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Electronic Supplementary Information

Discovery of new dibenzodiazepine derivatives as antibacterials against intracellular bacteria

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Table S1 Susceptibility of *S.* Typhimurium strains to SW33 and antibiotics in CAMHB

Drug	*Salmonella* Typhimurium					
	ATCC14028		CGC18		NL10	
	MIC (mg/L)	R/I/S	MIC (mg/L)	R/I/S	MIC (mg/L)	R/I/S
SW33	>64	-	>64	-	>64	-
Gentamicin	1	S	2	S	1	S
Tetracycline	4	S	>64	R	>64	R
Streptomycin	16	S	>64	R	>64	R
Chloramphenicol	2	S	64	R	16	R
Ampicillin	4	S	>64	R	16	R
Ciprofloxacin	<0.125	S	<0.125	S	8	R
Ofloxacin	<0.125	S	<0.125	S	8	R

The classification of antibiotic susceptibility is based on the CLSI 32nd edition guidelines, which categorize susceptibility results as resistant (R), intermediate (I), or sensitive (S) [1].

Biological materials and procedures

Cells

The human intestinal epithelial cell line INT-407 and murine macrophage cell lines RAW264.7 and J774.1 were cultured in DMEM (HyClone, Logan, U.S.A.) supplemented with 10% FBS (Gibco, Lafayette, U.S.A.). The human colon cell lines HT-29 and HCT-116 were cultured in McCoy's 5A medium (Gibco) supplemented with 10% FBS. The human peripheral blood mononuclear cell line THP-1 was cultured in Roswell Park Memorial Institute 1640 medium (RPMI; Gibco) supplemented with 10% FBS. THP-1-derived macrophages were obtained by treating cells with 100 ng/mL PMA (Cayman, Michigan, U.S.A.) for 72 h to induce the differentiation. All cells were cultured in T75 cell culture flasks at 37℃ with 5% CO_2 and passaged every 1–2 days. Cells were used only during the first 10 passages after thawing from the vapour phase of liquid nitrogen.

Bacterial strains

S. Typhimurium ATCC14028, CGC18, NL10, and *S*. Typhi Ty2 were cultured in Luria-Bertani (LB) broth (Bio Protech Inc., California, U.S.A.) at 37℃. The RFP-expressing *S*. Typhimurium strain was obtained by transforming the plasmid pBR-RFP.1 [2] into *S*. Typhimurium ATCC14028. *Y. enterocolitica* and *L. monocytogenes* clinical isolates were acquired from National Taiwan University Hospital and cultured in brain heart infusion (BHI) broth (BD, Franklin Lakes, U.S.A.) at 37℃.

Reagents

Loxapine (Sigma–Aldrich, St. Louis, U.S.A.) and olanzapine (Tokyo Chemical Industry, Japan) were dissolved in dimethyl sulfoxide (DMSO; Sigma–Aldrich). Gentamicin, ampicillin, tetracycline, streptomycin, ciprofloxacin, and ofloxacin were purchased from Bio Protech Inc. and dissolved in deionized ultra-pure water (ddH_2O). Ciprofloxacin and ofloxacin were prepared at a concentration of 1 mg/mL, and all other abovementioned antibiotics were prepared at a concentration of 10 mg/mL. Chloramphenicol was dissolved in ethanol to a concentration of 25 mg/mL. All antibiotics were sterilized by filtering through a 0.22 μm filter and were stored in a refrigerator at 4℃. 4',6-diamidino-2-phenylindole (DAPI) was purchased from AAT Bioquest Inc. and dissolved in ddH2O to a concentration of 2 mg/mL. MTT was purchased from Thermo Fisher Scientific Inc. and dissolved in phosphate-buffered saline (PBS; Gibco) to a concentration of 5 mg/mL. Triton X-100 was purchased from Bio Protech Inc. and diluted in PBS to a concentration of 0.1% on the day of use.

High-content analysis

RAW 264.7 macrophages were seeded in a black clear-bottom plate at a concentration of 10^4 cells/well in 100 µL of medium and cultured in a 37°C, 5% CO_2 incubator for 20 h. Meanwhile, five colonies of RFP-expressing *S.* Typhimurium were inoculated in 2 mL of LB broth supplemented with 100 µg/mL ampicillin and incubated at 37°C with agitation (200 rpm) for 16 – 18 h. The bacterial culture was then diluted 1:100 in 2 mL of fresh LB broth and incubated at 37°C with agitation (200 rpm) for another 2 h prior to centrifugation at 6,000 × g for 3 min. The bacterial pellet was suspended in PBS, and the OD_{600} was adjusted to 0.6 ± 0.01, which is equivalent to approximately 1.5×10^8 CFU/mL. The bacterial suspension was further diluted in cell culture medium and was then added to the RAW264.7 cell culture at a multiplicity of infection of 50. One hour post infection, the cell culture medium was removed, and the cells were washed twice with 200 µL of DMEM. The cells were then incubated with medium containing 100 µg/mL gentamicin for 1 h to eliminate extracellular bacteria prior to exposure to the test compounds at the desired concentrations in cell culture medium containing 20 µg/mL gentamicin. After 24 h of incubation, the cells were washed three times with DMEM and stained with 2.5 µM CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate; Thermo Fisher Scientific Inc., Lafayette, U.S.A.) dye for 40 min prior to recovery in cell culture medium for 20 min. The cells were then fixed with 3.7% formaldehyde (Sigma–Aldrich) at room temperature for 20 min, and the nuclei were stained with 100 µL of 2 µg/mL DAPI for 40 min. Finally, the numbers of nuclei and intracellular bacteria were determined using a HCA system (ImageXpress Micro 4, Molecular Devices, San Jose U.S.A.) equipped with a 10× objective lens.

CFU assay

RAW 264.7 cells were seeded at in a 6-well plate at 3×10^5 cells per well, and incubated at 37°C and 5% CO_2 for 20 h. Meanwhile, five bacterial colonies were inoculated in 2 mL of broth and incubated at 37°C with agitation (200 rpm) for 16 to 18 h. The bacterial culture was then diluted 1:100 in 2 mL of liquid medium and cultured for an additional 2 h. After centrifugation at 6,000 × g for 3 min, the supernatant was removed, and the bacterial pellet was suspended in PBS to an OD_{600} of 0.6 ± 0.01. The bacterial suspension was then diluted in the cell culture medium to achieve a multiplicity of infection of 10 and incubated for 1 h. Then the cells were treated with 100 µg/mL gentamicin for 1 h and then exposed to the test compounds at the designated concentrations in cell culture medium containing 20 µg/mL gentamicin. After 24 h of incubation, the cells were lysed with 0.1% Triton X-100 (Bio Basic, Markham, Canada) in PBS at 37°C for 10 min. The lysate was then serially diluted in PBS and plated onto agar plates. After incubation overnight at 37°C, the colonies on the plates were

enumerated. *Y. enterocolitica* and *L. monocytogenes* were cultured in BHI broth while LB broth was used for the other bacteria. EC_{50} values were calculated using CalcuSyn.

MTT cell viability assay

Initially, cells were adjusted to a concentration of 10^5 cells/mL in culture medium and seeded in a transparent 96-well plate (100 μL per well). The plate was then incubated at 37°C and 5% CO_2 for 20 h, after which the culture medium was removed and the cells were treated with the test compound at the designated concentrations for 24 h. The medium in each well was replenished with fresh medium containing 0.5 mg/mL MTT for 1 h, and the resulting formazan crystals were solubilized with DMSO. The absorbance at 570 nm was measured using a VersaMax microplate reader (Molecular Devices), and the CC_{50} of each compound was calculated using CalcuSyn (version 2.1, Biosoft, Cambridge, U.K.).

Bacterial growth analysis

An overnight-grown *S*. Typhimurium ATCC14028 culture in LB medium was inoculated into fresh CAMHB (BD), or DMEM supplemented with 10% FBS (Cell culture medium, CCM) to achieve a final concentration of 5×10^5 CFU/mL. The bacterial suspension was then exposed to various concentrations of SW33 in a flat-bottom 96-well plate and incubated at 37°C. Bacterial growth was monitored by measuring absorbance at 600 nm at designated times over a 24-hour period using a SpectraMax i3x microplate reader (Molecular Devices).

D2R affinity assay

This assay was conducted by Eurofins Panlabs Discovery Services Taiwan, Ltd., as a contract service. In brief, a total of 20 μg of human recombinant D2R protein produced in CHO cells was incubated with [^{3}H]-labeled spiperone (a known D2R antagonist) prior to the addition of loxapine or SW33 at designated concentrations in pH 7.4 Tris-HCl buffer for 2 h at 25°C. In addition, the background signal from nonspecific binding was determined by adding 10 μM Haloperidol (a D2R antagonist). After filtering and washing, the D2R protein was quantified, and the affinity of the test drug for the D2R protein was calculated by evaluating the inhibition of [^{3}H]-labelled spiperone binding to the D2R protein.

Antimicrobial susceptibility test

The susceptibility of bacteria to individual antibacterial agents was assessed using the broth microdilution method following the Clinical and Laboratory Standards Institute (CLSI) guidelines [1]. In brief, overnight bacterial cultures in LB broth were inoculated

into fresh CAMHB to achieve a final concentration of 5×10^5 CFU/mL. The bacterial cultures were then exposed to increasing concentrations of the test agents, ranging from 0.125 to 64 mg/L, in triplicate in 96-well plates at 37°C for 24 h. The minimal inhibitory concentration (MIC) of each individual compound was defined as the lowest concentration at which no bacterial growth was visible.

Statistical analysis

The data are expressed as the means ± standard deviations (SDs). To compare group means, a one-way analysis of variance (ANOVA) with Dunnett's test for multiple-comparisons of independent samples was performed. Statistical significance was assumed at a *P* value of < 0.05. All statistical analyses were performed using GraphPad Prism (version 6.0; GraphPad Software).

References

1. CLSI, *Performance Standards for Antimicrobial Susceptibility Testing — Eighteenth Informational Supplement: M100*, in *CLSI*. 2017.
2. Birmingham, C.L. and J.H. Brumell, *Autophagy recognizes intracellular Salmonella enterica serovar typhimurium in damaged Vacuoles.* Autophagy, 2006. **2**(3): p. 156-158.

Chemistry materials and characterization for compounds

Solvents and reagents were obtained from commercial suppliers and were used without further purification. Analytical thin-layer chromatography (TLC) was performed on precoated TLC sheets ALUGRAM Xtra SIL G/UV_{254} (Macherey Nagel) with visualization using ultraviolet light for monitoring reactions and column chromatography. All final compounds were determined by nuclear magnetic resonance (NMR), high-resolution mass spectrometry (HRMS), and high-performance liquid chromatography (HPLC). Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded at 400 MHz and 100 MHz, respectively, on a Bruker Avance III 400 using $CDCl_3$, CD_3OD, or DMSO-d_6 as solvents. Chemical shifts are reported in parts per million (ppm) with residual solvent signals as internal standard ($CDCl_3$, δ = 7.24 ppm for ^{1}H NMR, δ = 77.23 ppm for ^{13}C NMR; CD_3OD, δ = 3.31 ppm for ^{1}H NMR, δ = 49.15 ppm for ^{13}C NMR; DMSO-d_6, δ = 2.49 ppm for ^{1}H NMR, δ = 39.51 ppm for ^{13}C). Coupling constants (J) are quoted in hertz (Hz). Abbreviations used for multiplicity are as follows: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; br, broad; m, multiplet. The NMR spectra were shown in the following Figures S1-36. The high-resolution mass spectrum was recorded in the positive ion mode by electrospray ionization (ESI) using LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Bremen, Germany), Q Exactive Plus Hybrid Orbitrap mass spectrometer (Thermo Fisher Scientific), or Bruker maXis ultra-high-resolution time-of-flight mass spectrometer (Bruker Daltonics, Bremen, Germany). The purity of final compounds was determined by Agilent Technologies HPLC system (1260 Infinity II) equipped with a C-18 column (Kinetex® 2.6µm EVO C18 100 Å, LC Column 100 × 4.6 mm), eluting with different percentages mobile phase solution (Figures S37-47). Samples were dissolved to a concentration of 1000 ppm (Figures S37-47). Then, 1 µL sample solution was injected. An isocratic run was performed with a flow rate of 1 mL/min and monitored at 254 nm.

5-methyl-2-((2-nitrophenyl)amino)thiophene-3-carbonitrile (3). A suspension of sodium hydride (400 mg, 60% dispersion in mineral oil, 10.00 mmol, 2.00 equiv) in THF (3 mL) was stirred for 10 minutes under a nitrogen atmosphere at room temperature. Then, sodium hydride solution was added dropwise under 0 °C by a solution of 1-fluoro-2-nitrobenzene (**1**, 706 mg, 5.00 mmol, 1.00 equiv) and 2-amino-5-methylthiophene-3-carbonitrile (**2**, 691 mg, 5.00 mmol, 1.00 equiv) in THF (7 mL). The reaction mixture was stirred for 20 hours at room temperature, followed by acidification with 20% HCl solution to pH 7. The resulting mixture was diluted with water and extracted with EtOAc. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was

purified by column chromatography on silica using 4.8 % EtOAc in hexane to afford intermediate **3** (794 mg, 61% yield, reddish-brown solid). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.61 (s, 1H), 8.13 (dd, *J* = 8.8, 1.6 Hz, 1H), 7.62-7.58 (m, 1H), 7.04-7.01 (m, 3H), 2.42 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 149.1, 141.4, 136.4, 136.3, 134.3, 126.8, 124.1, 120.1, 116.3, 113.9, 104.8, 15.8 ppm. HRMS (ESI) *m*/*z* calculated for $C_{12}H_{10}N_3O_2S$ $[M+H]^+$: 260.0493. Found: 260.0499.

2-methyl-10H-benzo[b]thieno[2,3-e][1,4]diazepin-4-amine hydrochloride (4). A solution of intermediate **3** (420 mg, 1.62 mmol, 1.00 equiv) in ethanol (4 mL) was added dropwise by a solution of stannous chloride (1.536 g, 8.10 mmol, 5.00 equiv) in concentrated HCl (4 mL) at room temperature. The reaction mixture was refluxed for 3 hours and then concentrated under reduced pressure. The residue was cooled at 4 °C and the product precipitated out as a solid. The solid was filtered, washed with a small amount of cold water, and dried to afford intermediate **4** without further purification (211 mg, 49% yield, yellow solid). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.05 (br s, 1H), 9.53 (s, 1H), 9.08 (br s, 1H), 8.81 (br s, 1H), 7.08 (t, *J* = 7.6 Hz, 1H), 7.02-6.99 (m, 1H), 6.93 (d, *J* = 8.0 Hz, 1H), 6.84 (d, *J* = 8.0 Hz, 1H), 6.78 (s, 1H), 2.24 (s, 3H) ppm. ^{13}C NMR (100 MHz, CD_3OD) *δ* 164.7, 162.6, 142.6, 130.3, 130.1, 129.1, 126.3, 124.7, 122.5, 121.2, 109.1, 15.0 ppm. HRMS (ESI) *m*/*z* calculated for $C_{12}H_{12}N_3S$ $[M+H]^+$: 230.0752. Found: 230.0767.

General procedures for the synthesis of compounds SW38, 39, 41, and 42. A mixture of intermediate **4** (1.00 equiv) and the corresponding amine (20.23-20.99 equiv) was heated to 160 °C for 35 min under microwave irradiation. The mixture was diluted with water and extracted with DCM. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on triethylamine washed silica (silica gel slurry was basified with 2% triethylamine in starting flushing solvents) to afford **SW38**, **39**, **41**, and **42**.

2-methyl-N-(2-(piperidin-1-yl)ethyl)-10H-benzo[b]thieno[2,3-e][1,4]diazepin-4-amine (SW38). The title compound was prepared from intermediate **4** (80 mg, 0.30 mmol, 1.00 equiv) and 1-(2-aminoethyl)piperidine (784 mg, 6.11 mmol, 20.31 equiv). The residue was purified by column chromatography on triethylamine washed silica using 50% EtOAc in hexane to afford **SW38** (20.8 mg, 20% yield, light yellow solid). ^{1}H NMR (400 MHz, CD_3OD) δ 6.92-6.82 (m, 3H), 6.66 (d, *J* = 7.2 Hz, 1H), 6.53 (br s, 1H), 3.58 (t, *J* = 6.4 Hz, 2H), 2.68 (t, *J* = 6.4 Hz, 2H), 2.58 (br s, 4H), 2.30 (s, 3H), 1.66-1.61 (m, 4H), 1.51-1.49 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.5, 157.1, 145.3, 140.9, 128.6, 128.5, 125.3, 123.1, 120.2, 118.5, 56.7, 54.3, 38.6, 26.1, 24.5, 15.3 ppm. HRMS (ESI) *m*/*z* calculated for $C_{19}H_{25}N_4S$ $[M+H]^+$: 341.1794. Found: 341.1798.

N^1,N^1-dimethyl-N^2-(2-methyl-10H-benzo[b]thieno[2,3-e][1,4]diazepin-4-yl)ethane-1,2-diamine (SW39). The title compound was prepared from intermediate **4** (80 mg, 0.30 mmol, 1.00 equiv) and *N*,*N*-dimethylethylenediamine (540 mg, 6.13 mmol, 20.35 equiv). The residue was purified by column chromatography on triethylamine washed silica using 67% EtOAc in hexane to afford **SW39** (16.3 mg, 18% yield, yellow solid). ^{1}H NMR (400 MHz, CD_3OD) δ 6.89-6.79 (m, 3H), 6.63 (d, *J* = 7.2 Hz, 1H), 6.53 (br s, 1H), 3.53 (t, *J* = 6.4 Hz, 2H), 2.63 (t, *J* = 6.4 Hz, 2H), 2.32 (s, 6H), 2.28 (s, 3H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 158.3, 156.9, 145.1, 140.7, 128.4, 128.3, 125.0, 122.9, 120.0, 118.3, 59.4, 45.4, 40.2, 15.0 ppm. HRMS (ESI) *m*/*z* calculated for $C_{16}H_{21}N_4S$ $[M+H]^+$: 301.1481. Found: 301.1479.

2-methyl-N-(2-(piperazin-1-yl)ethyl)-10H-benzo[b]thieno[2,3-e][1,4]diazepin-4-amine (SW41). The title compound was prepared from intermediate **4** (90 mg, 0.34 mmol, 1.00 equiv) and 2-(piperazin-1-yl)ethan-1-amine (885 mg, 6.85 mmol, 20.23 equiv). The residue was purified by column chromatography on triethylamine washed silica using 7% MeOH in EtOAc to afford **SW41** (12.3 mg, 11% yield, orange viscous oil). ^{1}H NMR (400 MHz, CD_3OD) δ 6.93-6.91 (m, 3H), 6.69 (d, *J* = 7.6 Hz, 1H), 6.54 (br s, 1H), 3.58 (t, *J* = 6.0 Hz, 2H), 2.85 (br s, 4H), 2.69 (t, *J* = 6.0 Hz, 2H), 2.59 (br s, 4H), 2.29 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.6, 157.1, 145.1, 140.2, 128.6, 128.1, 125.2, 122.8, 120.2, 118.0, 56.1, 53.5, 45.4, 37.2, 15.1 ppm. HRMS (ESI) *m*/*z* calculated for $C_{18}H_{24}N_5S$ $[M+H]^+$: 342.1747. Found: 342.1745.

2-methyl-N-(3-(pyrrolidin-1-yl)propyl)-10H-benzo[b]thieno[2,3-e][1,4]diazepin-4-amine (SW42). The title compound was prepared from intermediate **4** (80 mg, 0.30 mmol, 1.00 equiv) and 3-(pyrrolidin-1-yl)propan-1-amine (810 mg, 6.32 mmol, 20.99 equiv). The residue was purified by column chromatography on triethylamine washed silica using 33% EtOAc in hexane to afford **SW42** (19.6 mg, 19% yield, brown viscous oil). ^{1}H NMR (400 MHz, CD_3OD) δ 6.89-6.82 (m, 3H), 6.64 (d, *J* = 8.0 Hz, 1H), 6.49 (br s, 1H), 3.46 (t, *J* = 6.4 Hz, 2H), 2.68-2.64 (m, 6H), 2.28 (s, 3H), 1.93-1.86 (m, 2H), 1.80 (br s, 4H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.6, 157.2, 145.3, 141.5, 128.7, 128.6, 125.3, 123.1, 120.3, 118.6, 56.9, 54.9, 44.3, 26.3, 24.1, 15.3 ppm. HRMS (ESI) *m*/*z* calculated for $C_{19}H_{25}N_4S$ $[M+H]^+$: 341.1794. Found: 341.1793.

5-chloro-2-nitrobenzoyl chloride (7). 5-chloro-2-nitrobenzoic acid (**6**, 1 g, 4.96 mmol, 1.00 equiv) was added by thionyl chloride (10.8 mL, 148.06 mmol, 29.84 equiv) under a nitrogen atmosphere. The reaction mixture was refluxed for 2 hours and then concentrated under reduced pressure to afford intermediate **7**, which was used for the following reaction step without further purification.

5-chloro-N-(2-chloropyridin-3-yl)-2-nitrobenzamide (8). A solution of intermediate **7** (1 g, 4.55 mmol, 1.00 equiv) in 1,4-dioxane (10 mL) was stirred for 10 minutes at room temperature and then added dropwise by a solution of 3-amino-2-chloropyridine (**5**, 585

mg, 4.57 mmol, 1.01 equiv) and pyridine (360 mg, 4.55 mmol, 1.00 equiv) in 1,4-dioxane (2 mL). The reaction mixture was refluxed for 16 hours, diluted with water, and extracted with EtOAc. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by recrystallization with EtOAc and hexane to afford intermediate **8** (973 mg, 69% yield, light brown solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (d, J = 7.6 Hz, 1H), 8.20 (br s, 1H), 8.14 (d, J = 8.4 Hz, 1H), 7.88 (s, 1H), 7.65-7.63 (m, 2H), 7.34 (dd, J = 8.0, 4.4 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4, 145.3, 144.6, 141.3, 140.7, 133.6, 131.6, 131.5, 130.1, 128.8, 126.7, 123.7 ppm. HRMS (ESI) *m*/*z* calculated for $C_{12}H_8Cl_2N_3O_3$ $[M+H]^+$: 311.9943. Found: 311.9953.

2-amino-5-chloro-N-(2-chloropyridin-3-yl)benzamide (9). A solution of intermediate **8** (1 g, 3.20 mmol, 1.00 equiv) in concentrated HCl (5 mL) was stirred for 10 minutes at room temperature and then added dropwise by a solution of stannous chloride (3.02 g, 15.93 mmol, 4.97 equiv) in concentrated HCl (5 mL). The reaction mixture was refluxed for 2 hours, cooled to room temperature, filtrated, and rinsed with water. The filtrate was collected, basified with 2 N NaOH, and then extracted with DCM. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to afford intermediate **9** (715 mg, 79% yield).

8-chloro-5,11-dihydro-6H-benzo[e]pyrido[3,2-b][1,4]diazepin-6-one (10). A solution of intermediate **9** (715 mg, 2.53 mmol, 1.00 equiv) in DEGMME (5 mL) was stirred for 16 hours at 150 °C. The reaction mixture was cooled at 0 °C, followed by the formation of crystals. The crystals were collected by filtration, rinsed with MeOH, and dried under reduced pressure to afford intermediate **10** (241 mg, 39% yield, yellow-brown solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.02 (s, 1H), 8.74 (s, 1H), 7.89 (dd, J = 4.8, 1.6 Hz, 1H), 7.64 (d, J = 2.8 Hz, 1H), 7.41 (dd, J = 8.8, 2.8 Hz, 1H), 7.29 (dd, J = 8.0, 1.6 Hz, 1H), 7.15 (d, J = 8.8 Hz, 1H), 6.96 (dd, J = 8.0, 4.8 Hz, 1H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.8, 149.1, 144.7, 141.0, 132.3, 130.2, 128.3, 124.0, 123.4, 122.0, 120.6, 117.9 ppm. HRMS (ESI) *m*/*z* calculated for $C_{12}H_9ClN_3O$ $[M+H]^+$: 246.0434. Found: 246.0449.

6,8-dichloro-11H-benzo[e]pyrido[3,2-b][1,4]diazepine (11). The intermediate **10** (280 mg, 1.14 mmol, 1.00 equiv) was added sequentially by phosphorus oxychloride (2.4 mL, 25.75 mmol, 22.59 equiv) and *N*,*N*-dimethylaniline (54 mg, 0.44 mmol, 0.39 equiv) under a nitrogen atmosphere. The resulting mixture was refluxed for 16 hours, concentrated under reduced pressure, diluted with water, and then extracted with toluene. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to afford intermediate **11**, which was used for the following reaction step without further purification.

General procedures for the synthesis of compounds SW21, 27, 29, and 30. A

solution of intermediate **11** (1.00 equiv) in xylene was stirred for 10 minutes at room temperature, and then the corresponding amine (8.00-12.50 equiv) was added dropwise. The reaction mixture was refluxed for 16 hours and basified with 2 N NaOH. The resulting mixture was diluted with water and extracted with EtOAc. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on triethylamine washed silica (silica gel slurry was basified with 2% triethylamine in starting flushing solvents) to afford **SW21**, **27**, **29**, and **30**.

8-chloro-N-(2-(piperidin-1-yl)ethyl)-11H-benzo[e]pyrido[3,2-b][1,4]diazepin-6-amine (SW21). The title compound was prepared from intermediate **11** (300 mg, 1.14 mmol, 1.00 equiv) in xylene (2.2 mL) and 1-(2-aminoethyl)piperidine (1.165 g, 9.09 mmol, 8.00 equiv). The residue was purified by column chromatography on triethylamine washed silica using 0.5% MeOH in DCM to afford **SW21** (272 mg, 67% yield, white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 3.6 Hz, 1H), 7.29-7.22 (m, 3H), 6.90-6.87 (m, 1H), 6.75 (d, *J* = 8.8 Hz, 1H), 5.87 (s, 1H), 5.59 (br s, 1H), 3.54 (d, *J* = 3.6 Hz, 2H), 2.58 (t, *J* = 6.0 Hz, 2H), 2.42 (br s, 4H), 1.57 (t, *J* = 5.2 Hz, 4H), 1.44 (br s, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.9, 152.2, 149.1, 141.4, 135.8, 134.6, 132.1, 128.0, 127.9, 127.2, 121.4, 120.6, 56.9, 54.5, 38.8, 26.3, 24.7 ppm. HRMS (ESI) *m*/*z* calculated for $C_{19}H_{23}N_5Cl$ $[M+H]^+$: 356.1636. Found: 356.1626.

N^1-(8-chloro-11H-benzo[e]pyrido[3,2-b][1,4]diazepin-6-yl)-N^2,N^2-dimethylethane-1,2-diamine (SW27). The title compound was prepared from intermediate **11** (250 mg, 0.95 mmol, 1.00 equiv) in xylene (2.5 mL) and *N*,*N*-dimethylethylenediamine (1.043 g, 11.83 mmol, 12.50 equiv). The residue was purified by column chromatography on triethylamine washed silica using 3% MeOH in DCM to afford **SW27** (85 mg, 28% yield, yellow solid). ^{1}H NMR (400 MHz, CD_3OD) δ 7.80 (d, *J* = 4.4 Hz, 1H), 7.51 (br s, 1H), 7.39 (d, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.03-6.98 (m, 2H), 3.82 (t, *J* = 5.2 Hz, 2H), 3.43 (t, *J* = 5.2 Hz, 2H), 2.99 (s, 6H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.0, 152.5, 148.9, 141.9, 135.0, 134.5, 132.6, 128.6, 128.5, 126.1, 121.3, 120.4, 58.0, 44.5, 38.0 ppm. HRMS (ESI) *m*/*z* calculated for $C_{16}H_{19}N_5Cl$ $[M+H]^+$: 316.1323. Found: 316.1334.

8-chloro-N-(3-(pyrrolidin-1-yl)propyl)-11H-benzo[e]pyrido[3,2-b][1,4]diazepin-6-amine (SW29). The title compound was prepared from intermediate **11** (245 mg, 0.93 mmol, 1.00 equiv) in xylene (2.5 mL) and 1-(3-aminopropyl)pyrrolidine (971 mg, 7.57 mmol, 8.16 equiv). The residue was purified by column chromatography on triethylamine washed silica using 0.5% MeOH in DCM to afford **SW29** (32 mg, 10% yield, light yellow solid). ^{1}H NMR (400 MHz, CD_3OD) δ 7.71 (dd, *J* = 5.2, 2.0 Hz, 1H), 7.37 (d, *J* = 2.8 Hz, 1H), 7.33 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.30 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.97 (d, *J* = 8.8 Hz, 1H), 6.94 (dd, *J* = 8.0, 5.2 Hz, 1H), 3.49 (t, *J* = 6.8 Hz, 2H),

2.69-2.63 (m, 6H), 1.97-1.90 (m, 2H), 1.83-1.79 (m, 4H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.8, 152.1, 148.9, 140.7, 135.9, 134.2, 131.6, 127.43, 127.42, 127.4, 121.0, 120.3, 56.2, 54.1, 43.6, 25.4, 23.3 ppm. HRMS (ESI) *m*/*z* calculated for $C_{19}H_{23}N_5Cl$ $[M+H]^+$: 356.1636. Found: 356.1637.

8-chloro-N-(2-(piperazin-1-yl)ethyl)-11H-benzo[e]pyrido[3,2-b][1,4]diazepin-6-amine (SW30). The title compound was prepared from intermediate **11** (300 mg, 1.14 mmol, 1.00 equiv) in xylene (3 mL) and 2-(piperazin-1-yl)ethan-1-amine (1.692 g, 13.10 mmol, 11.53 equiv). The residue was purified by column chromatography on triethylamine washed silica using 7% MeOH in EtOAc to afford **SW30** (16 mg, 4% yield, orange solid). ^{1}H NMR (400 MHz, CD_3OD) δ 8.15 (d, *J* = 4.0 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H),7.73 (br s, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.23-7.17 (m, 2H), 3.92 (t, *J* = 6.0 Hz, 2H), 3.39 (br s, 4H), 3.12 (br s, 6H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 163.8, 156.1, 150.6, 147.4, 136.8, 134.8, 131.7, 129.8, 124.9, 124.0, 121.2, 120.3, 55.9, 50.5, 44.0, 41.6 ppm. HRMS (ESI) *m*/*z* calculated for $C_{18}H_{22}N_6Cl$ $[M+H]^+$: 357.15890. Found: 357.15892.

2-((2-aminophenyl)amino)benzoic acid (14). A solution of 1,2-diamino-benzene (**12**, 541 mg, 5.00 mmol, 1.00 equiv), 2-bromobenzoic-acid (**13**, 1.005 g, 5.00 mmol, 1.00 equiv), K_2CO_3 (1.382 g, 10.00 mmol, 2.00 equiv), Cu powder (64 mg, 1.01 mmol, 0.20 equiv) in DMF (7 mL) was stirred for 16 hours at 170 °C. Subsequently, the reaction mixture was filtered and rinsed with DCM. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica using 0.5 % MeOH in DCM to afford intermediate **14** (599 mg, 53% yield, purple solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (br s, 1H), 7.84 (d, *J* = 7.2, 1H), 7.27 (t, *J* = 7.2, 1H), 7.02 (d, *J* = 7.6, 1H), 6.95 (t, *J* = 7.2, 1H), 6.79 (d, *J* = 7.6 Hz, 1H), 6.64 (t, *J* = 7.2, 1H), 6.59-6.57 (m, 2H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) *δ* 170.2, 149.2, 144.1, 133.9, 131.6, 126.2, 126.1, 124.9, 116.6, 115.9, 115.4, 113.2, 111.7 ppm. HRMS (ESI) *m*/*z* calculated for $C_{13}H_{13}N_2O_2$ $[M+H]^+$: 229.0977. Found: 229.0977.

5,10-dihydro-11H-dibenzo[b,e][1,4]diazepin-11-one (15). Concentrated H_2SO_4 (90 mg, 0.92 mmol, 0.23 equiv) was added in a solution of intermediate **14** (903 mg, 3.96 mmol, 1.00 equiv) in DMF (6 mL) under a nitrogen atmosphere. The reaction mixture was refluxed for 16 hours and then added by crushed ice to form precipitates. The precipitates were filtered, washed with water, and dried the solid to afford intermediate **15** (359 mg, 43% yield, grey solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.83 (s, 1H), 7.83 (s, 1H), 7.66 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.35-7.31 (m, 1H), 7.00-6.87 (m, 6H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) *δ* 167.9, 150.4, 139.9, 133.2, 132.1, 129.8, 124.5, 122.9, 122.8, 121.3, 120.7, 119.8, 119.0 ppm. HRMS (ESI) *m*/*z* calculated for $C_{13}H_{11}N_2O$ $[M+H]^+$: 211.0871. Found: 211.0872.

11-chloro-5H-dibenzo[b,e][1,4]diazepine (16). Phosphorus oxychloride (3.5 mL,

37.55 mmol, 26.31 equiv) was added in intermediate **15** (300 mg, 1.43 mmol, 1.00 equiv) under a nitrogen atmosphere. The resulting mixture was refluxed for 16 hours, concentrated under reduced pressure, diluted with water, and then extracted with toluene. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to afford intermediate **16**, which was used for the following reaction step without further purification.

General procedures for the synthesis of compounds SW33, 35, 44, and 45. A solution of intermediate **16** (1.00 equiv) in xylene (3 mL) was stirred for 10 minutes at room temperature, and then the corresponding amine (7.61-14.01 equiv) was added dropwise. The reaction mixture was refluxed for 16 hours and basified with 2 N NaOH. The resulting mixture was diluted with water and extracted with EtOAc. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on triethylamine washed silica (silica gel slurry was basified with 2% triethylamine in DCM) to afford **SW33**, **35**, **44**, and **45**.

N-(3-(pyrrolidin-1-yl)propyl)-5H-dibenzo[b,e][1,4]diazepin-11-amine (SW33). The title compound was prepared from intermediate **16** (300 mg, 1.31 mmol, 1.00 equiv) and 1-(3-Aminopropyl)pyrrolidine (1.28 g, 9.98 mmol, 7.61 equiv). The residue was purified by column chromatography on triethylamine washed silica using 0.5% MeOH in DCM to afford **SW33** (19.8 mg, 5% yield, orange viscous solid). ^{1}H NMR (400 MHz, CD_3OD) δ 7.37 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.32-7.28 (m, 1H), 7.00-6.83 (m, 6H), 3.52 (t, *J* = 6.8 Hz, 2H), 2.74-2.67 (m, 6H), 1.99-1.94 (m, 2H), 1.80 (br s, 4H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.7, 152.9, 141.6, 141.0, 131.4, 127.8, 127.1, 126.6, 124.2, 122.6, 122.4, 119.3, 119.1, 55.7, 54.0, 42.6, 26.4, 23.4 ppm. HRMS (ESI) *m*/*z* calculated for $C_{20}H_{25}N_4$ $[M+H]^+$: 321.2074. Found: 321.2068.

N-(2-(piperidin-1-yl)ethyl)-5H-dibenzo[b,e][1,4]diazepin-11-amine (SW35). The title compound was prepared from intermediate **16** (300 mg, 1.31 mmol, 1.00 equiv) and 1-(2-aminoethyl)piperidine (1.67 mg, 13.02 mmol, 9.93 equiv). The residue was purified by column chromatography on triethylamine washed silica using 0.5% MeOH in DCM to afford **SW35** (75.5 mg, 18% yield, light yellow solid). ^{1}H NMR (400 MHz, CD_3OD) δ 7.38 (d, *J* = 7.6 Hz, 1H), 7.30-7.26 (m, 1H), 6.99-6.81 (m, 6H), 3.62-3.58 (m, 2H), 2.69 (t, *J* = 6.4 Hz, 2H), 2.54 (br s, 4H), 1.63-1.61 (m, 4H), 1.48 (br s, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.6, 152.8, 141.11, 141.07, 131.7, 127.9, 127.0, 126.1, 124.2, 123.0, 122.8, 119.4, 119.3, 57.0, 54.2, 38.4, 25.9, 24.3 ppm. HRMS (ESI) *m*/*z* calculated for $C_{20}H_{25}N_4$ $[M+H]^+$: 321.2074. Found: 321.2070.

N-(2-(piperazin-1-yl)ethyl)-5H-dibenzo[b,e][1,4]diazepin-11-amine (SW44). The title compound was prepared from intermediate **16** (130 mg, 0.57 mmol, 1.00 equiv) and 2-(piperazin-1-yl)ethan-1-amine (600 mg, 4.68 mmol, 8.23 equiv). The residue was

purified by column chromatography on triethylamine washed silica using 8% MeOH in DCM to afford **SW44** (16 mg, 9% yield, yellow solid). ^{1}H NMR (400 MHz, CD_3OD) δ 7.40 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.33-7.28 (m, 1H), 7.00-6.82 (m, 6H), 3.61 (t, *J* = 6.4 Hz, 2H), 2.88 (t, *J* = 5.2 Hz, 4H), 2.72 (t, *J* = 6.4 Hz, 2H), 2.59 (br s, 4H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 161.8, 155.5, 144.5, 141.6, 133.3, 129.7, 127.5, 126.7, 124.8, 124.7, 123.6, 120.8, 120.5, 58.6, 54.6. 46.2, 39.6 ppm. HRMS (ESI) *m*/*z* calculated for $C_{19}H_{24}N_5$ $[M+H]^+$: 322.2026. Found: 322.2024.

N*^{1}*-(5H-dibenzo[b,e][1,4]diazepin-11-yl)-N*^{2}*,N*^{2}*-dimethylethane-1,2-diamine (SW45). The title compound was prepared from intermediate **16** (100 mg, 0.44 mmol, 1.00 equiv) and *N*,*N*-dimethylethylenediamine (540 mg, 6.13 mmol, 14.01 equiv). The residue was purified by column chromatography on triethylamine washed silica using 4% MeOH in DCM to afford **SW45** (20 mg, 16% yield, yellow viscous oil). ^{1}H NMR (400 MHz, CD_3OD) δ 7.41 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.31-7.27 (m, 1H), 6.99-6.82 (m, 6H), 3.60 (t, *J* = 6.8 Hz, 2H), 2.70 (t, *J* = 6.8 Hz, 2H), 2.35 (s, 6H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 161.7, 155.5, 144.4, 142.1, 133.2, 129.7, 127.5, 126.9, 124.8, 124.5, 123.6, 120.7, 120.4, 59.2, 45.6, 40.3 ppm. HRMS (ESI) *m*/*z* calculated for $C_{17}H_{21}N_4$ $[M+H]^+$: 281.1761. Found: 281.1763.

Fig. S1 ^{1}H NMR spectrum of **3** (400 MHz, DMSO-d_6)

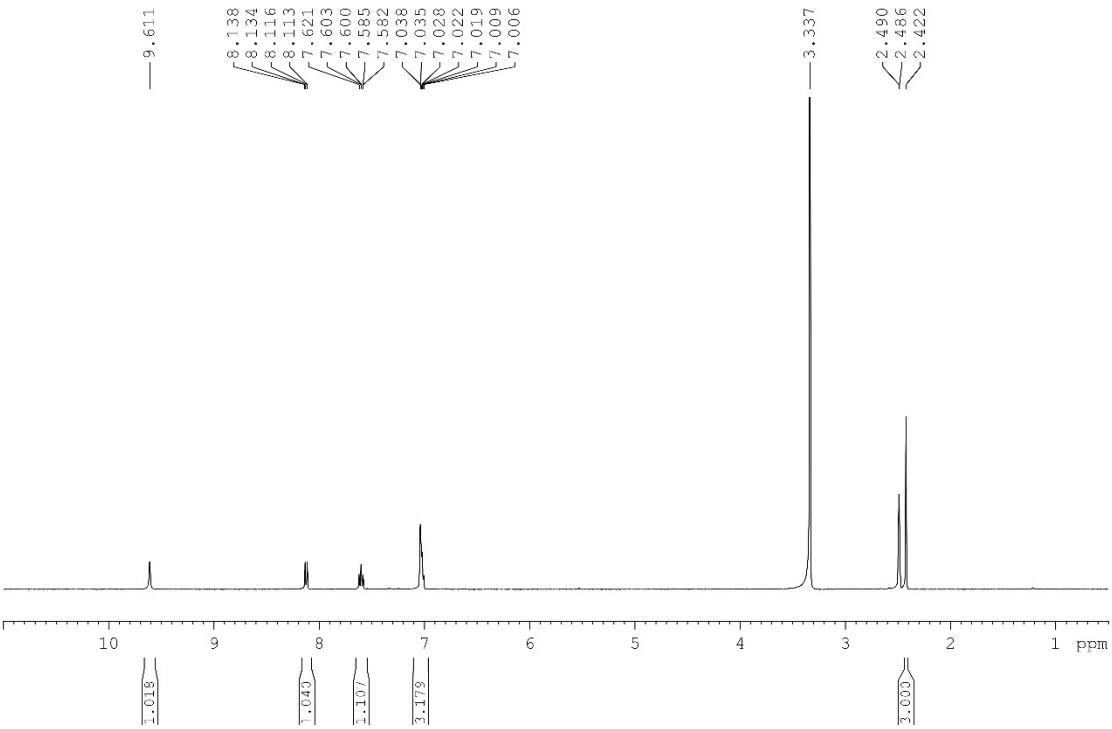


Fig. S2 ^{13}C NMR spectrum of **3** (100 MHz, $CDCl_3$)

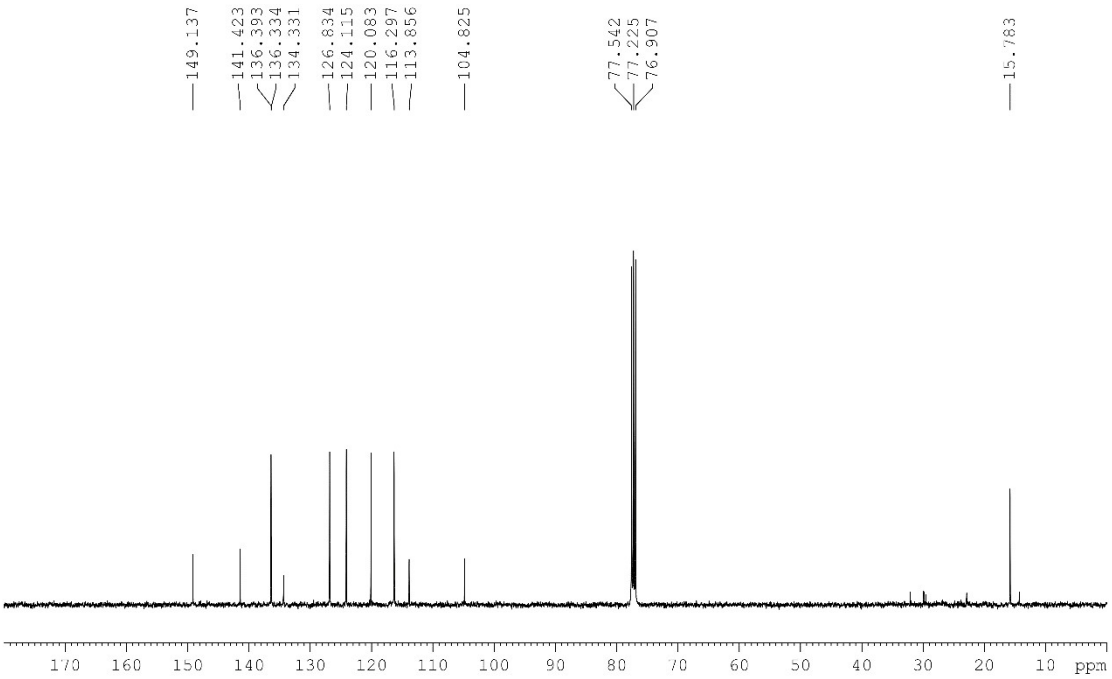


Fig. S3 ^{1}H NMR spectrum of **4** (400 MHz, DMSO-*d6*)

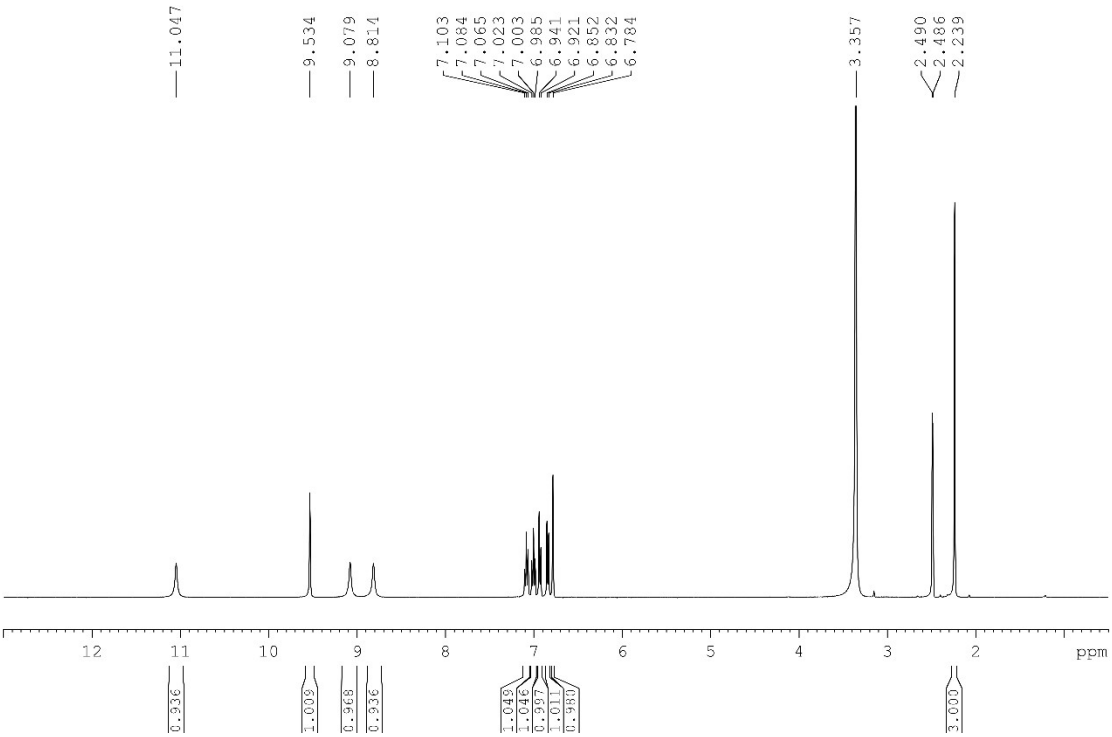


Fig. S4 ^{13}C NMR spectrum of **4** (100 MHz, CD_3OD)

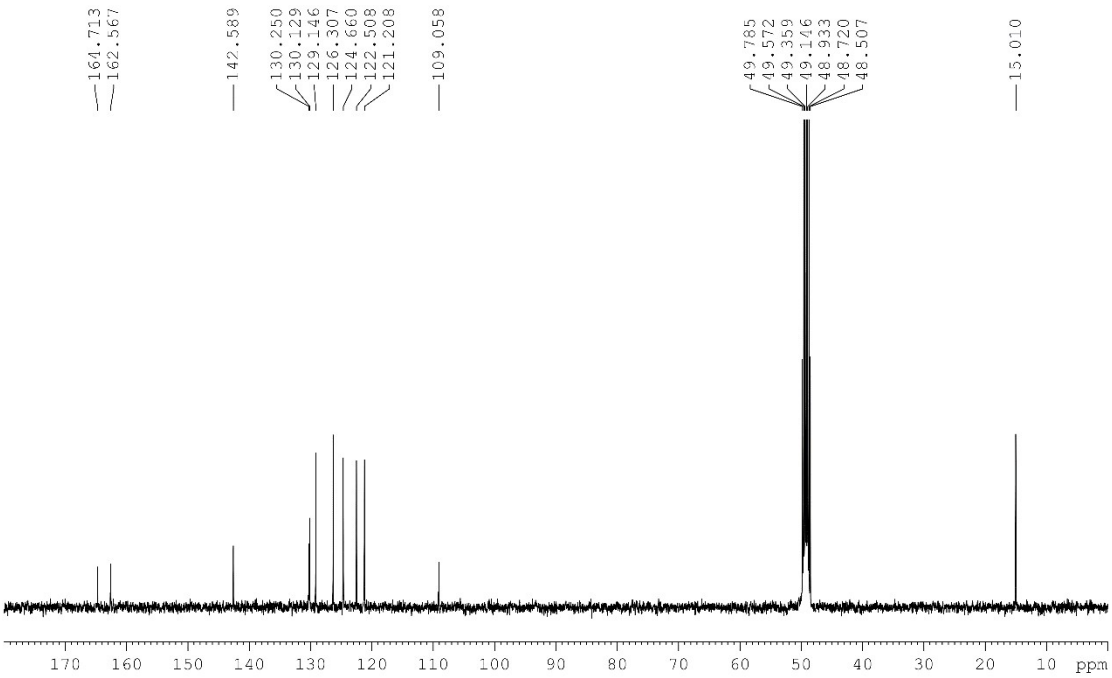


Fig. S5 ^{1}H NMR spectrum of **SW38** (400 MHz, CD_3OD)

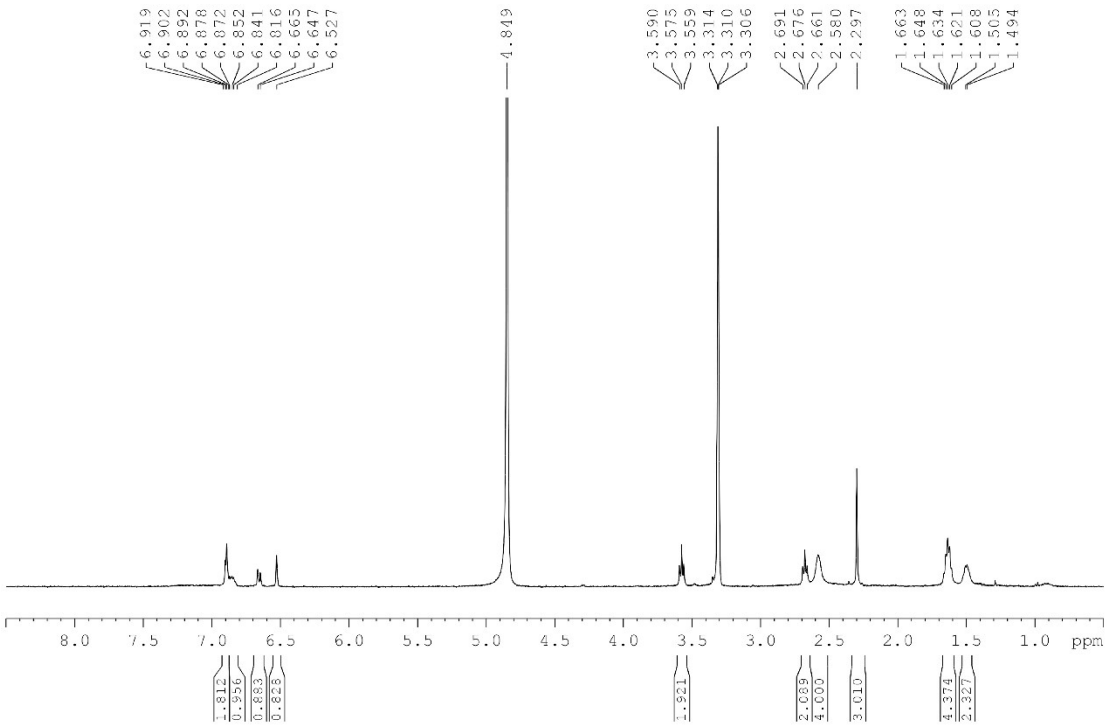


Fig. S6 ^{13}C NMR spectrum of **SW38** (100 MHz, $CDCl_3$)

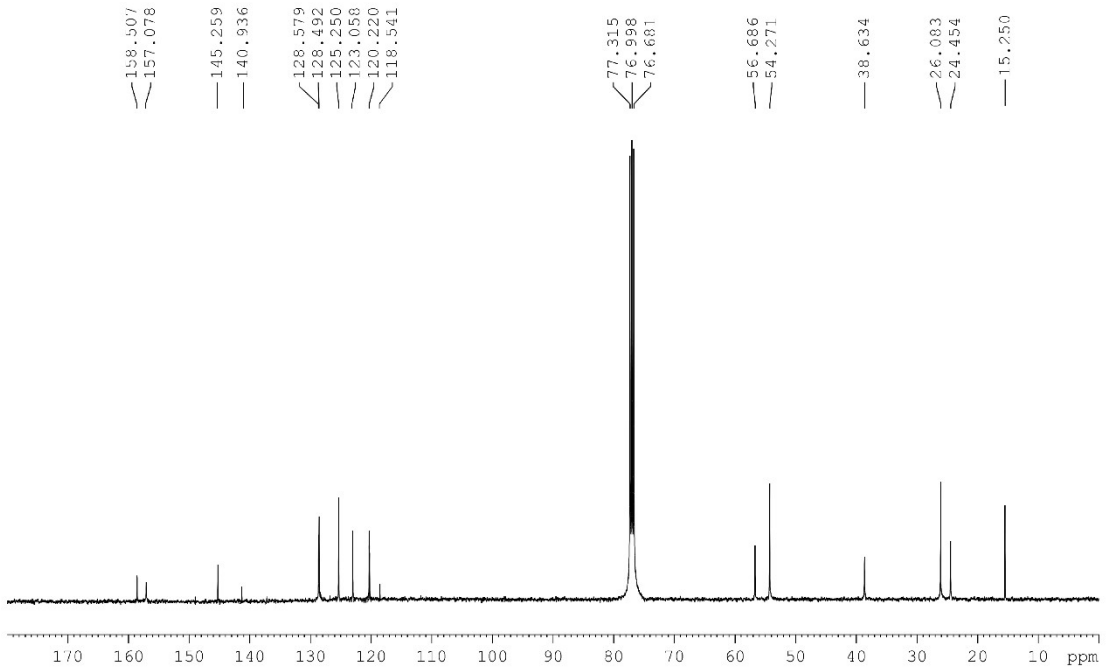


Fig. S7 ^{1}H NMR spectrum of **SW39** (400 MHz, CD_3OD)

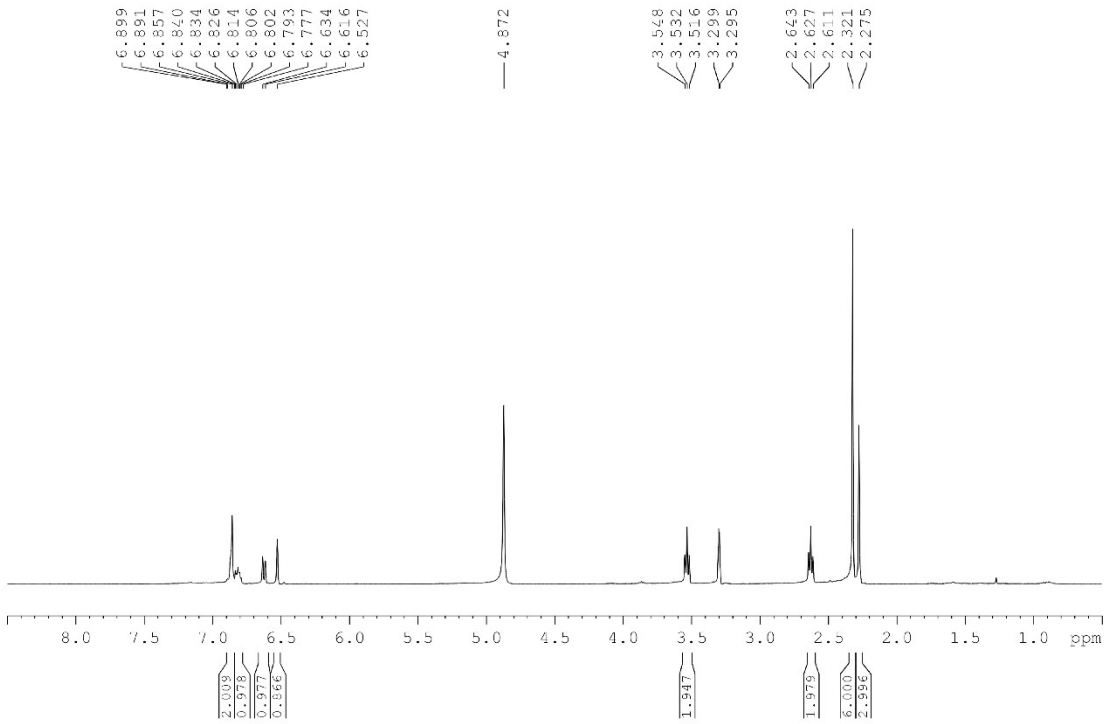


Fig. S8 ^{13}C NMR spectrum of **SW39** (100 MHz, CD_3OD)

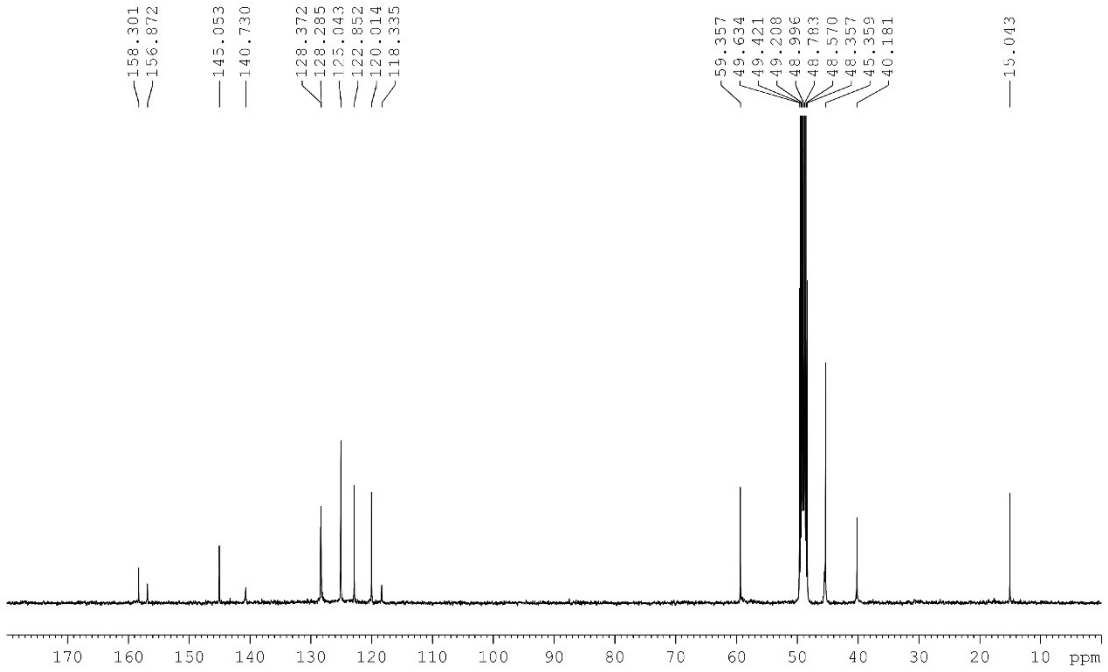


Fig. S9 ^{1}H NMR spectrum of **SW41** (400 MHz, CD_3OD)

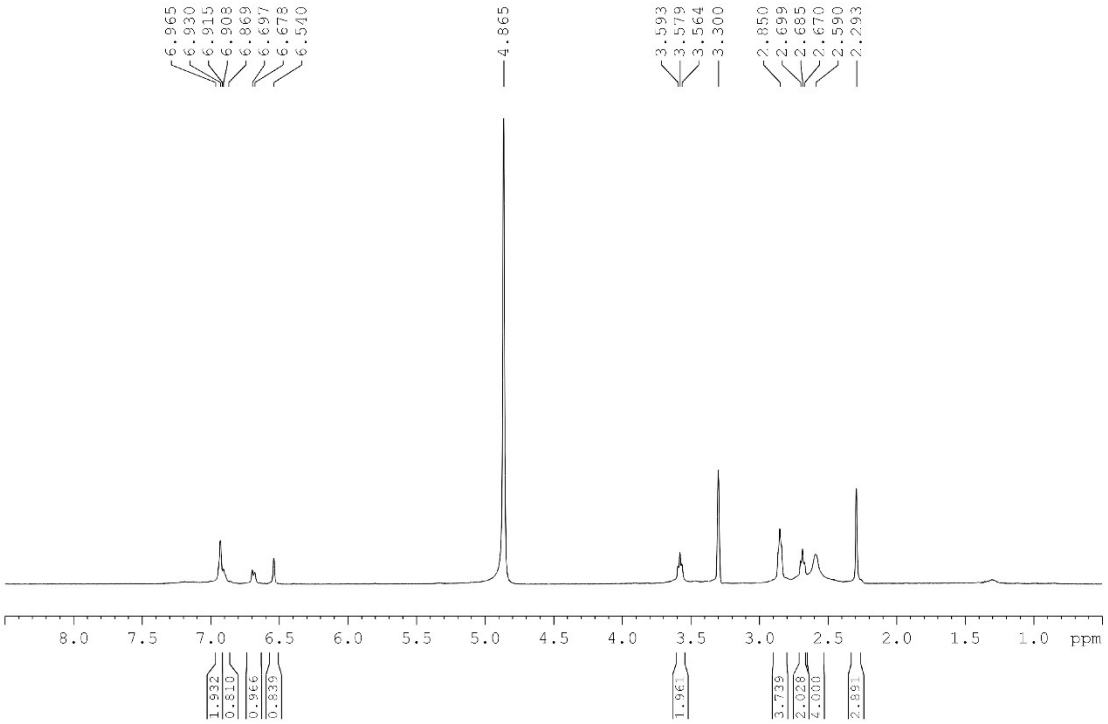


Fig. S10 ^{13}C NMR spectrum of **SW41** (100 MHz, $CDCl_3$)

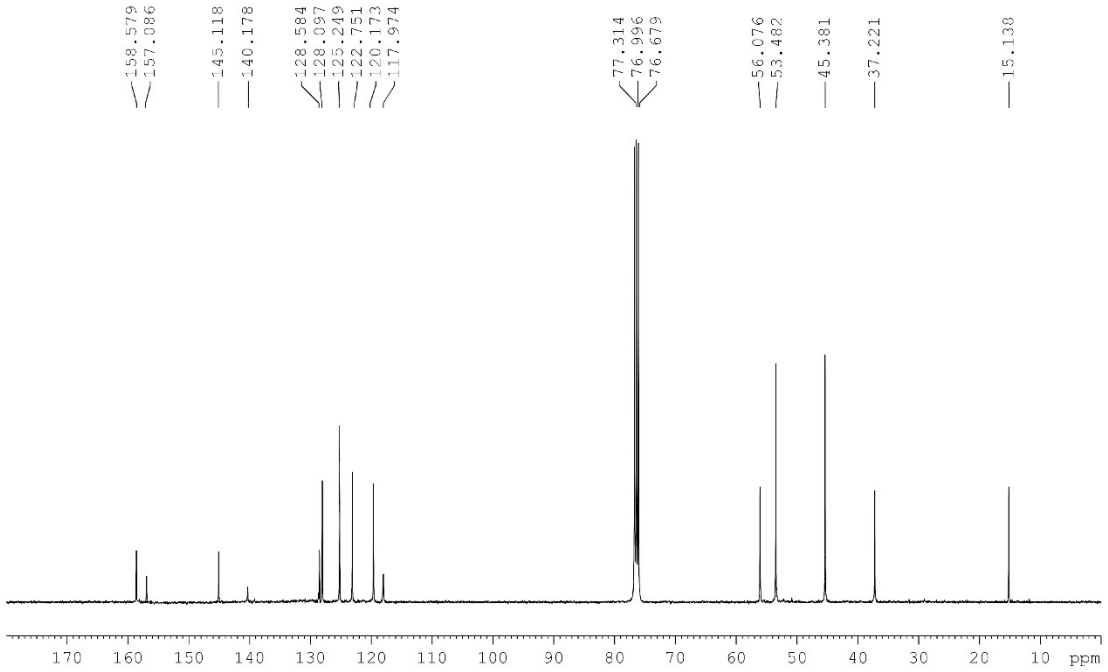


Fig. S11 ^{1}H NMR spectrum of **SW42** (400 MHz, CD_3OD)

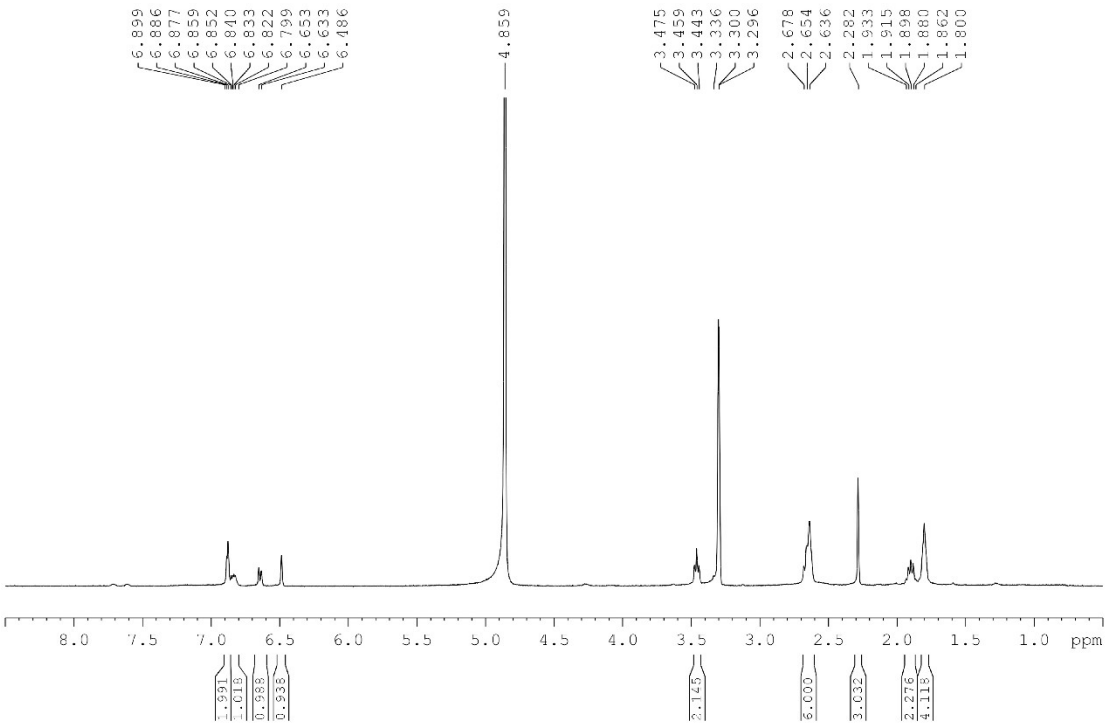


Fig. S12 ^{13}C NMR spectrum of **SW42** (100 MHz, $CDCl_3$)

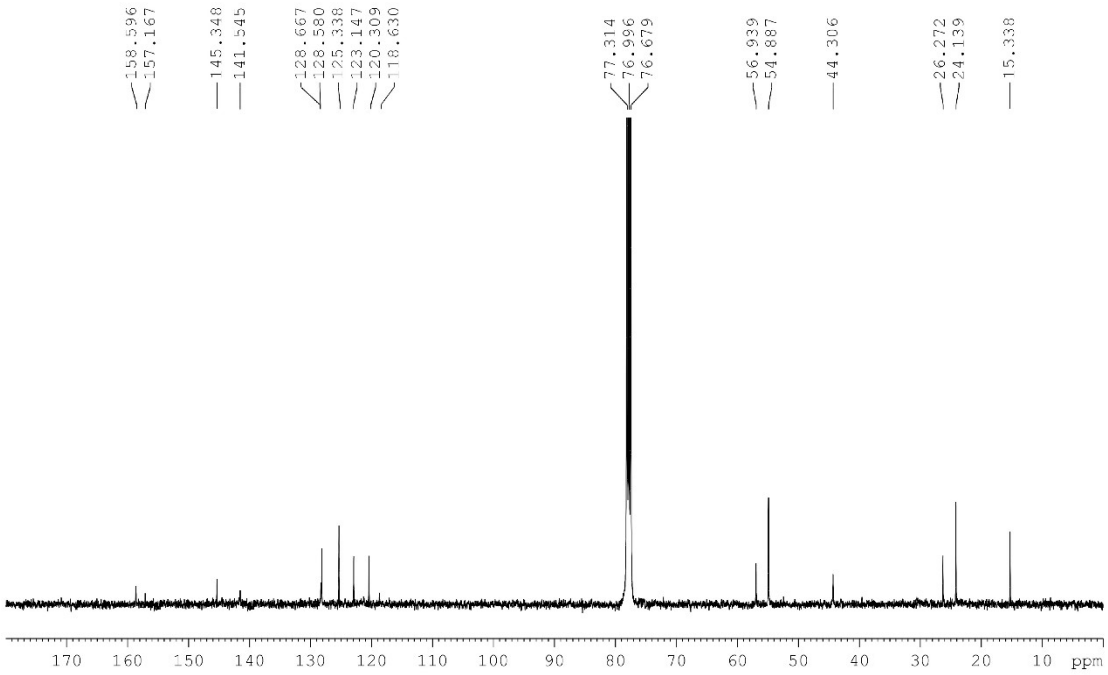


Fig. S13 ^{1}H NMR spectrum of **8** (400 MHz, $CDCl_3$)

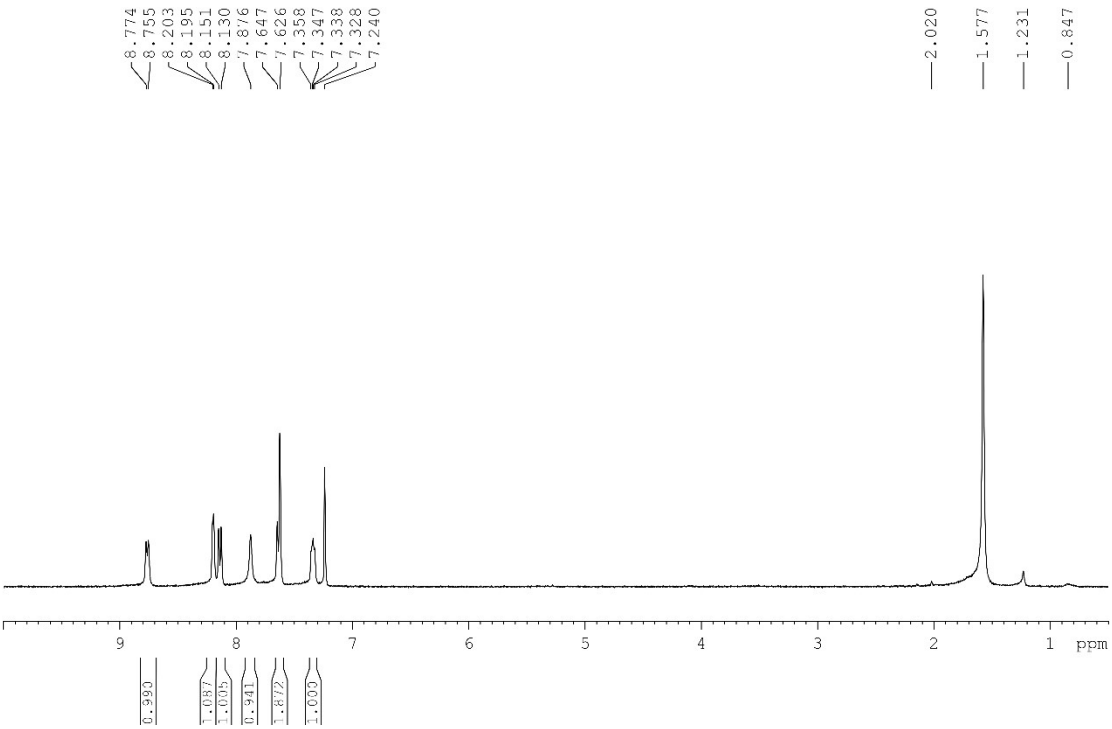


Fig. S14 ^{13}C NMR spectrum of **8** (100 MHz, $CDCl_3$)

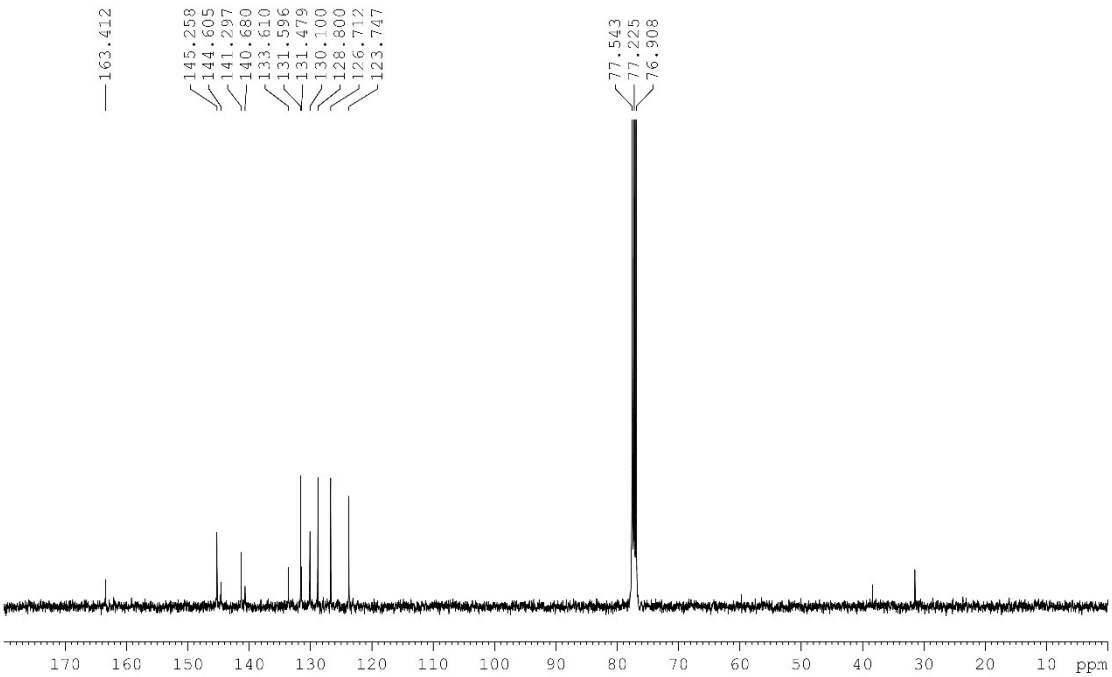


Fig. S15 ^{1}H NMR spectrum of **10** (400 MHz, DMSO-d_6)

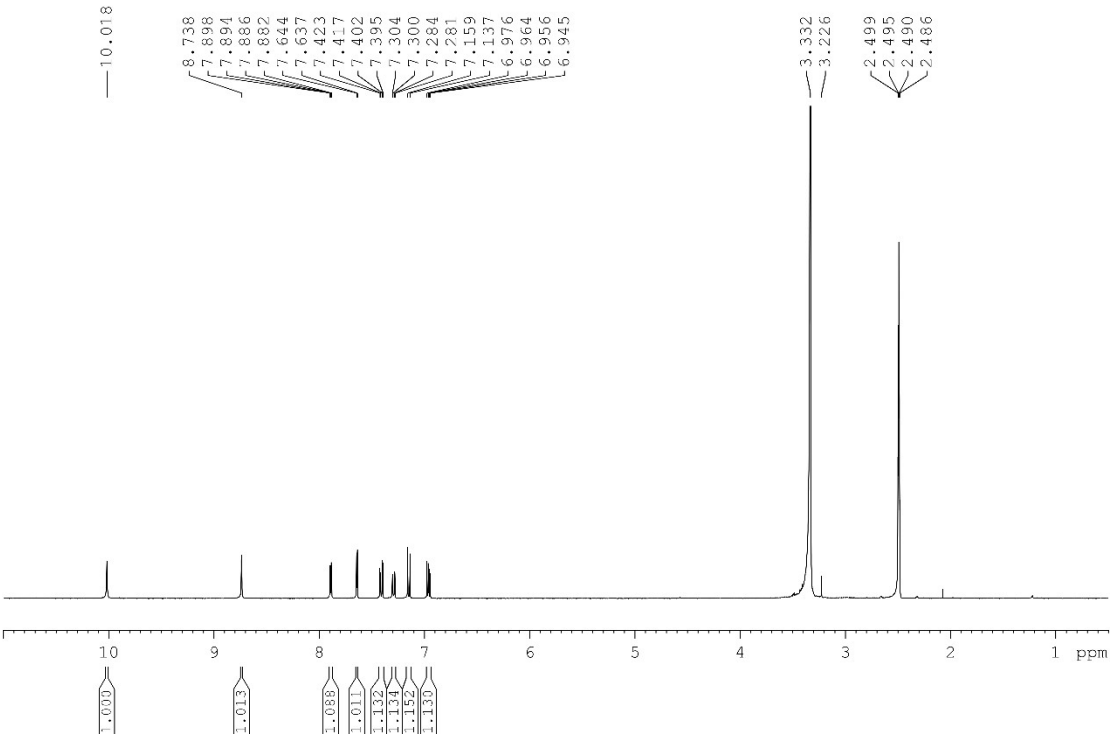


Fig. S16 ^{13}C NMR spectrum of **10** (100 MHz, DMSO-d_6)

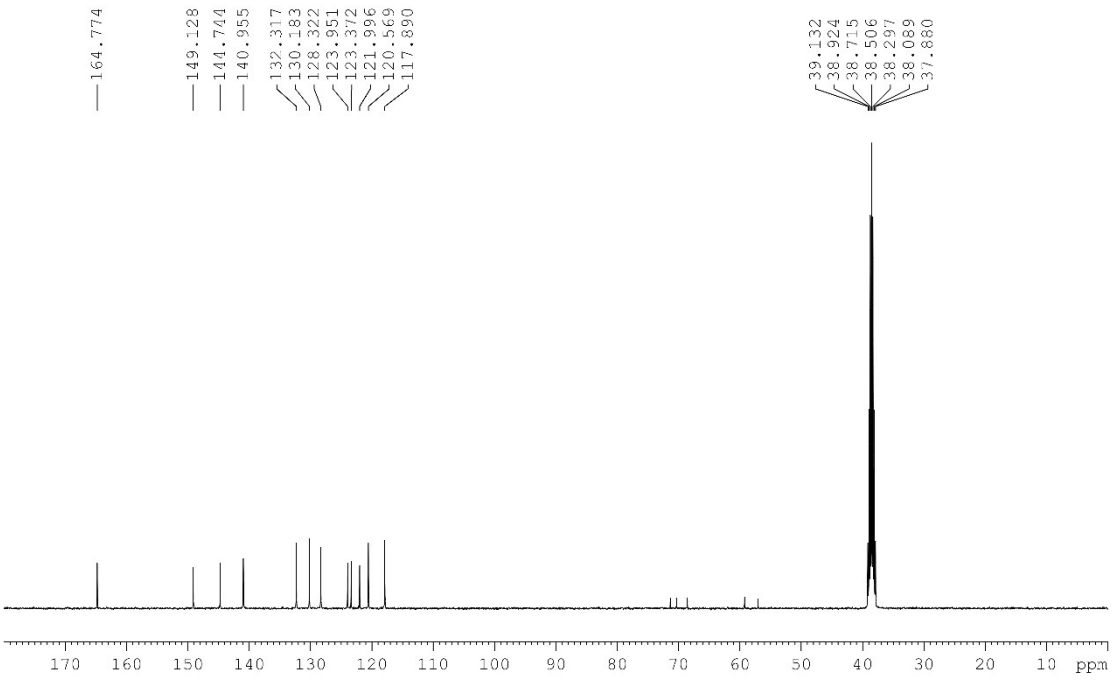


Fig. S17 ^{1}H NMR spectrum of **SW21** (400 MHz, $CDCl_3$)

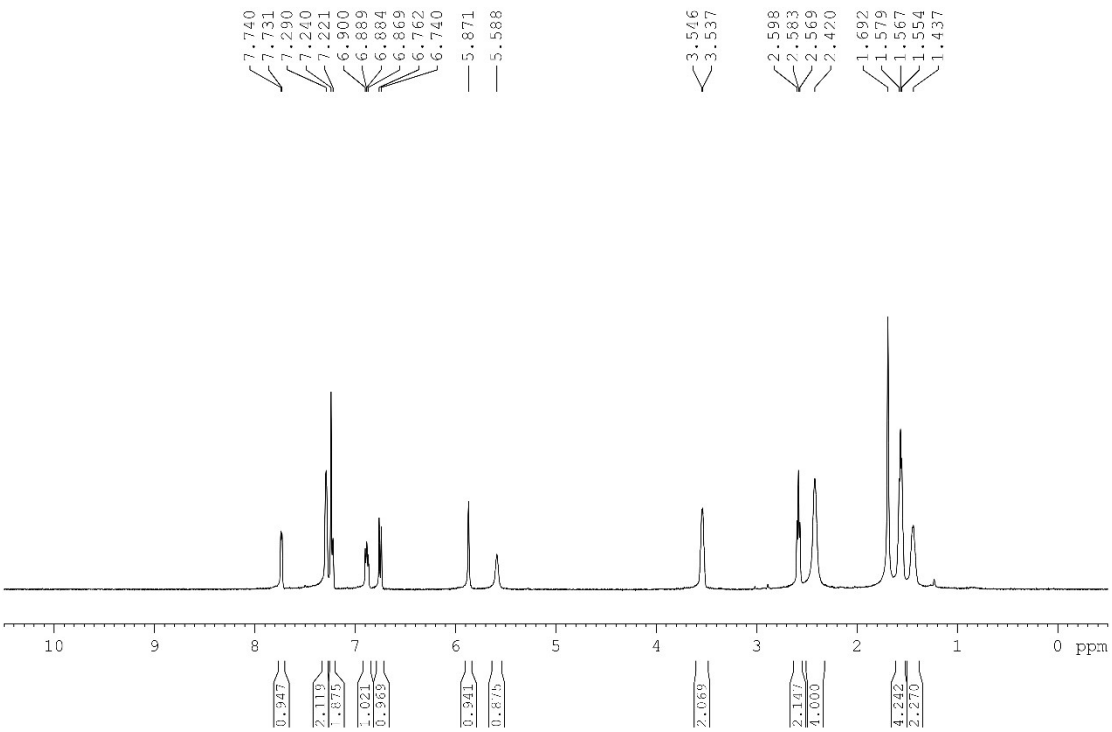


Fig. S18 ^{13}C NMR spectrum of **SW21** (100 MHz, $CDCl_3$)

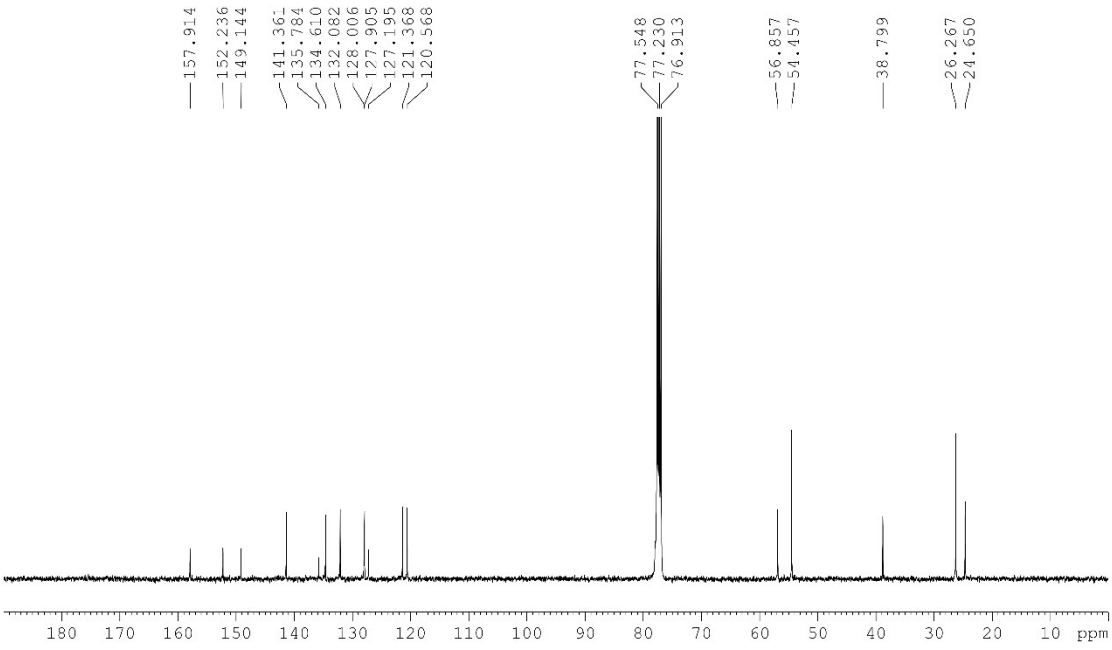


Fig. S19 ^{1}H NMR spectrum of **SW27** (400 MHz, CD_3OD)

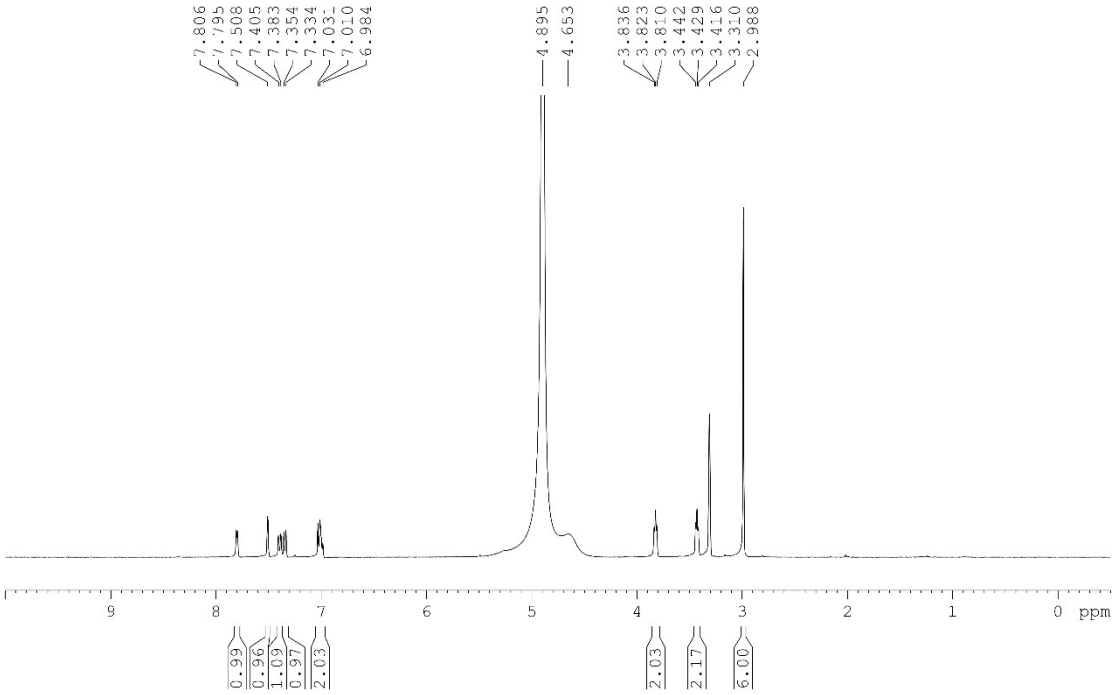


Fig. S20 ^{13}C NMR spectrum of **SW27** (100 MHz, $CDCl_3$)

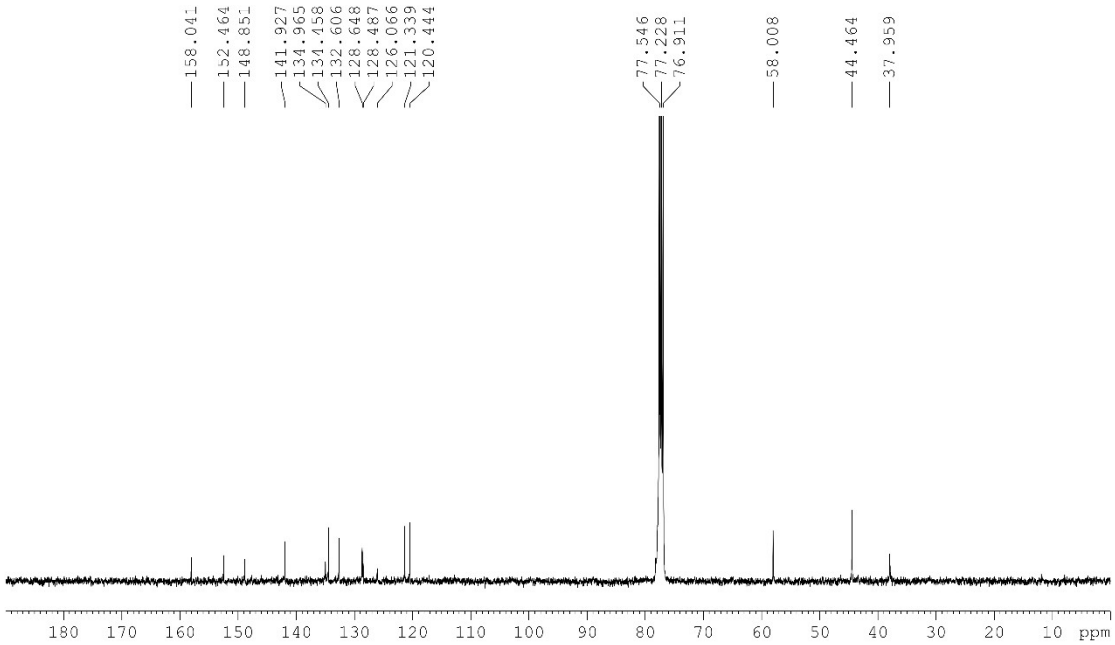


Fig. S21 ^{1}H NMR spectrum of **SW29** (400 MHz, CD_3OD)

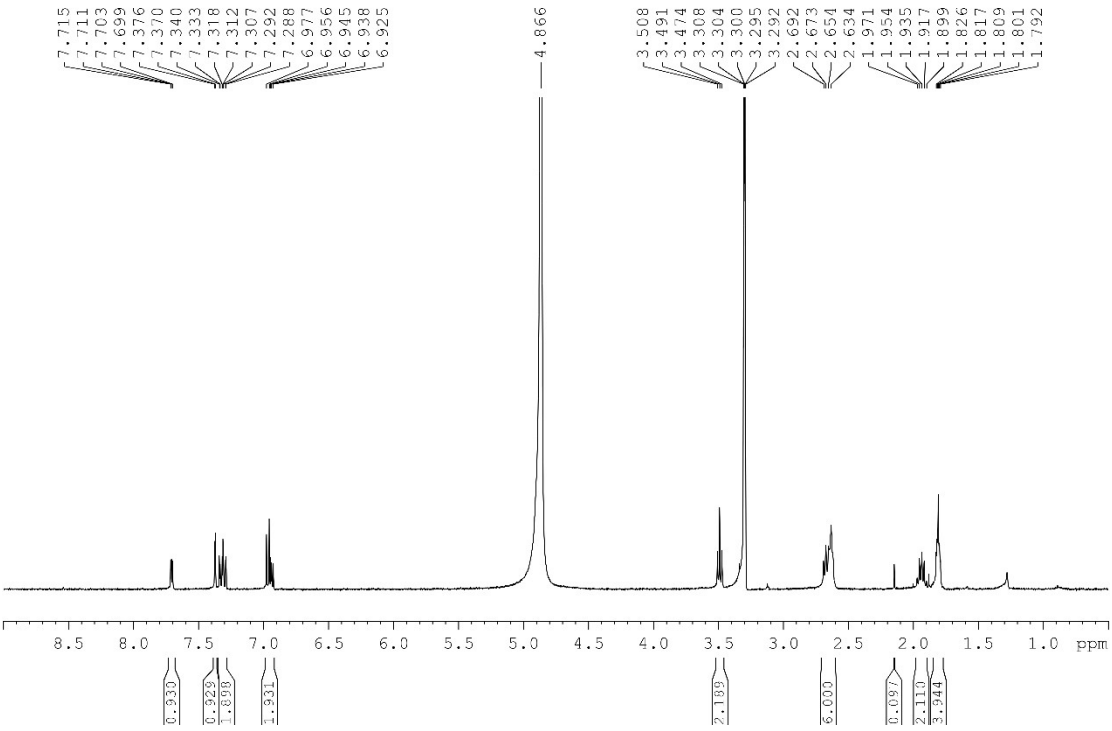


Fig. S22 ^{13}C NMR spectrum of **SW29** (100 MHz, $CDCl_3$)

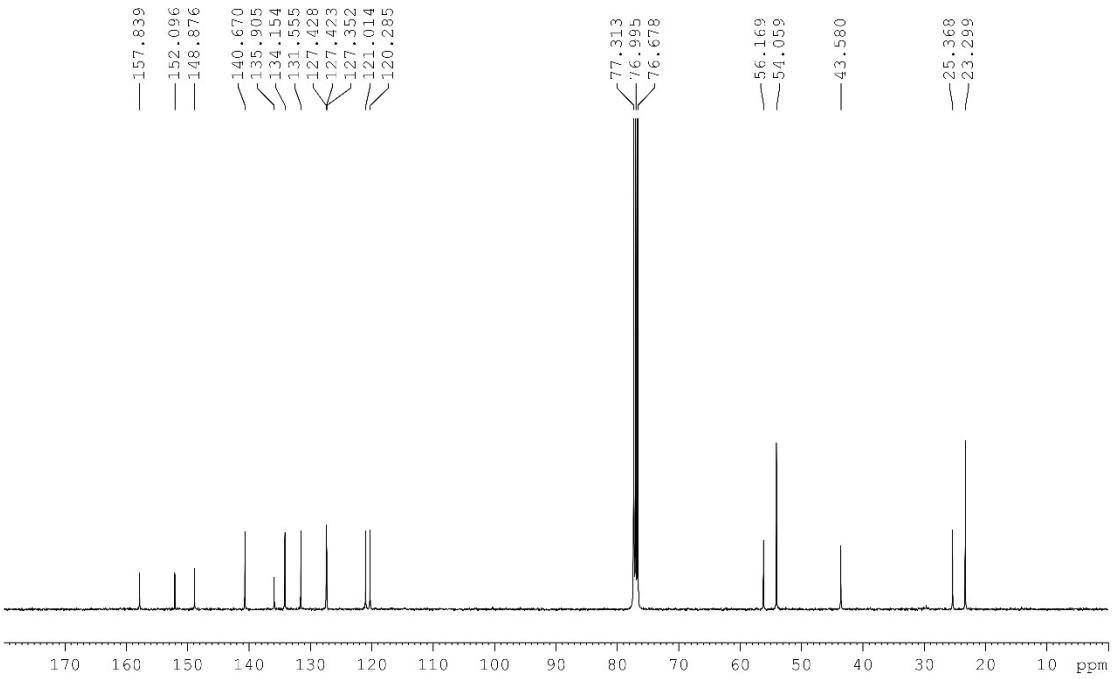


Fig. S23 ^{1}H NMR spectrum of **SW30** (400 MHz, CD_3OD)

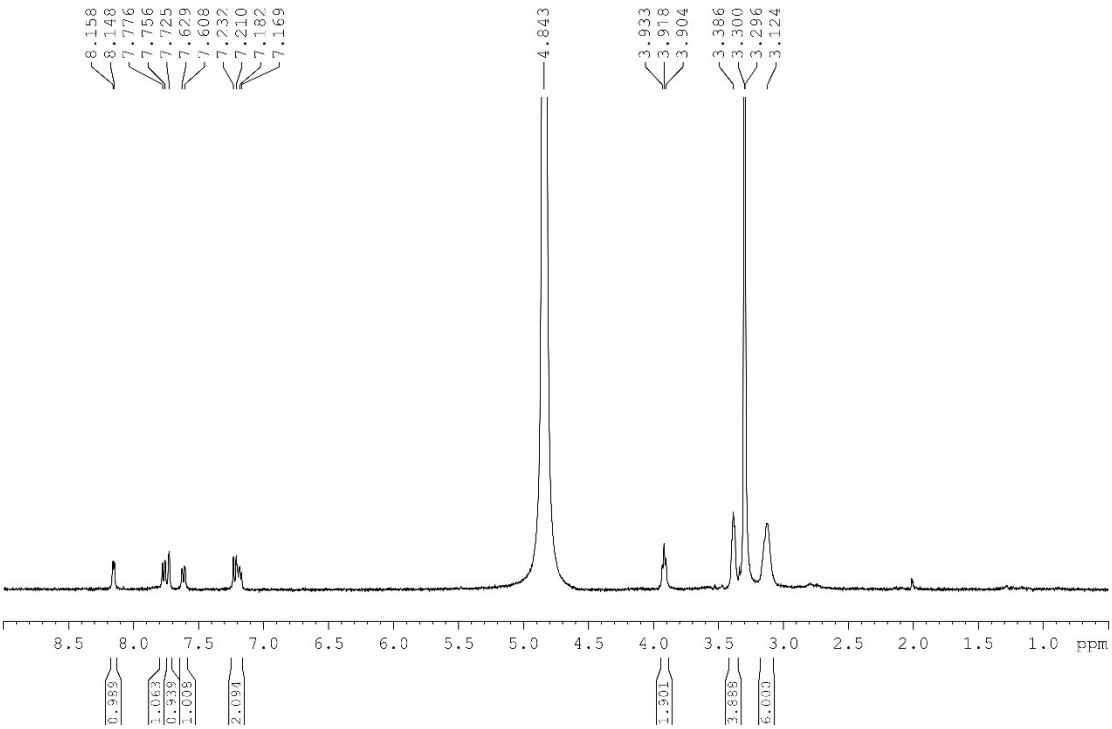


Fig. S24 ^{13}C NMR spectrum of **SW30** (100 MHz, CD_3OD)

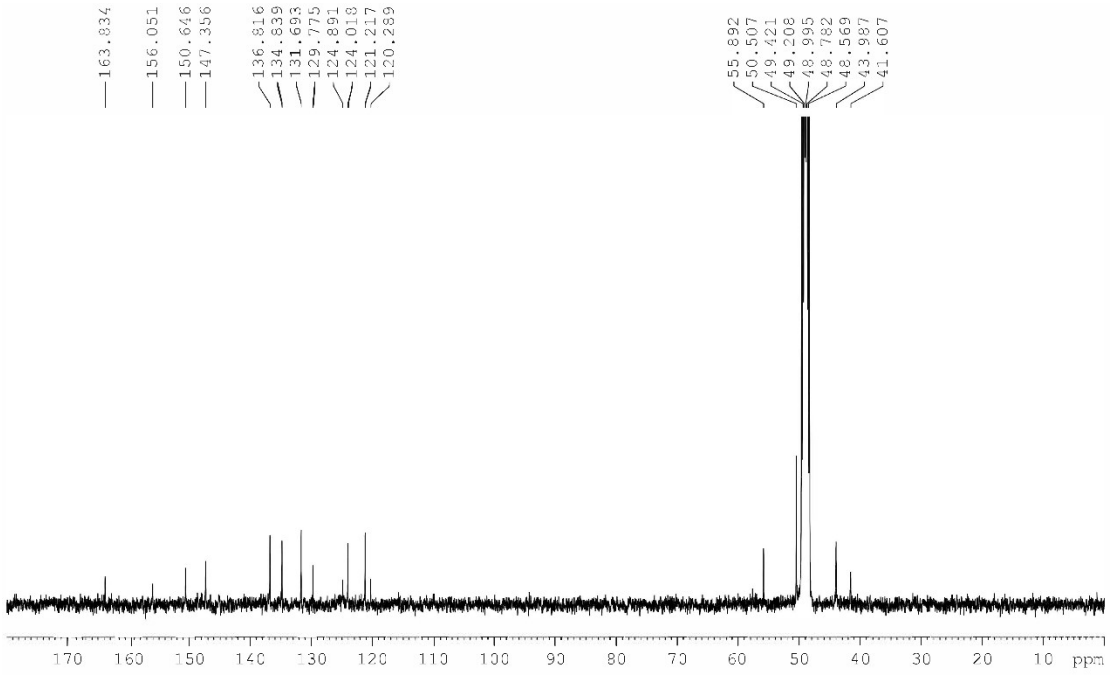


Fig. S25 ^{1}H NMR spectrum of **14** (400 MHz, DMSO-d_6)

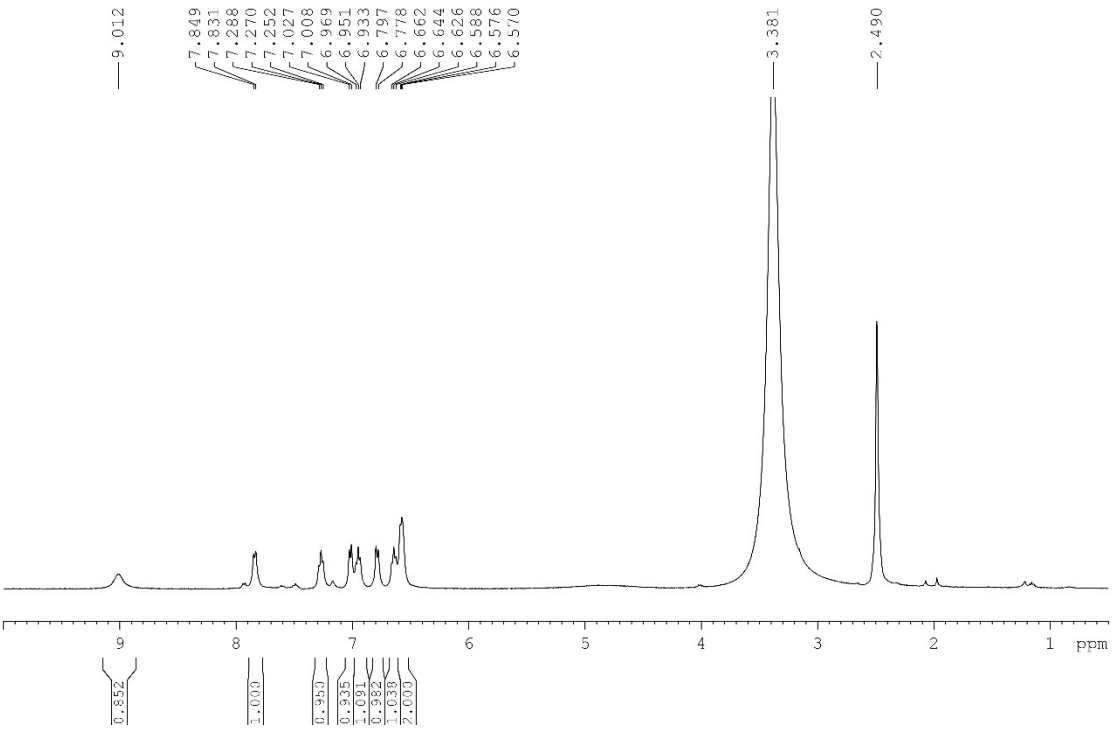


Fig. S26 ^{13}C NMR spectrum of **14** (100 MHz, DMSO-d_6)

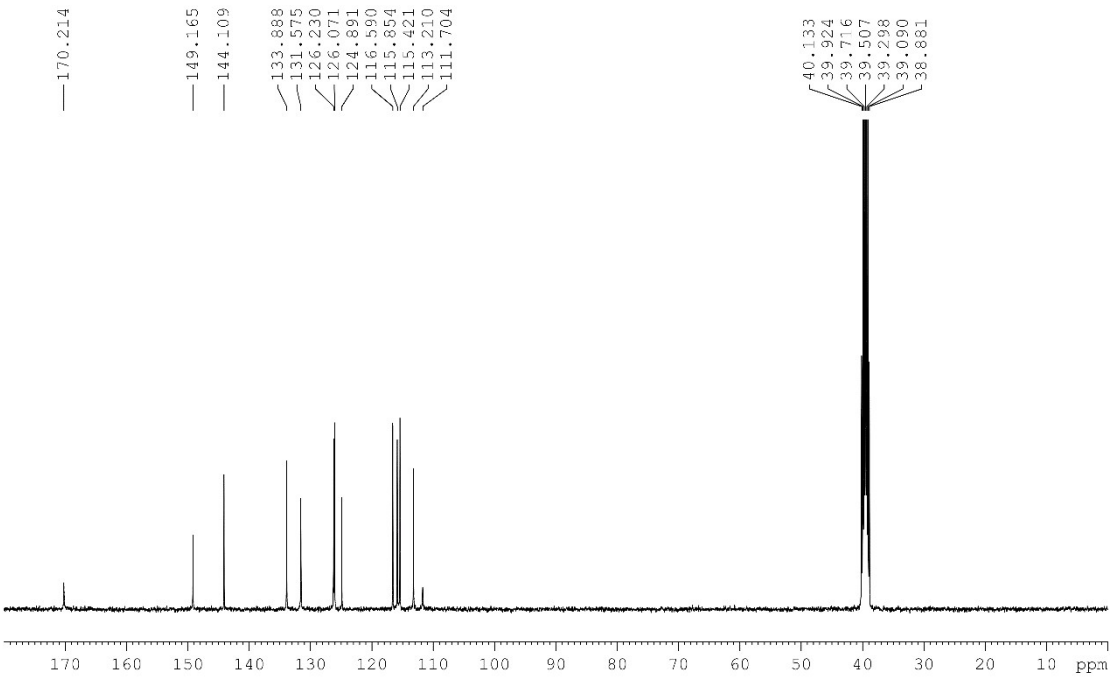


Fig. S27 ^{1}H NMR spectrum of **15** (400 MHz, DMSO-d_6)

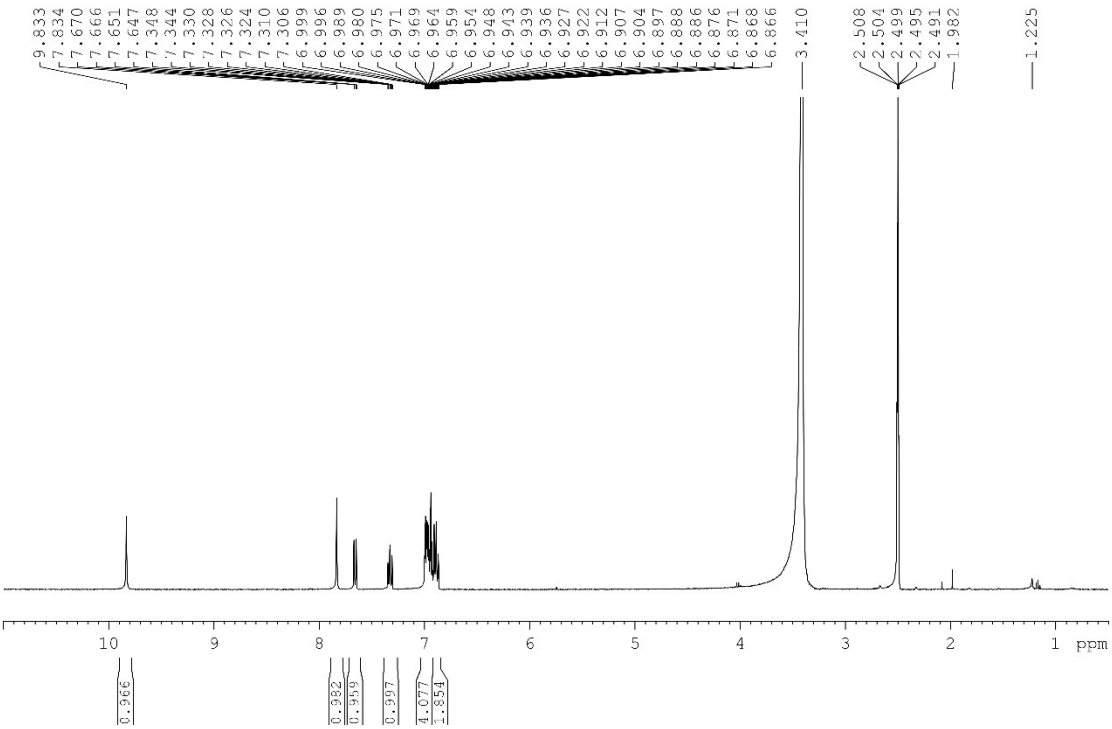


Fig. S28 ^{13}C NMR spectrum of **15** (100 MHz, DMSO-d_6)

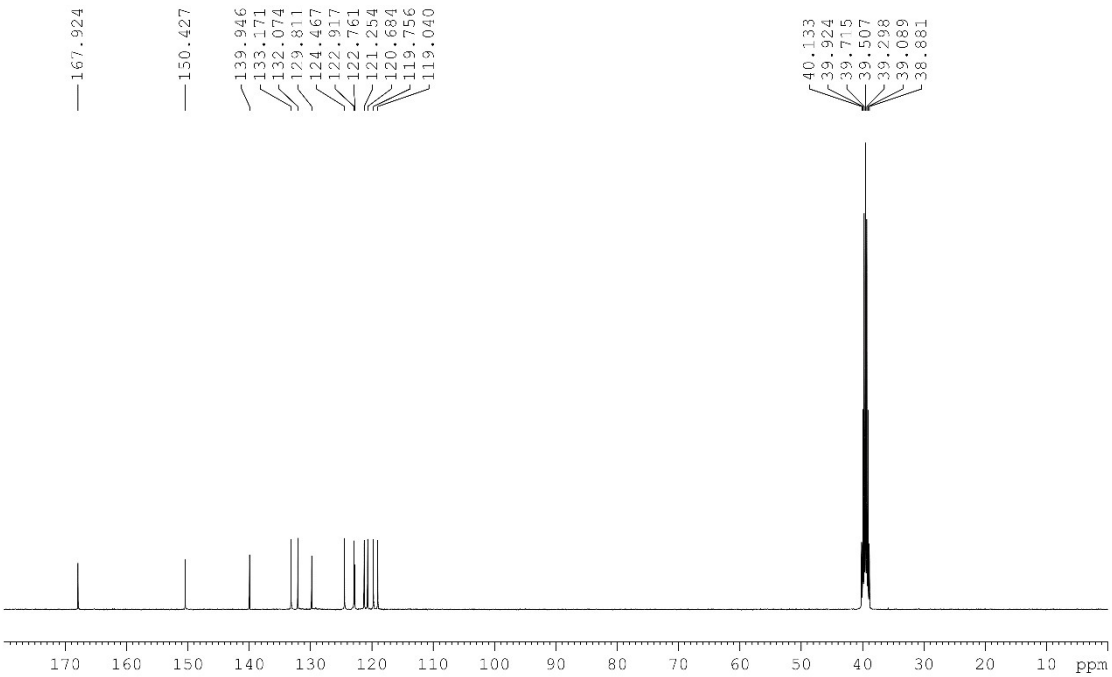


Fig. S29 ^{1}H NMR spectrum of **SW33** (400 MHz, CD_3OD)

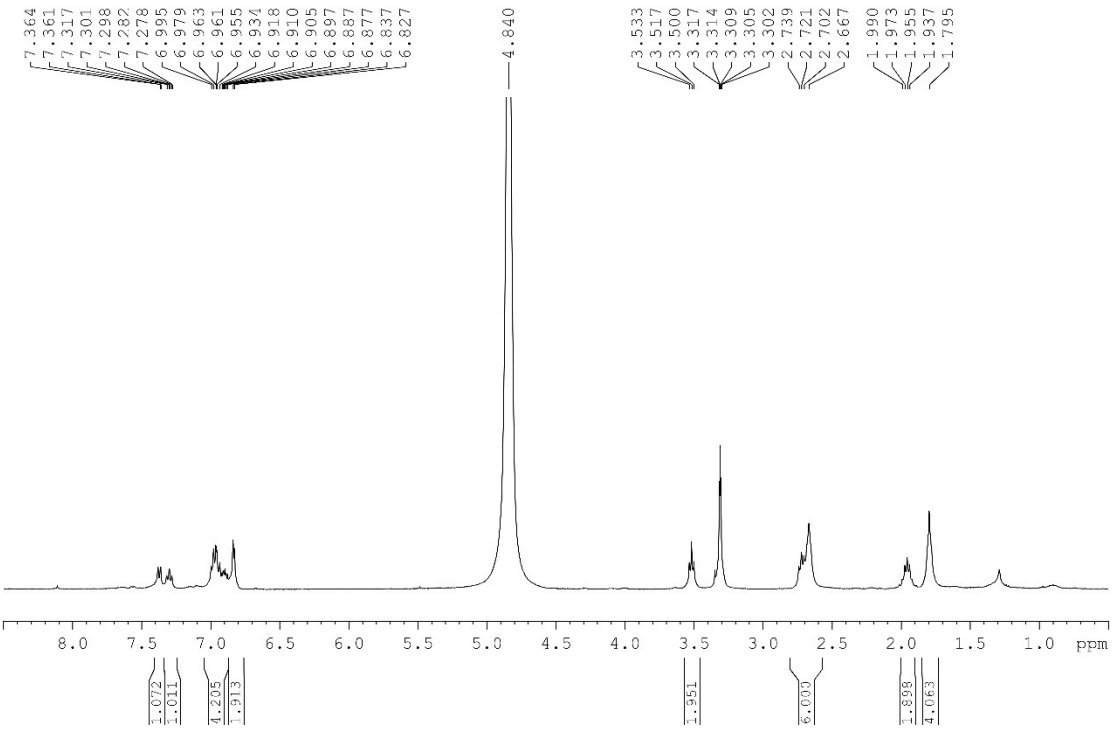


Fig. S30 ^{13}C NMR spectrum of **SW33** (100 MHz, $CDCl_3$)

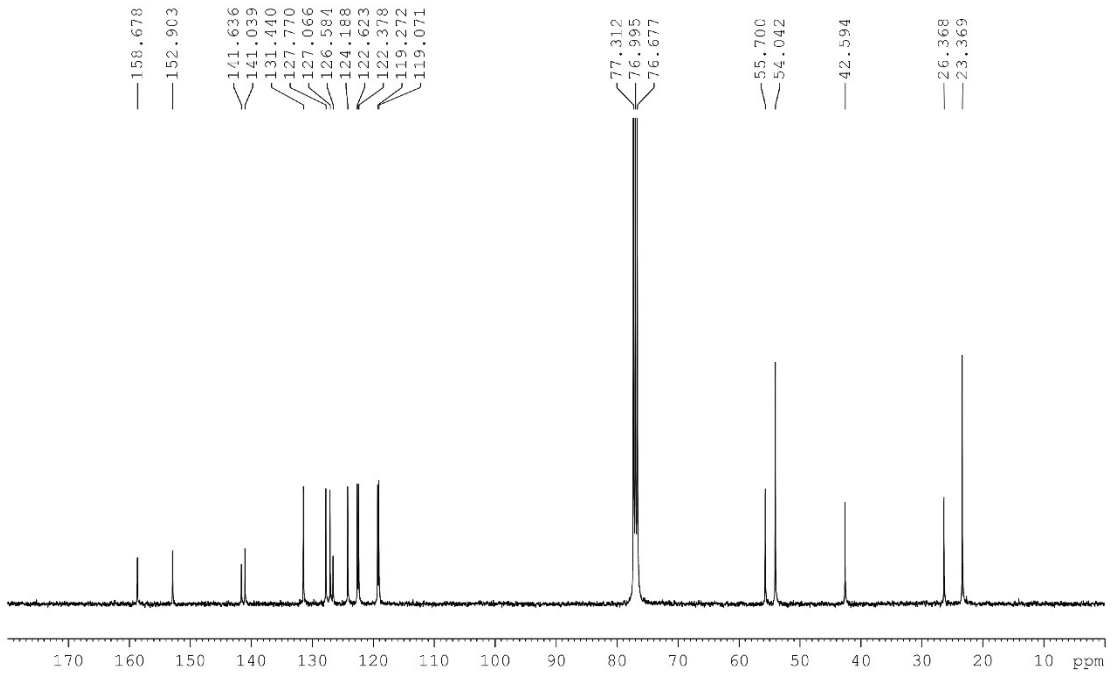


Fig. S31 ^{1}H NMR spectrum of **SW35** (400 MHz, CD_3OD)

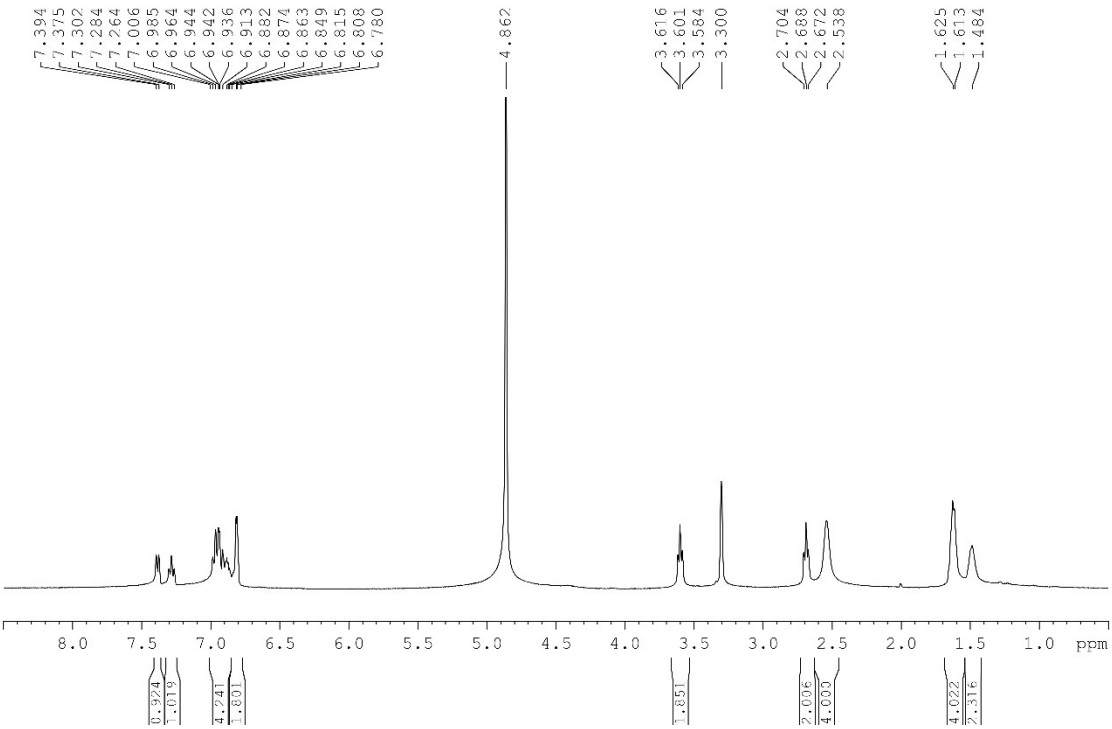


Fig. S32 ^{13}C NMR spectrum of **SW35** (100 MHz, $CDCl_3$)

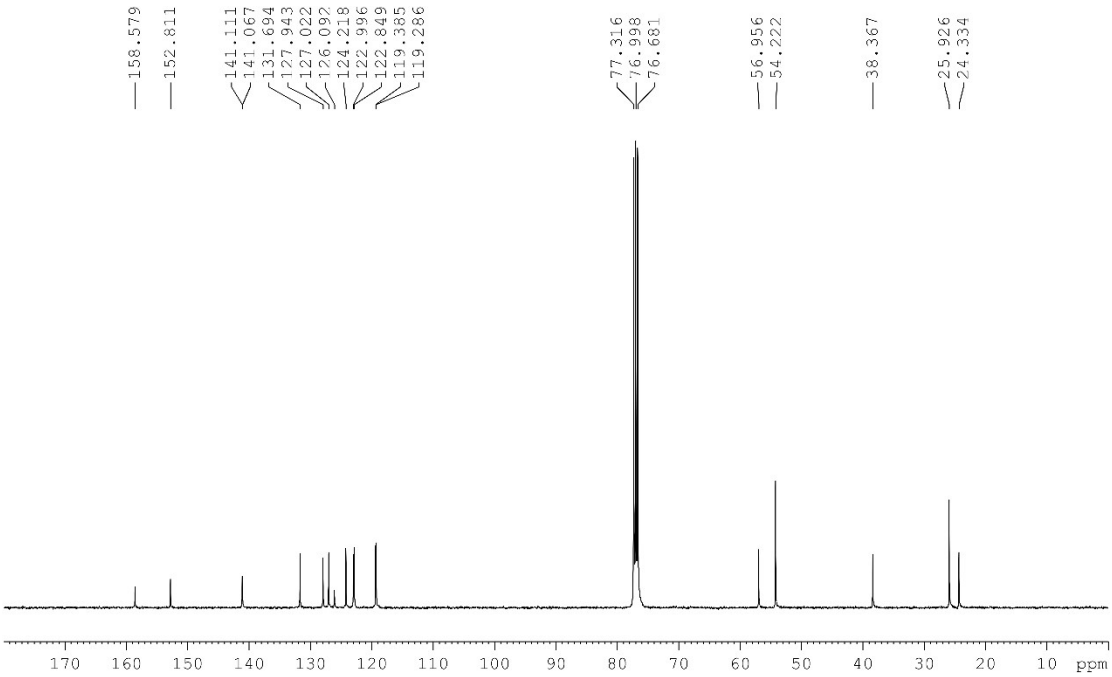


Fig. S33 ^{1}H NMR spectrum of **SW44** (400 MHz, CD_3OD)

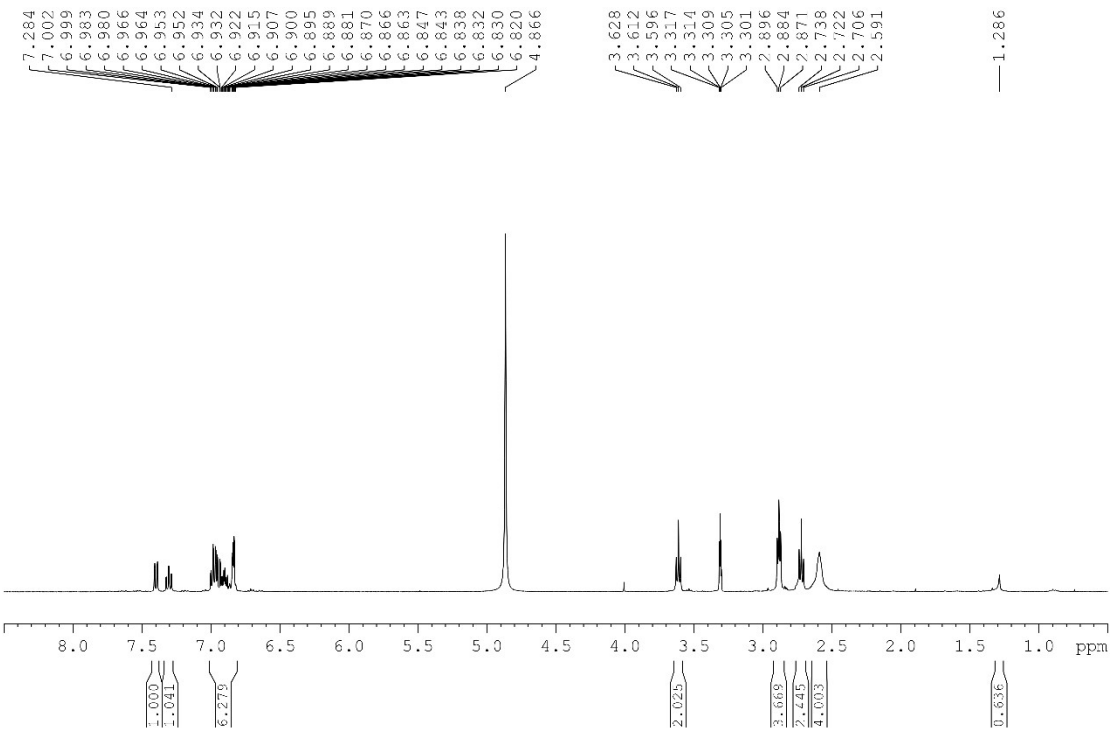


Fig. S34 ^{13}C NMR spectrum of **SW44** (100 MHz, CD_3OD)

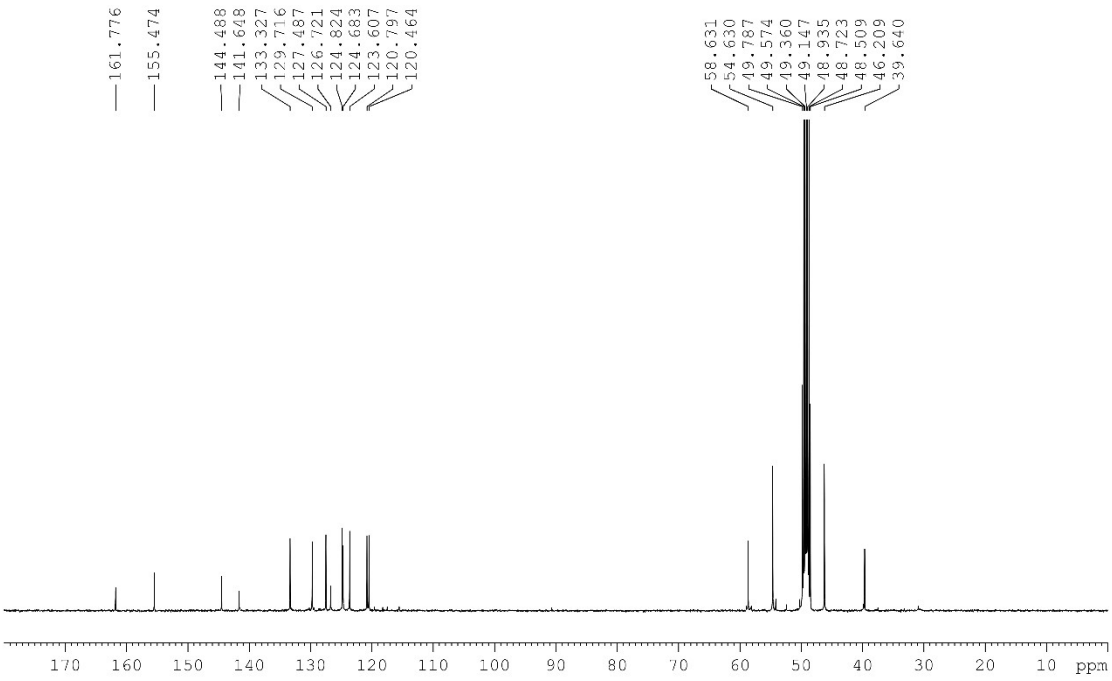


Fig. S35 ^{1}H NMR spectrum of **SW45** (400 MHz, CD_3OD)

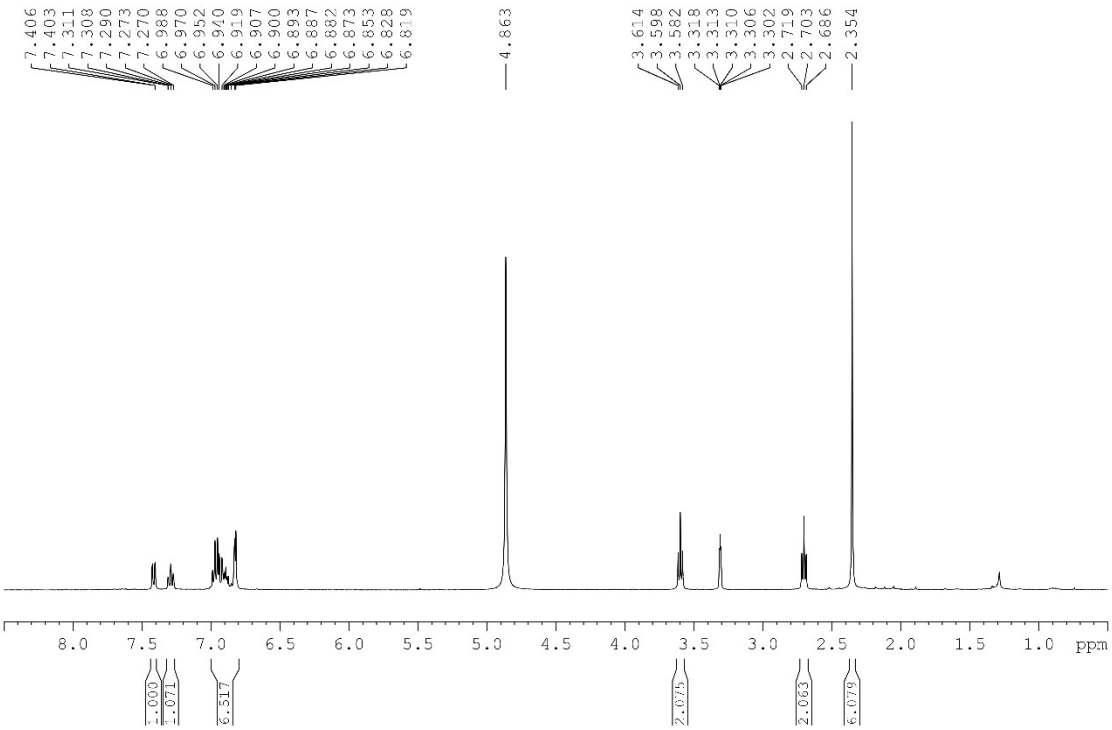


Fig. S36 ^{13}C NMR spectrum of **SW45** (100 MHz, CD_3OD)

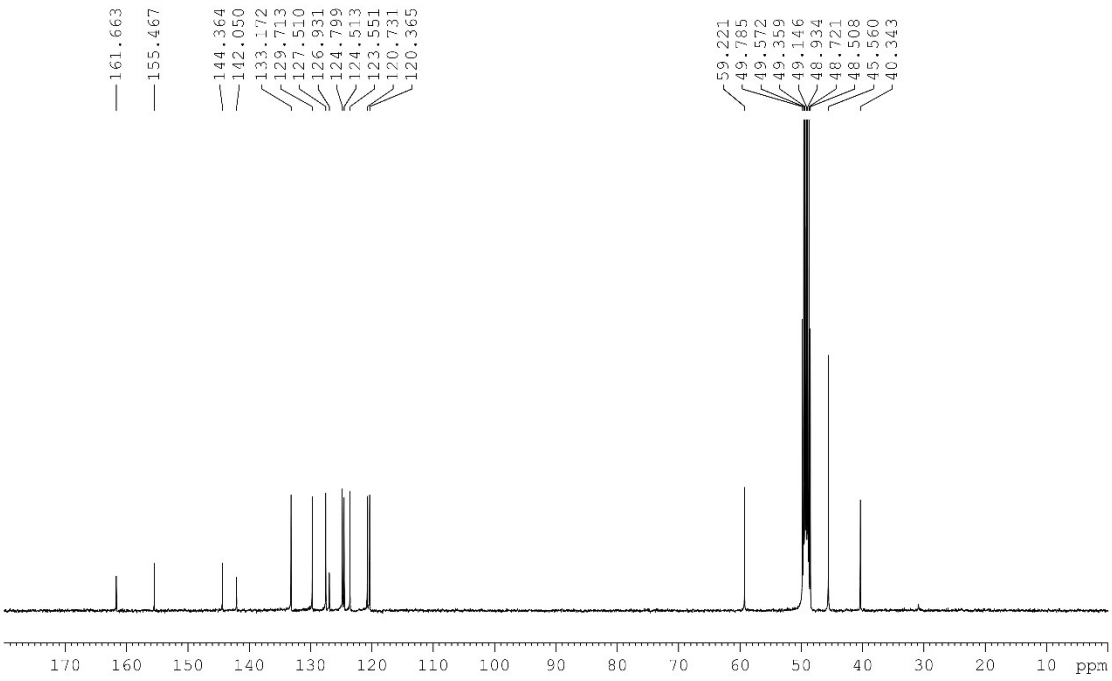
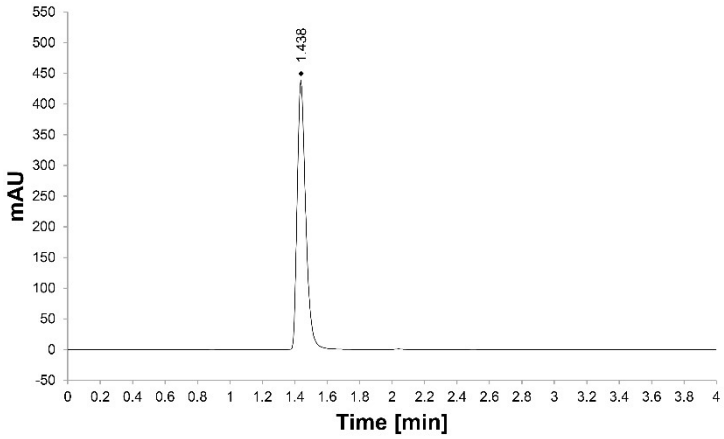


Fig. S37 HPLC chromatogram of **SW38**

Sample: SW38
Sample pretreatment: DMSO: ACN: H_2O = 2:29.4:68.6, v/v/v
Elution method: H_2O (0.1% formic acid): ACN (0.1% formic acid) = 80:20, v/v

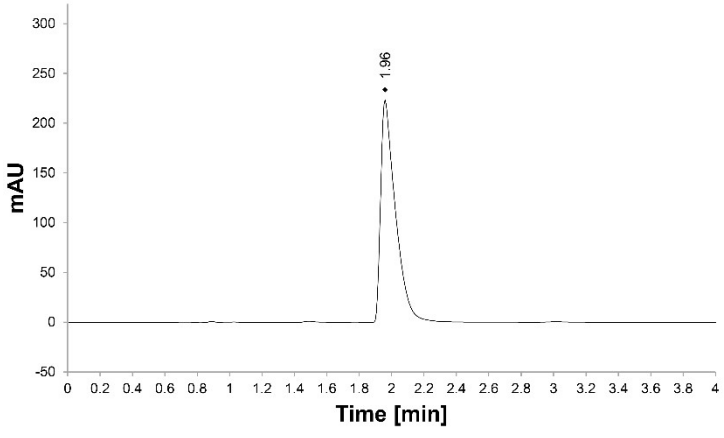


Signal: Sig = 254 nm

RT [min]	Area	Height	Area %
1.438	1609.7423	439.8401	100.0000

Fig. S38 HPLC chromatogram of **SW39**

Sample: SW39
Sample pretreatment: DMSO: ACN: H_2O = 2:29.4:68.6, v/v/v
Elution method: H_2O (0.1% formic acid): ACN (0.1% formic acid) = 80:20, v/v



Signal: Sig = 254 nm

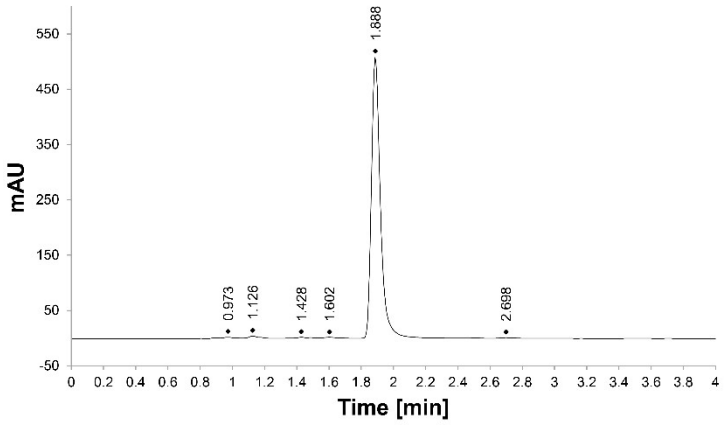
RT [min]	Area	Height	Area %
1.960	1415.3573	223.5697	100.0000

Fig. S39 HPLC chromatogram of **SW41**

Sample: SW41

Sample pretreatment: DMSO: ACN: H_2O = 2:29.4:68.6, v/v/v

Elution method: H_2O (0.1M CH_3COONH_4 and 0.1M NaCl): ACN = 79:21, v/v



Signal: Sig = 254 nm

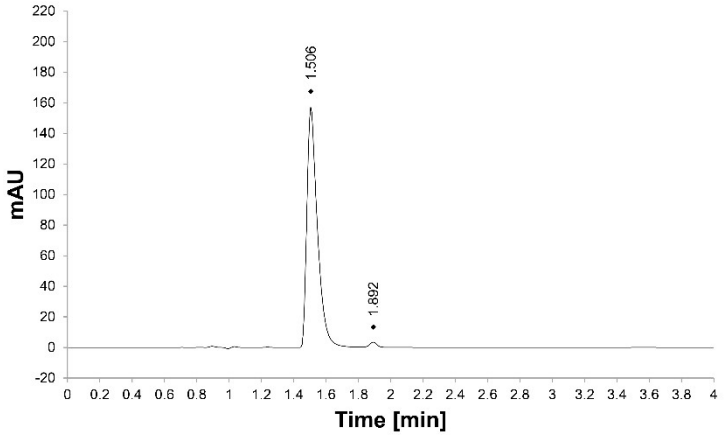
RT [min]	Area	Height	Area %
0.973	10.8641	2.2813	0.5327
1.126	19.9783	4.3344	0.9796
1.428	5.0286	1.5908	0.2466
1.602	8.4416	1.4551	0.4139
1.888	1987.8467	509.2690	97.4716
2.698	7.2525	1.3562	0.3556

Fig. S40 HPLC chromatogram of **SW42**

Sample: SW42

Sample pretreatment: DMSO: ACN: H2O = 2:63.7:34.3, v/v/v

Elution method: H_2O (0.1% formic acid): ACN (0.1% formic acid) = 82:18, v/v

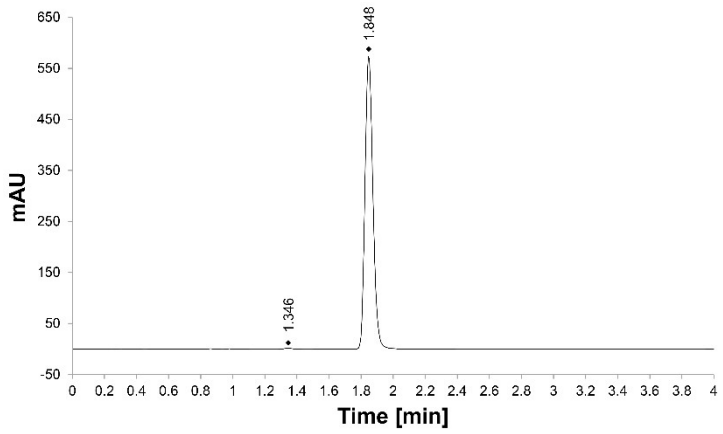


Signal: Sig = 254 nm

RT [min]	Area	Height	Area %
1.506	713.3449	157.4367	98.5616
1.892	10.4102	3.3253	1.4384

Fig. S41 HPLC chromatogram of **SW27**

Sample: SW27
Sample pretreatment: DMSO: ACN = 2:98, v/v
Elution method: H_2O (0.1M CH_3COONH_4 and 0.1M NaCl): ACN = 70:30, v/v

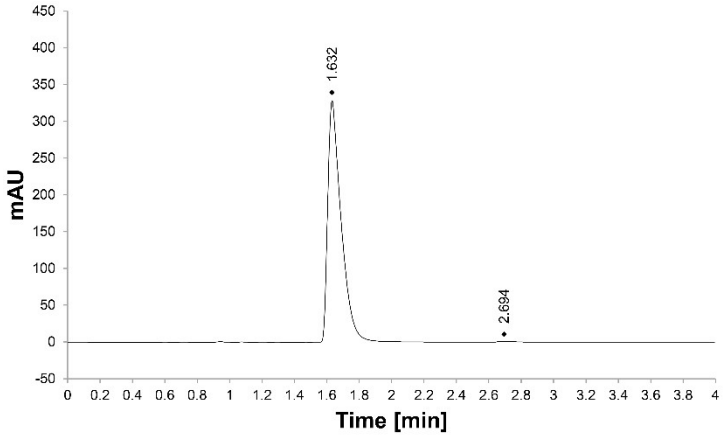


Signal: Sig = 254 nm

RT [min]	Area	Height	Area %
1.346	7.9383	2.4064	0.3980
1.848	1986.3574	577.5507	99.6020

Fig. S42 HPLC chromatogram of **SW29**

Sample: SW29
Sample pretreatment: DMSO: ACN: H_2O = 2:29.4:68.6, v/v/v
Elution method: H_2O (0.1% formic acid): ACN (0.1% formic acid) = 90:10, v/v



Signal: Sig = 254 nm

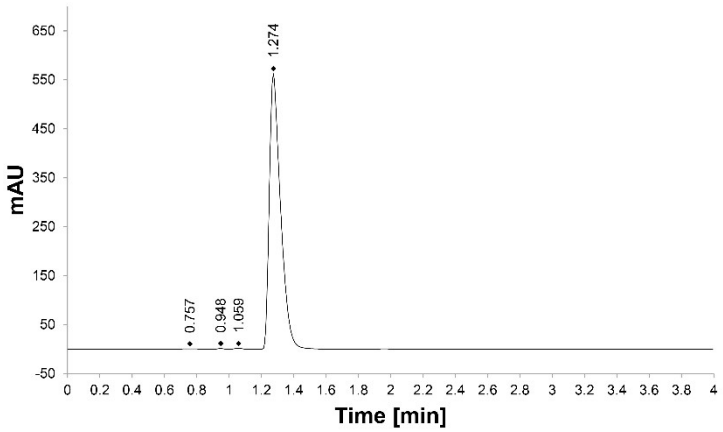
RT [min]	Area	Height	Area %
1.632	1892.1515	329.2071	99.6072
2.694	7.4625	1.2283	0.3928

Fig. S43 HPLC chromatogram of **SW30**

Sample: SW30

Sample pretreatment: DMSO: ACN: H_2O = 2:29.4:68.6, v/v/v

Elution method: H_2O (0.1% formic acid): ACN (0.1% formic acid) = 90:10, v/v



Signal: Sig = 254 nm

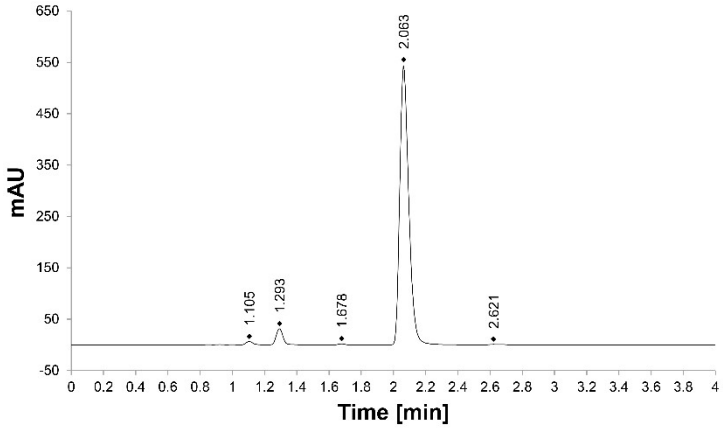
RT [min]	Area	Height	Area %
0.757	9.2818	1.0193	0.3393
0.948	5.2756	1.7220	0.1928
1.059	9.3546	1.9234	0.3419
1.274	2711.8606	562.7664	99.1260

Fig. S44 HPLC chromatogram of **SW33**

Sample: SW33

Sample pretreatment: DMSO: ACN: H_2O = 2:29.4:68.6, v/v/v

Elution method: H_2O (0.1M CH_3COONH_4 and 0.1M NaCl): ACN = 65:35, v/v



Signal: Sig = 254 nm

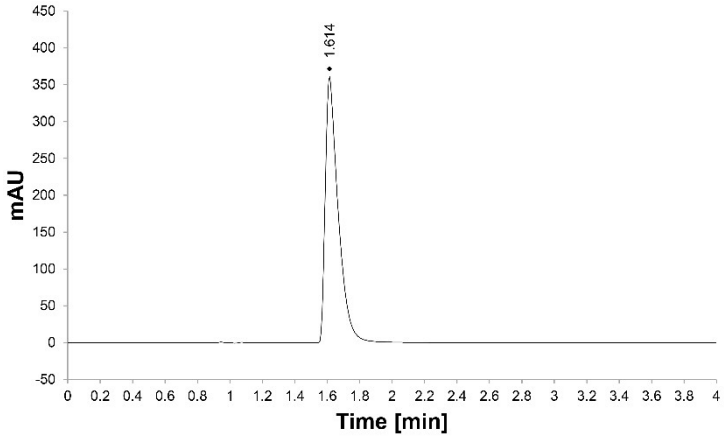
RT [min]	Area	Height	Area %
1.105	20.1388	6.5794	0.8972
1.293	92.9923	31.6239	4.1430
1.678	7.6530	2.1676	0.3410
2.063	2116.5369	545.5126	94.2971
2.621	7.2189	1.1210	0.3216

Fig. S45 HPLC chromatogram of **SW35**

Sample: SW35

Sample pretreatment: DMSO: ACN: H_2O = 2:29.4:68.6, v/v/v

Elution method: H_2O (0.1% formic acid): ACN (0.1% formic acid) = 90:10, v/v



Signal: Sig = 254 nm

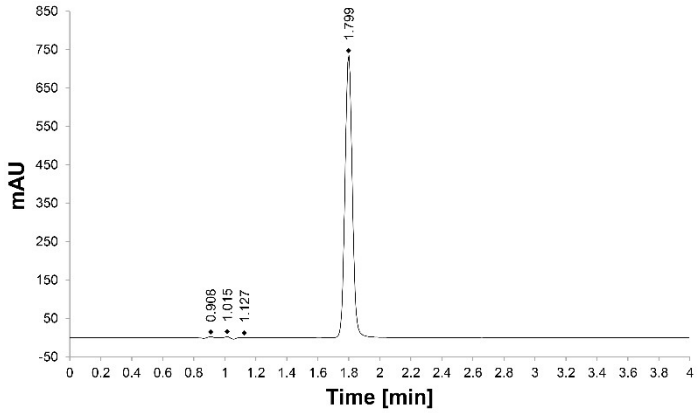
RT [min]	Area	Height	Area %
1.614	2000.3433	361.4542	100.0000

Fig. S46 HPLC chromatogram of **SW44**

Sample: SW44

Sample pretreatment: DMSO: MeOH = 2:98, v/v

Elution method: H_2O (0.1M CH_3COONH_4 and 0.1M NaCl): ACN = 78:22, v/v



Signal: Sig = 254 nm

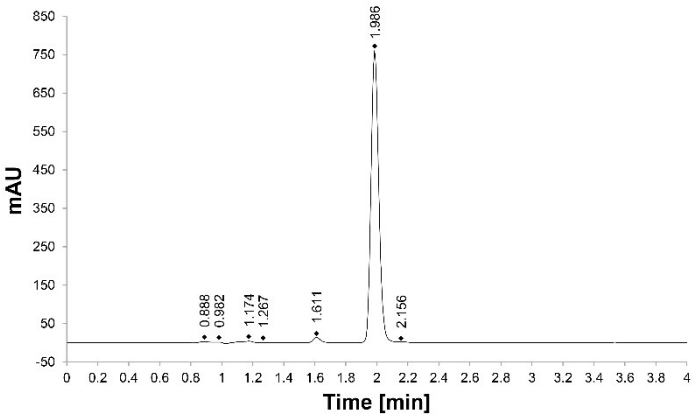
RT [min]	Area	Height	Area %
0.908	18.3354	4.4505	0.7430
1.015	17.7547	5.6157	0.7194
1.127	11.1185	1.9674	0.4505
1.799	2420.6729	737.1699	98.0871

Fig. S47 HPLC chromatogram of **SW45**

Sample: SW45

Sample pretreatment: DMSO: MeOH = 2:98, v/v

Elution method: H_2O (0.1M CH_3COONH_4 and 0.1M NaCl): ACN = 78:22, v/v



Signal: Sig = 254 nm

RT [min]	Area	Height	Area %
0.888	23.0137	4.2641	0.8246
0.982	11.6326	3.5517	0.4168
1.174	43.7288	5.8608	1.5668
1.267	11.2392	2.1263	0.4027
1.611	47.1795	13.8228	1.6904
1.986	2646.0042	761.9946	94.8039
2.156	8.2318	1.5865	0.2949

List of chemical abbreviations

ACN, acetonitrile; DCM, dichloromethane; DEGMME, diethylene glycol monomethyl ether; DMA, *N*,*N*-dimethylaniline; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EtOAc, ethyl acetate; EtOH, ethanol; MeOH, methanol; THF, tetrahydrofuran.