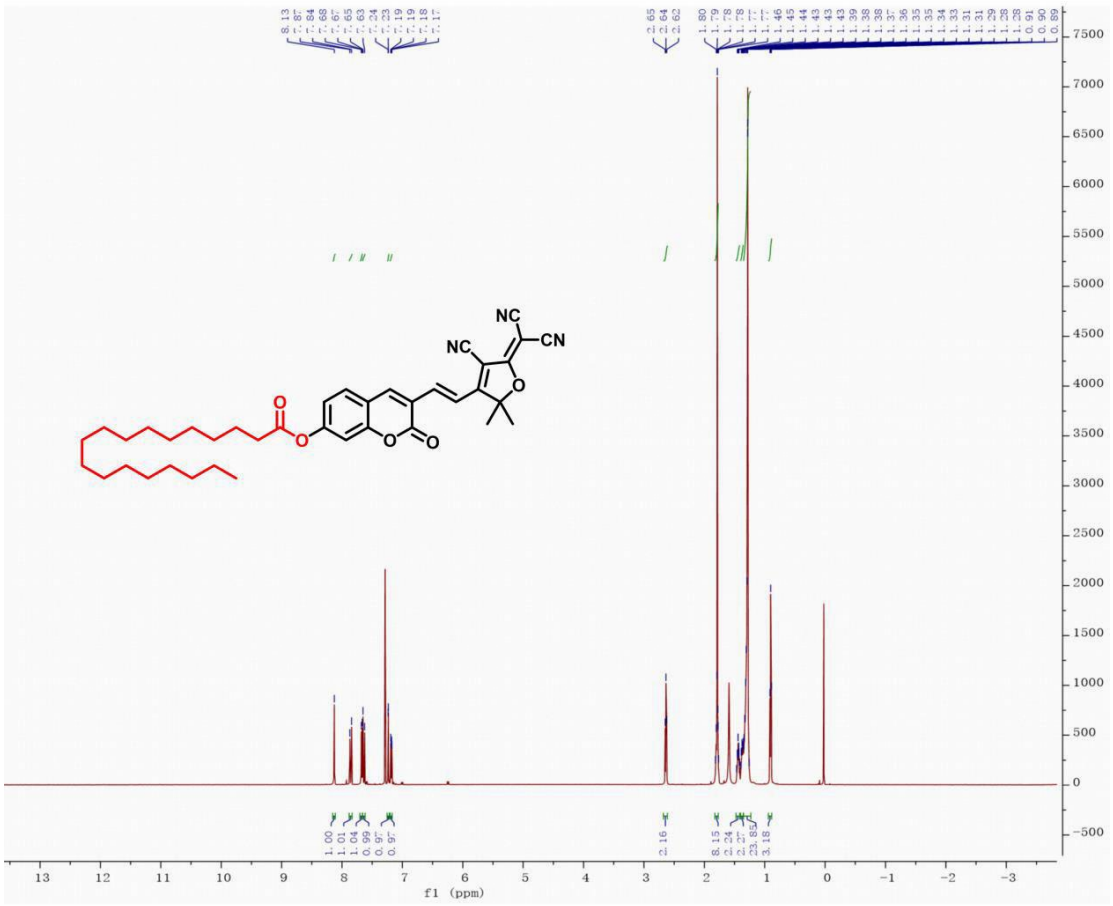


Fig. S31. ^{1}H NMR spectra of compound **L-18C.**

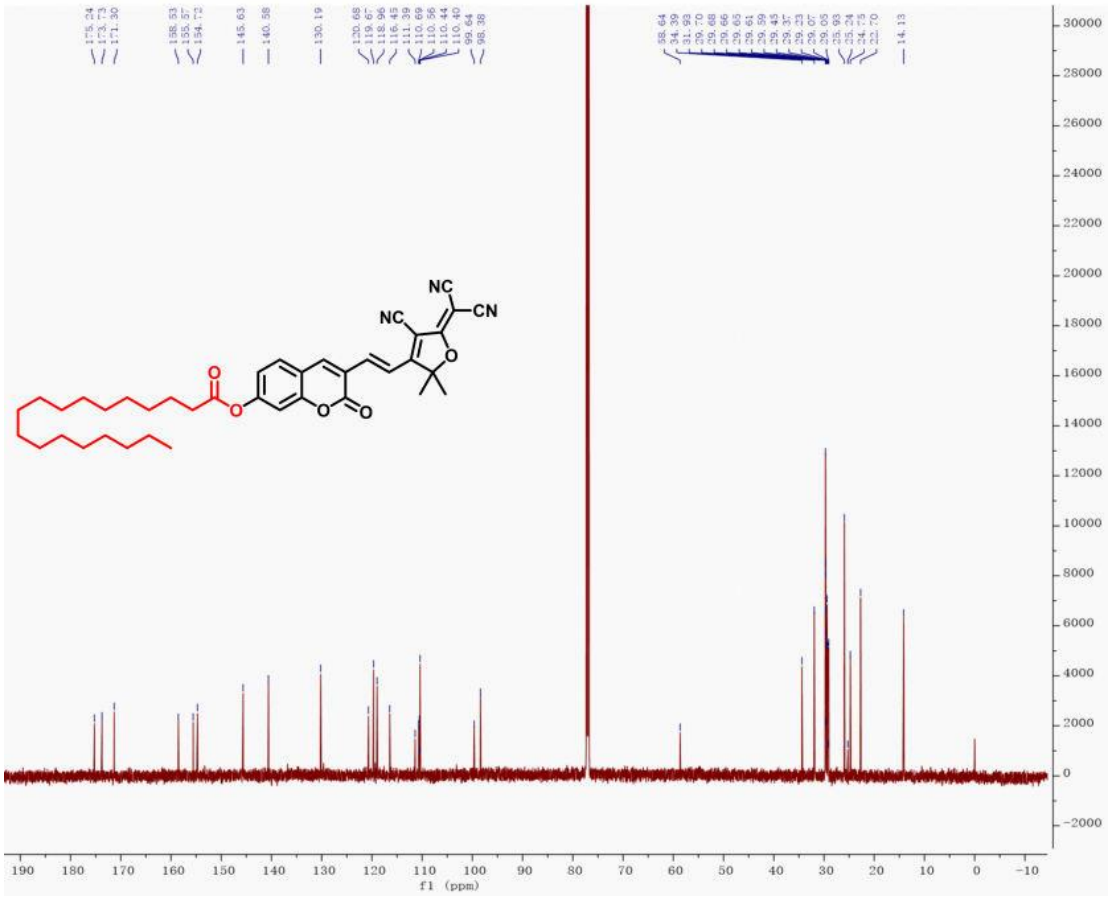


Fig. S32. ^{1}C NMR spectra of compound **L-18C.**

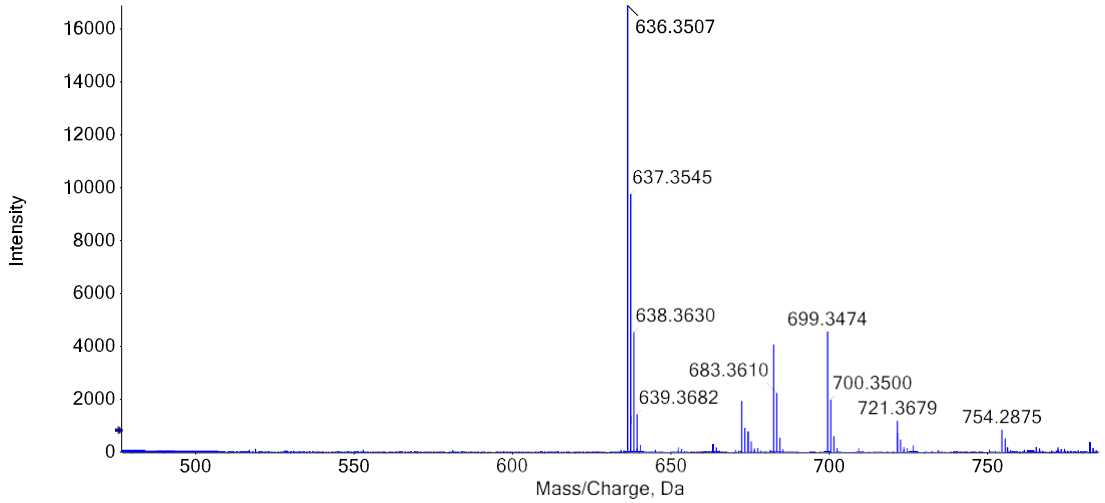


Fig. S33. HRMS spectrum of compound **L-18C.**

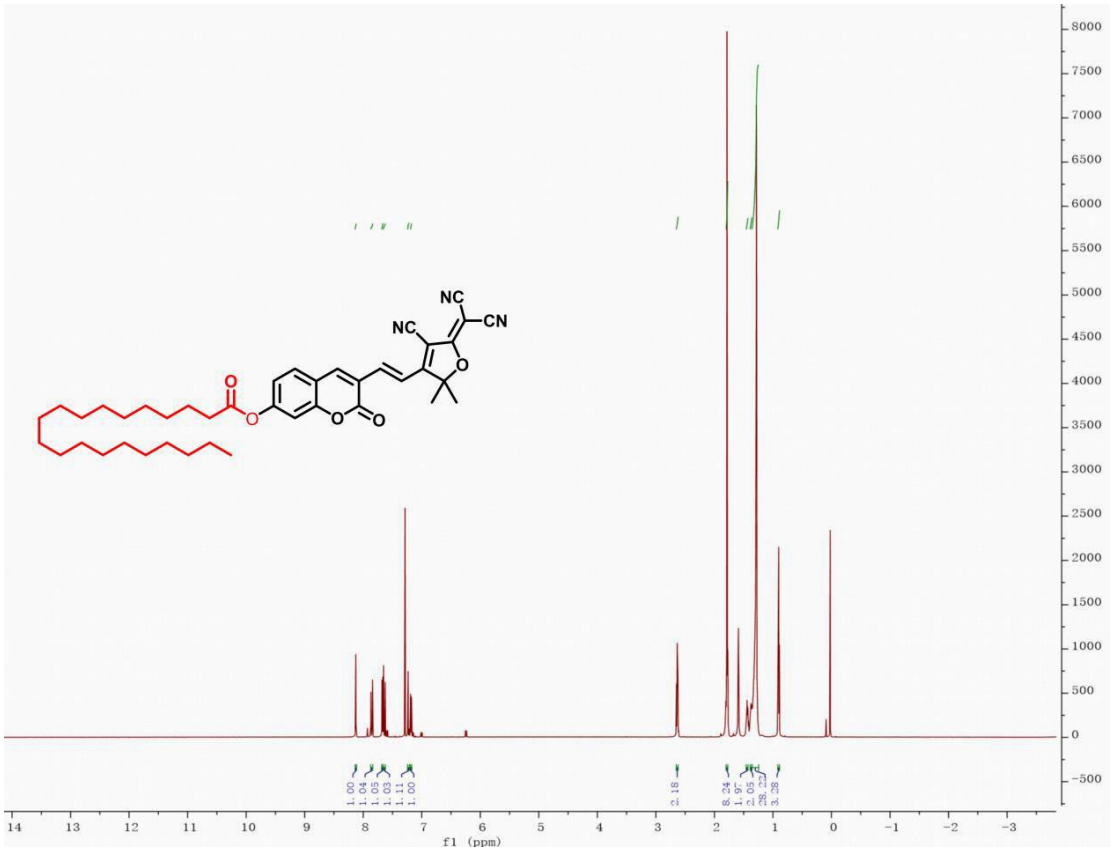


Fig. S34. ^{1}H NMR spectra of compound **L-22C.**

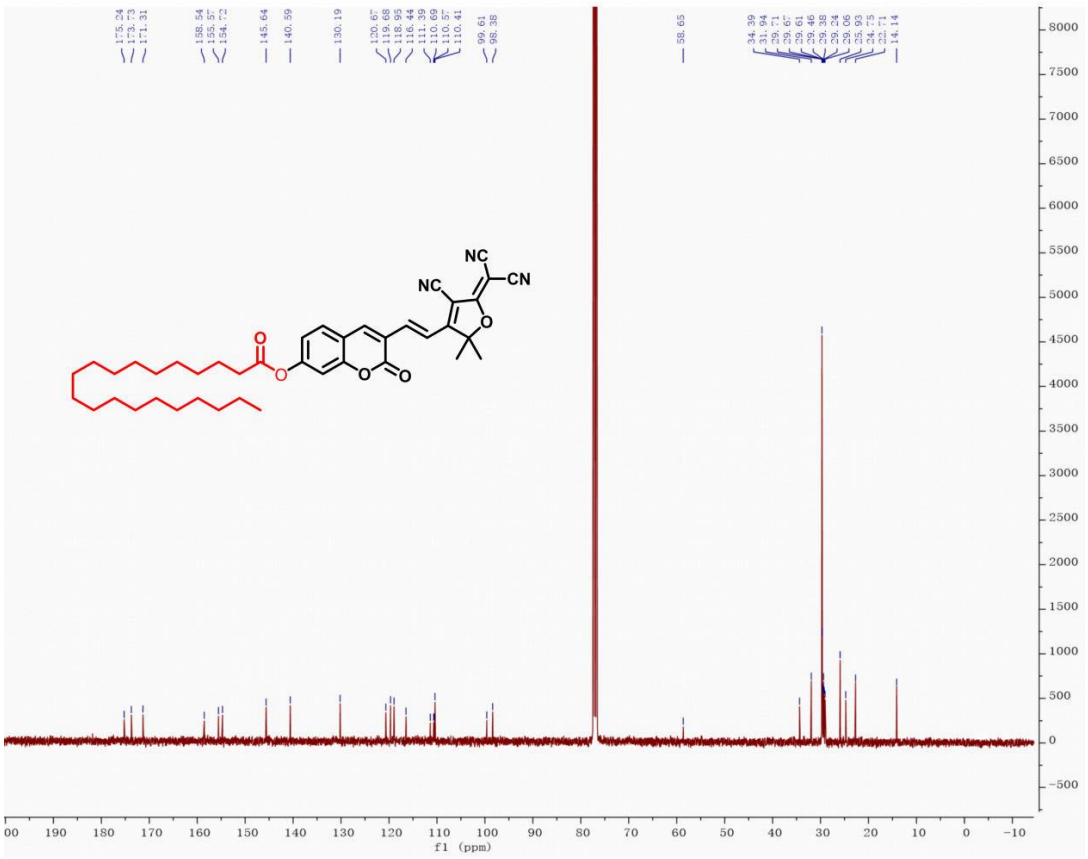


Fig. S35. [1]C NMR spectra of compound **L-22C.**

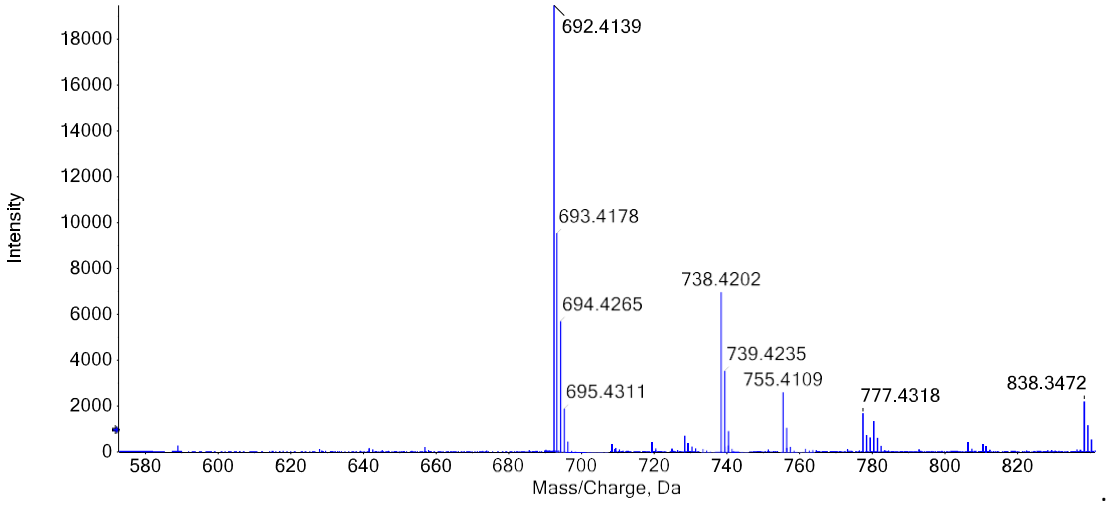


Fig. S36. HRMS spectrum of compound **L-22C.**

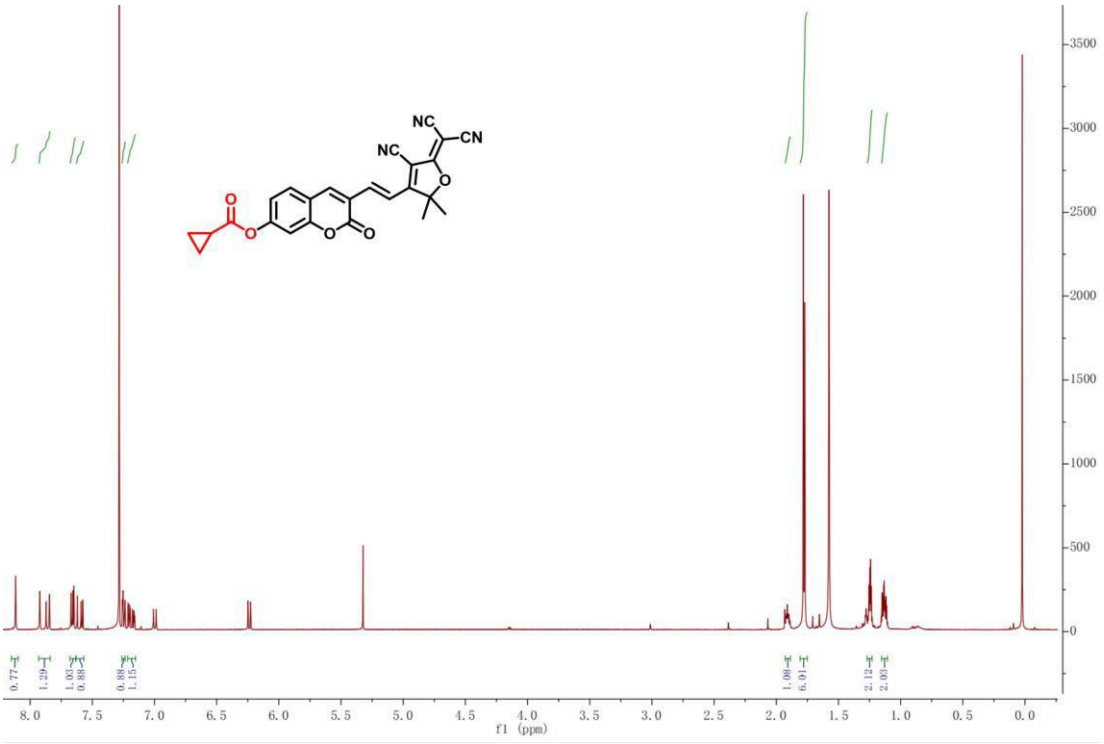


Fig. S37. [1]H NMR spectrum of compound **L-CP.**

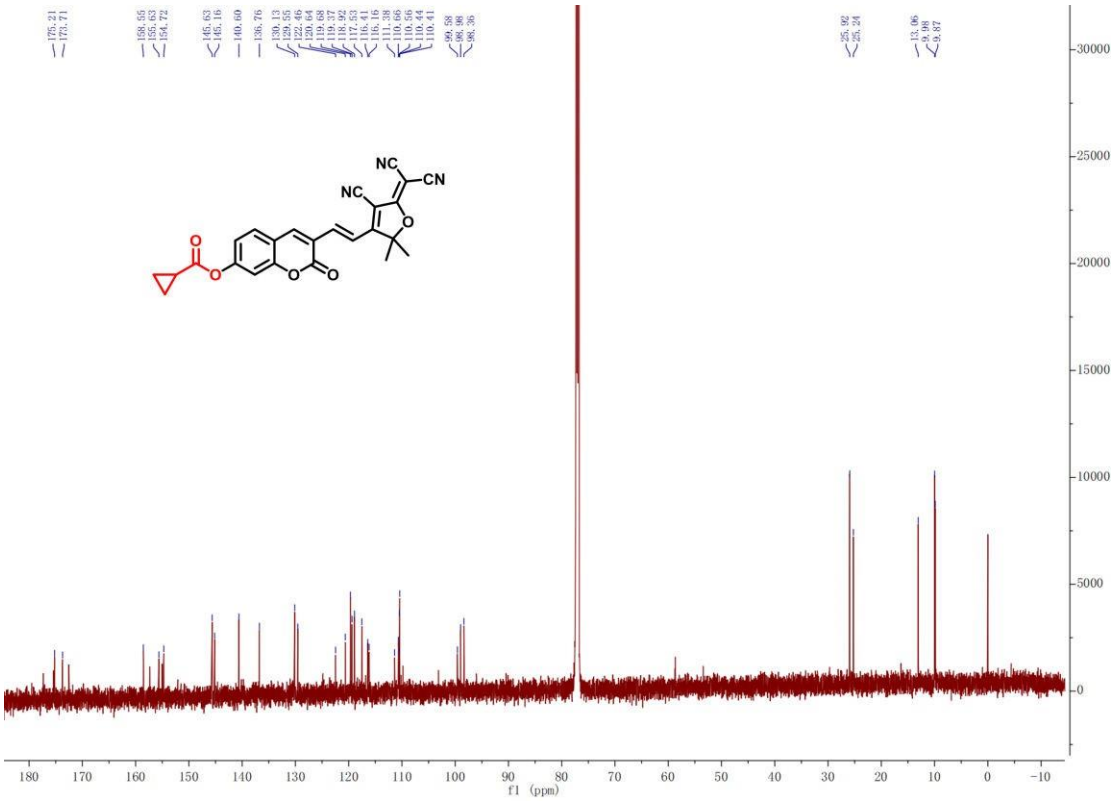


Fig. S38. [1]C NMR spectrum of compound **L-CP.**

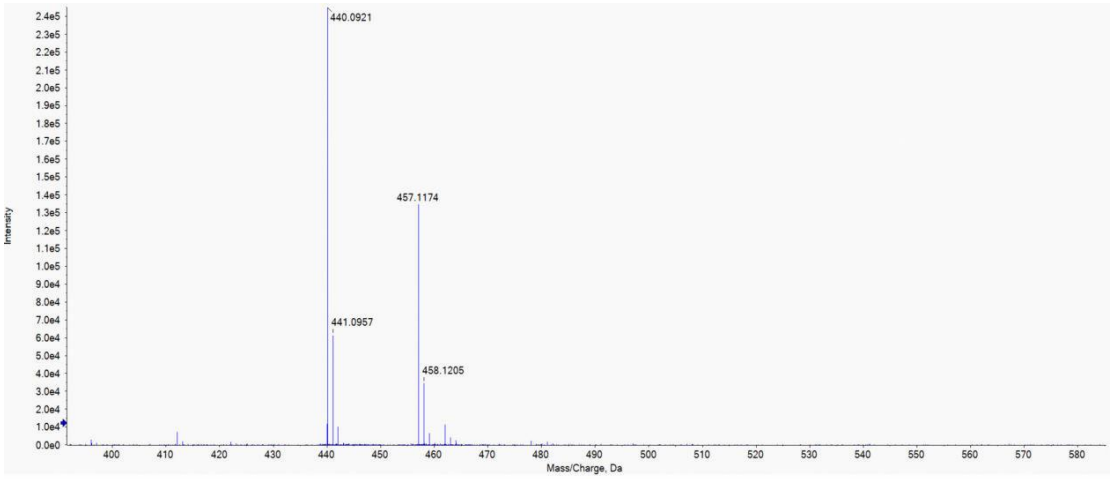


Fig. S39. [1]C HRMS spectrum of compound **L-CP.**

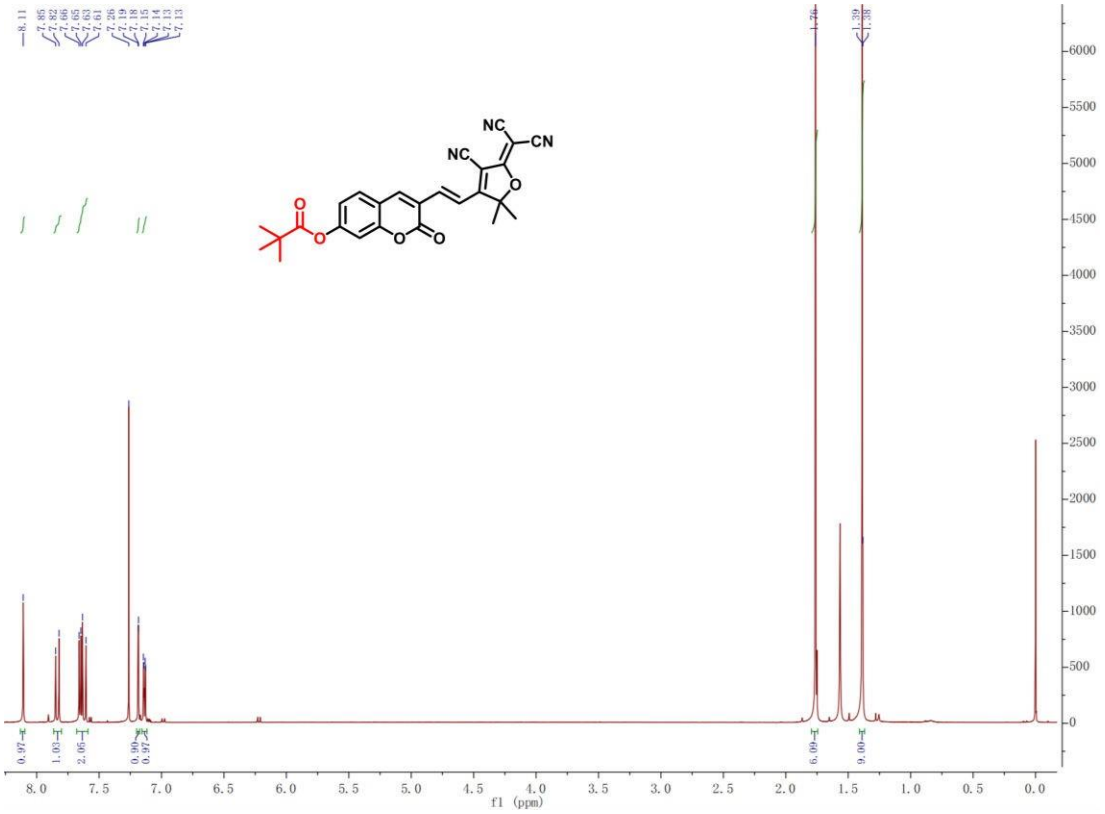


Fig. S40. [1]H NMR spectrum of compound **L-PV.**

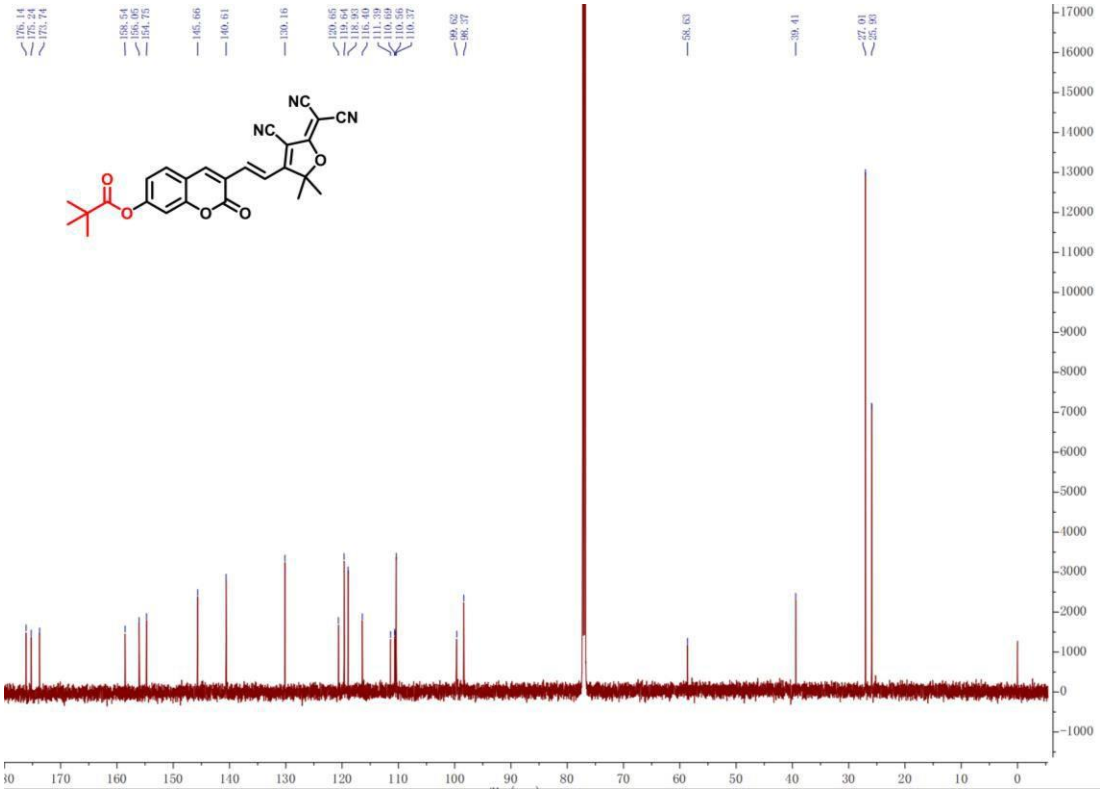


Fig. S41. [1]C NMR spectrum of compound **L-PV.**

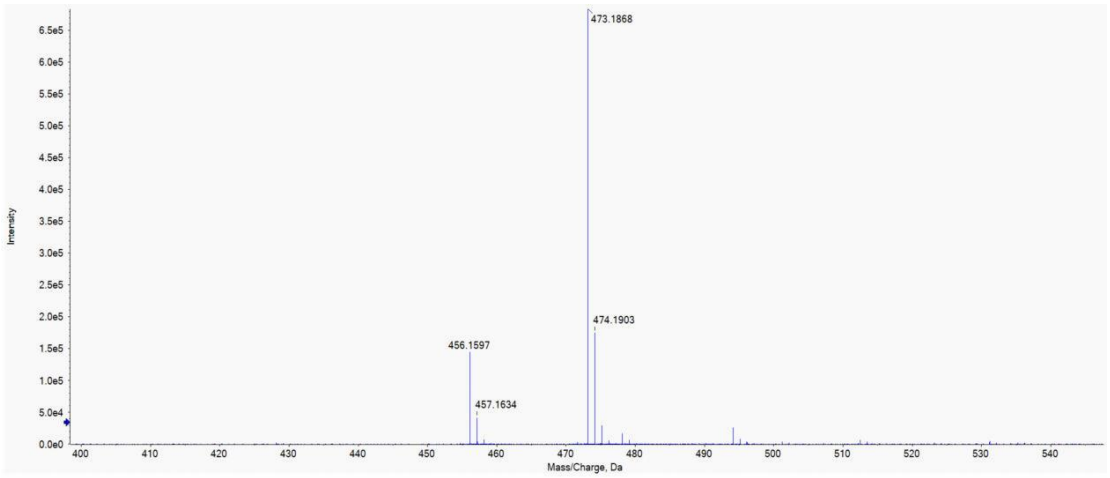


Fig. S42. HRMS spectrum of compound **L-PV.**

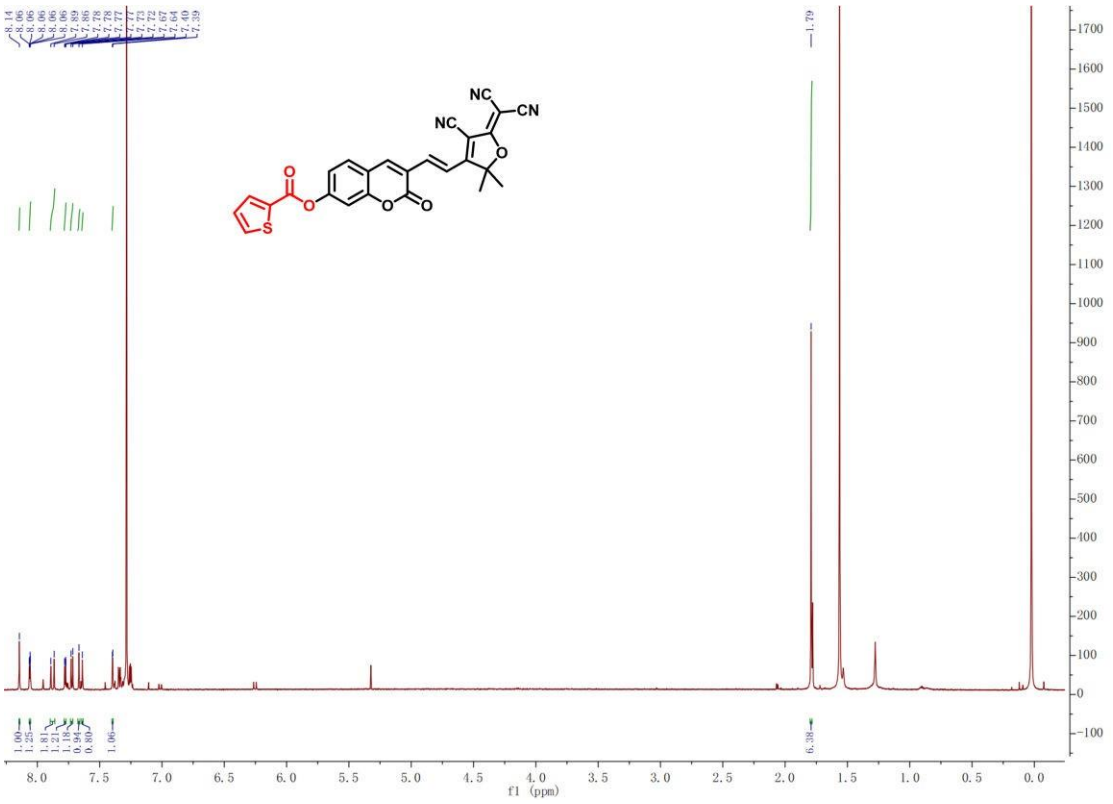


Fig. S43. ^{1}H NMR spectrum of compound **L-TH.**

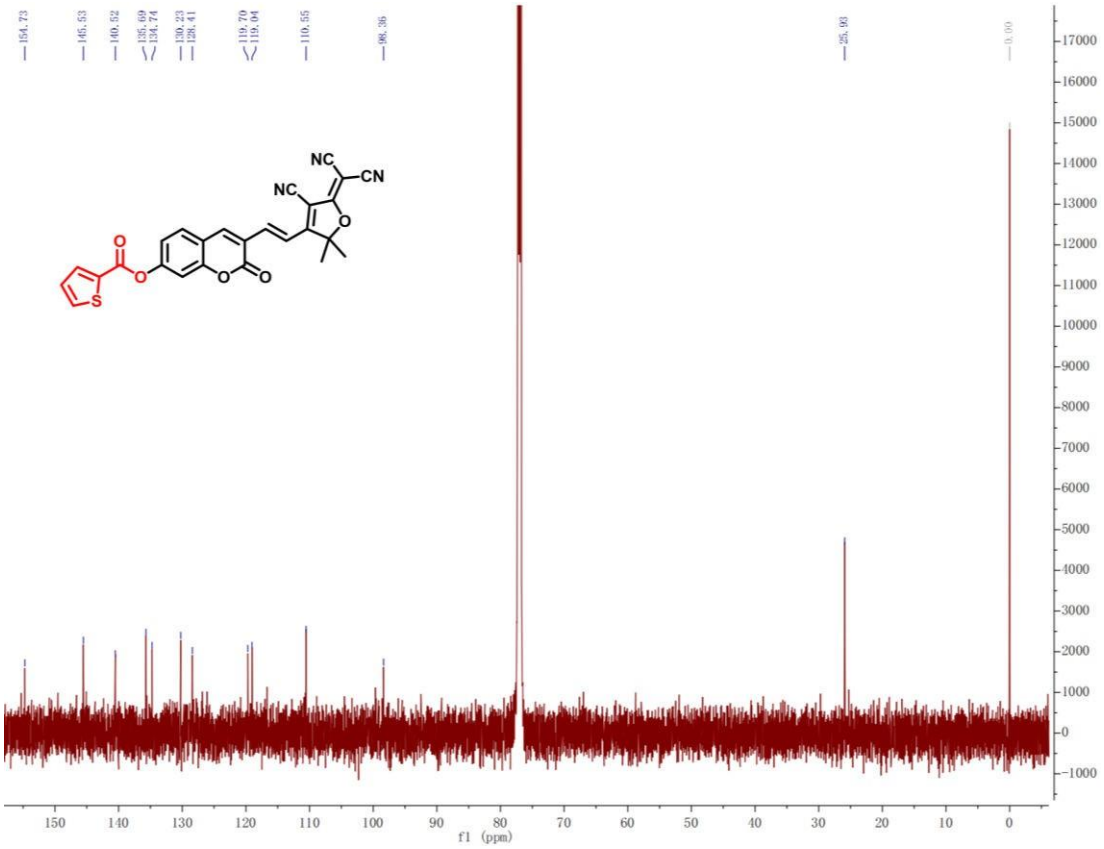


Fig. S44. [1]C NMR spectrum of compound **L-TH.**

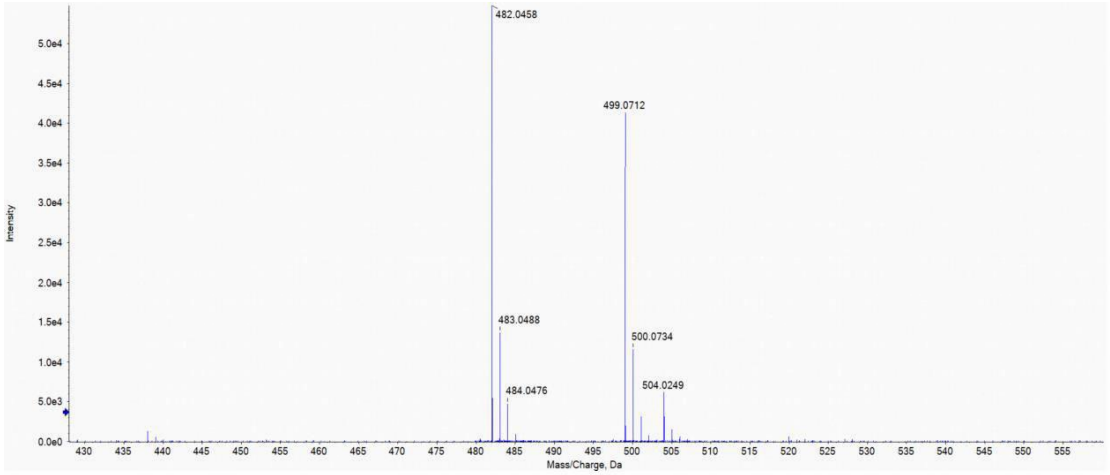


Fig. S45. HRMS spectrum of compound **L-TH.**

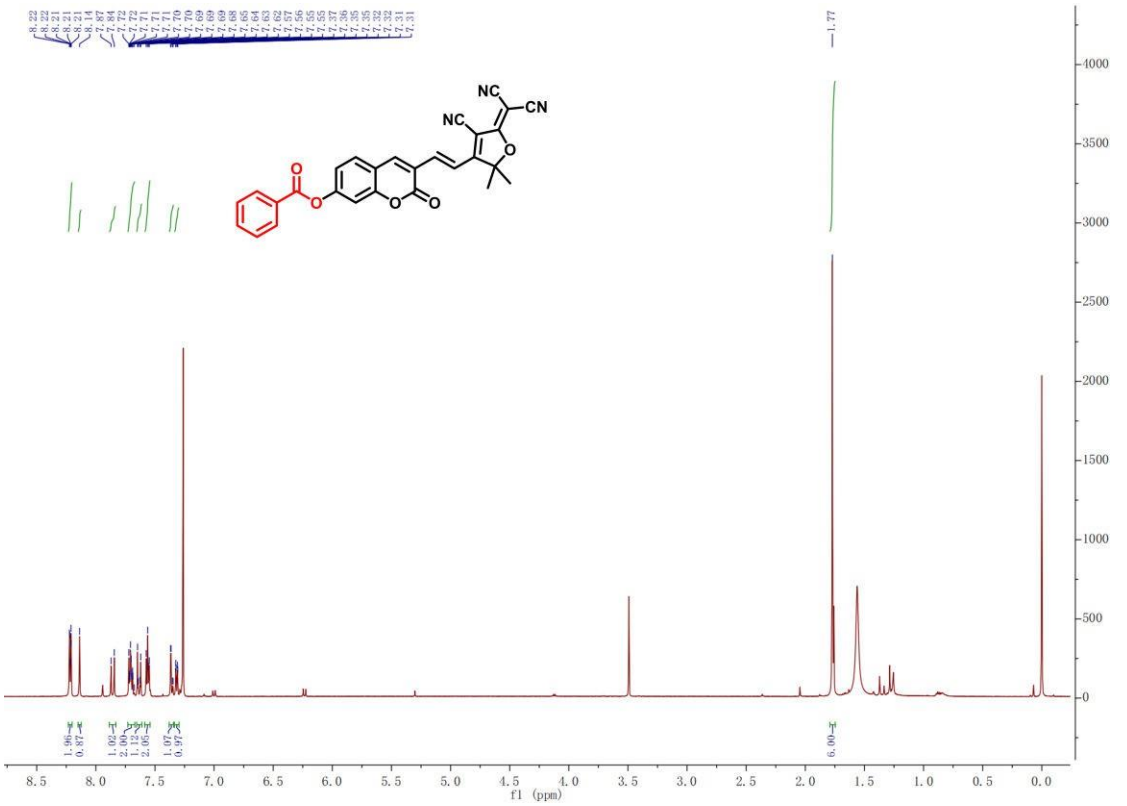


Fig. S46. ^{1}H NMR spectrum of compound **L-Ph.**

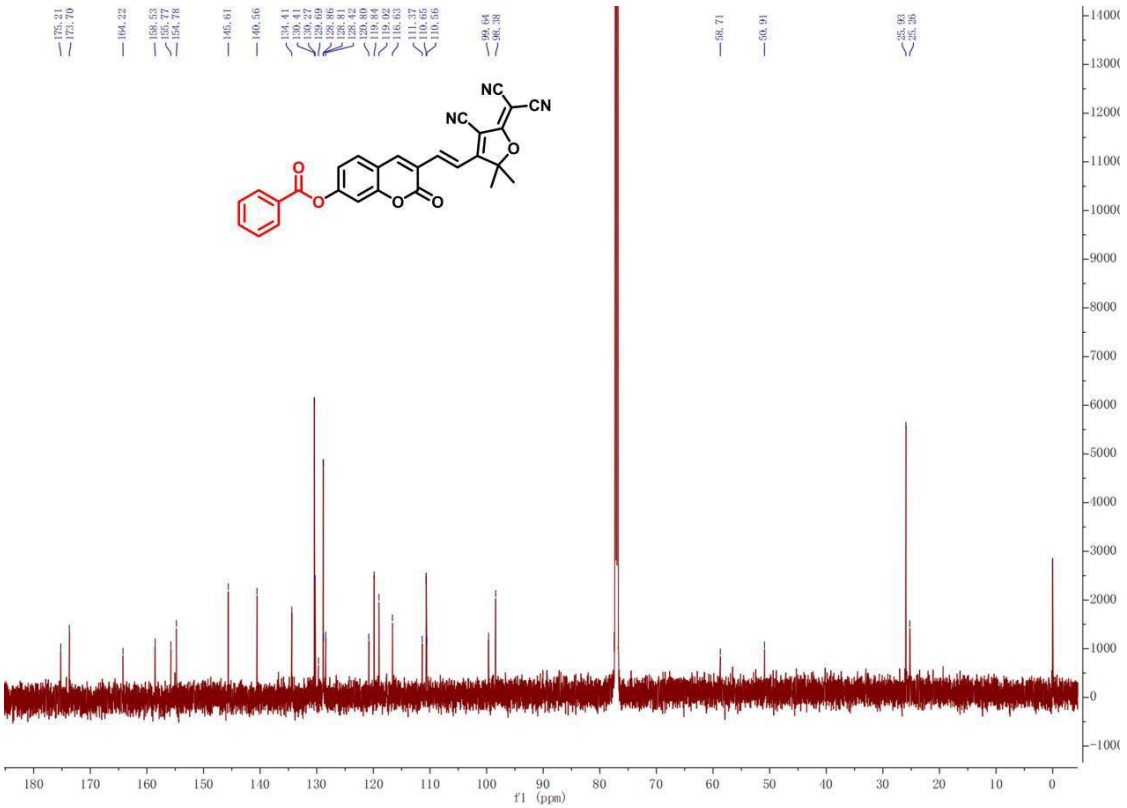


Fig. S47. ^{1}C NMR spectrum of compound **L-Ph.**

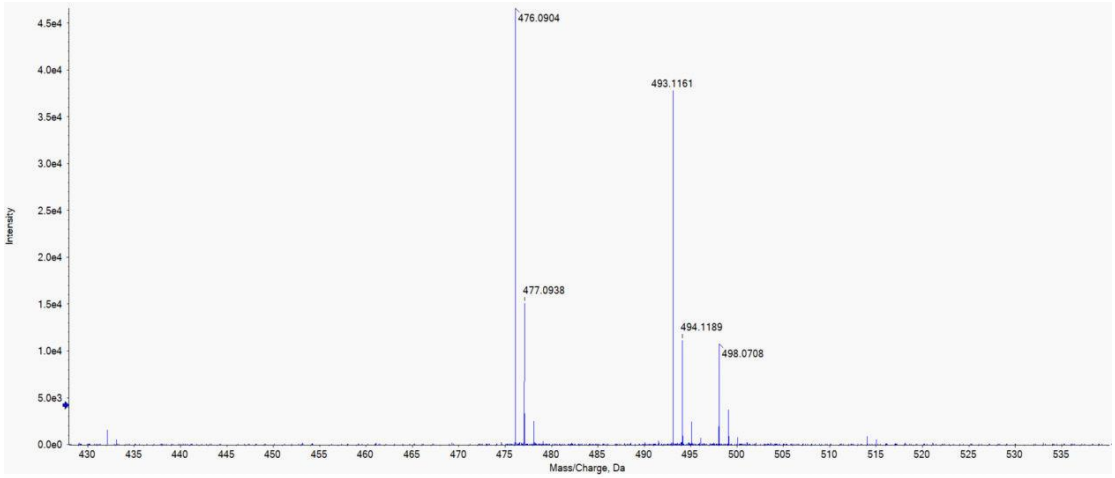


Fig. S48. HRMS spectrum of compound **L-Ph.**

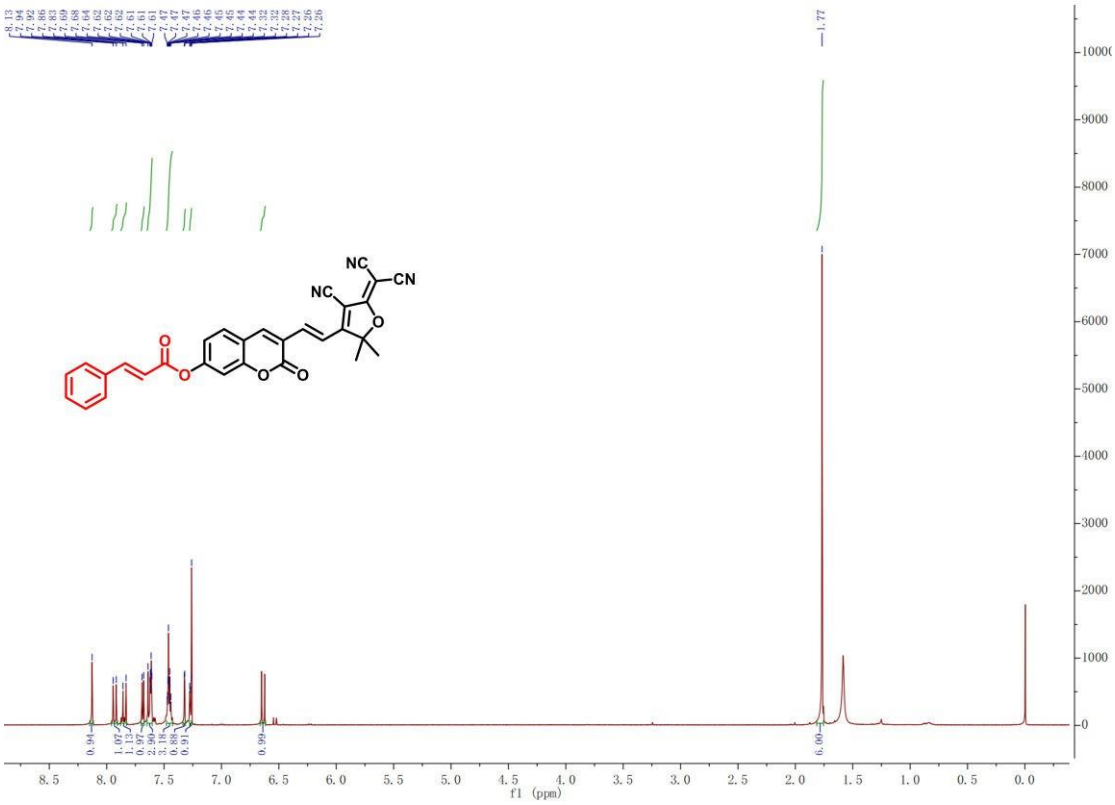


Fig. S49. ^{1}H NMR spectrum of compound **L-Ci.**

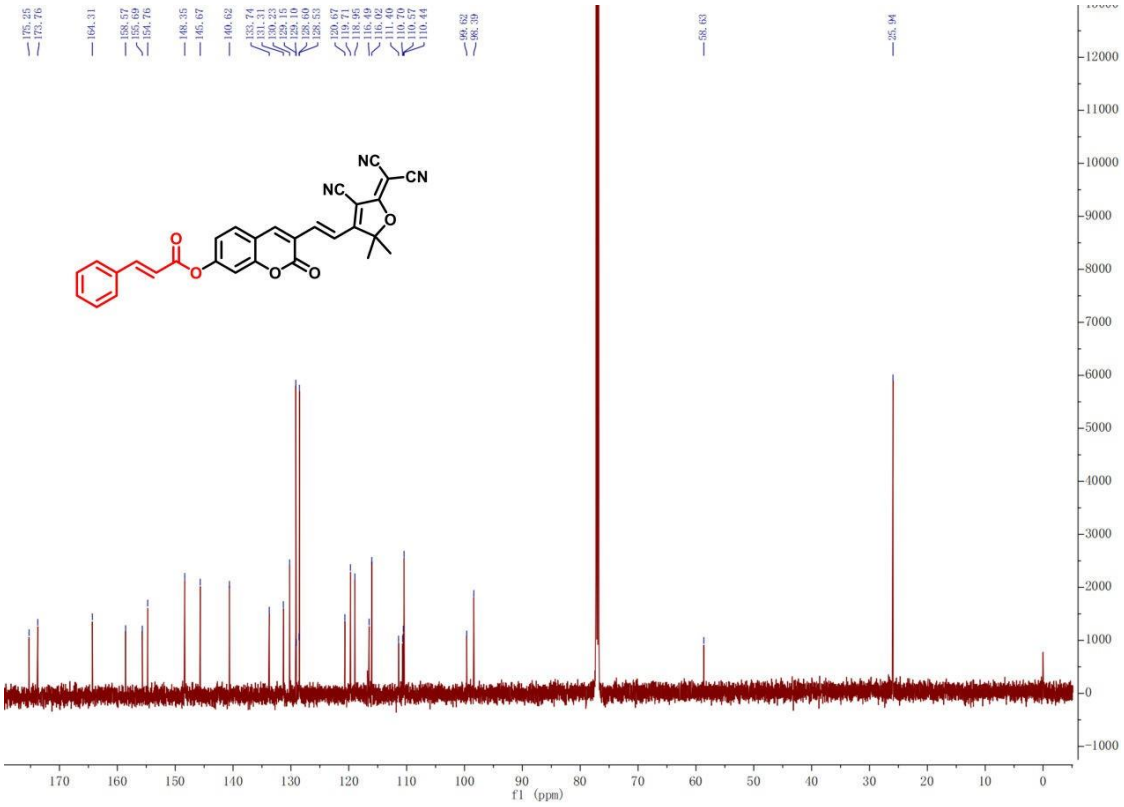


Fig. S50. ^{1}C NMR spectrum of compound **L-Ci.**

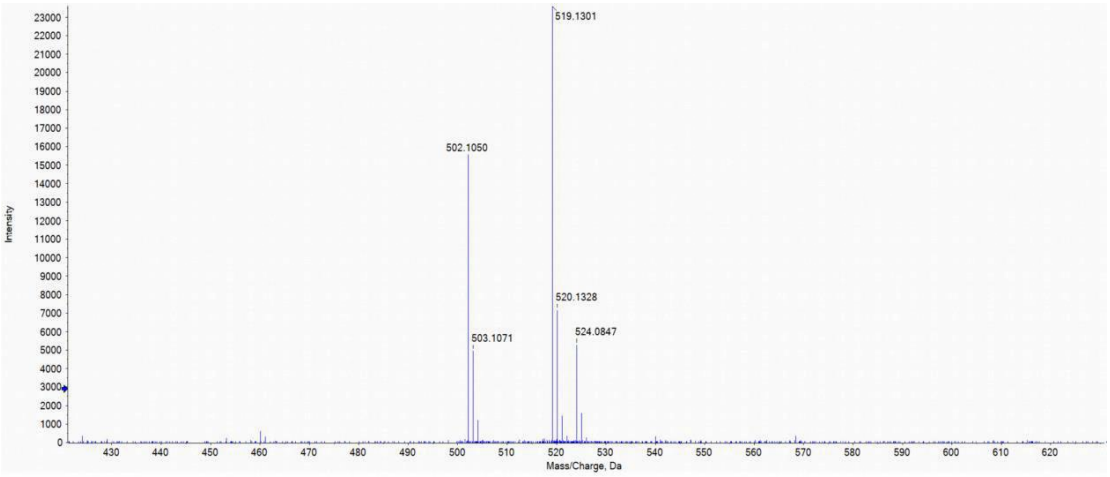


Fig. S51. HRMS spectrum of compound **L-Ci.**

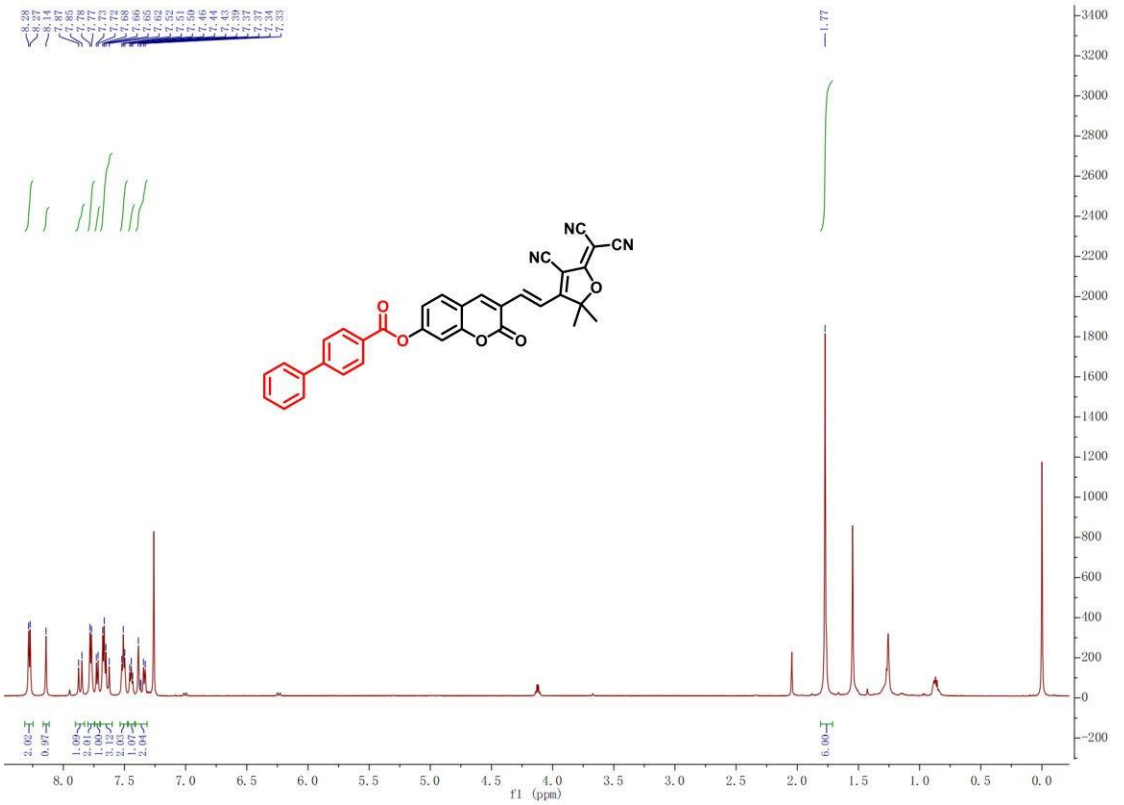


Fig. S52. ^{1}H NMR spectrum of compound **L-BB.**

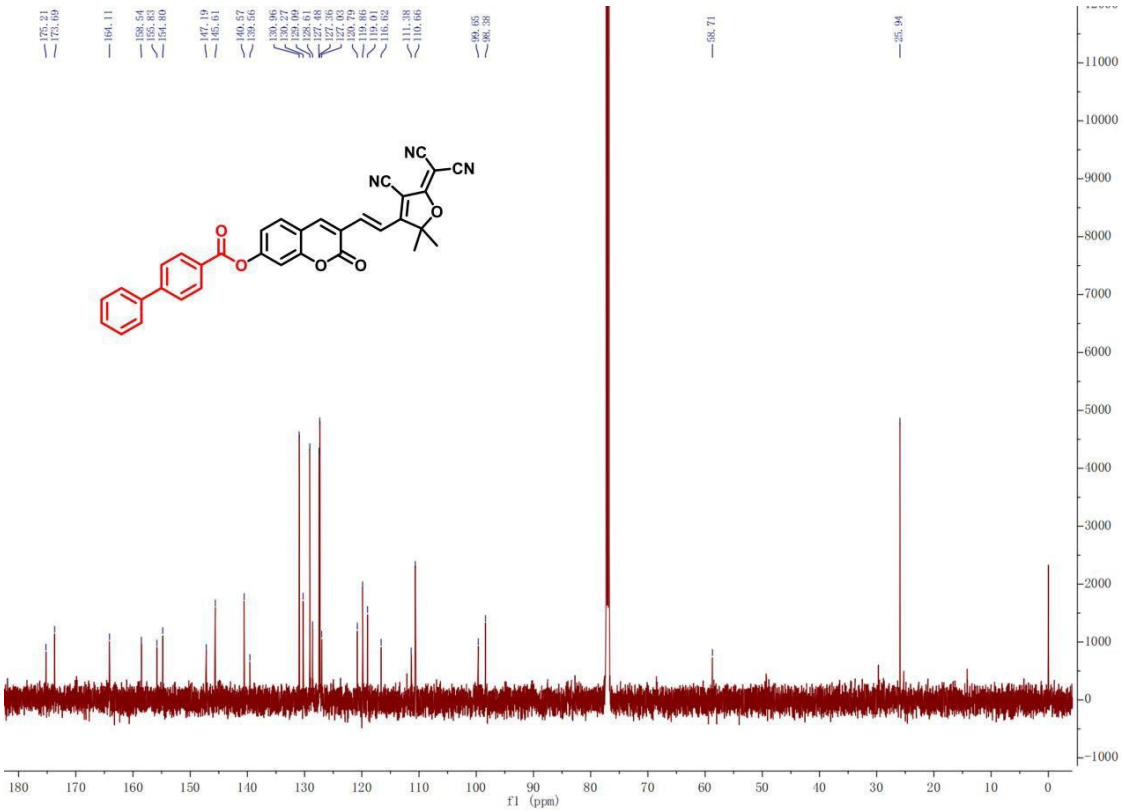


Fig. S53. ^{1}C NMR spectrum of compound **L-BB.**

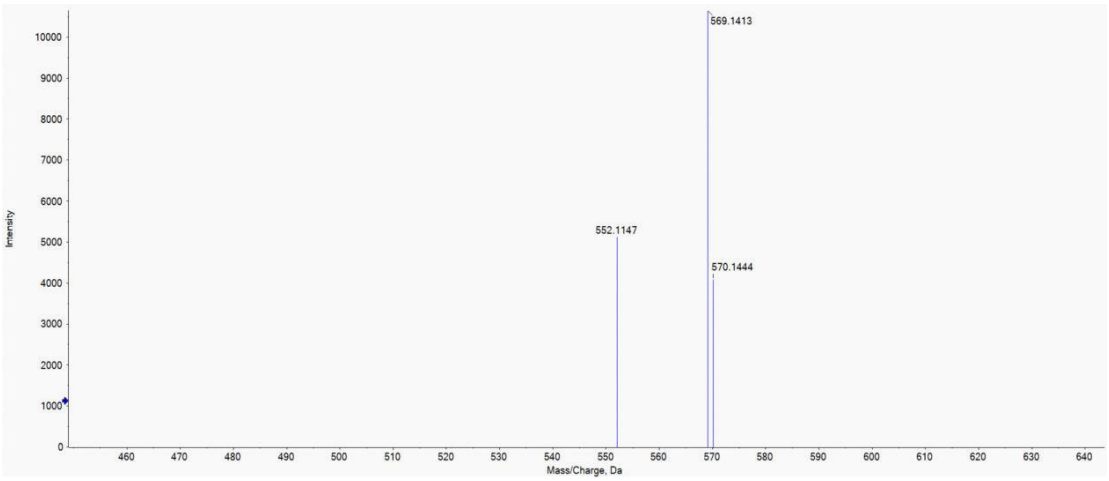


Fig. S54. HRMS spectrum of compound **L-BB.**

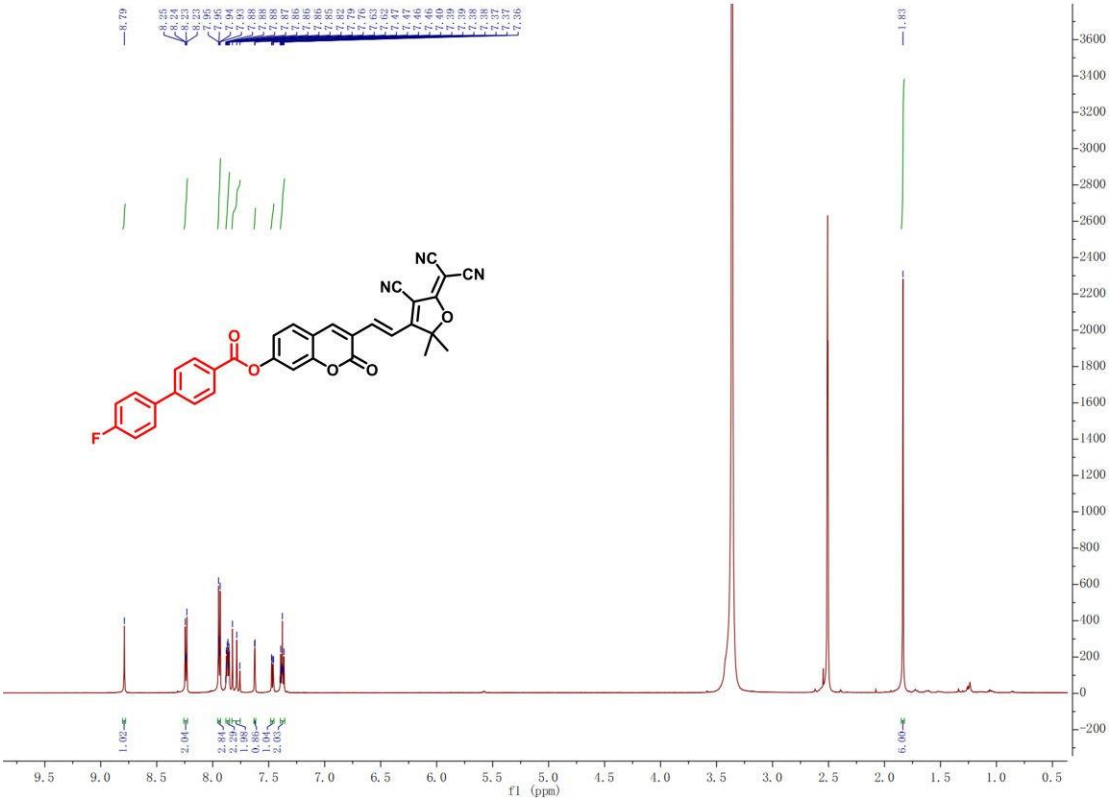


Fig. S55. ^{1}H NMR spectrum of compound **L-FB.**

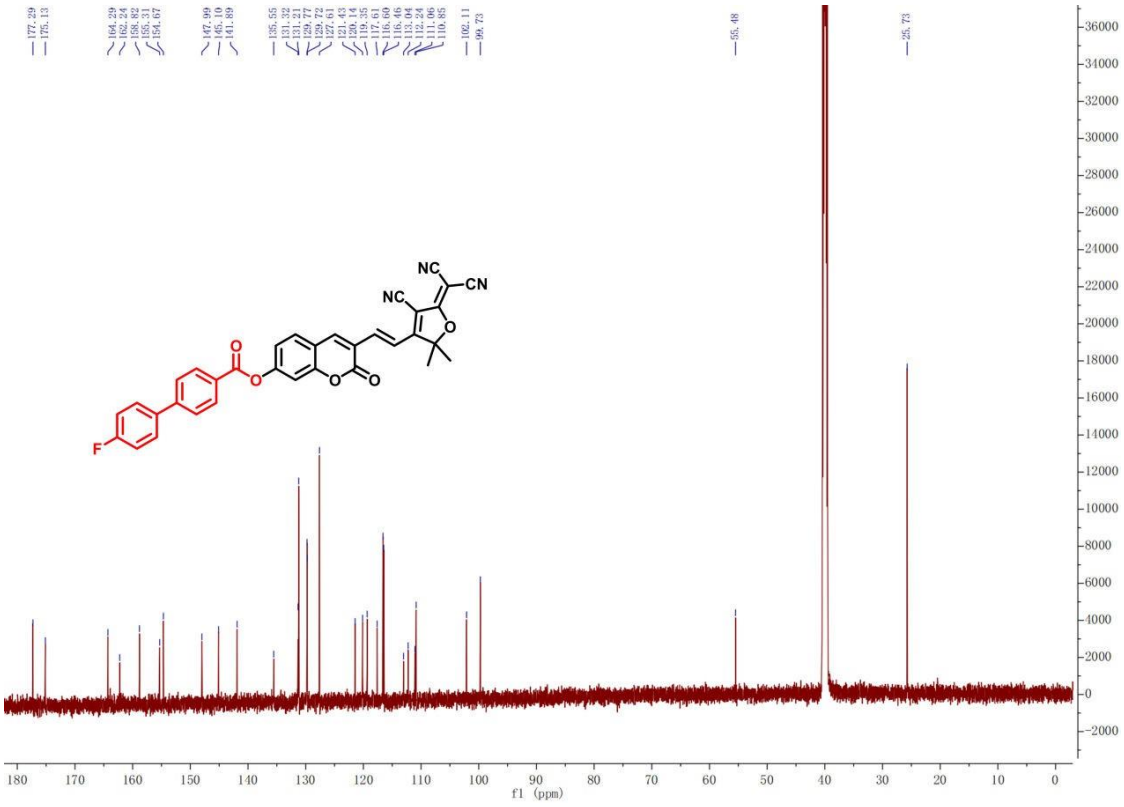


Fig. S56. [1]C NMR spectrum of compound **L-FB.**

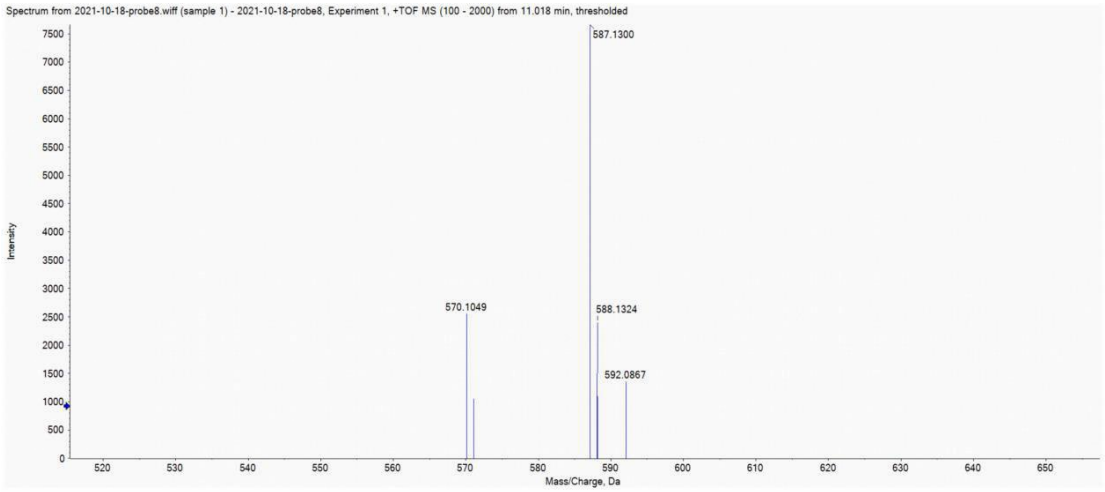


Fig. S57. HRMS spectrum of compound **L-FB.**

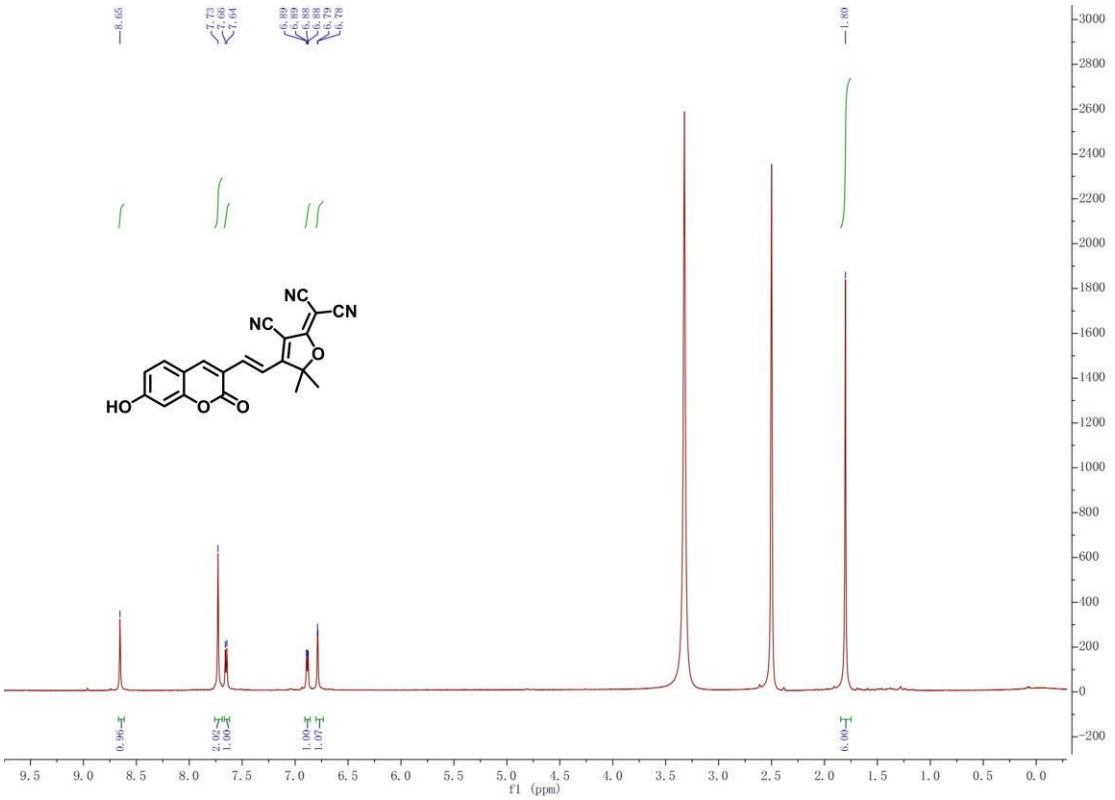


Fig. S58. [1]H NMR spectrum of compound **7-HTCF.**

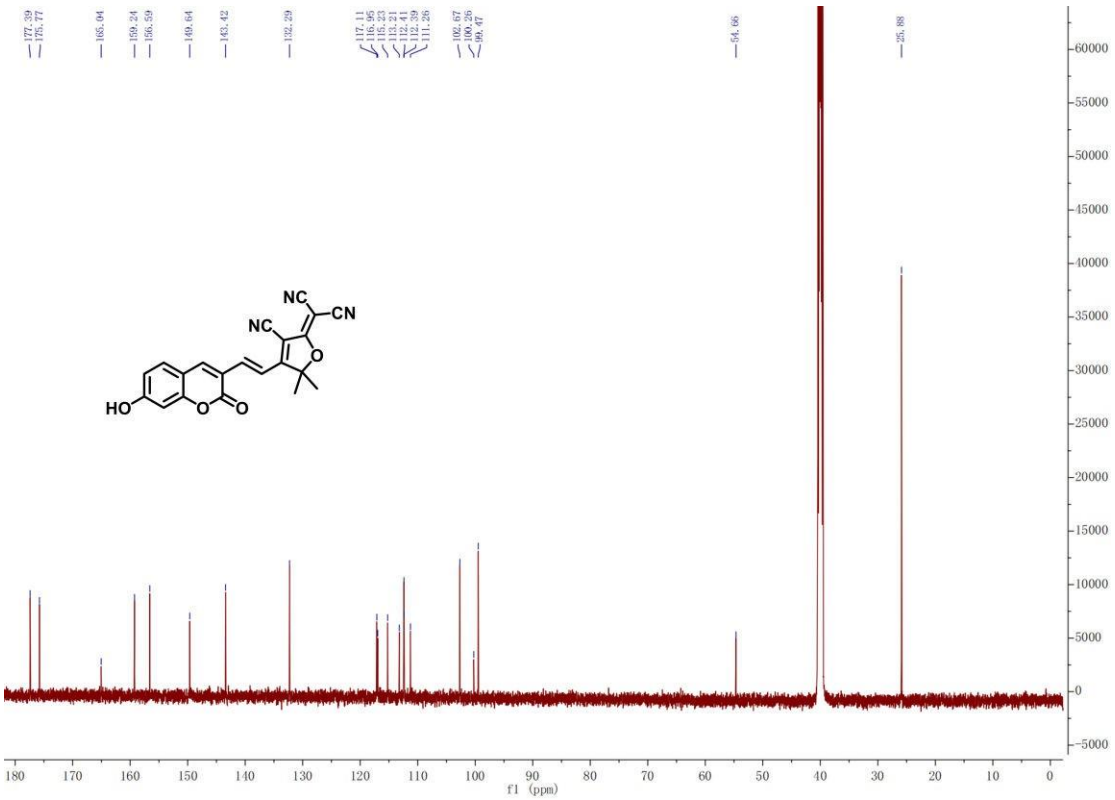


Fig. S59. [1]C NMR spectrum of compound **7-HTCF.**

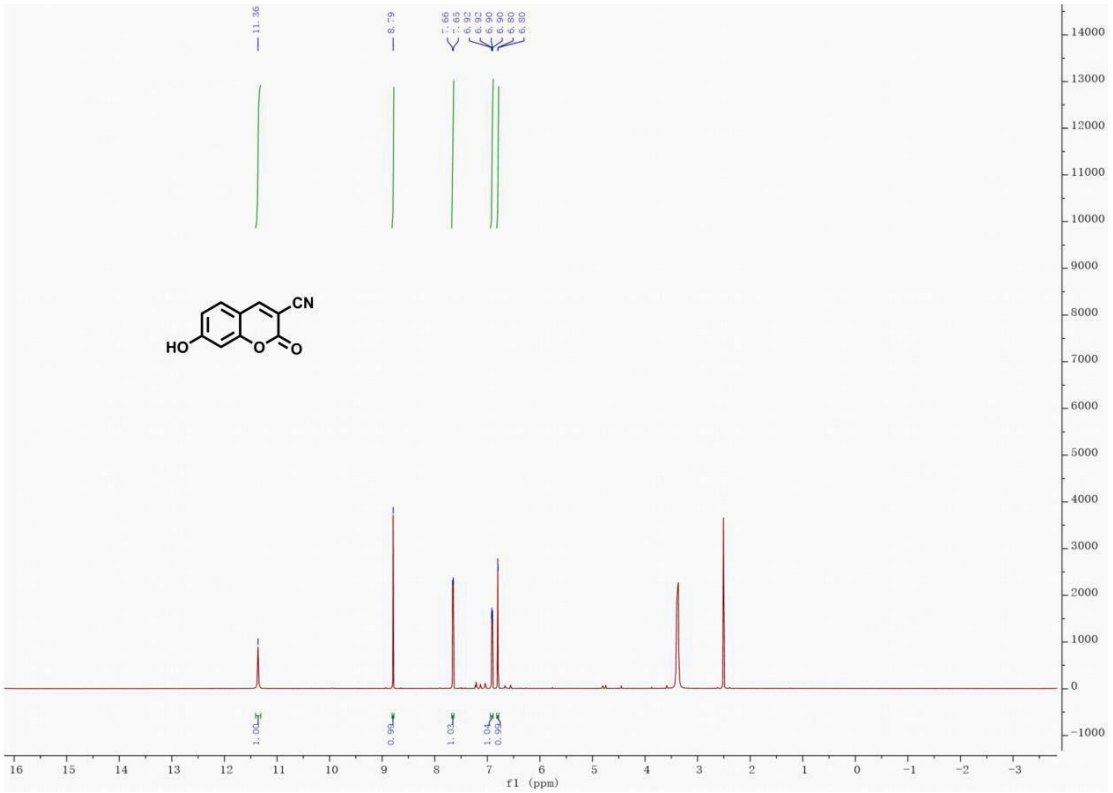


Fig. S60. [1]H NMR spectrum of compound **Compound 2**.

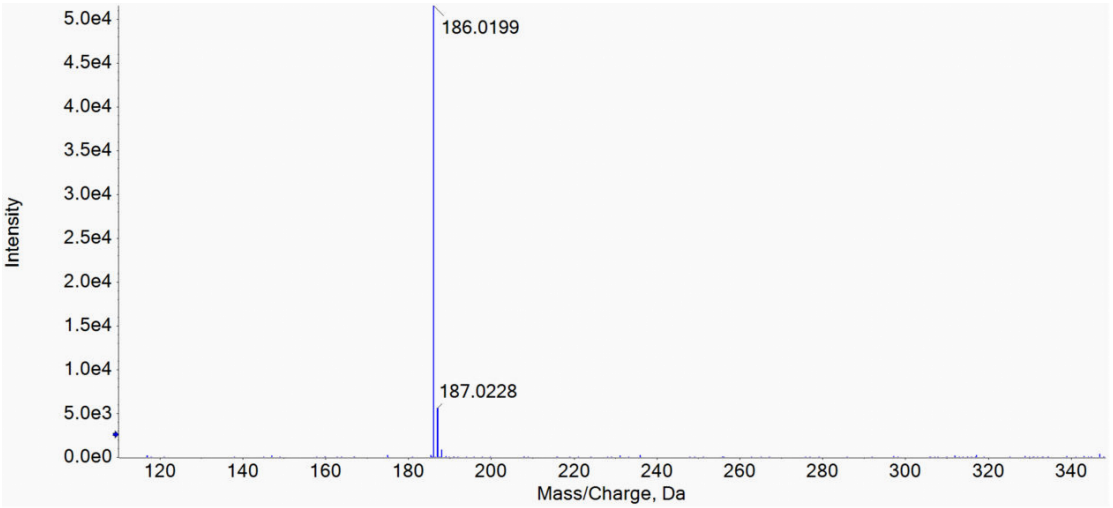


Fig. S61. HRMS spectrum of **Compound 2**.

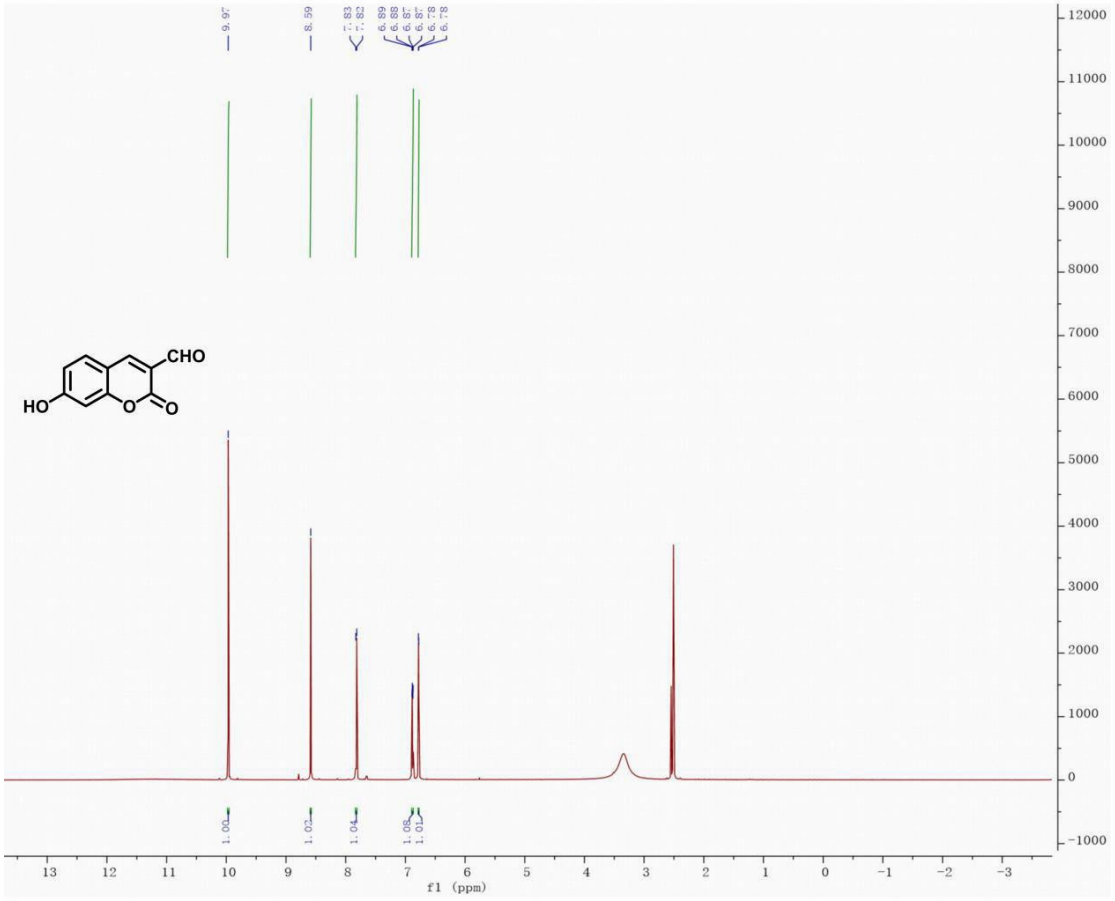


Fig. S62. [1]H NMR spectrum of compound **Compound 3**.

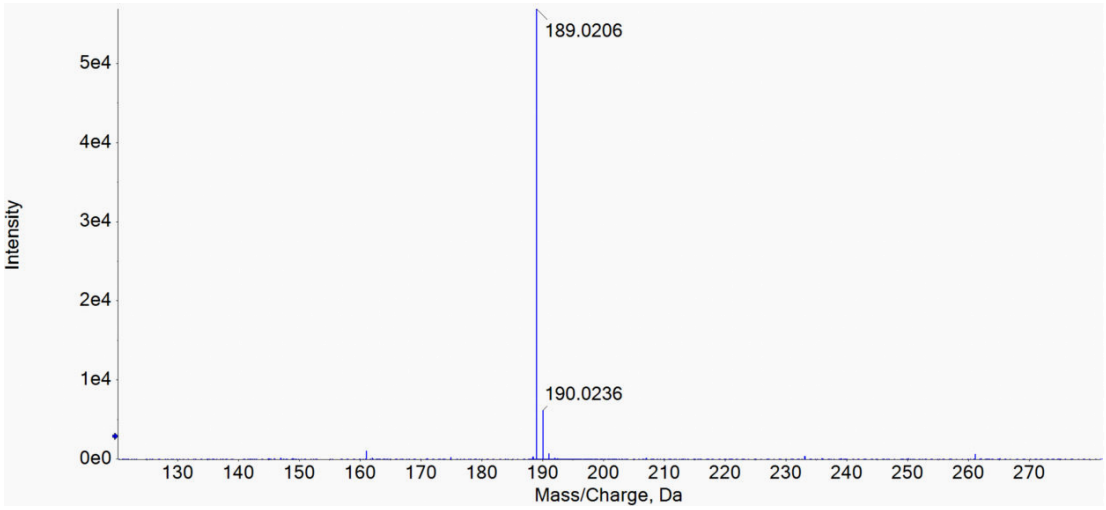
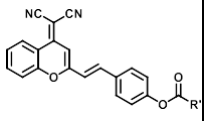
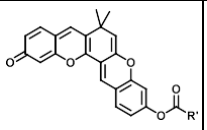
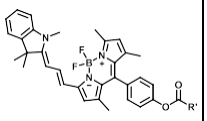
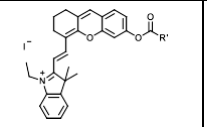
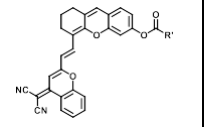
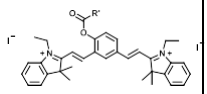
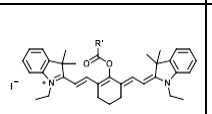
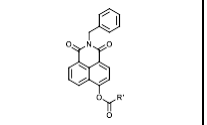
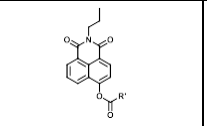
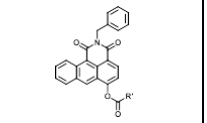
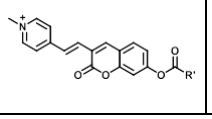


Fig. S63. HRMS spectrum of **Compound 3.**

Table S1. Computational screening and discovery of fluorescent substrates for 6 serine hydrolases.

Fluorophore		Distance (Å)						Affinity towards hPL (kcal/mol)
		hCES1A	hCES2A	Notum	AchE	BchE	hPL	
A		8.50	7.73	6.84	12.95	10.71	12.74	-11.71
B		10.08	4.86	10.22	14.46	12.28	16.22	-12.66
C		6.21	12.46	14.20	10.05	12.09	16.22	-13.04
D		10.75	5.30	13.55	12.67	10.48	9.22	-12.68
E		10.56	7.09	7.77	14.63	12.95	10.14	-12.16
F		10.78	10.05	14.77	10.91	12.32	13.25	-12.93
G		6.88	9.05	12.40	11.02	7.67	12.06	-13.15
H		8.30	8.41	11.78	9.38	10.17	13.92	-10.37
I		10.16	9.90	13.74	7.75	10.51	9.98	-11.34
J		9.32	11.90	9.35	11.03	10.86	10.98	-12.00
K		10.61	8.40	10.59	15.27	10.57	12.26	-12.04

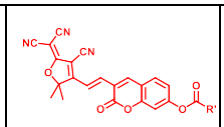
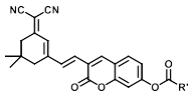
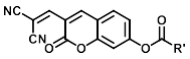
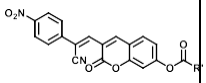
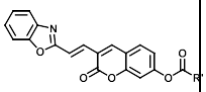
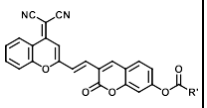
L		**6.08**	**8.72**	**14.98**	**14.31**	**10.40**	**3.60**	**-11.58**
M		**8.94**	**10.37**	**10.07**	**8.01**	**10.35**	**10.66**	**-12.87**
N		**9.68**	**9.37**	**10.15**	**10.20**	**6.90**	**13.05**	**-11.22**
O		**9.10**	**10.05**	**10.25**	**15.25**	**10.32**	**7.69**	**-11.49**
P		**6.86**	**10.18**	**14.60**	**13.26**	**8.90**	**12.98**	**-11.54**
Q		**9.64**	**9.80**	**5.59**	**15.02**	**10.59**	**12.91**	**-12.49**

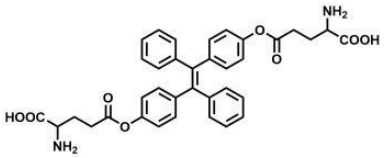
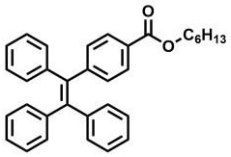
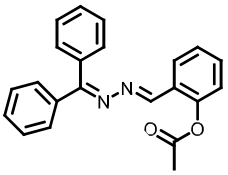
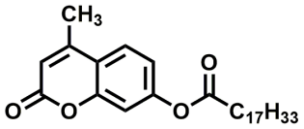
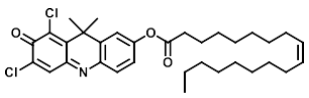
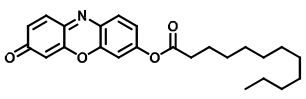
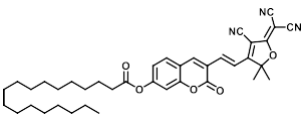
Table S2. The names for the herbs.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**Blank**	**orlistat**	*Aconiti Lateralis Radix Praeparata*	*Semen Coicis*	*Angelica dahurica*	*Lindera aggregata*	*Eupatorium japonicum Thunb*	*Polygonum aviculareL*	*Semen Armeniacae Amarum*	*Glycyrrhiza uralensis Fisch*	*Eupatorium fortunei Turcz*	*Mentha haplocalyx Briq*
B	*Glycine max (L.)Merr.*	*Caulispolygonimultiflori*	*Commelina communis*	*Ophicalcitum*	*Heracleum hemsleyanum*	*Euphorbia humifusa Willd*	*Puerariae Lobatae Radix*	*Plantago asiatica L*	*Arisaema Cum Bile*	*Citri Reticulatae Pericarpium Viride*	*Panax quinquefolius*	*Tetradium ruticarpum*
C	*Silktree Albizia Bark*	*Centella asiatica (L.) Urban*	*Equisetumhyemale L*	*Setariae Fructus Germinatus*	*Radix glycyrrhizae preparata*	*Pumex*	*Polygonatum sibiricum F. Delaroche*	*Trogopterori Faeces*	*Triticum aestivum L*	*Morus alba L.*	*Sepiella maindronide Rochebrune*	*Lygodium japonicum*
D	*Magnoliae Officinalis Cortex*	*Itoa orientalis*	*Fritillaria thunbergii*	*Sophora japonica Linn*	*Cnidium monnieri (L.) Cuss*	*Paris polyphylla*	*Vitex trifolia L*	*Chloriti Lapis*	*Imperata cylindrica*	*Pyrola calliantha H. Andres*	*Juncus effusus L*	*Citri Reticulatae Pericarpium*
E	*Acorus gramineus Aiton*	*Actinidia arguta*	*Citrus maxima*	*Lasiosphaera seu Calvatia*	*Zingiber officinale Roscoe*	*Ilex pubescensHook. et Arn*	*Semen Nelumbinis*	*Arisaema heterophyllum Blume*	*Fructus Kochiae Scopariae*	*Caulis Sinomenii*	*Haematitum*	*Ilex cornuta Lindl.ex Paxt*
F	*Smilaxhayatae*	*Prunella vulgaris L*	*Citri sarcodactylis Fructus*	*Armeniaca mume Sieb*	*Bubali Cornu*	*MeretrixmeretrixLinnaeus*	*Cyrtomium fortunei J. Sm*	*Herba Centipedae*	*Morus alba L*	*Radic aconiti kusnezoffii preparata*	*Semen Sojie Preparatum*	*Angelica dahurica*
G	*Poria cum Radix Pini*	*Herba Lophatheri*	*Herba Taraxaci*	*Oryza sativa L*	*Notopterygium incisum Ting ex H. T. Chang*	*Malva verticillata Linn*	*Lemnaminor L*	*Ampelopsis japonica*	*Citrus tangerina Hort.et Tanaka C.erythrosa Tanaka*	*Ginkgo biloba*	*Dendranthema morifolium (Ramat.）Tzvel*	*Cyperusrotundus L.*
H	*Peucedanum praeruptorum Dunn*	*Stachyuri Medulla*	*Concretio Silicea Bambusae*	*FineleafSchizonepetaHerb*	*Cynanchum atratum Bunge*	*Akebiae Caulis*	*ChineseBuckeyeSeed*	*Radix oryzae glutinosae*	*Curcumae Rhizoma*	*Andrographis paniculata*	*Pericarpium Trichosanthis*	*Camellia angustifolia (Pu-er tea)*

Table S3. Identification of the chemical composition of pu-er tea.

No.	RT (min)	$[M–H]^-$	$[M+H]^+$	Molecular formula	Identification
1	0.92	191.06	193.07	$C_7H_{12}O_6$	Ellagic acid
2	1.15	173.09	175.11	$C_7H_{10}O_5$	Shikimic acid
3	1.40	128.03	128.03	C_5H_7NOS	Goitrine
4	1.94	169.01	--	$C_7H_6O_5$	Gallic acid
5	3.62	305.07	307.08	$C_{15}H_{14}O_7$	Unknown
6	5.50	--	181.07	$C_7H_8N_4O_2$	Theobromine
7	8.12	305.07	307.08	$C_{15}H_{14}O_7$	Epigallocatechin
8	8.31	289.07	291.09	$C_{15}H_{14}O_6$	Catechin
9	9.49	353.09	--	$C_{16}H_{18}O_9$	chlorogenic acid
10	10.51	353.09	--	$C_{16}H_{18}O_9$	Cryptochlorogenic acid
11	10.54	--	195.09	$C_8H_{10}N_4O_2$	Caffeine
12	11.06	457.08	459.09	$C_{22}H_{18}O_{11}$	Gallocatechin gallate
13	11.87	289.07	289.07	$C_{15}H_{14}O_6$	Epicatechin
14	12.67	457.08	459.09	$C_{22}H_{18}O_{11}$	Epigallocatechin gallate
15	14.88	441.08	443.10	$C_{22}H_{18}O_{10}$	Catechin gallate
16	15.91	441.08	443.10	$C_{22}H_{18}O_{10}$	Epicatechin gallate
17	18.44	301.00	303.05	$C_{14}H_6O_8$	Ellagic acid
18	18.78	609.15	611.16	$C_{27}H_{30}O_{16}$	Rutin
19	21.07	447.09	449.11	$C_{21}H_{20}O_{11}$	Astragalin
20	23.80	301.04	303.05	$C_{15}H_{10}O_7$	Quercetin
21	26.90	285.04	287.05	$C_{15}H_{10}O_6$	Luteolin
22	27.16	285.04	287.05	$C_{15}H_{10}O_6$	Kaempferol
23	30.56	193.09	195.09	$C_{10}H_{10}O_4$	Ferulic acid

Table S4. Comparison of reported PL fluorescent substrates with the substrates in this paper.

Fluorescent substrate	Target enzyme	Detection conditions	LOD	Ref.
	PPL	λ_{ex}=360 nm λ_{em}=453 nm	0.13 U/L	[2]
	PPL	λ_{ex}=350 nm λ_{em}=420 nm	0.1 mg/mL	[3]
	pPL	λ_{ex}=380 nm λ_{em}=562 nm	0.05 U/L	[4]
	hPL	λ_{ex}=360 nm λ_{em}=460 nm	N.D.	[5]
	hPL	λ_{ex}=600 nm λ_{em}=660 nm	0.40 μg/mL	[6]
	hPL	λ_{ex}=570 nm λ_{em}=590 nm	0.369 μg/mL	[7]
	hPL	λ_{ex}=570 nm λ_{em}=670 nm	0.06 μg/mL	This study

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