**Supporting Information**

# Development of a Nitric Oxide-Releasing Cephalexin-based Hybrid Compound for Enhanced Antimicrobial Efficacy and Biofilm Disruption

Sumit Kumar[a], Myddelton C. Parker[a], Yi Wu[a], Anastasia Marx[a], Hitesh Handa[a, b], Elizabeth J. Brisbois[a, *]

[a]*School of Chemical, Materials, and Biomedical Engineering, College of Engineering, University of Georgia, Athens, GA 30602, United States*

[b]*Pharmaceutical and Biomedical Science Department, College of Pharmacy, University of Georgia, Athens, GA 30602, United States*

***Correspondence**

Elizabeth J. Brisbois, School of Chemical, Materials, and Biomedical Engineering, College of Engineering, University of Georgia, 302 E Campus Rd., Athens, GA 30602, USA.

**Email:** ejbrisbois@uga.edu

## S Materials and Methodology

### S.1 Materials

A diverse range of substances and materials were acquired for investigations. The following chemicals were obtained from Sigma-Aldrich in St. Louis, Missouri: acetic anhydride, anhydrous magnesium sulfate, chloroform, iso-propanol, EDTA (ethylenediaminetetraacetic acid), glutaraldehyde, HMDS (hexamethyldisilazane), hexanes, hydrochloric acid, methanol, Lysogeny broth and agar (LB and LA), N-acetyl penicillamine, Dimethyl sulfoxide-d-$_6$, phosphate-buffered saline (PBS), pyridine, sulfuric acid, glacial acetic acid, sodium nitrate ($NaNO_2$), tetrahydrofuran (THF), Griess reagent (modified), and MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl-tetrazolium bromide). Penicillin-Streptomycin (5,000 U/mL) was purchased from Fisher Scientific. Trypsin EDTA, Corning®, Trypsin EDTA 1X 0.25% Trypsin/2.21 mM EDTA was purchased from Corning. In addition, Corning DMEM ( Dulbeccos Modification of Eagles Medium) [+] 4.5 g/L glucose, L-glutamine, sodium pyruvate and Avantor® Seradigm, Select Grade Fetal Bovine Serum 89510-186, and calcium- and magnesium-free phosphate-buffered saline (CMF-PBS) and Cephalexin hydrate were obtained from VWR International. In microbiological research, two distinct strains of bacteria were employed. The Gram-positive bacterium *Staphylococcus aureus* (*S. aureus* ATCC 6538) and the Gram-negative bacterium *Pseudomonas aeruginosa* (*P. aeruginosa* ATCC 9027) were obtained from the American Type Culture Collection (ATCC) located in Manassas, Virginia. The human fibroblast cells were also obtained from ATCC (BJ CRL-2522).

### S.2 Chemical Synthesis

The covalent conjugation of S-nitroso-N-acetyl-D-penicillamine (SNAP) with Cephalexin (CEX) was performed in three steps. First, N-acetyl-D-penicillamine (NAP, 2.5 g, 13 mmol) was converted to its self-protected thioacetone derivative, NAPTH (3-acetamido-4,4-dimethylthietan-2-one), following a reported protocol.[1] NAP was dissolved in ice-cooled dry pyridine (10 mL), followed by the addition of acetic anhydride ($Ac_2O$, 4.63 g, 45 mmol) under stirring. The reaction mixture was stirred at room temperature for 14 h. Upon completion, the solvent was removed under reduced pressure. The crude residue was redissolved in chloroform (30 mL) and washed three times with 1 M HCl. The organic layer was dried over anhydrous magnesium sulfate ($MgSO_4$), filtered, and concentrated. The resulting solid was triturated with cold hexane in an ice bath to yield white precipitates, which were filtered, washed, and dried under vacuum to afford NAPTH as a white solid (yield: 34%).

Next, CEX (0.5 g, 1.37 mmol) was dissolved in water (5 mL) and added to a solution of NAPTH (0.22 g, 1.25 mmol) in $CHCl_3$ (5 mL) and pyridine (10 mL). The reaction mixture was stirred at ambient temperature for 48 h. The crude product was isolated and purified by recrystallization using a 10% ethyl acetate in hexane mixture at 0 °C, affording the NAP-conjugated CEX.

The NAP-conjugated CEX (0.2 g, 0.38 mmol) was nitrosated to yield SNAP_CEX. The compound was dissolved in methanol (10 mL) and added to ice-cold 1 M HCl (10 mL) under dark conditions. After 10 min of stirring, concentrated $H_2SO_4$ (2 mL) was added dropwise,

followed by a slow addition of aqueous $NaNO_2$ solution (3.0 equiv., in 2 mL deionised water). The reaction mixture was stirred for 1 h at 0 °C. The final product was extracted with $CHCl_3$ (3 × 30 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The resulting green-colored SNAP_CEX was obtained in 78% yield and stored at –20 °C, protected from light.

The control molecule for the study, SNAP, was also synthesized. Briefly, 2 g (10.45 mmol) of N-acetyl-d-penicillamine (NAP) was dissolved in a mixture of methanol (40 mL), concentrated sulfuric acid (2 mL), and HCl (8 mL). The reaction mixture was then charged with a sodium nitrite solution (0.73 g (10.56 mmol) in 5 mL of DI water). After stirring for 15 min, the mixture was ice-cooled and filtered to obtain a green-colored precipitate, which was washed with deionized water and vacuum desiccated overnight to give green SNAP crystals (1.6 g, 70%). The purity was analyzed using a Sievers 290i nitric oxide analyzer and recorded at >95%.

## S.3 Characterization

### S.3.1 NMR Characterization

These compounds were investigated by nuclear magnetic resonance spectroscopy on an Advance III HD Nanobay 400 spectrometer equipped with Ascend 400 MHz magnet (Bruker, Billerica, MA). The samples were dissolved in deuterated DMSO. 1H NMR spectra were created using 64 scans, while 13C NMR spectra were generated by accumulating 1024 scans. The DEPT NMR studies were carried out to assign the peak values of $CH_2$ and CH groups and other carbons.

### S.3.2 ESI Mass Analysis

Nitrosation from NAP-CEX to SNAP_CEX was authenticated using a Bruker Esquire 3000 Plus Ion spectrometer, which used ESI-MS analysis in negative mode. The samples were dissolved in DMSO before injection.

### S.3.3 Spectroscopic Data

#### S.3.3.1 (6*R*,7*R*)-7-((2*R*)-2-(2-acetamido-3-mercapto-3-methylbutanamido)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (NAP_CEX):

NAP_CEX was obtained as a white solid with a 75% yield. $^1$H NMR (400 MHz, DMSO-d$^6$) $\delta$ 9.15 (d, $J$ = 8.1 Hz, H-1), 8.64 (d, $J$ = 7.1 Hz, H-2), 7.93 (d, $J$ = 9.4 Hz, H-3), 7.45 – 7.29 (m, H-4), 5.64 – 5.58 (m, H-5), 4.94 (d, $J$ = 4.6 Hz, H-6), 4.74 (d, $J$ = 9.4 Hz, H-7), 3.44 (d, $J$ = 18.5 Hz, H-8), 3.24 (d, $J$ = 18.2 Hz, H-9), 2.80 (s, H-10), 1.98 (s, H-11), 1.91 (s, H-12), 1.39 (d, $J$ = 6.7 Hz, H-13). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 170.9, 169.8, 169.4, 164.4, 163.9, 150.0, 138.0, 136.6, 130.2, 128.6, 127.9, 124.4, 123.2, 60.7, 59.0, 57.7, 56.6, 46.7, 30.1, 29.8, 29.3, 22.9, 19.8.

#### S.3.3.2 (6*R*,7*R*)-7-((2*R*)-2-(2-acetamido-3-methyl-3-(nitrosothio)butanamido)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (SNAP_CEX):

SNAP_CEX as a green powder with a 78% yield. $^{1}$H NMR (400 MHz, DMSO-d$^{6}$) $\delta$ 9.16 (d, $J$ = 8.1 Hz, H-1), 9.06 (d, $J$ = 7.1 Hz, H-2), 8.34 (d, $J$ = 9.8 Hz, H-3), 7.48 – 7.30 (m, H-4), 5.62 (d, $J$ = 7.3 Hz, H-5), 5.45 (d, $J$ = 9.7 Hz, H-6), 4.94 (d, $J$ = 4.5 Hz, H-7), 3.42-3.26 (m, H-8, 9), 2.11 (s, H-10), 1.98 (s, 6H, H-11), 1.81 (s, H-12). $^{13}$C NMR (101 MHz, DMSO-d$^{6}$) $\delta$ 170.8, 169.7, 168.5, 164.4, 163.9, 137.7, 130.2, 128.7, 128.2, 128.1, 123.2, 60.7, 58.9, 57.7, 56.7, 29.3, 27.5, 25.2, 22.7, 19.8.

### S.3.4 Nitric Oxide (NO) Determination Using the Griess Assay

The adapted Griess assay was used for measuring the concentrations of nitrite, $NO_2^-$, produced from reactions involving NO. SNAP_CEX and SNAP compounds were dissolved in 2% DMSO within CMF-PBS to prepare the sample solutions. The reason behind using this specific CMF-PBS solution is to avoid interference with nitrite measurement. Calcium and magnesium ions form complexes with specific reagents in the assay, potentially reducing their availability for reacting with nitrite (Griess Reagent System Technical Bulletin TB229).[2] The modified Griess reagent was diluted with 2% DMSO within PBS to a final 20 mg/mL concentration in the 96-well plate. Both the sample solutions in different concentrations (10 µL) and modified Griess reagent (90 µL) were mixed in a 96-well plate. The mixtures were then incubated at room temperature for 15 min for color development. The absorbance was measured at 540 nm using a BioTek Cytation 5 plate reader. A standard curve was developed using known concentrations of sodium nitrite, which helped quantify the nitrite level in the samples. The mean nitrite concentration was presented ± standard deviation (S.D.) for three independent trials (N = 3).

### S.3.5 UV Stability Analysis

To assess the stability of SNAP and its conjugate hybrid SNAP_CEX, a UV–vis spectroscopic study was performed. Solid samples of both compounds were stored at room temperature under ambient conditions in the dark to simulate standard laboratory storage. At predetermined intervals (Day 1, 2, 7, 14, 21, and 28), 50 mg of each sample was dissolved in DMSO, and the absorbance was measured using a Genesis 10S UV–vis spectrophotometer. The characteristic NO-related absorption bands at 340 nm ($\pi \rightarrow \pi$)* and 590 nm ($n \rightarrow \pi$)* were monitored. To avoid spectral overlap with CEX, data interpretation focused primarily on the 590 nm peak for three independent trials (N = 3). The percentage degradation was calculated by comparing absorbance values over time relative to Day 1.

## S.4 Antibacterial Efficacy

### S.4.1 Minimal Inhibitory Concentration (MIC) Determination

The minimum inhibitory concentrations (MICs) for SNAP, CEX, and SNAP_CEX were assessed during a 24 h bacterial growth study against *P. aeruginosa* and *S. aureus*. The compounds were serially diluted by 50%. A 16 mM stock solution was prepared for SNAP. The other two compounds, CEX and SNAP_CEX, were initially tested at various concentrations, starting with 100 µM, and subsequently diluted by half to determine the MIC against *S. aureus*. CEX was prepared at a concentration of 32 mM, followed by serial 50%

dilutions to assess its MIC against *P. aeruginosa*. SNAP_CEX was tested at an initial concentration of 4 mM, with subsequent 50% dilutions.

To prepare the bacterial solution, a single colony was inoculated into LB and incubated at 37 °C and 150 rpm until it reached the log phase of growth (Optical density of ~0.7OD). The bacterial suspension was then collected, rinsed with PBS, and resuspended in LB. The absorbance at 600 nm was measured to adjust bacterial counts to approximately 0.2OD. Next, equal volumes of bacterial solution and treatmentwere added in a 96-well plate for a final optical density of 0.1OD. Initial absorbance readings were taken to ensure a proper experimental setup. The plates were then placed in a shaking incubator at 37 °C and 150 rpm for 24 h while protected from light. After 24 h, the absorbance of the well was measured at 600 nm using the BioTek Cytation 5 plate reader. The growth curves' final absorbances were plotted against treatment concentration, with media and treatment blanks subtracted for analysis. Each treatment was tested in four wells, and experiments were done in biological triplicates to confirm the results.

**S.4.2 Biofilm Reduction Assay**

Biofilms were cultivated for 48 h on 8 mm diameter CarboSil films and treated with SNAP, CEX, and SNAP_CEX solutions. *P. aeruginosa* and *S. aureus* solutions were prepared by incubating a single bacterial colony in LB at 37 °C and 150 rpm until reaching ~0.7OD. The bacterial pellet was collected, rinsed with PBS, resuspended in LB media, and diluted to a working bacterial solution of $10^7$ CFU $mL^{-1}$ (OD) of 0.1. Further, 1 mL of the bacterial solution was added to each well of a 48-well plate containing a sterilized CarboSil film. The plate was sealed, protected from light, and incubated at 37 °C for 48 h. After 24 h, the medium was replaced with fresh LB to maintain nutrient levels without disturbing biofilm growth. After 48 h, the films were rinsed with PBS and transferred to a new plate. Control films were treated with PBS while SNAP, CEX, and SNAP_CEX solutions were added (1 mL per well). Based on MIC study results, *P. aeruginosa* biofilms were treated with 3 mM, and *S. aureus* biofilms with 7 μM. The plate was incubated for another 24 h at 150 rpm and 37 °C.

*Biofilm Biomass Quantification (Crystal Violet Assay):* To evaluate total biomass, treated films were stained with 0.1% crystal violet for 15 min, washed thoroughly with PBS, and air-dried. The dye retained by the biofilm was solubilized in 30% acetic acid, and absorbance was measured at 590 nm to quantify the extent of biomass remaining post-treatment.

*Viable Cell Enumeration (CFU Count):* Treated films were rinsed with PBS, homogenized using an Omni-TH ultrasonic homogenizer at 25,000 rpm, and vortexed for 60 s to dislodge biofilm-embedded bacteria. Serial dilutions were plated on LB agar and incubated at 37 °C for 24 h. Colony-forming units (CFUs) were counted, and bacterial load was normalized to the surface area of the films. Three biological replicates were used for each treatment group.

*Planktonic Viability Assay:* The surrounding media from each well was collected post-treatment, diluted appropriately, and plated on LB agar to determine planktonic bacterial CFUs. Plates were incubated under the same conditions as above. This assay provided insight into the treatment's effectiveness against detached or non-adherent bacterial populations.

### S.4.3 Confocal Microscopy

Biofilm coverage was assayed by confocal laser scanning microscopy (CLSM) to visualize and quantify biofilm integrity after the treatments. After treatment, biofilms were gently washed with sterile PBS to remove nonadherent cells. The samples were stained using a live/dead bacterial viability kit from Invitrogen, differentiating live cells with green fluorescence from dead cells with red fluorescence. Stained samples were observed by a confocal laser scanning microscope (Zeiss LSM 900 Confocal Microscope with AI Sample Finder) using a 20× objective.

### S.4.4 Scanning Electron Microscopy (SEM)

To better understand how the treatment affects biofilm structure, the experiment was repeated specifically for imaging. After 24 hours of treatment, the CarboSil-coated films were carefully taken out of the culture media, gently rinsed with PBS to remove any loose debris, and then fixed in 3% glutaraldehyde to preserve the attached bacterial biofilm. The samples were gradually dehydrated using a graded ethanol series and finally dried using hexamethyldisilazane (HMDS) to maintain structural integrity. Once dried, the films were mounted on scanning electron microscopy (SEM) stubs and coated with a thin layer (10 nm) of gold-palladium using a Leica sputter coater. High-resolution SEM images were captured using a FEI Teneo instrument operated at 5.00 kV to visualize biofilm disruption at the surface level.

### S.5 Biocompatibility Evaluation

An *in vitro* cytotoxicity experiment was conducted to evaluate the biocompatibility of three drugs (SNAP, CEX, and SNAP_CEX) using human fibroblast cells (BJ CRL-2522) by ISO-10993–5 criteria. BJ fibroblast cells were revived from cryopreserved stocks and cultivated in DMEM with 10% FBS and 1% Penicillin-Streptomycin at 37 °C in a 5% $CO_2$-humidified incubator. The cells were grown for a maximum of 10 passages and divided when they attained more than 70% confluency.

In cytocompatibility tests, cells were dissociated using trypsin-EDTA, gathered, quantified, and seeded in 96-well plates (8000 cells/well) to achieve an estimated 70% confluency within 24 h. Media was substituted with new media with different concentrations of all three chemicals. The following 24 h later, the medium was removed, and the cells were rinsed with PBS. The cells were then placed in a solution of PBS containing MTT reagent at a concentration of 0.5 mg/mL. Following incubation, the formazan crystals formed during the MTT assay were solubilized in dimethyl sulfoxide (DMSO). The absorbance of the cells was measured at a wavelength of 570 nm, with a reference measurement taken at 690 nm. The relative cell viability was determined by calculating the difference in absorbance between treated and untreated cells. The results are the average percentage of viable cells and the standard deviation obtained from three experiments.

### S.6 Fractional Inhibitory Concentration (FIC) Assay

The fractional inhibitory concentration (FIC) assay was performed to evaluate the interaction between SNAP and CEX when used in combination against bacterial strains. The assay followed a standard checkerboard design conducted in 96-well microtiter plates.

To prepare the wells, serial two-fold dilutions of each antimicrobial agent—SNAP and CEX—were first prepared independently. For combination wells, each well received 37.5 µL of the diluted SNAP solution and 37.5 µL of the diluted CEX solution, totaling 75 µL of antimicrobial mixture. This ensured that each well in the combination assay maintained the same final volume as those containing single agents.

An additional 75 µL of bacterial suspension was added to each well, bringing the final volume per well to 150 µL. Concentration combinations ranged from 2×MIC to 0×MIC for each agent. Plates were incubated at 37 °C for 24 hours under static conditions. Absorbance at 600 nm was measured using BioTek Cytation 5 plate reader at 0 h and 24 h to assess bacterial growth inhibition.

The FIC index was calculated using the following equation:

$$FIC = \left(MIC\ of\ CEX\ in \frac{combination}{alone}\right) + (MIC\ of\ SNAP\ in \frac{combination}{alone})$$

Interpretation of FIC values was as follows: FIC ≤ 0.5 indicated synergy, values between 0.5 and 4.0 indicated additive or indifferent effects, and FIC > 4.0 suggested antagonism. All experiments were performed in triplicate, and data were expressed as mean ± SD.

**S.7 Statistical Analysis**

All data are expressed as mean ± S.D. ANOVA was used for statistical analysis of NOA studies at each time point, and antimicrobial and biocompatibility studies were analyzed using one-way ANOVA with Tukey post hoc correction and two-way ANOVA, respectively. A *p*-value of < 0.05 was considered statistically significant for all experiments.

**S. 8 Fractional Inhibitory Concentration (FIC) Analysis**

To evaluate the interaction between SNAP and CEX, a checkerboard Fractional Inhibitory Concentration (FIC) assay was conducted against both *S. aureus* and *P. aeruginosa*. The maximum concentration used for SNAP is 10 mM (2 MIC *vs. S. aureus*) and 16 mM (2 MIC *vs. P. aeruginosa*) for CEX it was 2 µM (2 MIC *vs. S. aureus*) and 32mM (2 MIC *vs. P. aeruginosa*). The results revealed clear synergistic effects (FIC ≤ 0.5) in both strains, but with distinct concentration dependencies (**Fig. S10**). The growth inhibition plots (left) show a concentration-dependent decline in viability for both pathogens, with corresponding FIC heatmaps (right) indicating distinct synergy patterns. For *S. aureus*, a significant reduction in bacterial growth was observed even at low concentrations of the SNAP and CEX combination (as low as 0.125 µM CEX with 0.625 mM SNAP), with percent viability falling below 80% and decreasing steadily across higher combinations (**Fig. S10**). The FIC heatmap supports this, displaying broad areas of synergy (FICI ≤ 0.5) particularly between 0.25–2 µM CEX and 1.25–5 mM SNAP (**Fig. S10**). These results are consistent with the known clinical efficacy of CEX

against Gram-positive pathogens such as *S. aureus*, and the enhanced activity observed here likely reflects the additive contribution of NO donor.

In contrast, *P. aeruginosa* required substantially higher concentrations to achieve a similar effect. The viability plot shows minimal growth inhibition at lower doses, with significant reduction emerging only at ≥6 mM SNAP in combination with ≥8 µM CEX (**Fig. S10**). The FIC heatmap for *P. aeruginosa* reflects this trend—synergy is limited to a narrow band at high SNAP (6–8 mM) and high CEX (16–32 µM) concentrations. Lower concentrations either resulted in indifference (FICI > 1) or a borderline additive effect. Different combination concentrations were employed to see the synergistic effect of both the compounds. The observed results were consistent with the poor intrinsic activity of CEX against Gram-negative bacteria. While synergy with SNAP was still evident, it required higher concentrations of both agents to overcome the bacterial defenses.

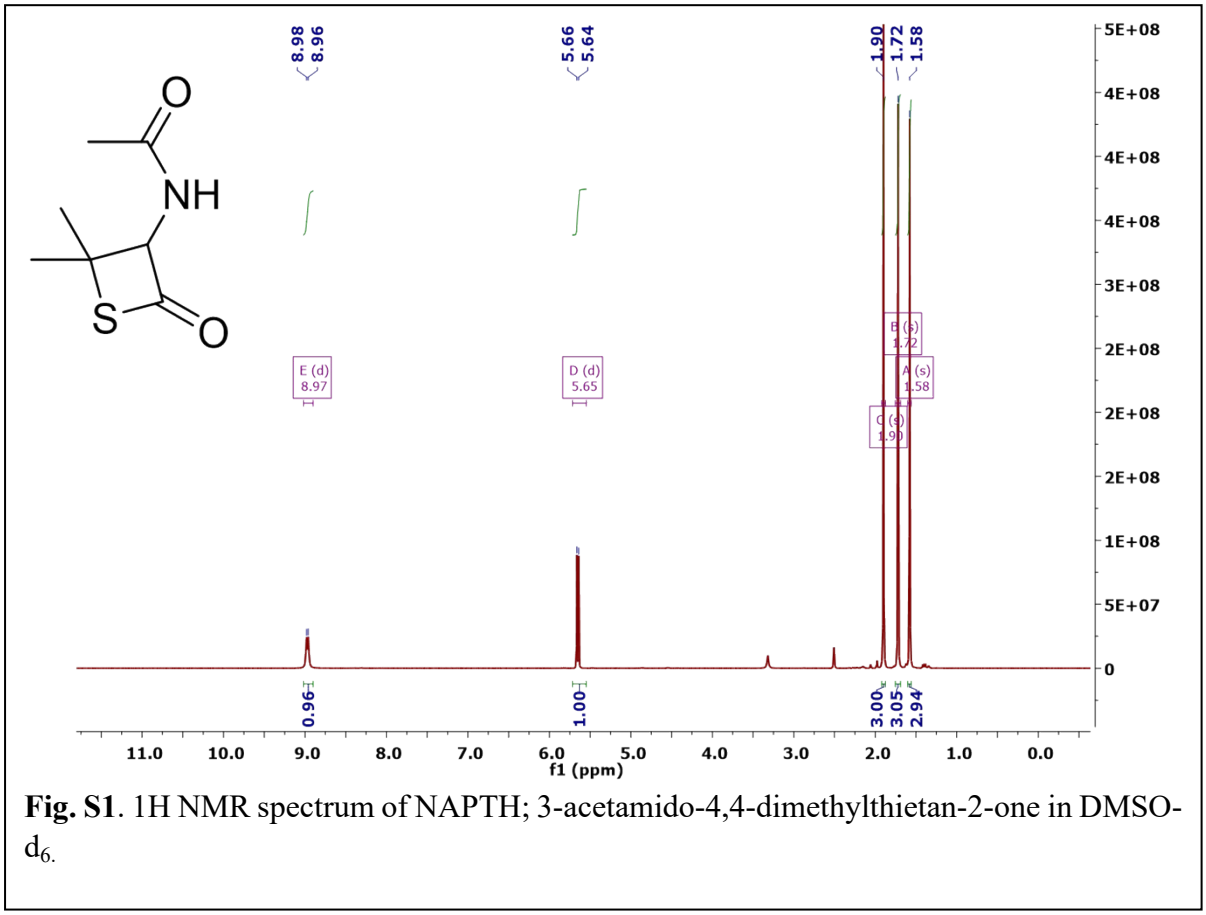


**Fig. S1**. 1H NMR spectrum of NAPTH; 3-acetamido-4,4-dimethylthietan-2-one in DMSO-$d_6$.

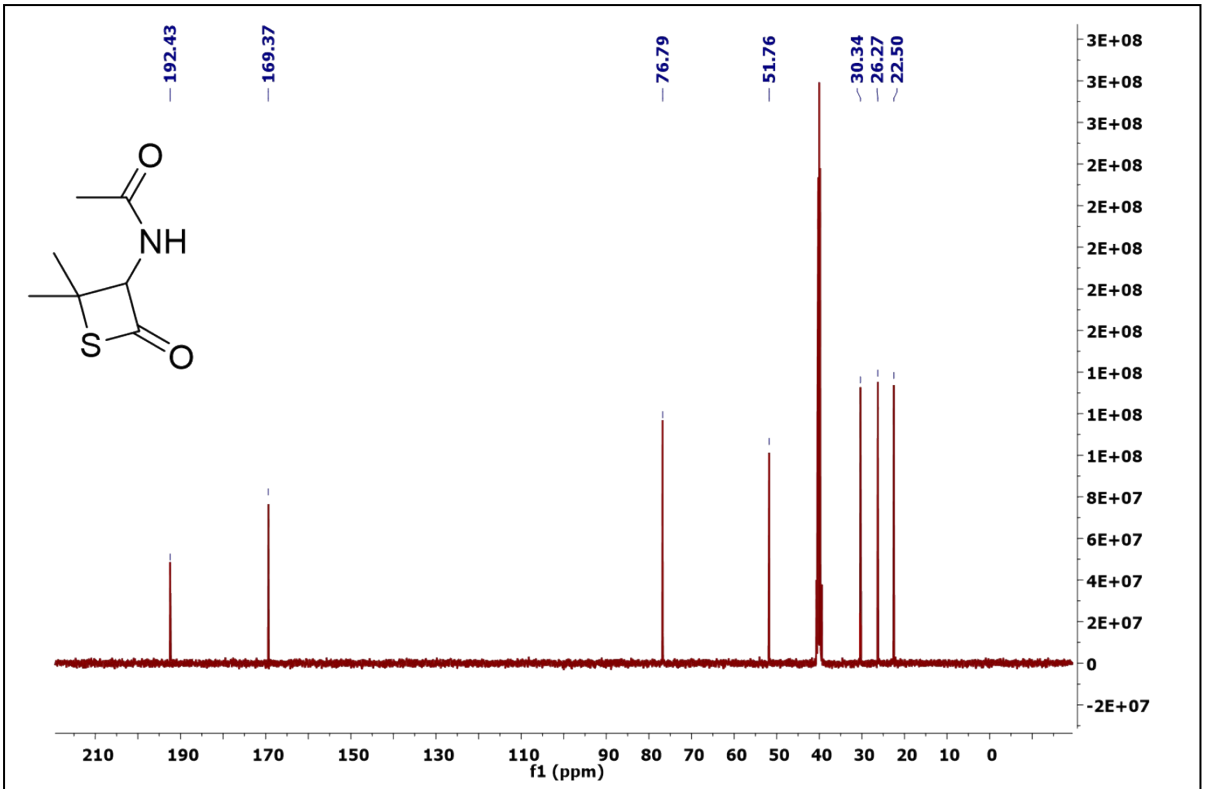


**Fig. S2**. 13C NMR spectrum of NAPTH; 3-acetamido-4,4-dimethylthietan-2-one in DMSO-$d_6$.

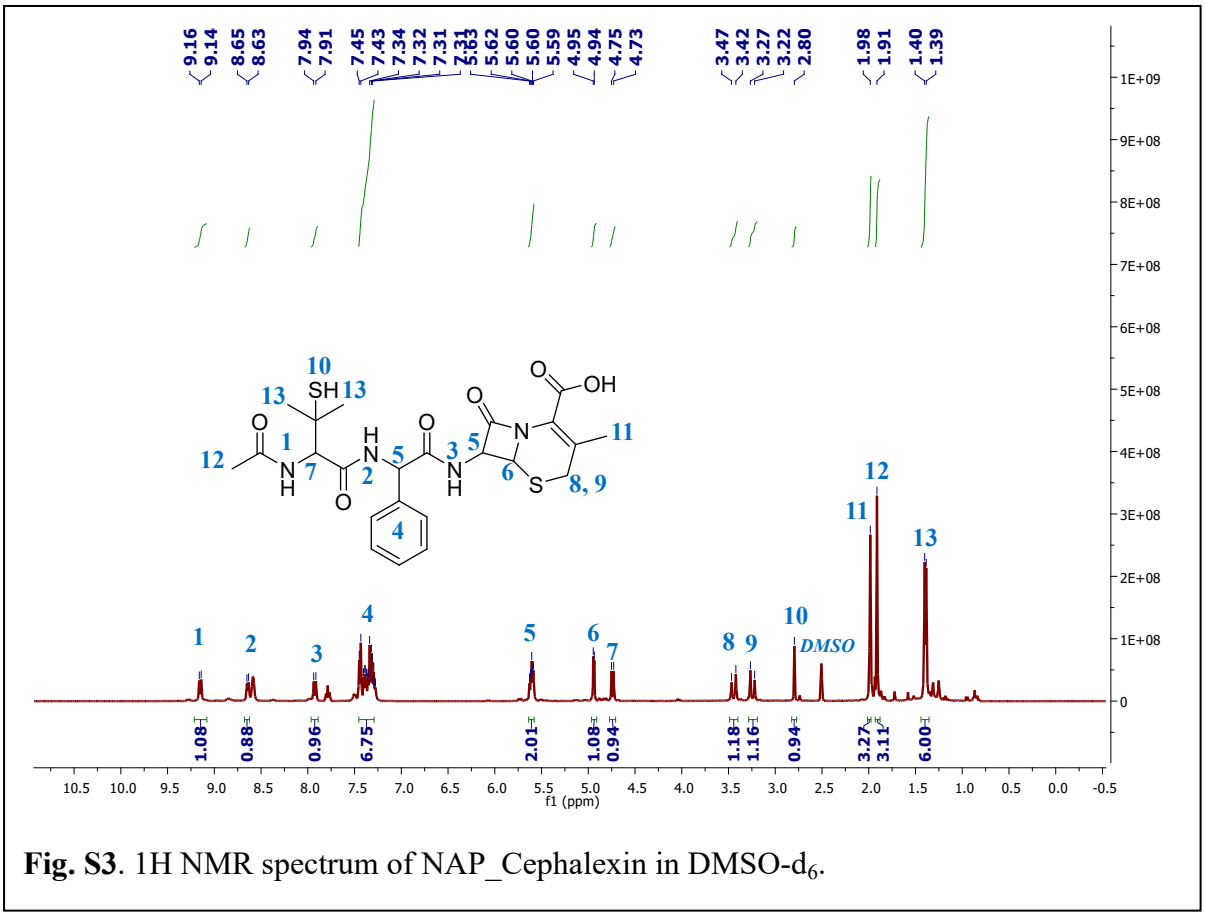


**Fig. S3**. 1H NMR spectrum of NAP_Cephalexin in DMSO-$d_6$.

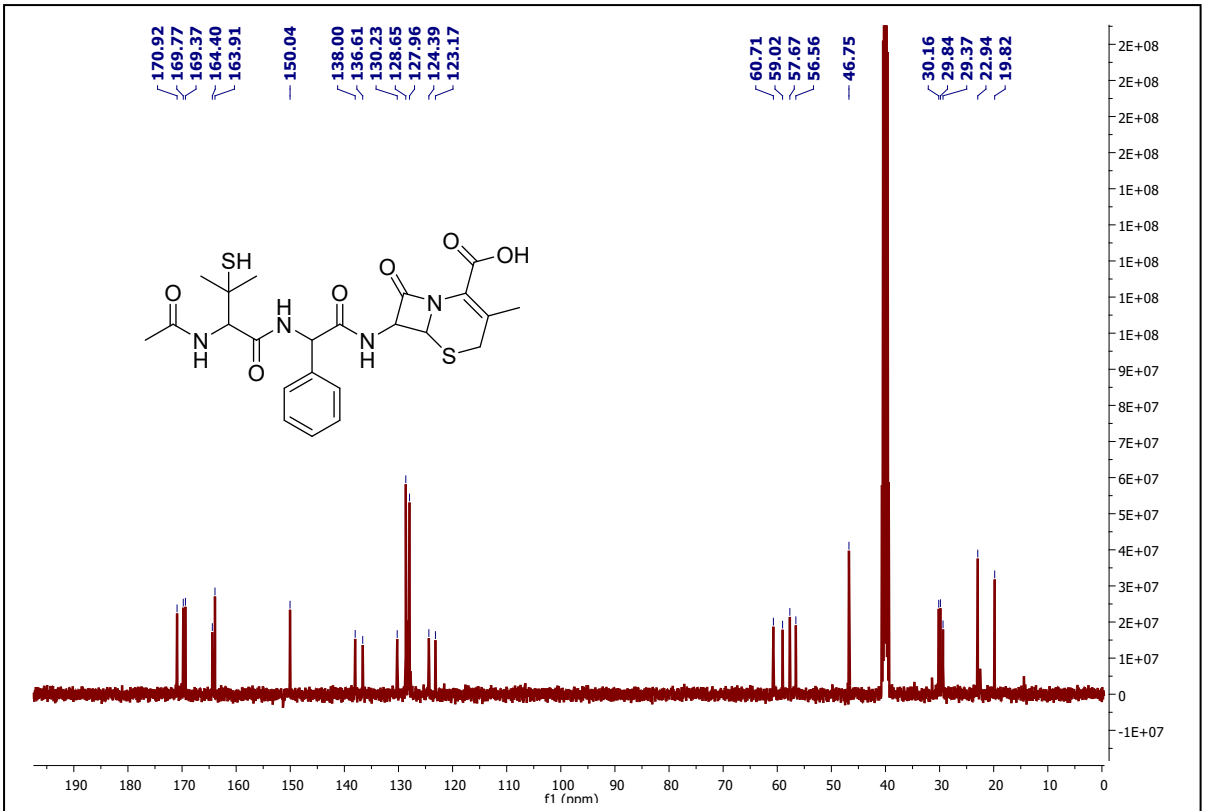


**Fig. S4**. 13C NMR spectrum of NAP_Cephalexin in DMSO-$d_6$.

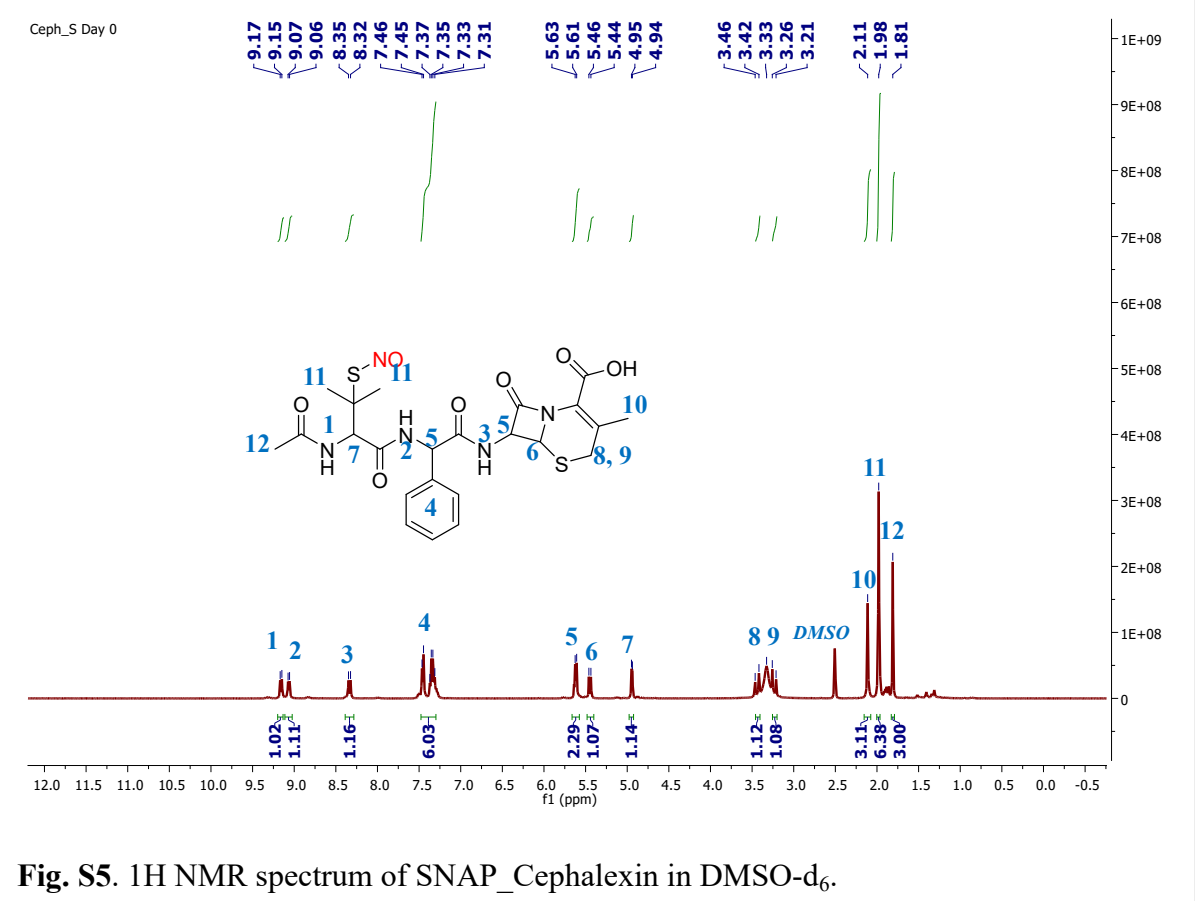


**Fig. S5**. 1H NMR spectrum of SNAP_Cephalexin in DMSO-$d_6$.

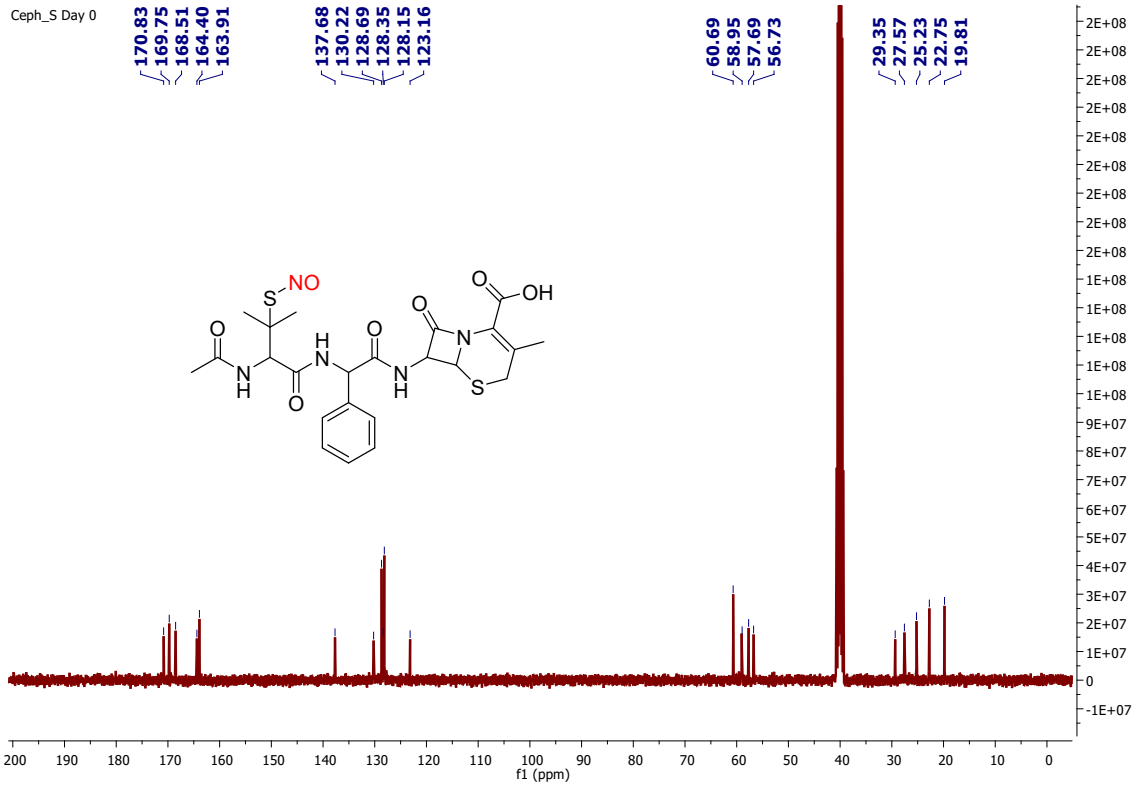


**Fig. S6**. 13C NMR spectrum of SNAP_Cephalexin in DMSO-$d_6$.

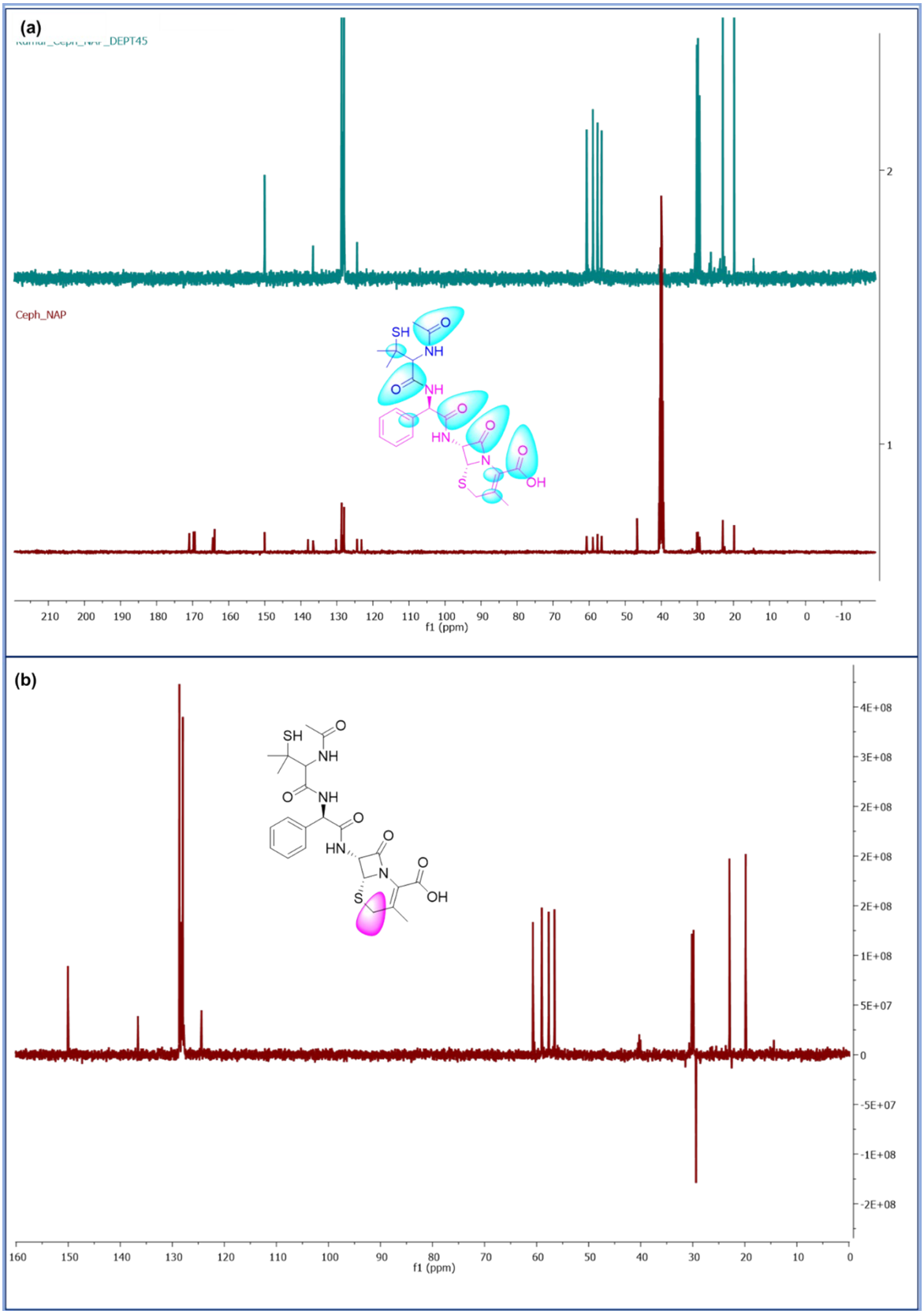


**Fig. S7**. DEPT_45 and DEPT_135 analyses of NAP_CEX indicated the absence of carbons without attached protons (**7a**) and the presence of a single $CH_2$ group (**7b**).

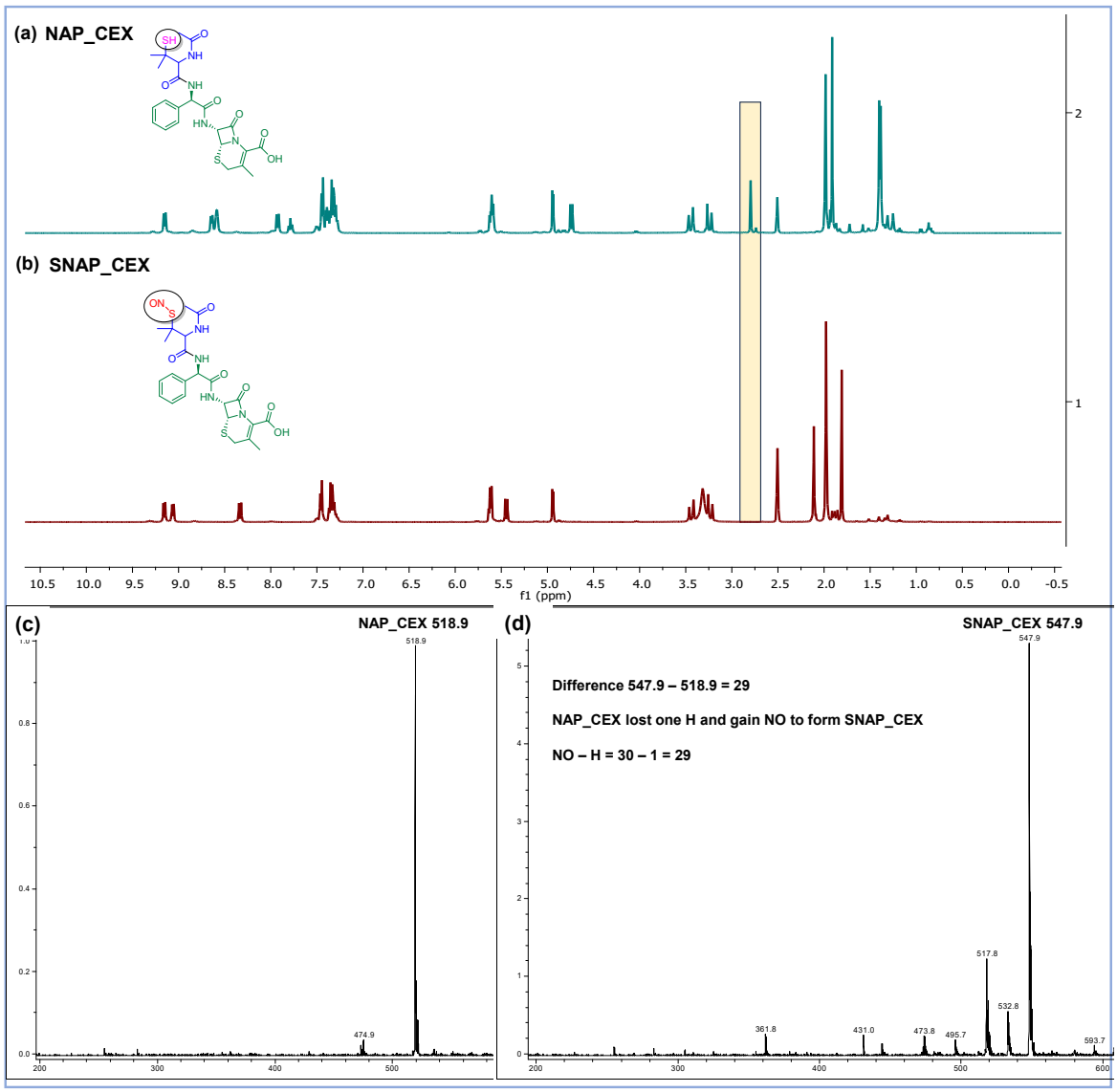


**Fig. S8.** (**a-b**) Characterization of the conversion of thiol to nitroso group in the target compound: Proton NMR comparison for NAP_CEX (**1a**) and SNAP_CEX (**1b**). (**c-d**) ESI-Mass analysis indicates the clear conversion of NAP_CEX (**1b**) to SNAP_CEX (**1d**).

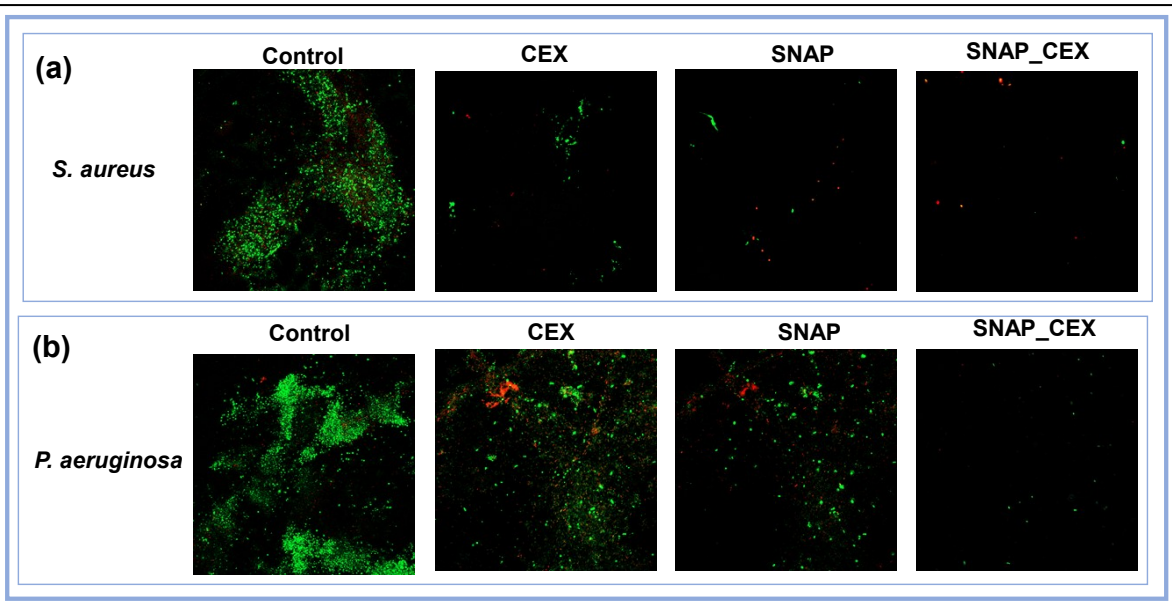


**Fig. S9.** (**a-b**) Confocal images confirmed biofilm disruption and bacterial lysis, highlighting SNAP_CEX's enhanced dual-action mechanism.

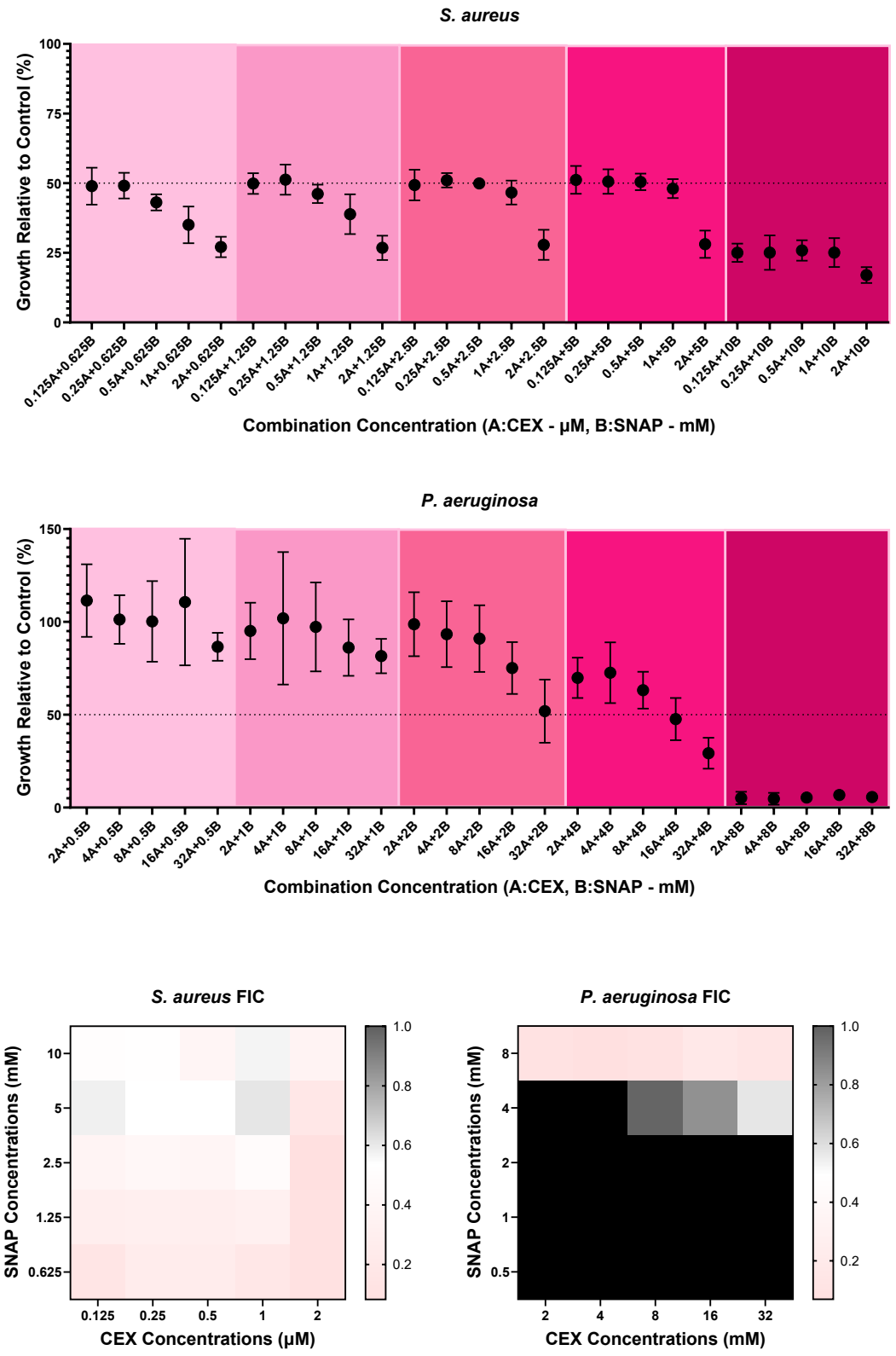


**Fig. S10.** Fractional inhibitory concentration (FIC) analysis of SNAP and CEX combination against *S. aureus* and *P. aeruginosa*. Growth inhibition plots showing percentage growth relative to control across increasing combinations of SNAP and CEX. FIC heatmaps illustrating synergistic interaction (FICI ≤ 0.5, pink), additive effect (FICI > 0.5 to ≤ 1.0, gray), and indifference (FICI > 1.0 to ≤ 4.0, black) based on checkerboard assay.

**Table S1.** Average daily NO release for SNAP and SNAP_CEX, calculated from Griess assay nitrite data, expressed in μg/mL/day and μM/day.

| Compound | Interval (days) | Δ Nitrite (μg/mL) | Avg. daily release (μg/mL/day) | Avg. daily release (μM/day) |
|---|---|---|---|---|
| SNAP | 1–5 | 13.91 | 3.478 | 75.598 |
| SNAP | 5–14 | 13.99 | 1.554 | 33.792 |
| SNAP | 14–21 | 14.10 | 2.014 | 43.789 |
| SNAP | 21–30 | 0.30 | 0.033 | 0.723 |
| SNAP_CEX | 1–5 | 34.10 | 8.525 | 185.326 |
| SNAP_CEX | 5–14 | 37.00 | 4.111 | 89.370 |
| SNAP_CEX | 14–21 | 41.00 | 5.857 | 127.326 |
| SNAP_CEX | 21–30 | 1.20 | 0.133 | 2.898 |

## References

1. Y. Zhou, J. Tan, Y. Dai, Y. Yu, Q. Zhang and M. E. Meyerhoff, *Chem. Commun.*, 2019, **55**, 401-404.
2. X. Huang, F. Xu, H. Hou, J. Hou, Y. Wang and S. Zhou, *Nano Res.*, 2019, **12**, 1361-1370.