Electronic Supplementary Information (ESI)

A dual covalent binder for labelling and inhibiting serine and metallo-carbapenemases

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1. Materials and Methods

1.1. Materials

All reagents used in this study were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO, USA), Energy Chemical (shanghai, China) and Adamas Reagent (shanghai, China). All other chemicals were analytical grade. *Escherichia coli* BL21 (DE3) cells were purchased from Wolsen Co. Ltd. (Xi'an, China).

1.2. Instrumentations

^{1}H and ^{13}C NMR spectra were measured with a 400 MHz Bruker NMR. The residual signals from DMSO-d_6 (^{1}H: δ 2.50 ppm; ^{13}C: δ 39.52 ppm) or $CDCl_3$ (^{1}H: δ 7.26 ppm; ^{13}C: δ 77.00 ppm) were used as internal standards. CLSM images were observed using a confocal laser scanning microscope (CLSM, LEICA TCS SP8, Germany). ICP-MS was analyzed using Agilent 7500a, Agilent Technologies (CA, USA). HR-MS spectra were measured at the LC-30A+Triple TOF 5600+ (AB SCIEX). ITC experiment was performed on a Malvern MicroCal iTC 200 instruments. All fluorescence data measurements were obtained using a fluorospectrophotometer (F-4600, Japan). The inhibition activity was tested on an Agilent UV8453 spectrometer.

1.3. Synthesis of compounds

To a solution of D01 (1.0 mmol) in anhydrous acetonitrile was added KSeCN (1.0 mmol). The mixture was stirred at 80 ℃ for 18 h. Then the mixture was cooled to 25 ℃ and filtered. The filter cake was washed with acetonitrile and dried under vacuum to obtain the brown solid D02. The isolated solid was used without purification for further reactions.

To a solution of D03 (1.0 eq) in DCM (5 mL) and DMF (5 mL) was added EDCI (1.2 eq.), HOBT (1.2 eq.) and TEA (3.0 eq.). The mixture was stirred at 25 ℃ for 30 min under nitrogen atmosphere. Then D02 (1.2 eq.) was added into the mixture. The mixture was stirred at 25 ℃ for 16 h under inert atmosphere. TLC showed the reaction was complete. The mixture was diluted with H_2O (20 mL), the aqueous layer was extracted with DCM, the combined organic layer was washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography

to obtain the desire compound **DS01**, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.88 (d, *J* = 6.2 Hz, 1H), 8.60 (s, 1H), 7.35 (d, *J* = 9.0 Hz, 1H), 6.59 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.43 (d, *J* = 2.4 Hz, 1H), 3.56 (q, *J* = 6.2 Hz, 2H), 3.39 (q, *J* = 7.1 Hz, 4H), 3.07 (t, *J* = 6.9 Hz, 2H), 2.14 (p, *J* = 6.7 Hz, 2H), 1.18 (d, *J* = 7.1 Hz, 6H).^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.13, 162.83, 157.74, 152.76, 148.32, 131.24, 110.11, 109.66, 108.36, 102.41, 96.59, 45.13, 38.14, 31.35, 27.72, 12.42. $C_{18}H_{21}N_3O_3Se$, $[M+H]^+$ cal: 408.0826, found: 408.0820.

D02 (2 mmol) was dissolved in a small amount of DMF, subsequently, added to 20 mL of DCM, followed by triethylamine (3 mmol) and D04 (2 mmol), the reaction was carried out under an ice bath. The reaction was monitored by TLC until the end of the reaction. The solvent was spun off and purified by column chromatography to give **DS02**, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.93 (t, *J* = 6.2 Hz, 1H), 8.67 (s, 1H), 7.42 (d, *J* = 9.0 Hz, 1H), 6.65 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.49 (d, *J* = 2.4 Hz, 1H), 3.60 (q, *J* = 6.3 Hz, 2H), 3.45 (q, *J* = 7.1 Hz, 4H), 3.04 (t, *J* = 7.1 Hz, 2H), 2.15 (q, *J* = 6.7 Hz, 2H), 1.24 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.83, 162.86, 157.72, 152.73, 148.32, 131.24, 112.27, 110.09, 109.71, 108.35, 96.57, 45.12, 37.38, 31.79, 30.35, 12.42. $C_{18}H_{21}N_3O_3S$, $[M+H]^+$ cal: 360.1382, found: 360.1383.

To a solution of D05 (1.0 eq) in DCM (10 mL) was added EDCI (1.2 eq.), HOBT (1.2 eq.) and TEA (3.0 eq.). The mixture was stirred at 25 ℃ for 30 min under nitrogen atmosphere. Then D02 (1.2 eq.) was added into the mixture. The mixture was stirred at 25 ℃ overnight. The mixture was diluted with H_2O (20 mL), the aqueous layer was extracted with DCM, the combined organic layer was washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography to obtain the desire compound **DC01**, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (t, *J* = 5.5 Hz, 1H), 8.23 (s, 1H), 7.93 (dd, *J* = 7.9 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 1H), 5.04 (s, 2H), 3.44-3.39 (m, 2H), 3.13 (t, *J* = 7.3 Hz, 2H), 2.07 (p, *J* = 6.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.23, 157.23, 133.85, 130.15, 129.98, 121.70, 105.17, 70.42, 38.93, 31.52, 28.15. $C_{12}H_{13}BN_2O_3Se$, $[M+H]^+$ cal: 325.0263, found: 325.0255.

1.4. Over-expression and purification of serine and metallo-carbapenemases

Over-expression and purification of metallo-carbapenemases NDM-1, VIM-2 (subclass B1), ImiS (subclass B2), L1 (subclass B3) and serine carbapenemases KPC-2 (class A), OXA-48 (class D) was overexpressed and purified as previously described.[1-3] *E. coli* BL21 cells were first transformed with the over-expression plasmid pET26b and the cells were plated on LB-agar plates containing kanamycin. A single colony was used to inoculate LB containing kanamycin. After preculture overnight at 37 °C, 10 mL cells in LB was used to inoculate 4 × 1 L of LB containing 25 μg/mL kanamycin. The cells were allowed to grow at 37 °C with shaking until the cells reached an OD_{600} of 0.6-0.8. Protein production was induced with 1 mM IPTG, and the cells were shaken at 25 °C for 3~4 h. Then cells were collected by centrifugation and resuspended in 25 mL of 30 mM Tris, pH 8.0. The cells were lysed by ultrasonication, and the cell debris was separated by centrifugation. The cleared supernatant was dialyzed versus 30 mM Tris, pH 8.0, for 36 h at 4 °C, centrifuged to remove insoluble matter, and loaded onto an equilibrated Q/SP-Sepharose column. Bound proteins were eluted with a 0-500 mM NaCl gradient in 30 mM Tris, pH 8.0, at 2 mL/min. Fractions (2 mL) containing protein were pooled and concentrated with an Amicon ultrafiltration cell equipped with a YM10 membrane. Protein purity was ascertained by SDS PAGE and protein concentration was determined using Beer's law.

1.5. Determination of inhibition activity

The inhibitor concentrations causing a 50% decrease of enzyme activity (IC_{50}) were determined. The IC_{50} values of the inhibitors against enzymes were determined in triplicate after pre-mixing for 1 hour, varying the concentrations of the compounds between 0 and 20 μg/mL. 50 μM meropenem was used as the substrate, and the substrate hydrolysis was monitored at 300 nm on an Agilent UV8453 spectrometer at 25 °C. All experimental hydrolytic rates were determined in triplicate. The percent inhibition was calculated by equation $\%I = 1 - (V_i/V_0)$, and IC_{50} values were calculated by plotting the average percentage inhibition against the inhibitor concentration and sigmoidal fitting of the data. All the kinetic and inactivation parameters were determined using GraphPad Prism.[1-2]

1.6. Fluorescence spectra measurements

All fluorescence experiments were performed at room temperature in PBS buffer solution. Any changes in the fluorescence intensity were monitored using a fluorescence spectrometer (λ_{ex} = 430 nm, λ_{em} = 470 nm, slit widths: W_{ex} = 5 nm, W_{em} = 5 nm). Selectivity was measured with 3 eq. NDM-1(class B1), VIM-2(class B1), ImiS (class B2), L1 (class B3), and other proteins.[4]

1.7. SDS-PAGE assays

The enzyme (5 μM) was added to a solution of DS01 (5 μM) in 10 mM Tris-HCl buffer (pH 7.5) at 25 °C. After 1 h, labeled protein was solubilized in 2 × SDS gel loading buffer (100 mM Tris-HCl buffer, pH 6.8, 2.5% SDS, 20% glycerol) and resolved by SDS-PAGE. The images of the gels were then captured or irradiating the gel with UV light at 365 nm. The gels were stained with Coomassie Brilliant Blue, and images of the stained gels were captured.[4]

1.8. Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy was used for investigating the recognition of DS01 by *E. coli*-BL21 cells expressing NDM-1. *E. coli*-BL21-NDM-1 cells (OD_{600} = 0.15, prepared as described for the measurement of MH) were incubated with 1 μg/mL DS01 in tubes at 37 °C for 0-30 min. Next, the cells were repeatedly washed with PBS for three times (10,000 rpm, 5 min), suspended in PBS (2 mL) and then transferred to the chambered coverglass. The bacterial cells were spotted on glass slides, immobilized by the coverslips, and the cell imaging tests were conducted for CLSM.[4]

1.9. Construction of the NDM-1 C221A mutant

The NDM-1 C221A mutant was constructed as previously described.[5] The wild-type NDM-1encoding pET26b plasmid was used to introduce C221A mutation by site-directed mutagenesis. The forward primer CGCGTTCGGCGGTGCCCTGA TTAAAGATAGAAAGC and the reverse was primer was GGCACCGCCGAACGCGAT GTCGGTGCCACAATGCCG. The mutated gene was cloned between the Nde I and Xho I restriction sites into pET-26b harboring a kanamycin resistance gene. The constructed plasmid encoding the C221A mutant were

transferred into *E. coli* BL21 (DE3). The expression was determined by SDS-PAGE analysis.

1.10. The determination of zinc ion content by ICP-MS

Purified NDM-1 (10 μM) dissolved in trace-metal-free buffer containing 50 mM HEPES, pH 7.0, was incubated with 0-40 μM DS01 at 25 °C for 5 h with mild shaking. The sample was subsequently dialyzed in ICP-MS buffer to remove unbound-metal ions and was then acidified and analyzed using an ICP-MS.[5]

1.11. LC MS/MS characterization

Samples were prepared for mass spectrometry as follows: NDM-1/KPC-2 (0.5 mM) and labeling agent DS01 and DC01 (3.0 mM) were incubated in Tris-HCl (2 mL, 50mM) at pH 7.2 for 2 h at room temperature, and then the reaction was stored at 4°C overnight. Prior to high resolution mass spectrometry analysis on digestion and bottom-up mass spectrometry analysis, after incubation of the NDM-1 protein with the inhibitor, 40 μg of the complex protein was prepared for digestion by performing a buffer exchange by using a 10 kDa molecular weight cut-off filter. The filtered protein was diluted in NH_4HCO_3 (50 mM) buffer. Firstly, for Trypsin digestion, 10 μL Trypsin (0.1 μg/μL) was added to 40 μg of buffer-exchanged protein and incubated overnight at 37°C. Following digestion, the peptides were desalted on C18 spin columns (Thermo/Pierce) and reconstituted in 0.1% formic acid in water for LC MS/MS analysis. NDM-1/KPC-2 alone is digested as above. LC MS analysis was undertaken by using an Eksigent II nano LC system operated in a reversed-phase nano-liquid chromatography mode at a flow rate of 300 nL/min. Separation was performed with eluent A, consisting of 0.1% formic acid in water, and eluent B, consisting of 0.1% formic acid in CH_3CN, with a 30 min linear gradient from 2– 40% eluent B at a flow rate of 300 nL/min. LC MS/MS analysis was performed on an Thermo Fisher Orbitrap mass spectrometer (Onbitrap FusionTM LumosTM TribridTM mass spectrometer).[1]

1.12. ITC experiments.

Isothermal titration calorimetry (ITC) experiments were performed on a Malvern MicroCal iTC 200 instruments by a single injection mode.[6] The purified NDM-1 and KPC-2

enzyme and substrate were prepared in 30 mM Tris, pH 7.0, with 1% addition of DMSO. 38 µL of meropenem (1 mM) in the syringe was titrated into the sample cell filled with 210 µL of protein solution (10 µM). Heat flow (microcalories/second) was recorded as a function of time. Data were collected every 1 s until the signal returned to the baseline. According to this relationship, the rates of substrate hydrolysis are calculated. The enzyme catalyzed hydrolysis progress curves of meropenem in the absence and presence of DC01 were obtained by fitting initial velocity versus substrate concentration at each inhibitor concentration using MicroCal Analysis Launcher Origin 7.[6]

1.13. Thermal shift assay

The thermal shift assay was performed in clear 96-well plates (Invitrogen) using SYPRO Orange (Invitrogen Darmstadt, Germany) as dye. 3.2 µg/mL 4 µL compounds in assay buffer (50 mM HEPES, pH 7.5, containing 0.01% Triton X-100 for NDM-1) was mixed with 5 µM 32 µL enzyme and 4 µL of 2.5× SYPRO Orange. The temperature dependent fluorescence increase reporting protein denaturation was measured in triplicate in an ICycler (Bio-Rad) from 20 to 80 °C in steps of 0.2 °C at 300 nm excitation and 570 nm emission wavelength. The first derivative of the protein melting curve was calculated using the Graph Pad software.[7]

1.14. Docking studies

Docking studies of DC01 into the active site of NDM-1 (PDB: 4EYL) and KPC-2 (PDB: 6J8Q) was performed by AutoDock 4.2. The grid and docking parameter files were prepared using Zn(II) van der Waals parameters = 0.25 kcal/mol and r0 = 1.95 Å. NDM-1 was treated as a rigid receptor. The grid box was centered between the two active-site Zn(II) ions, with dimensions of 50 x 50 x 50 grid points with grid points spaced at 0.375 Å. The mutation rate and crossover rates were set at 0.02 and 0.8, respectively, while the maximum energy evaluations and generations' numbers were set at 2,500,000 and 27,000, respectively. Default values were kept for all other parameter and no constraints were used. Fifty conformations were generated according to the Lamarckian genetic algorithm and grouped into clusters based on a root mean square deviation (RMSD) tolerance of 2.0 Å. The

conformations are shown in Fig. S9 are the highest ranked (lowest energy) conformations. Subsequently, the Zn(II) ion oordinated with Cys221 was removed from the active sites, DC01 without CN moiety was covalently docked to the active site of NDM-1, based on examples provided by the AutoDock website (http://autodock.scripps.edu/resources/covalentdocking?searchterm=covalent), using the flexible residue method.[7]

1.15. MIC determination

MICs of meropenem plus compounds against *E. coli* BL21 (DH3) expressing serine and metallo-carbapenemases (NDM-1, VIM-2, ImiS, KPC-2 and OXA-48) were determined by using the Clinical and Laboratory Standards Institute (CLSI) method. The MIC was interpreted as the lowest concentration of the drug that completely inhibited the visible growth of bacteria after incubating the plates for 16−18h at 37 °C. Each inhibitor was tested in triplicate, and the highest MIC value was reported.[8-9]

1.16. Toxicity assays

L929 cells were cultured in the medium containing DMEM. The cells were plated in 96-well plates, the final cell number was 5×10^4 cells/well. After gradient dilution with the medium, the compounds were added to the 96-well plate of L929 cells and then incubated for 24 h. After incubation, MTT at a concentration of 0.5 mg/ mL in PBS MTT (10 μL/well) was further added and then incubated for the other 4 h and added 150 μL/well DMSO for 10 min. The optical density (OD) of each well was measured at 490 nm using a Microplate Reader.[9-10]

1.17. Fluorescence microscopy

L929 cells maintained in complete DMEM media supplemented with 10% FBS and penicillin–streptomycin solution, were seeded into the wells of a 96-well plate like the operation of the cytotoxic and then treated with 20 μM DC01. The negative control group was set to be changed only by an equal amount of medium. After the test plate was incubated for 24 h, all the treated and untreated cells were washed once with 1 × PBS and stained with 2 μM calcein AM and 4 μM propidium iodide (PI) for 30 min at 37 °C under 5%

CO_2. Finally, the cells were washed and images were captured with a 10 × objective in Nikon A1 fluorescence microscope, with an excitation and emission wavelengths are 535 nm and 615 nm, respectively.[10]

1.18. Determination of acute toxicity

Kunming mice weighing 20–22 g were obtained from Experimental Animal Center, Health Science Center of Xi'an Jiaotong University. The animals were kept in a room temperature. Then, the animals were randomly divided into 6 groups of 6 animals, and DC01 was intraperitoneally injected (200 μL per mouse) at doses of 0, 6.25, 12.5, 25, 50, 100, and 200 mg/kg, respectively. All the mice experiments were conducted in accordance with the guidelines of the Laboratory Animal Center of Xi'an Jiaotong University and approved by the Animal Ethics Committee of Xi'an Jiaotong University (approved ID: NO. XJTULAC20191234), and all applicable institutional and governmental regulations concerning the ethical use of animals were followed.[10]

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Supporting Figures

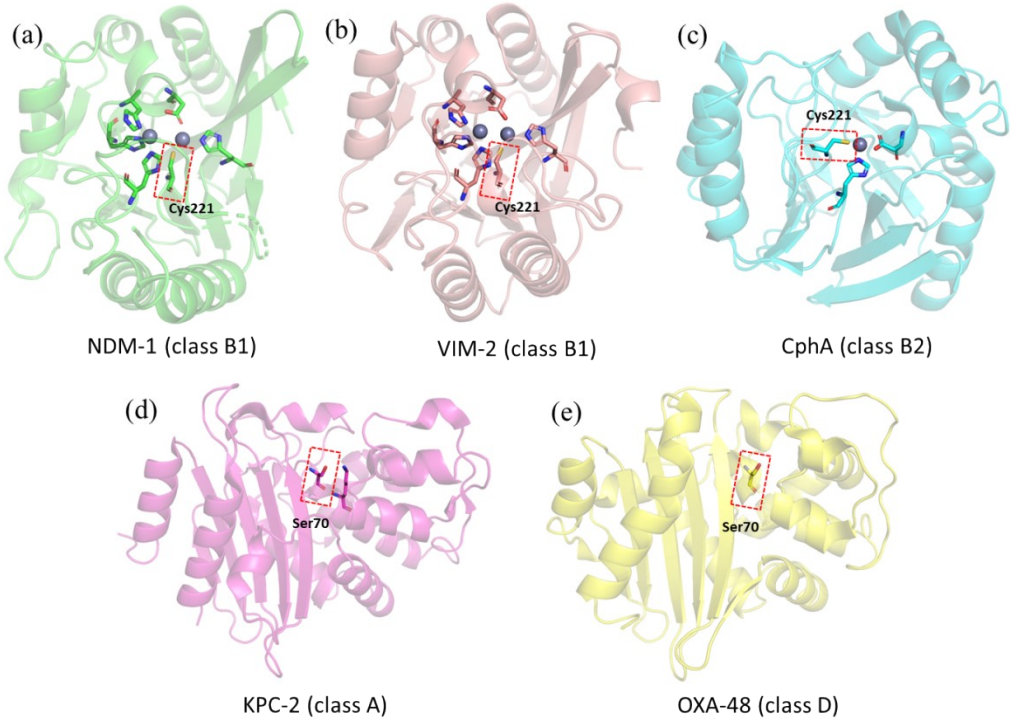

Fig. S1. The overview of the active site of metallo-carbapenemases: NDM-1, VIM-2, CphA, and serine-carbapenemases: KPC-2, OXA-48; Residue Cys221 and Ser70 are marked with the red boxes; PDB code: (a) 3spu, (b) 4nq2, (c) 2qds, (d) 5mgi, (e) 7jhq.

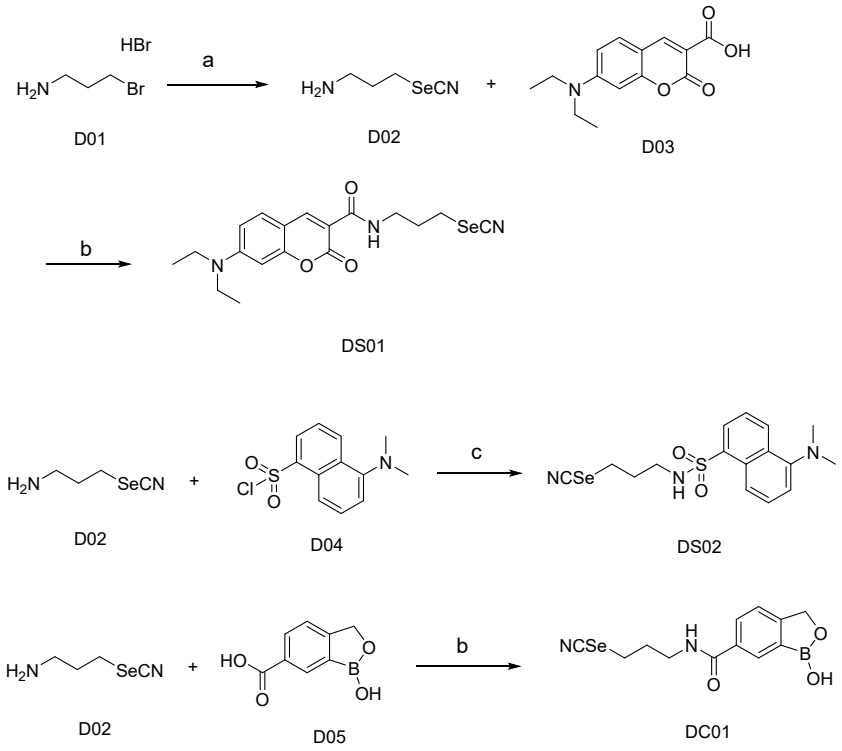

Fig. S2. The synthetic route of compound DS01, DS02, and DC01; (a) KSeCN, CH_3CN, 80 ℃, 12 h; (b) EDCI, HOBT, TEA, DCM/DMF, r. t., overnight; (c) Et_3N, 0 ℃, DCM/DMF, 3 h,.

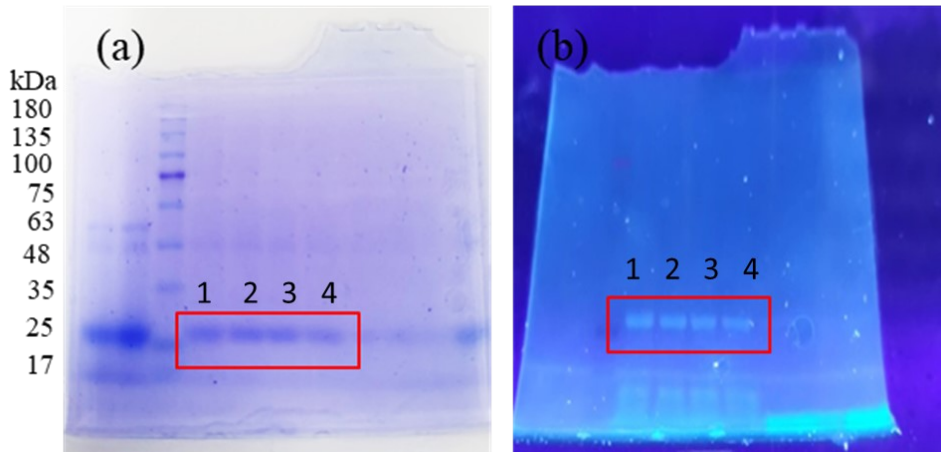

Fig. S3. The image of Coomassie blue staining (a) and in-gel fluorescence (b) of SDS-PAGE run at 120 V with 5 µM NDM-1 upon treatment with 5 µM DS01, incubation time: 2 h, $\lambda_{ex}/\lambda_{em}$ = 365/470 nm, 1-4: NDM-1 (class B1).

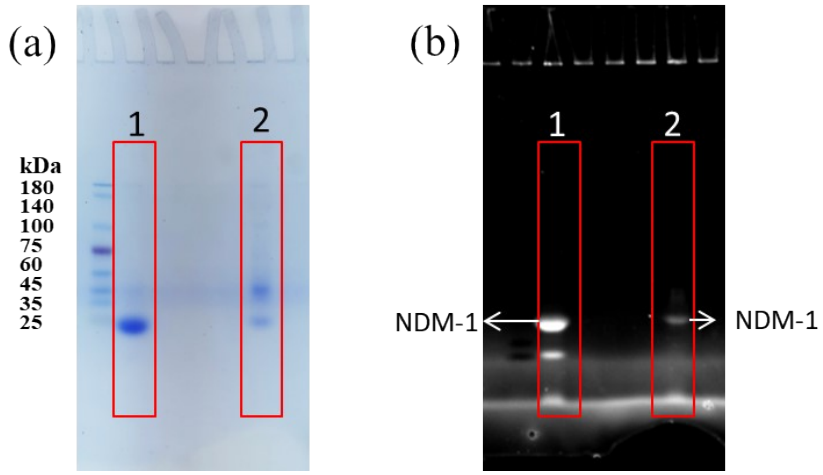

Fig. S4. The image of Coomassie blue staining (a) and in-gel fluorescence (b) of SDS-PAGE run at 120 V for 120 min with NDM-1 and lysate of *E. coli* cells upon treatment with 2.5 µM DS01, incubation time: 2 h, $\lambda_{ex}/\lambda_{em}$ = 365/470 nm, 1: NDM-1 (class B1), 2: lysate of *E. coli*-BL21-NDM-1.

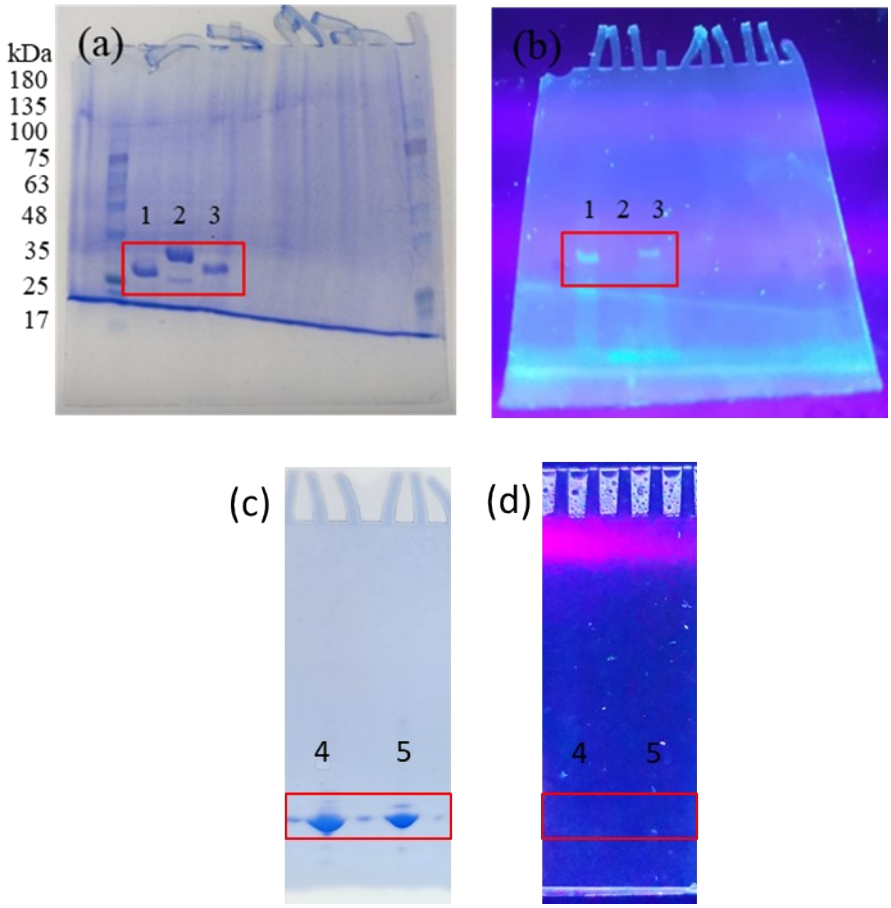

Fig. S5. The image of Coomassie blue staining (a, c) and in-gel fluorescence (b, d) of SDS-PAGE run at 120 V with different carbapenemases upon treatment with 5 µM DS01, incubation time: 2 h, $\lambda_{ex}/\lambda_{em}$ = 365/470 nm, 1: NDM-1 (subclass B1), 2: L1 (subclass B3), 3: ImiS (subclass B2), 4: KPC-2 (class A), 5: OXA-48 (class D).

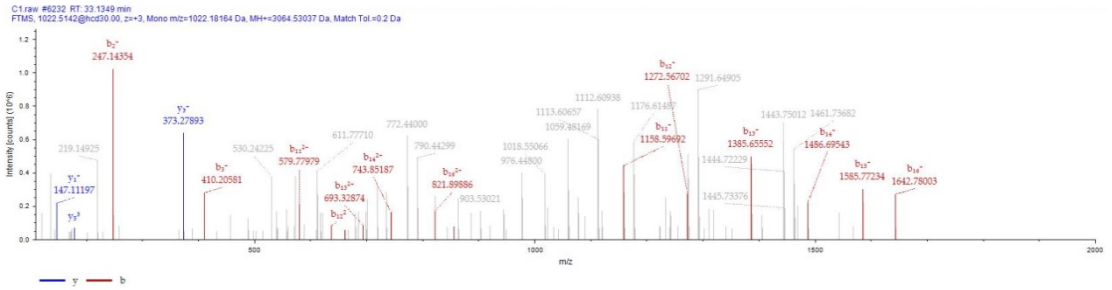

Fig. S6. LC-MS/MS scan of the peptide (VFYPGPGHTSDNITVGIDGTDIAFGGCLIK) containing Cys221 residue of NDM-1.

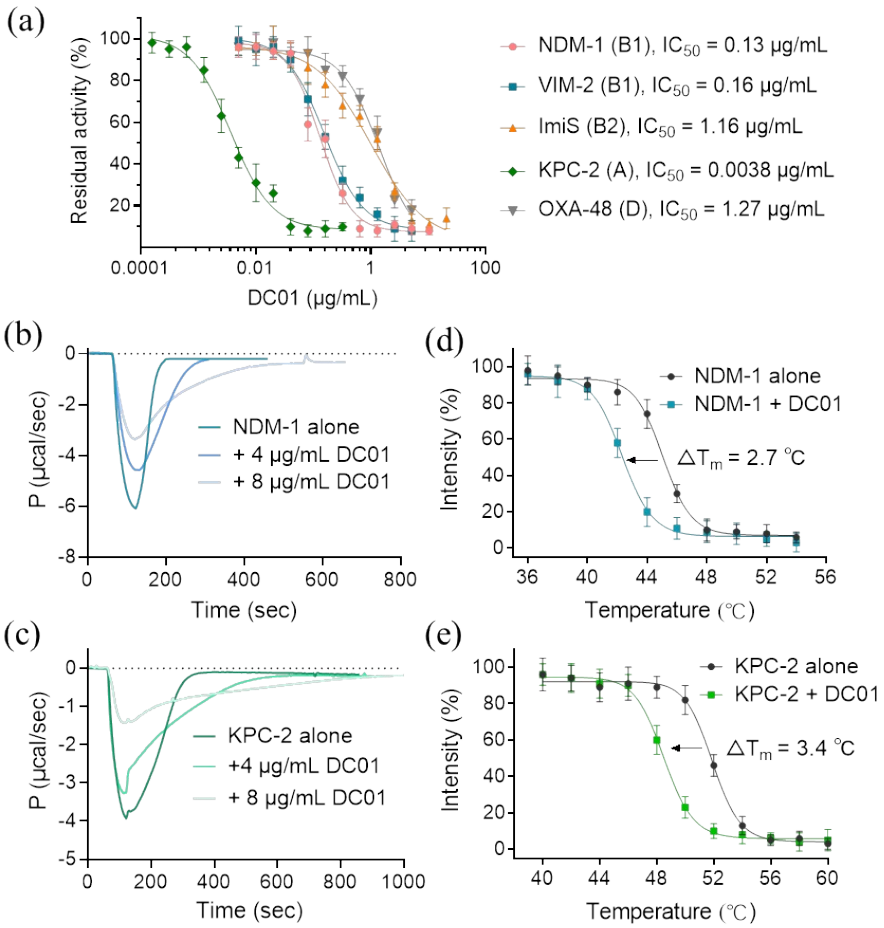

Fig. S7. Dual inhibition profiles for serine and metallo-carbapenemases by DC01 with IC50 values of 0.13, 0.16, 1.16, 0.0038 and 1.27 μg mL^{-1} for NDM-1, VIM-2, ImiS, KPC-2 and OXA-48, respectively (a). Heat flow curves of meropenem (0.15 mM) hydrolysis with wild-type NDM-1 (b) and KPC-2 (c) inhibited by DC01 (0, 4, 8 μg mL^{-1}); thermal shift assays (TSA) indicating that 3.2 μg mL^{-1} DC01 bound to NDM-1 (d) and KPC-2 (e) resulting in the protein-melting temperature (Tm) shift of the enzyme. The Tm of NDM-1/KPC-2 was shifted from 45.1/51.9 to 42.4/48.5 °C for the control and DC01-treated group, respectively. (a), (d), and (e), the data are presented as mean ± SEM, n = 3.

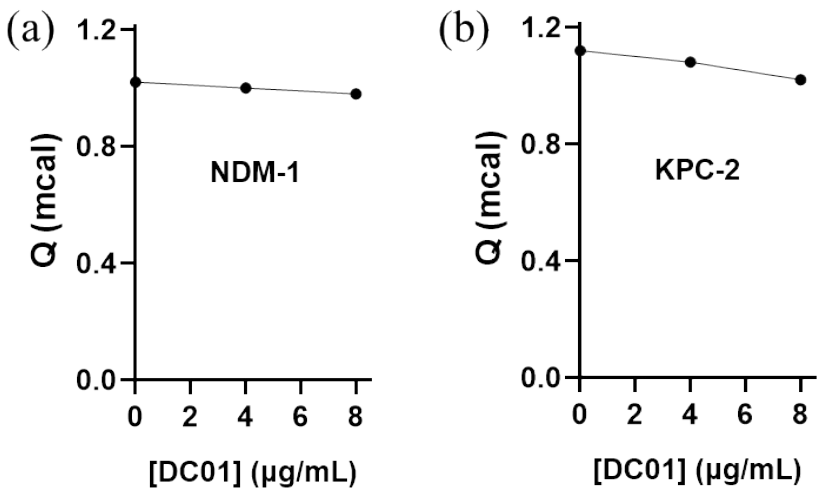

Fig. S8. The change of Q over the dilution in the presence of DC01 at various concentrations for NDM-1 (a) and KPC-2 (b), respectively.

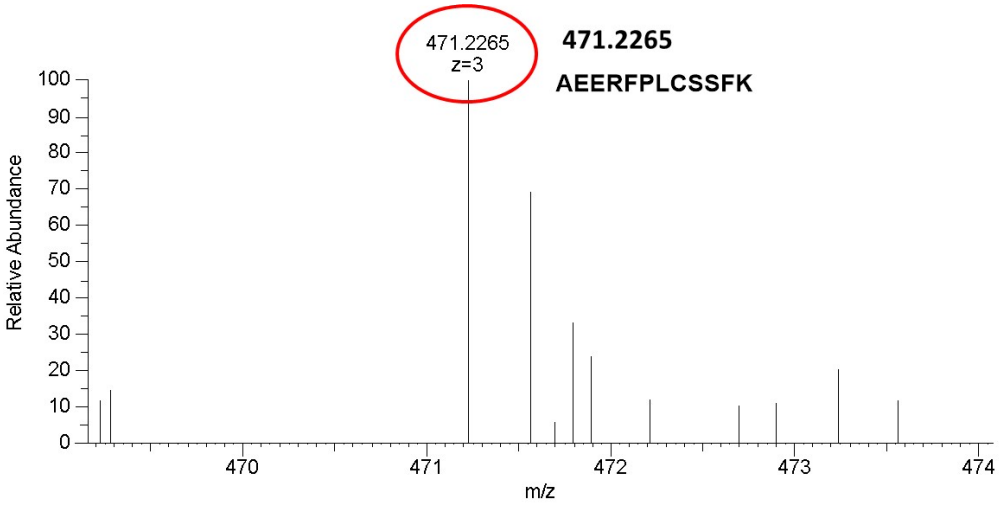

Fig. S9. LC-MS scan of the peptide (AEERFPLCSSFK) containing Ser70 residue of KPC-2 (expected: $[M]^{3+}$, 471.2033; observed: $[M]^{3+}$, 471.2265).

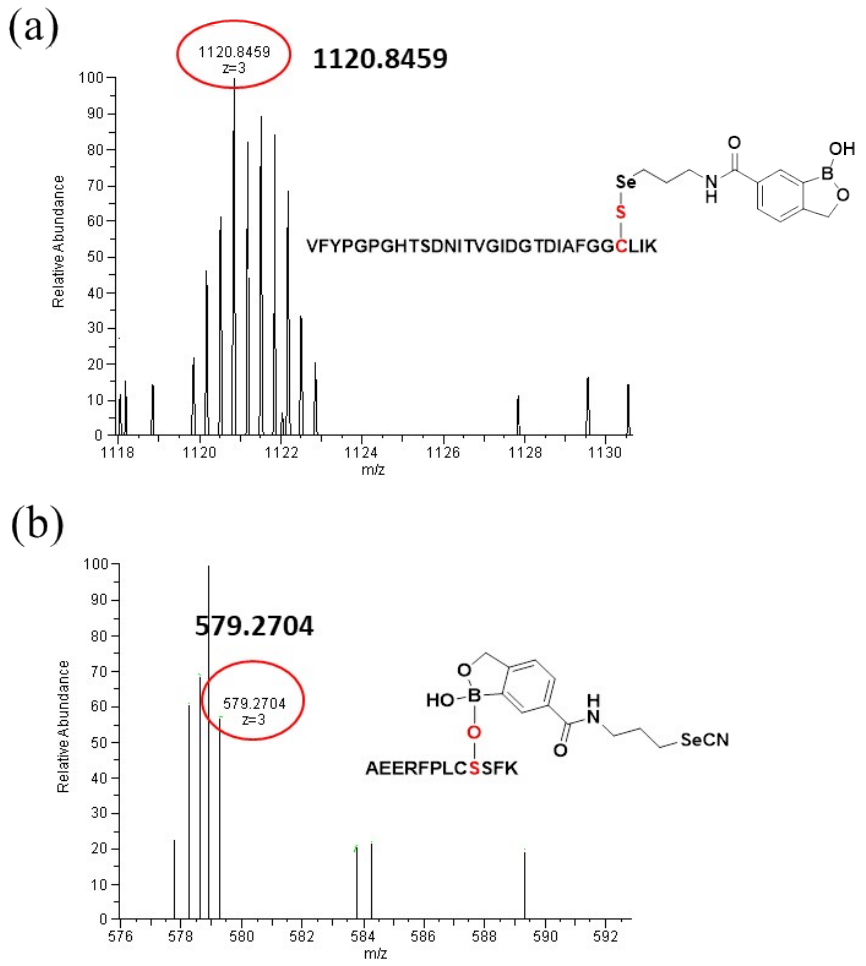

Fig. S10. LC-MS scan of the peptide (VFYPGPGHTSDNITVGIDGTDIAFGGCLIK) containing Cys221 of NDM-1 after DC01 treatment (expected: $[M]^{3+}$, 1120.8413; observed: $[M]^{3+}$, 1120.8459, a) and (AEERFPLCSSFK) containing Ser70 of KPC-2 after DC01 treatment (expected: $[M]^{3+}$, 579.2095; observed: $[M]^{3+}$, 579.2704, b).

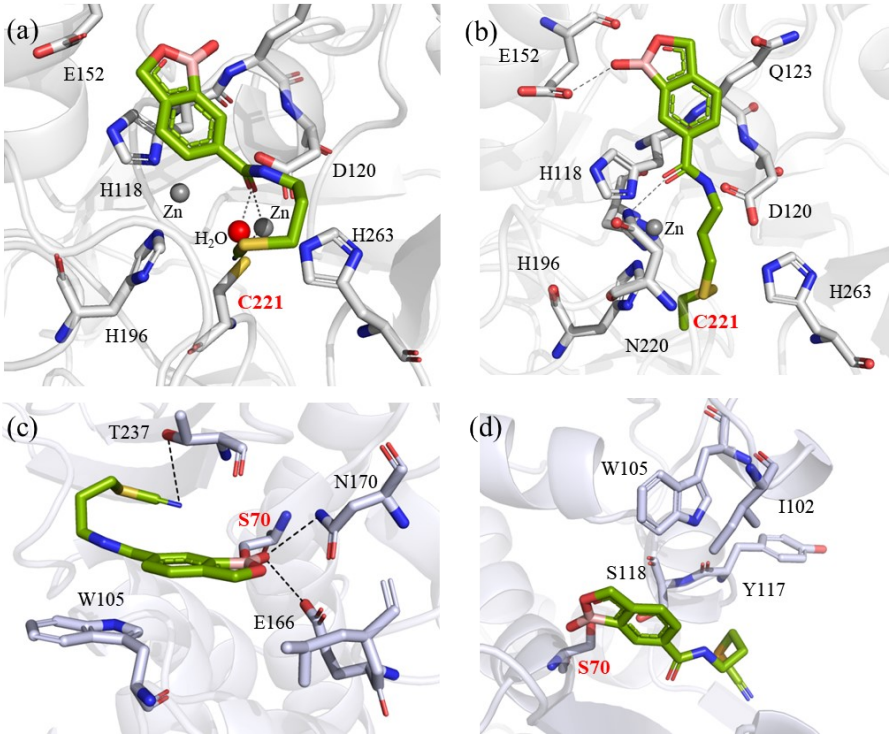

Fig. S11. The predicted binding mode of DC01 with NDM-1 (a, b), KPC-2 (c) and OXA-48 (d); one possible noncovalent binding complex (a) and covalent complex (b) are described; these modes suggests that the DC01 is likely to form a covalent bond with the catalytically residue C221 of NDM-1, S70 of KPC-2 and OXA-48, respectively. Figure created using the PyMOL Molecular Graphics System.

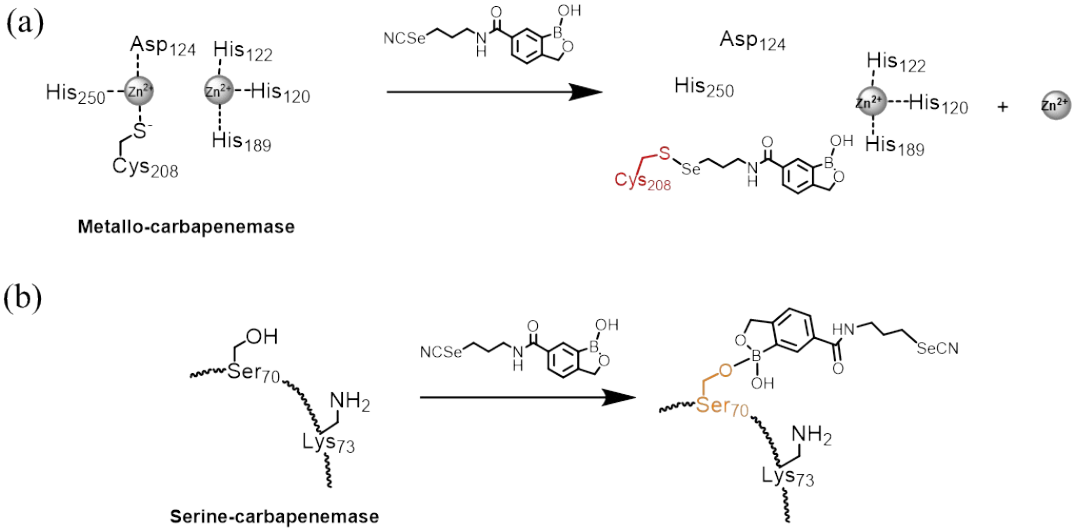

Fig. S12. The proposed dual inhibition mechanism of NDM-1 (c) and KPC-2 (d) by DC01.

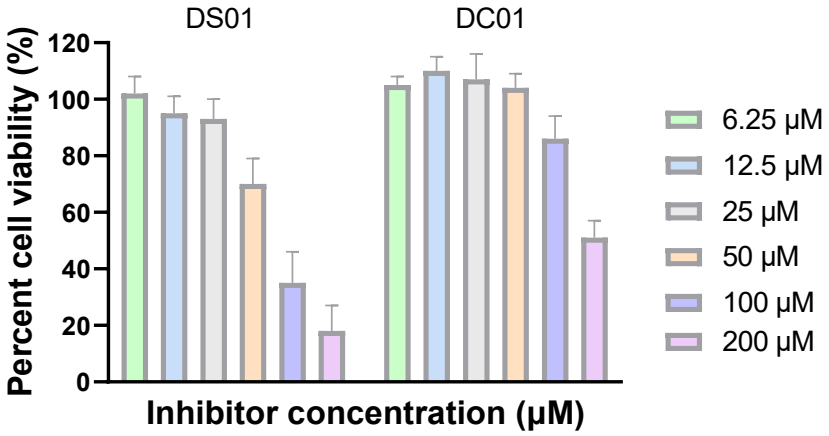

Fig. S13. Cytotoxicity assay in L929 cells were treated with DS01 and DC01 at a concentration ranging from 6.25 to 200 µM.

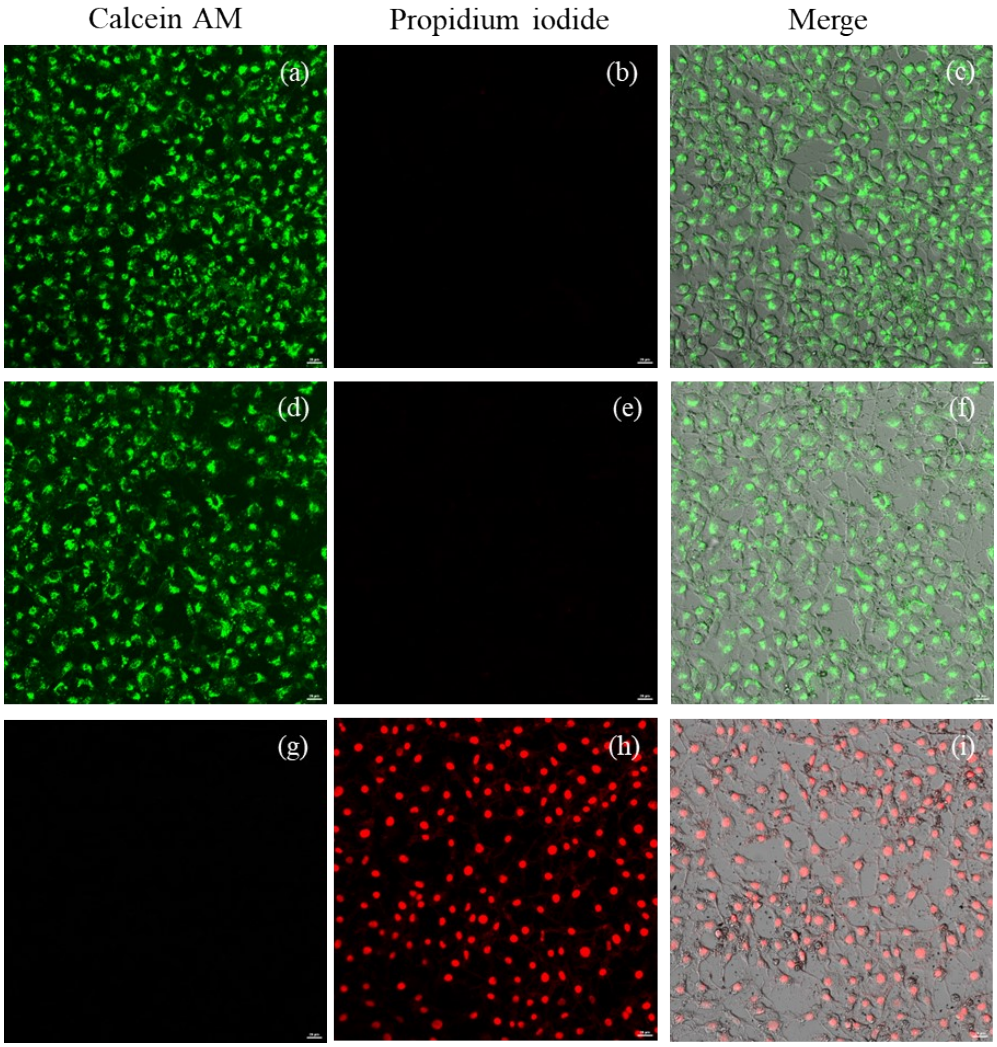

Fig. S14. Fluorescence microscopy images of L929 cells after treatment with DC01 for 24 h and staining with Calcein AM/Propidium iodide. (a-c) non-treated cells; (d-f) cells treated with DC01 (20 µM); (g-i) cells treated with 0.1% Triton-X; Scale bar, 5 µm.

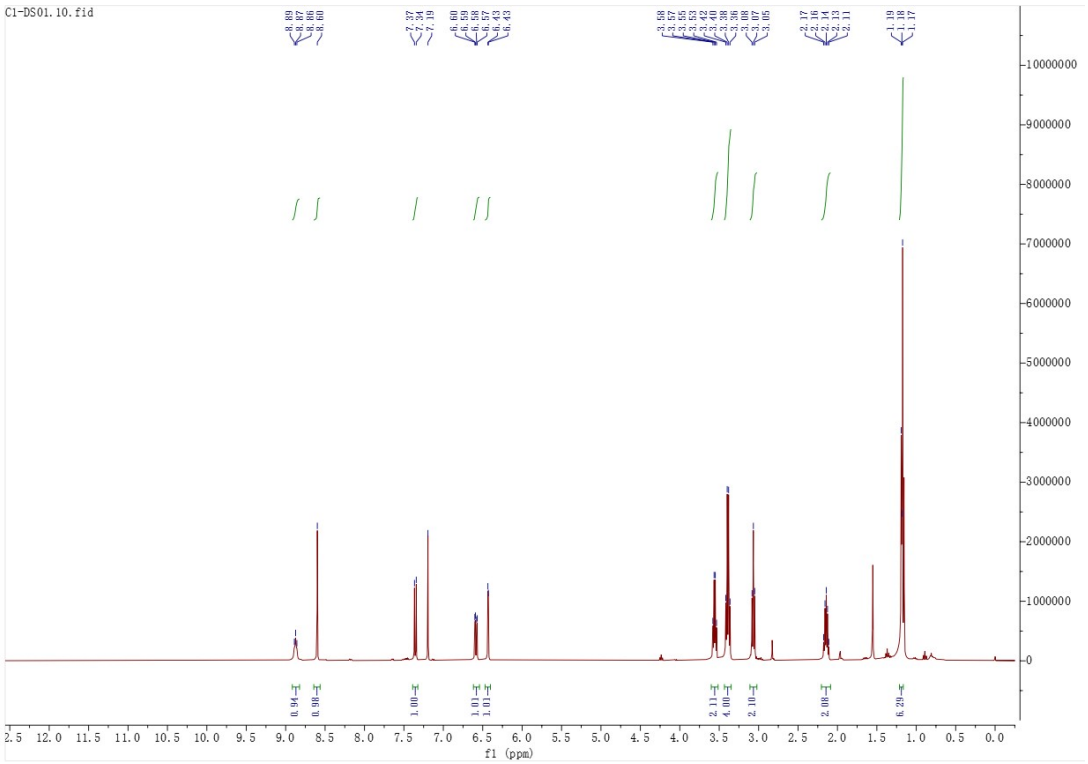

Fig. S15. ^{1}H NMR spectrum of DS01.

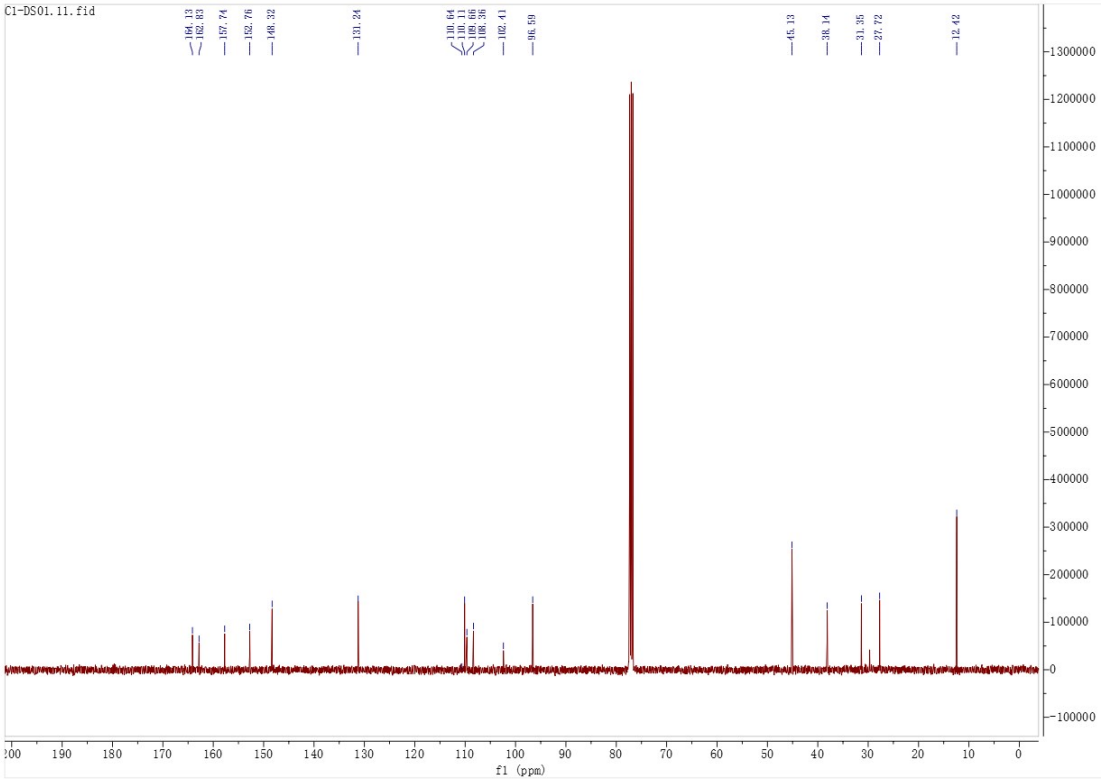

Fig. S16. ^{13}C NMR spectrum of DS01.

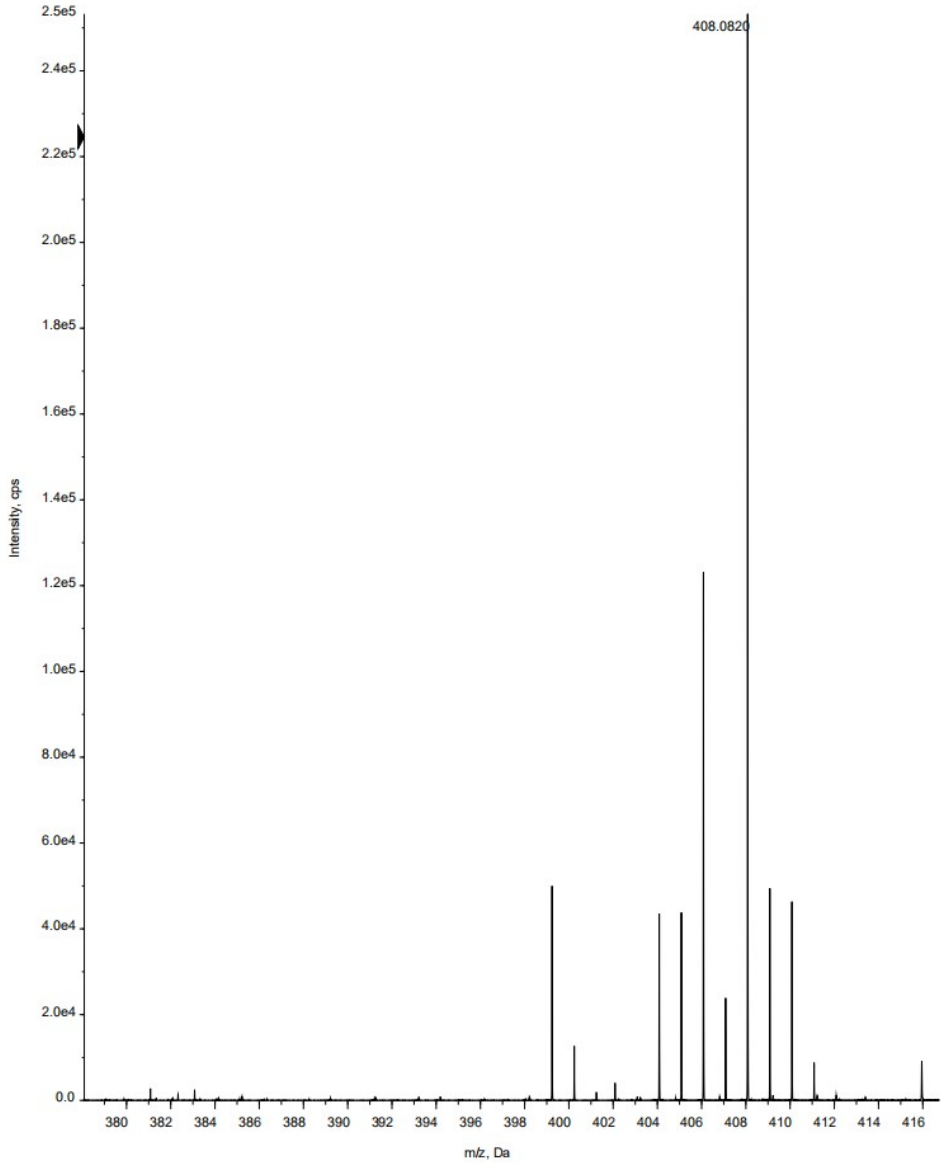

Fig. S17. HR-MS spectrum of DS01.

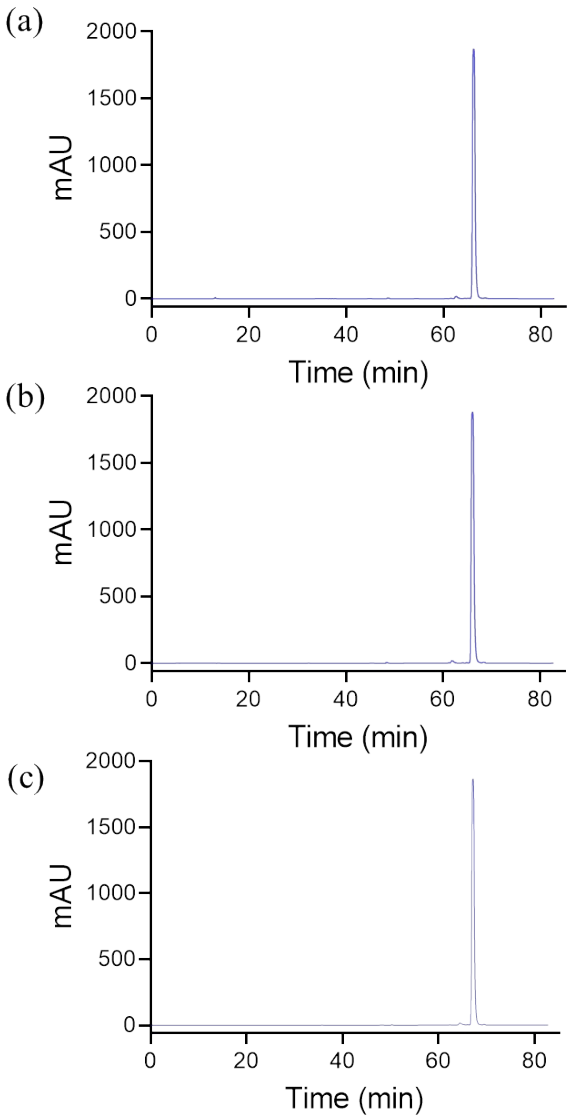

Fig. S18. The monitoring of aqueous stability and purity of DS01, 0 h (a), 24 h (b), 48 h (c), determined by HPLC (Agilent 1200 Infinity), acetonitrile/H_2O = 5% - 95%, 1.0 mL/min, λ = 430 nm; t_R = 66 min. The results indicate that DS01 is very stable in the aqueous solution over 48 h.

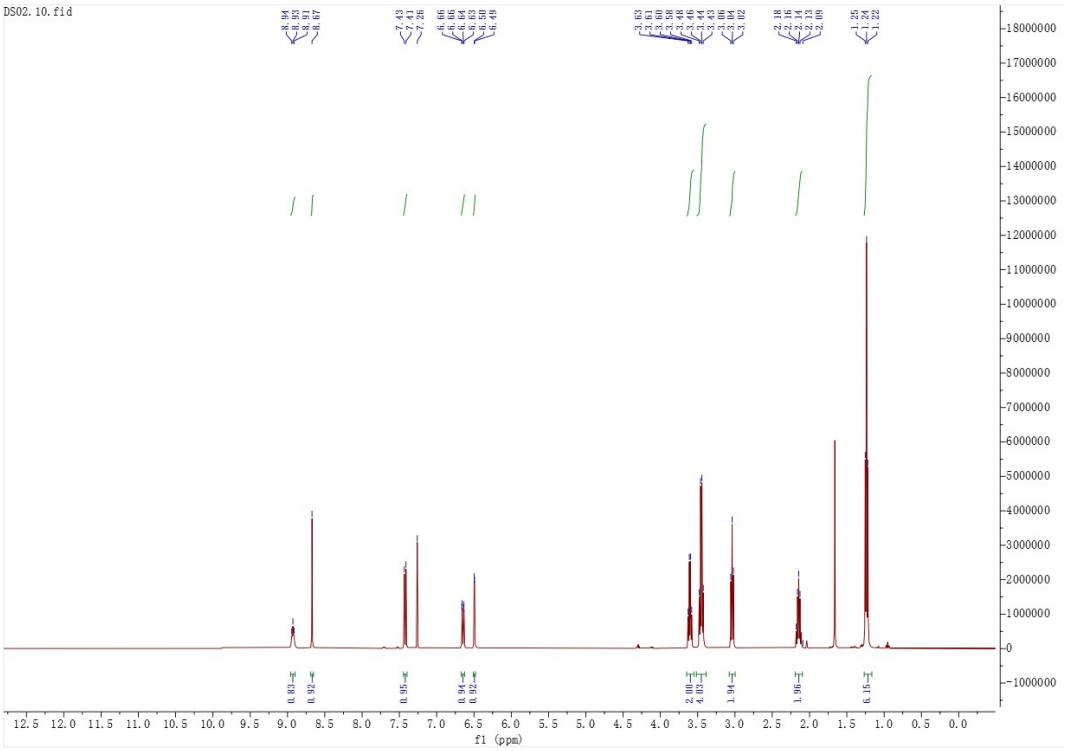

Fig. S19. ^{1}H NMR spectrum of DS02.

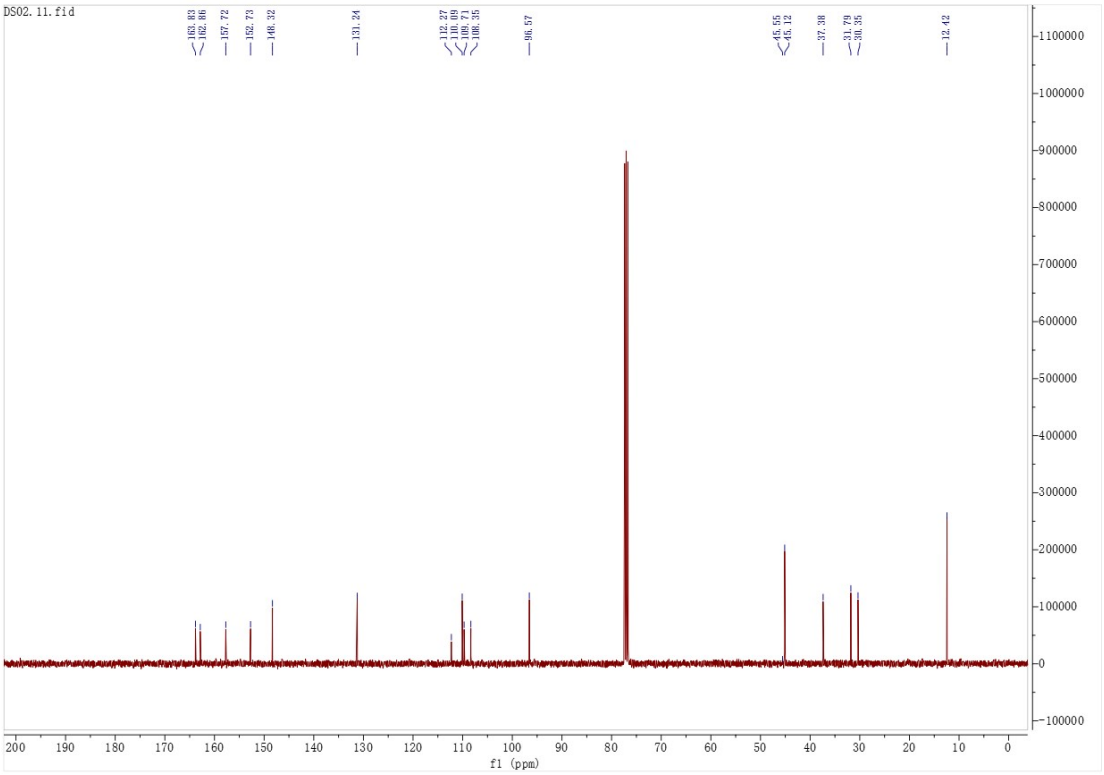

Fig. S20. ^{13}C NMR spectrum of DS02.

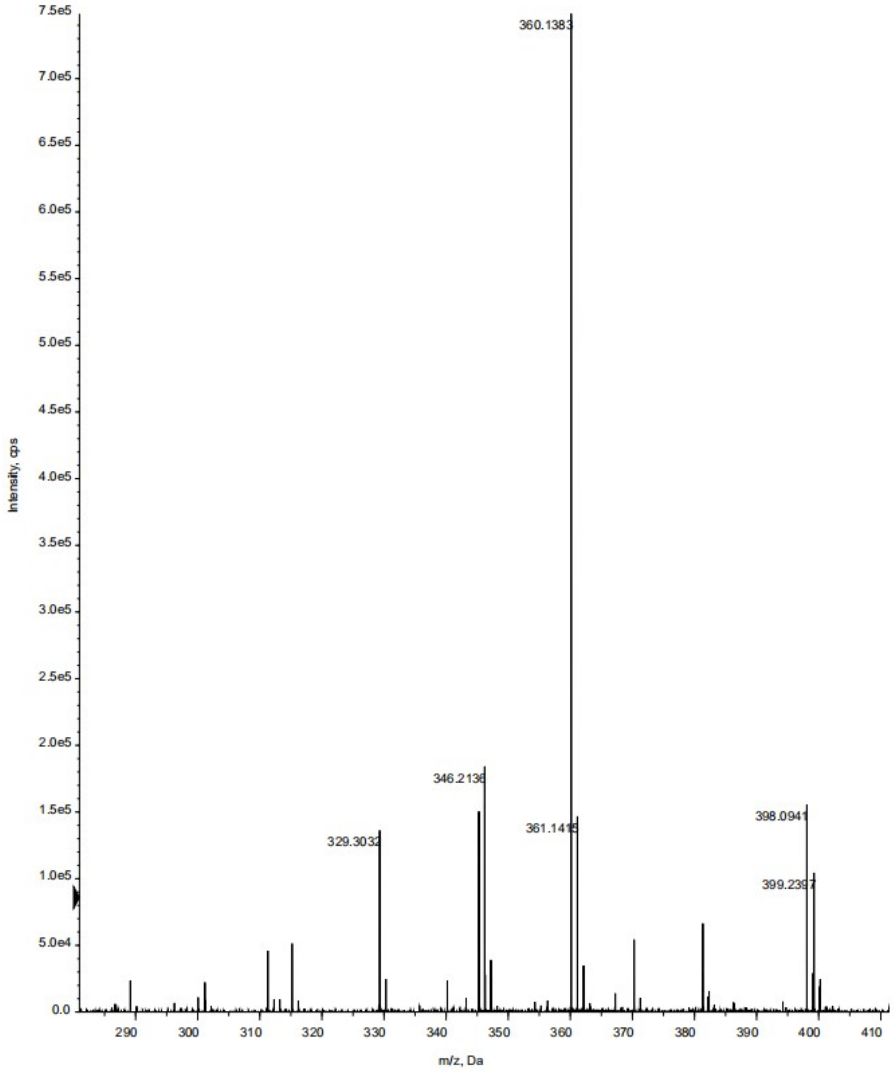

Fig. S21. HR-MS spectrum of DS02.

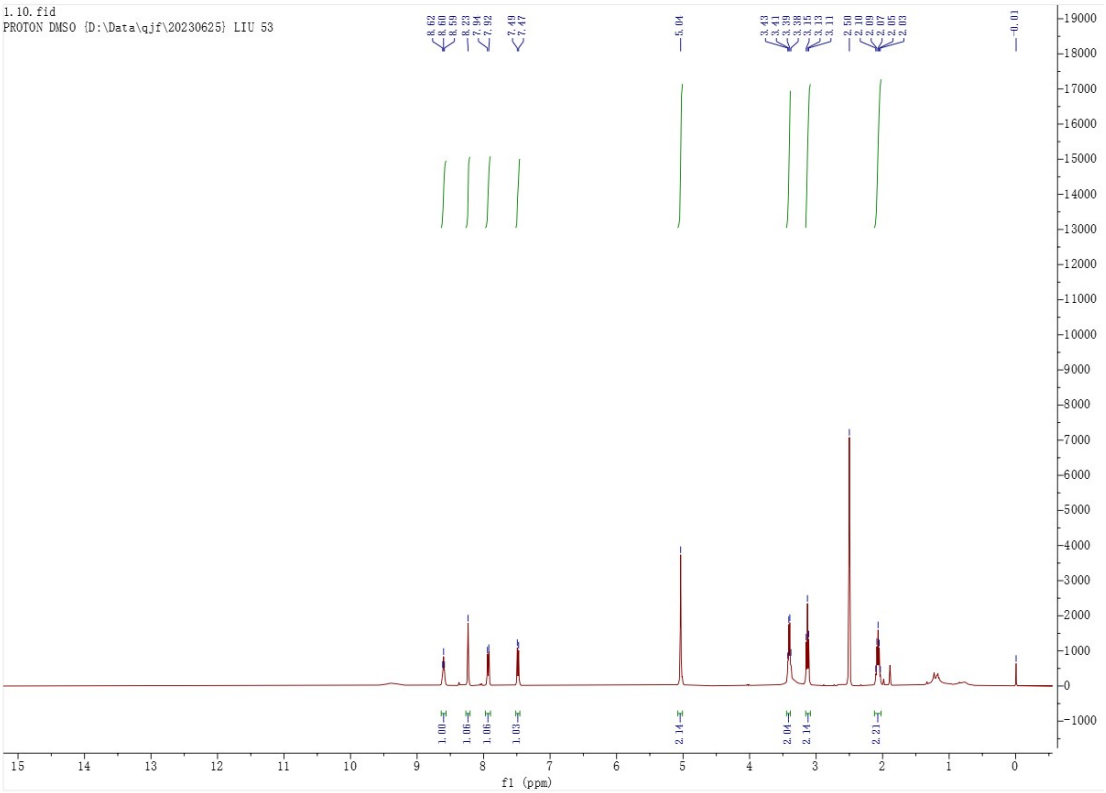

Fig. S22. ^{1}H NMR spectrum of DC01.

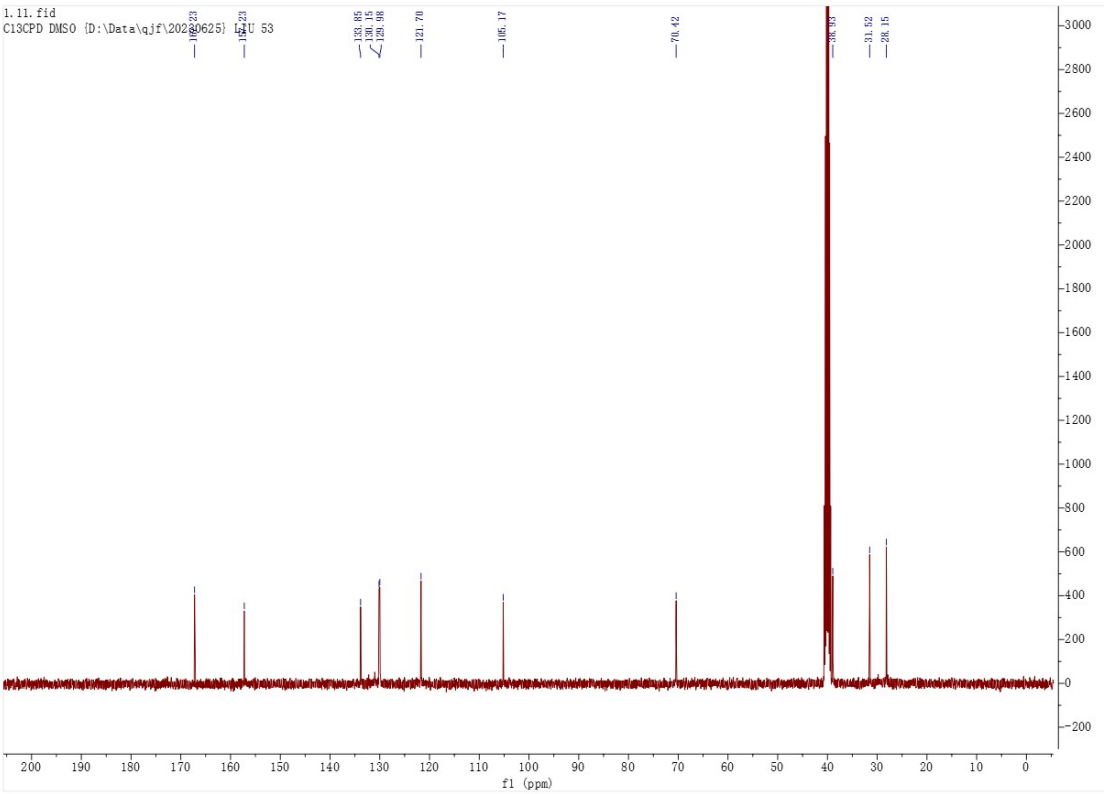

Fig. S23. ^{13}C NMR spectrum of DC01.

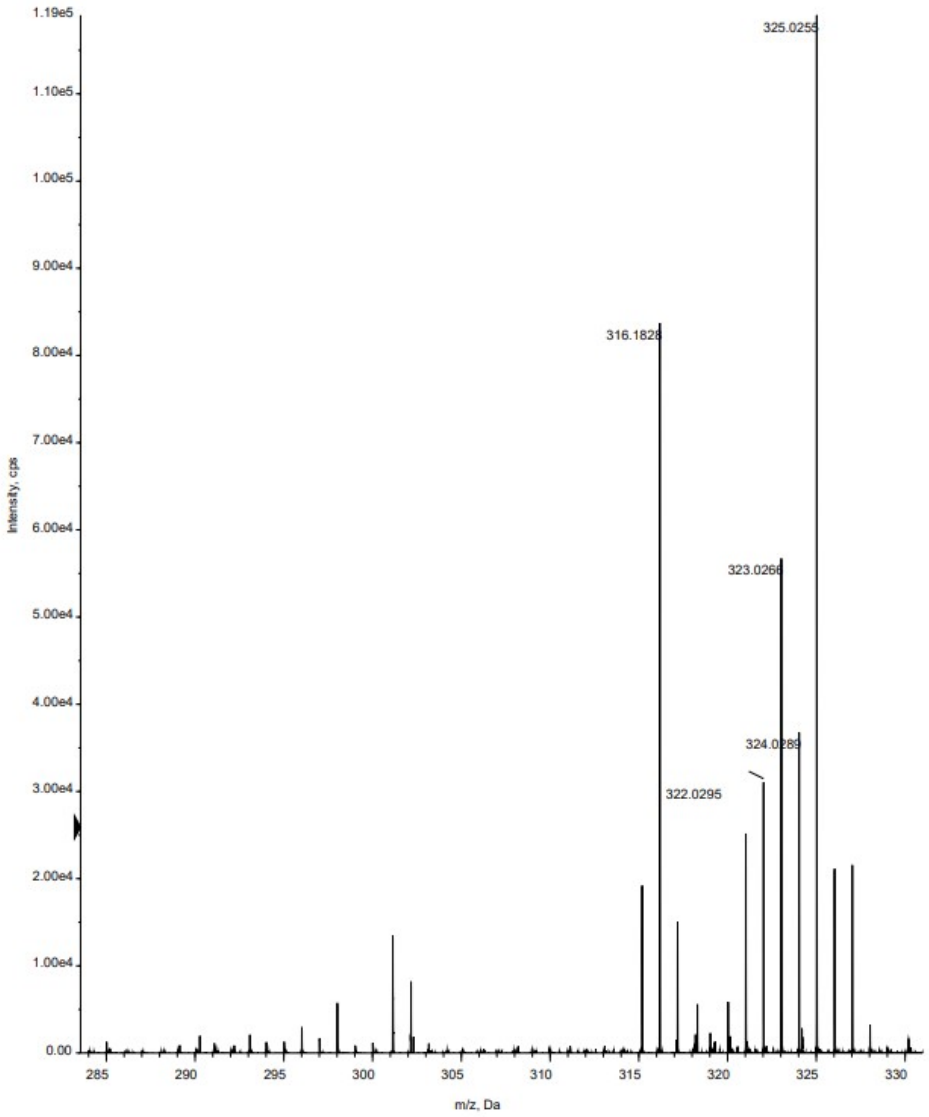

Fig. S24. HR-MS spectrum of DC01.

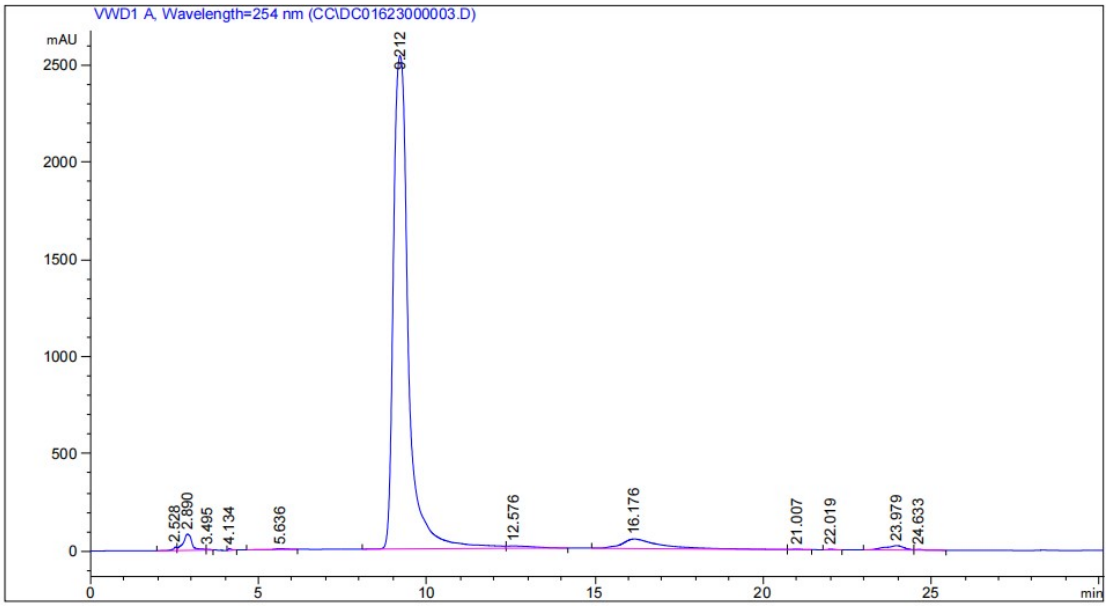

Fig. S25. The purity of DC01 determined by HPLC (Agilent 1200 Infinity), acetonitrile/H_2O = 25% - 95%, 1.0 mL/min, λ = 254 nm; t_R = 9.2 min.

Supporting Tables

Table S1. Inhibitory activity (IC_{50}, μg/mL) of DS01, DC01 against clinical relevant serine and metallo-carbapenemases.

IC_{50} (μg/mL)	DS01	DC01	Avibactam	Disulfiram
NDM-1 (subclass B1)	0.081	0.13	> 20	0.043
VIM-2 (subclass B1)	0.12	0.16	> 20	> 20
ImiS (subclass B2)	0.56	1.16	> 20	1.12
L1 (subclass B3)	> 20	> 20	> 20	> 20
KPC-2 (class A)	> 20	0.0038	0.0026	> 20
OXA-48 (class D)	> 20	1.27	0.069	> 20

Table S2. Susceptibility of strains producing serine and metallo-carbapenemases against meropenem and DS01 combination.

Strains	MER MIC (µg/mL) in the presence of DS01 at concentration stated (µg/mL)						
	0	2	4	8	16	16 µg/mL Avibactam	16 µg/mL Disulfiram
***E. coli*-BL21**	0.125	0.125	0.125	0.125	0.125	0.125	0.125
***E. coli*-NDM-1**	32	16	8	4	2	32	4
***E. coli*-VIM-2**	64	16	16	4	2	64	32
***E. coli*-ImiS**	128	32	8	2	1	128	16
***E. coli*-L1**	32	32	32	32	32	32	32
***E. coli*-KPC-2**	16	16	16	16	16	<0.25	16
***E. coli*-OXA-48**	8	8	8	16	8	<0.25	8

Table S3. Susceptibility of strains producing serine and metallo-carbapenemases against meropenem and DS02 combination.

Strains	MER MIC (μg/mL) in the presence of DS02 at concentration stated (μg/mL)						
	MER	2	4	8	16	16 μg/mL Avibactam	16 μg/mL Disulfiram
***E. coli*-NDM-1**	32	16	8	8	4	32	4
***E. coli*-VIM-2**	64	32	16	8	4	64	32
***E. coli*-ImiS**	128	128	64	16	8	128	16
***E. coli*-L1**	32	32	32	32	32	32	32
***E. coli*-KPC-2**	16	16	16	16	16	<0.25	16
***E. coli*-OXA-48**	8	8	8	8	8	<0.25	8

Table S4. Susceptibility of strains producing serine and metallo-carbapenemases against meropenem and DC01 combination.

Strains	MER MIC (μg/mL) in the presence of DC01 at concentration stated (μg/mL)						
	0	2	4	8	16	16 μg/mL Avibactam	16 μg/mL Disulfiram
***E. coli*-BL21**	0.125	0.125	0.125	0.125	0.125	0.125	0.125
***E.coli*-NDM-1**	32	8	8	4	1	32	4
***E. coli*-VIM-2**	64	16	8	2	2	64	32
***E. coli*-ImiS**	128	64	32	16	4	128	16
***E. coli*-L1**	32	32	32	32	32	32	32
***E. coli*-KPC-2**	16	8	4	1	0.5	<0.25	16
***E.coli*-OXA-48**	8	8	4	1	1	<0.25	8

Table S5. Acute toxicity of DC01 in mice.

Dose (mg/kg)	No. of mice	Intraperitoneal injection	
		No. of dead mice	Survival
0	6	0	100 %
6.25	6	0	100 %
12.5	6	0	100 %
25	6	0	100 %
50	6	0	100 %
100	6	1	83.3 %

Table S6. Related information of bacterial strains.

Strain	Phenotype	Source
E. coli BL21(DE3)	Engineering strain	In house
C221A-BL21	*E. coli* BL21 (DE3)(pET-26b-NDM-1-C221A);	In house
E. coli-NDM-1	*E. coli* BL21(DE3) (pET-26b-NDM-1); MER^R	In house
E. coli-VIM-2	*E. coli* BL21(DE3) (pET-26b-VIM-2); MER^R	In house
E. coli-ImiS	*E. coli* BL21(DE3) (pET-26b-ImiS); MER^R	In house
E. coli-L1	*E. coli* BL21(DE3) (pET-26b-L1); MER^R	In house
E. coli-KPC-2	*E. coli* BL21(DE3) (pET-26b-KPC-2); MER^R	This study
E. coli-OXA-48	*E. coli* BL21(DE3) (pET-26b-OXA-48); MER^R	This study