

## **Supporting Information**

### **A Prospective Antibacterial for Drug-resistant Pathogens: A Dual Warhead Amphiphile Designed to Track Interactions and Kill Pathogenic Bacteria by Membrane Damage and Cellular DNA Cleavage**

*Durairaj Thiyagarajan,<sup>a</sup> Sudeep Goswami,<sup>a</sup> Chirantan Kar,<sup>b</sup> Gopal Das\*<sup>b</sup> and Aiyagari Ramesh\*<sup>a</sup>*

<sup>a</sup>Department of Biotechnology

Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India

E-mail: aramesh@iitg.ernet.in (Aiyagari Ramesh)

<sup>b</sup>Department of Chemistry

Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India

E-mail: gdas@iitg.ernet.in (Gopal Das)

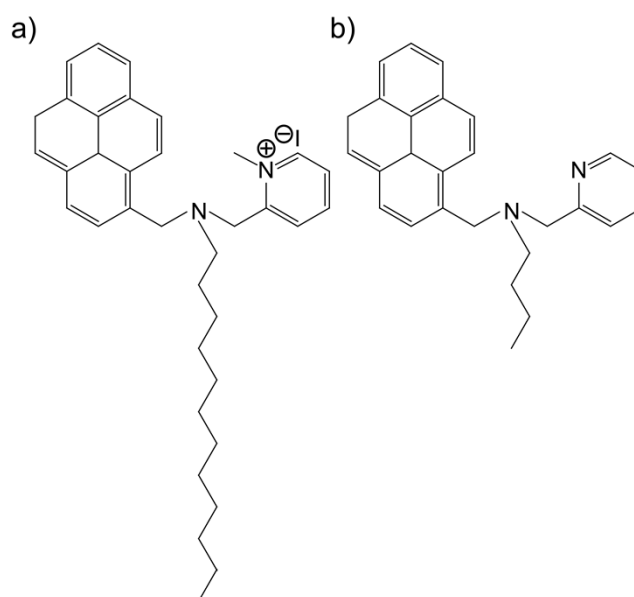
## EXPERIMENTAL SECTION

### 1.0 Materials

1-Pyrenecarboxaldehyde and 2- (Chloromethyl) pyridine hydrochloride were purchased from Sigma-Aldrich (USA). Dodecylamine was purchased from Fluka (Sweden) and Iodomethane was purchased from Merck (Germany). 5 (and 6)-carboxyfluorescein diacetate succinimidyl ester (cFDA-SE), ethidium bromide (EtBr), propidium iodide (PI), carbonyl cyanide m-chlorophenylhydrazone (CCCP), valinomycin, polymyxin B, GenElute plasmid DNA miniprep kit, PCR purification kit, Dulbecco's Modified Eagle Medium (DMEM), trypsin-EDTA and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were procured from Sigma-Aldrich (USA). Calf thymus DNA (CT-DNA) and cesium chloride purified pUC18 plasmid DNA was procured from Bangalore Genei, India. Nutrient Broth (NB), Brain-Heart Infusion (BHI) broth and Luria-Bertani (LB) broth were procured from HiMedia, Mumbai, India. Dimethyl sulfoxide (DMSO) was obtained from Merck, India. N-2-hydroxyethyl piperazine N-2 ethane sulphonic acid (HEPES buffer) was procured from Sisco Research Laboratories SRL, Mumbai, India. Fetal bovine serum (FBS) was procured from PAA Laboratories, USA.

### 2.0 Synthesis of Amphiphiles

The general structure of the amphiphiles used in the present study is shown in Figure S1.

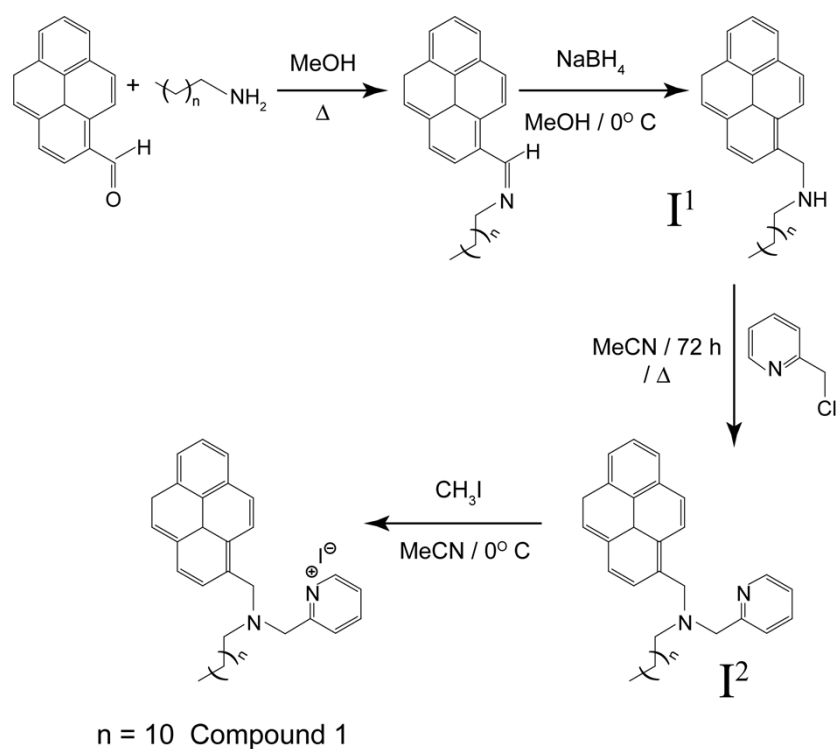


**Figure S1.** Structure of **compound 1** and **compound 2**.

Synthesis of the amphiphiles was accomplished as follows:

### 2.1. Compound 1

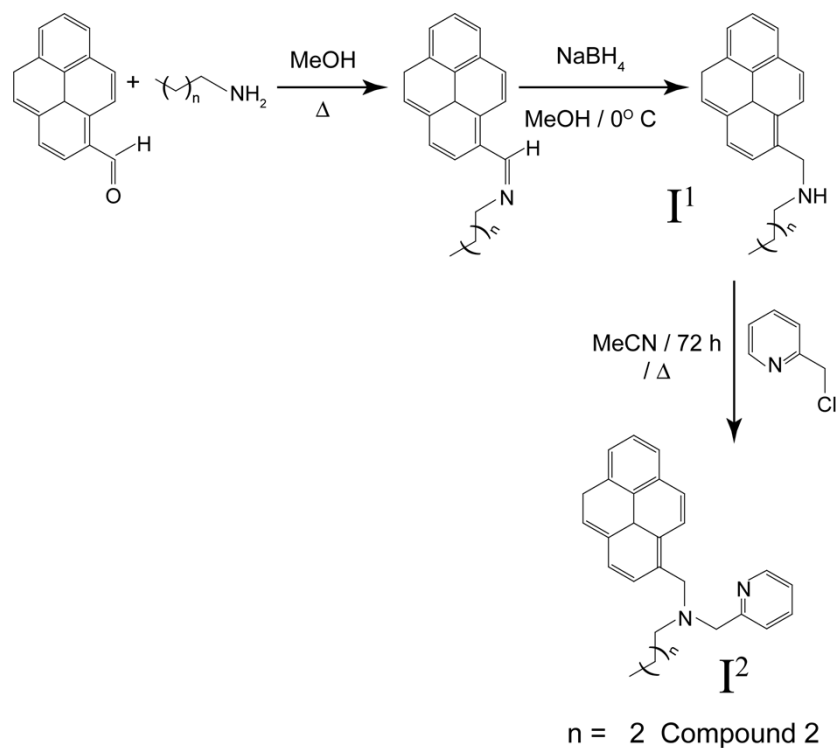
The synthesis route of **compound 1** is indicated in Scheme S1. The precursor molecule **I**<sup>2</sup> was synthesized following the reported procedure.<sup>[1]</sup> 100 mg of 1-pyrenealdehyde was refluxed with 88 mg (1.1 eqv.) of dodecylamine in methanol to afford the desired Schiff base N-((pyren-6-yl) methylene) dodecan-1-amine in 70 % yield. The Schiff base was reduced by gradual addition of NaBH<sub>4</sub> in its methanolic solution to obtain N-((pyren-6-yl) methyl) dodecan-1-amine (**I**<sup>1</sup>). The intermediate **I**<sup>2</sup> was directly synthesized from N-((pyren-6-yl) methyl) dodecan-1-amine by refluxing it with 1-chloromethyl (pyridine hydrochloride) in the presence of K<sub>2</sub>CO<sub>3</sub> in CH<sub>3</sub>CN for 72 hours. Crude product was purified by column chromatography with 55% yield. **Compound 1** was synthesized by reacting **I**<sup>2</sup> with CH<sub>3</sub>I in MeCN solvent.



**Scheme S1.** Synthesis route of **compound 1**.

## 2.2. Compound 2

The synthesis route of **compound 2** is indicated in Scheme S2. 230.26 mg of 1-pyrenealdehyde was refluxed with 80.44 mg (1.1 eqv.) of butylamine in methanol to afford the desired Schiff base N-((pyren-6-yl) methylene) butane-1-amine in 80% yield. The Schiff base was reduced by gradual addition of NaBH<sub>4</sub> in its methanolic solution to obtain N-((pyren-6-yl) methyl) butane-1-amine. **Compound 2** was directly synthesized from N-((pyren-6-yl) methyl) butane-1-amine by refluxing it with 1-chloromethyl (pyridine hydrochloride) in the presence of K<sub>2</sub>CO<sub>3</sub> in CH<sub>3</sub>CN for 48 hours. Crude product was purified by column chromatography with 68% yield.



**Scheme S2.** Synthesis route of **compound 2**.

### 3.0 Bacterial Strains and Growth Conditions

The target bacterial strains consisted of Gram-positive *Staphylococcus aureus* MTCC 96 (*S. aureus*), *Listeria monocytogenes* Scott A (*L. monocytogenes*), *Bacillus subtilis* MTCC 441 (*B. subtilis*) and *Micrococcus luteus* NCIM 2379 (*M. luteus*) and Gram-negative *Escherichia coli* MTCC 433 (*E. coli*), *Enterobacter aerogenes* MTCC 2822 (*E. aerogenes*), *Pseudomonas aeruginosa* MTCC 2488 (*P. aeruginosa*) and *Yersinia enterocolitica* MTCC 859 (*Y. enterocolitica*). *B. subtilis* MTCC 441, *E. coli* MTCC 433, *P. aeruginosa* MTCC 2488 and *E. aerogenes* MTCC 2822 were grown in NB medium at 37°C and 180 rpm for 12 h whereas *S. aureus* MTCC 96, *L. monocytogenes* Scott A, *M. luteus* NCIM 2379 and *Y. enterocolitica* MTCC 859 were propagated in BHI broth at 37°C and 180 rpm for 12 h.

### 4.0 Stock Solutions of Synthetic Amphiphiles

Stock solution of **compound 1** and **compound 2** (10 mg/mL each) were prepared in DMSO and stored at -20°C. From the stock, amphiphile solutions were freshly reconstituted to the concentrations required for the specific experiments.

### 5.0 Screening of Antibacterial Activity of Synthetic Amphiphiles

Bactericidal activity of the amphiphiles was ascertained against a group of Gram-positive and Gram-negative bacteria as mentioned in section 3.0. Target bacterial strains were grown in requisite growth media in presence of varying concentrations of **compound 1** or **compound 2** (50 µg/mL and 100 µg/mL each) for 24 h. The growth of amphiphile-treated bacterial cells was determined by recording the absorbance at 600 nm from replica samples in a spectrophotometer (CARY 300 Bio, Varian) and was expressed as percentage growth compared to control (untreated cells).

### 6.0 Minimum Inhibitory Concentration (MIC) and Minimum Killing Concentration (MKC) of Compound 1

MIC and MKC of **compound 1** was determined against Gram-positive bacteria *L. monocytogenes* Scott A and *S. aureus* MTCC 96 and Gram-negative bacteria *E. coli* MTCC 433 and *E. aerogenes* MTCC 2822. The bacterial cultures were inoculated at 1% level in microtitre wells (approximately  $5 \times 10^5$  CFU/well) having 100 µL of the specific growth medium and propagated overnight at 37°C and 180 rpm in presence of varying concentrations of **compound 1**. The growth of the bacterial strains was verified by measuring absorbance at

600 nm in a microtitre plate reader (Infinite M200, TECAN, Switzerland). MIC of **compound 1** was defined as the minimum amphiphile concentration that resulted in growth inhibition of the target bacteria ( $A_{600} < 0.1$ ). An aliquot (1% v/v) from all the wells that indicated a lack of cell growth ( $A_{600} < 0.1$ ) was re-inoculated into separate microtitre wells with fresh growth medium in the absence of amphiphile and incubated overnight at 37°C and 180 rpm. MKC of **compound 1** was defined as the lowest amphiphile concentration that inhibited the growth of the target bacterial cells following re-inoculation ( $A_{600} < 0.1$ ). MIC and MKC values were determined from six independent experiments and expressed as average values.

### 7.0 Fluorescence-based Tracking of Compound 1-Bacteria Interaction

Cells of *S. aureus* MTCC 96 were suspended in sterile phosphate buffered saline (PBS) in separate sets ( $10^6$  CFU/mL in each set) and incubated with various concentrations of **compound 1** (1.5  $\mu$ M, 3.0  $\mu$ M, 4.5  $\mu$ M and 6.0  $\mu$ M) at 37°C for 3h in a shaking incubator. Subsequently the cells were collected by centrifugation at 8000 rpm for 5 minutes. The cell pellet representing cell-bound **compound 1** was resuspended in 1.0 mL sterile PBS and the supernatant representing unbound **compound 1** was collected in a sterile tube. The fluorescence emission spectra of all the samples (**compound 1** alone, cell-bound **compound 1** and unbound **compound 1**) were measured by exciting at 340 nm and the emission was collected from 360-600 nm.

For fluorescence microscope analysis, cells of *S. aureus* MTCC 96 were suspended in 1.0 mL sterile PBS ( $10^6$  CFU/mL) and incubated with 45  $\mu$ M of **compound 1** at 37°C for 3h in a shaking incubator. The cells were then separated by centrifugation at 8000 rpm for 5 minutes. The supernatant was discarded and the cell pellet was resuspended in PBS. An aliquot of the resuspended cells was then smeared on a clean glass slide, dried and observed under a fluorescence microscope (Eclipse Ti-U, Nikon, USA) in UV, blue, green and white light. Images of the cells under each excitation were recorded.

### 8.0 Mode of Action of Compound 1

The following experiments were conducted to determine the mechanism of action of **compound 1**:

### 8.1. Field Emission-Scanning Electron Microscope (FE-SEM) Analysis

Overnight grown cells of *S. aureus* MTCC 96 and *E. coli* MTCC 433 were collected by centrifugation, washed twice with sterile PBS and resuspended in the same. The cells were then treated with 40  $\mu$ M of **compound 1** for 6h at 37°C. Untreated cells were also incubated in sterile PBS under the same conditions as control samples. Following 6h incubation, untreated as well as treated cells were collected by centrifugation, washed with sterile PBS and sterile MilliQ water and finally suspended in sterile MilliQ water. A 10  $\mu$ L aliquot of each sample was spotted on separate aluminium foil covered glass SEM grid and air dried in the laminar hood. The samples were then mounted on a carbon tape covered metal grid and gold (Au) coating was done twice. Finally the samples were analyzed in a field emission scanning electron microscope (Zeiss Sigma, USA) at 1.5-3.0 Kv.

### 8.2. cFDA- SE Leakage Assay

A stock solution of cFDA-SE (500  $\mu$ M) was prepared in ethanol and stored at -20°C. Cells of *S. aureus* MTCC 96 and *E. coli* MTCC 433 were harvested from overnight grown cultures by centrifugation at 8000 rpm for 5 min. The cell pellet was washed twice with sterile PBS, resuspended in the same to achieve a cell concentration of 10<sup>6</sup> CFU/mL and labelled with cFDA-SE (final concentration of 50  $\mu$ M) for 20 min at 37°C. The cells were then centrifuged, washed twice with sterile PBS to remove excess cFDA-SE molecules and resuspended in 1.0 mL of sterile PBS. Various concentrations of **compound 1** (4.0  $\mu$ M, 8.0  $\mu$ M, 12  $\mu$ M and 16  $\mu$ M ) was then added to separate sets of cFDA-SE labelled *S. aureus* MTCC 96 and *E. coli* MTCC 433 cells and incubated at 37°C and 180 rpm. As a control sample, only DMSO solution devoid of **compound 1** was also added to cFDA-SE labelled cells and incubated under the same conditions. At intermittent periods of incubation (1h, 2h and 3h), cells were harvested by centrifugation and leakage of carboxyfluorescein from the cells was determined by measuring fluorescence of the cell free supernatant at an excitation wavelength of 488 nm and emission wavelength of 518 nm in a spectrofluorimeter (FluoroMax-3, HORIBA). The fluorescence measurements were recorded after subtracting the fluorescence of effluxed dye from control samples. Fluorescence measurements were recorded from three independent experimental samples for every amphiphile concentration and control sample.

### 8.3. Membrane Depolarization Assay

*S. aureus* MTCC 96 and *E. coli* MTCC 433 cells were grown till mid-logarithmic phase ( $A_{600} = 0.4-0.5$ ), harvested by centrifugation, washed and resuspended in HEPES buffer (5.0 mM HEPES, 20 mM glucose, pH 7.2). The cell suspensions ( $A_{600}$  of 0.05) were then incubated with 0.4  $\mu\text{M}$  DiSC<sub>3</sub>5 for 1 h at 37°C followed by the addition of KCl (final concentration of 100 mM) and further incubated for 15 min.<sup>[2]</sup> The cell suspension was then placed in a cuvette to which varying concentrations of **compound 1** (4.0  $\mu\text{M}$ , 8.0  $\mu\text{M}$ , 12  $\mu\text{M}$  and 16  $\mu\text{M}$ ) was added and the fluorescence emission spectra ( $\lambda_{\text{Ex}} = 622$  nm and  $\lambda_{\text{Em}} = 670$  nm) was recorded in short time intervals in a spectrofluorimeter (FluoroMax-3, HORIBA) with excitation and emission slit width set at 10 nm each. In a parallel set, cells treated with valinomycin (30  $\mu\text{M}$ ) were used as positive control. All fluorescence measurements were taken for three independent experimental samples.

### 8.4. PI Uptake Assay

A stock solution of PI (1.5 mM) was prepared in sterile MilliQ water and stored at 4°C. Overnight grown cells of *S. aureus* MTCC 96 and *E. coli* MTCC 433 were harvested by centrifugation, washed twice with sterile PBS and resuspended in the same to achieve a cell concentration of  $10^6$  CFU/mL. In separate sets, the cells were then incubated with varying concentrations of **compound 1** (4.0  $\mu\text{M}$ , 8.0  $\mu\text{M}$ , 12  $\mu\text{M}$  and 16  $\mu\text{M}$ ) at 37°C and 180 rpm for 1h, 2h and 3h. In case of control sample, only DMSO was added to the cells and incubated under the same conditions. Following incubation, cells were washed with sterile PBS, resuspended in the same and incubated with PI (final concentration of 30  $\mu\text{M}$ ) for 30 min at 37°C in a circulating water bath incubator (Amersham, USA). Subsequently, the cells were centrifuged, washed with sterile PBS to remove excess dye and resuspended in the same. The fluorescence emission spectra of the samples was then measured in a spectrofluorimeter (FluoroMax-3, HORIBA) at an excitation wavelength of 535 nm and emission wavelength of 617 nm. Fluorescence measurements were acquired from three independent experimental samples.

## 9.0 DNA Binding Studies with Amphiphiles

### 9.1. UV-visible Spectroscopy

A stock solution of CT-DNA was prepared in sterile nuclease-free MilliQ water and the molar extinction coefficient value of  $6600 \text{ M}^{-1} \text{ cm}^{-1}$  at 260 nm was used to determine the molarity of DNA.<sup>[3]</sup> In separate sets of experiments, varying concentrations of CT-DNA



(0.06  $\mu\text{M}$ -0.6  $\mu\text{M}$ ) was added drop-wise to either **compound 1** or **compound 2** (5.0  $\mu\text{M}$  each), mixed gently and then the absorbance of the solution was recorded in a spectrophotometer (CARY 300 Bio, Varian) in scanning mode from 235 nm to 600 nm. Control samples consisted of only DNA solution or amphiphiles. Absorbance measurements were acquired from three independent experimental samples. The binding constants ( $K_b$ ) for **compound 1** and **compound 2** were determined by monitoring the change in absorbance at 260 nm with increasing concentration of DNA and calculating the ratio of the slope to the y intercept in the plots of  $[\text{DNA}]/(\epsilon_a - \epsilon_f)$  versus  $[\text{DNA}]$ . [4]

### 9.2. Fluorescence Spectroscopy

For the fluorescence emission spectroscopic measurements, varying concentrations of CT-DNA (15 nM-75 nM) prepared in sterile nuclease-free MilliQ water was added drop-wise to separate sets of **compound 1** and **compound 2** (150 nM each). The contents of the tubes were gently mixed and then the fluorescence emission spectra of the samples were recorded in a spectrofluorometer (FluoroMax-3, HORIBA) in a scanning mode from 360 nm to 600 nm by setting the excitation wavelength at 340 nm. The scan rate was set at 1s/nm and the excitation and emission slit width were kept as 1nm and 5 nm, respectively. Control samples consisting of only DNA solution or amphiphiles were also included in the experiments. Fluorescence measurements were obtained from three independent experimental samples.

### 9.3. Ethidium Bromide (EtBr) Displacement Assay

Several sets of CT-DNA solution (0.75  $\mu\text{M}$ ) was incubated with 0.15  $\mu\text{M}$  EtBr solution for 30 min in an amber color microcentrifuge tube. The fluorescence emission spectra of the resulting solution was measured in a spectrofluorometer (FluoroMax-3, HORIBA) in scanning mode from 530 nm to 720 nm (scan rate of 1s/nm, slit width 1/5) by exciting at 515 nm. Subsequently, varying concentrations of **compound 1** or **compound 2** (0.3  $\mu\text{M}$ -4.5  $\mu\text{M}$  of each amphiphile) were gradually dispensed into the tubes, incubated for 5 min at room temperature and then the fluorescence emission spectra of the samples were again recorded in scanning mode from 530 nm to 720 nm by setting the excitation wavelength at 515 nm in a spectrofluorometer. Emission spectra were also recorded for DNA-bound EtBr alone in the absence of the amphiphiles. Fluorescence measurements were obtained from three independent experimental samples. The quenching constant ( $K_q$ ) for **compound 1** and **compound 2** was determined from a standard Stern-Volmer plot. [4]

## 10.0 Plasmid DNA Cleavage Studies

*E. coli* DH5 $\alpha$  cells harbouring pUC18 plasmid DNA were grown overnight in Luria-Bertani (LB) broth in presence of 100  $\mu$ g/mL ampicillin and pUC18 plasmid DNA was then isolated from the cells using a plasmid DNA isolation kit (Sigma-Aldrich, USA) following the manufacturer instructions. The concentration of plasmid DNA was determined by measuring the absorbance at 260 nm. To determine DNA cleavage activity of the amphiphiles, purified pUC18 plasmid DNA (60  $\mu$ M) was taken in sterile nuclease-free MilliQ water and incubated with varying concentrations of **compound 1** or **compound 2** in separate sets (6.0  $\mu$ M, 12  $\mu$ M, 18  $\mu$ M, 24  $\mu$ M and 30  $\mu$ M of each amphiphile) for 1h at 37°C. Cleavage reactions were also performed in the presence of NaN<sub>3</sub> and DMSO as described in an earlier study. [5] Following incubation, the cleavage reactions were terminated by adding loading dye (0.05% bromophenol blue, 50% glycerol and 2 mM EDTA) to the reaction mixture. The samples were then analyzed by agarose gel (0.8%) electrophoresis followed by staining the gel with EtBr solution and the DNA bands were visualized in a gel documentation system (Gel Doc XR + System, Bio-Rad). Quantification of band intensity was accomplished by ImageJ analysis (<http://rsb.info.nih.gov/ij/>).

## 11.0 Plasmid DNA Cleavage and Ligation Studies

In separate sets of experiments, cesium chloride purified pUC18 plasmid DNA (1.0  $\mu$ g) was incubated with either *Eco*RI enzyme (Fermentas, Lithuania) or **compound 1** (30  $\mu$ M) at 37°C for 1h. A negative control sample was also included, which consisted of pUC18 plasmid DNA alone. The reaction mixtures were then purified using a PCR purification kit (Sigma-Aldrich, USA). Cleavage of pUC18 plasmid DNA by *Eco*RI enzyme and **compound 1** was verified by agarose gel electrophoresis of the samples. Subsequently, ligation reactions were set up with **compound 1**-treated plasmid DNA, *Eco*RI-treated plasmid DNA and untreated plasmid DNA (50 ng of plasmid DNA in each) using T4 DNA ligase (New England Biolabs) at 16°C for 16h. Negative control experiments of the same samples in the absence of T4 DNA ligase enzyme were also set up in parallel. The ligation reaction mixtures were used to transform *E. coli* DH5 $\alpha$  cells following a previously described protocol. [6] The transformed colonies were enumerated to measure transformation efficiency.

## 12.0 Uptake and Binding of Compound 1 with Bacterial Cellular DNA

Overnight grown cells of *E. coli* MTCC 433 were harvested by centrifugation, washed twice with sterile PBS and resuspended in the same to achieve a cell concentration of  $10^6$  CFU/mL. In separate sets, the cells were then incubated with 15  $\mu$ M **compound 1** or 0.5  $\mu$ g/mL polymyxin B for 3h to induce membrane damage. The cells were then washed twice with sterile PBS (pH 7.4), resuspended in 1.0 mL of the same and stained with PI following the method described earlier in section 8.4. To ascertain membrane damage by either **compound 1** or polymyxin B treatment and subsequent uptake and intercalation of PI with bacterial cellular DNA, the fluorescence emission spectra of the cell suspensions were recorded in a spectrofluorometer (FluoroMax-3, HORIBA) at an excitation wavelength of 535 nm and emission wavelength of 617 nm. Fluorescence measurements were acquired from three independent experimental samples. Subsequently, varying concentrations of **compound 1** (15  $\mu$ M, 30  $\mu$ M and 45  $\mu$ M) was further added separately to either **compound 1**- or polymyxin B-treated and PI stained cell suspensions and the change in the fluorescence emission intensity was again measured at intervals of 2 min over a period of 20 min, at an excitation wavelength of 535 nm and emission wavelength of 617 nm.

## 13.0 Cleavage of Intracellular Plasmid DNA by Compound 1

Overnight grown cells of *E. coli* DH5 $\alpha$  harbouring pUC18 plasmid DNA were harvested by centrifugation, washed twice with sterile PBS and resuspended in the same. In two separate sets, either polymyxin B (0.5  $\mu$ g/mL) or **compound 1** (45  $\mu$ M) was added to the cell suspensions of *E. coli* DH5 $\alpha$ . At two different time periods of 3h and 6h, the cells were centrifuged, washed twice with sterile PBS and then pUC18 plasmid DNA was isolated from the cells using the plasmid DNA isolation kit (Sigma-Aldrich, USA). A control sample consisting of *E. coli* DH5 $\alpha$  cells alone was also subjected to the same experimental conditions. pUC18 plasmid DNA isolated from all the samples were analyzed by agarose gel electrophoresis.

## 14.0 Cytotoxicity Assay for Compound 1

The *in vitro* cytotoxic potential of **compound 1** was tested on human cervical carcinoma (HeLa) cell lines, human colon adenocarcinoma (HT-29) cell lines and human breast cancer (MCF-7) cell lines by standard MTT assay according to the manufacturer instruction (Sigma-Aldrich, MO, USA). The cell lines were initially propagated in Dulbecco's Modified Eagle

Medium (DMEM) supplemented with 10% fetal calf serum (FCS), penicillin (100 µg/mL) and streptomycin (100 µg/mL) at 37°C in a CO<sub>2</sub> incubator under a humidified atmosphere of 5% CO<sub>2</sub>. The cells were then seeded in 96 well plates (10<sup>4</sup> cells/well) and varying concentrations of **compound 1** (6.0 µM, 12 µM, 24 µM) made in DMEM were added to the cells and incubated for 24 hours under 5% CO<sub>2</sub> at 37°C. Control samples (cells treated with DMSO alone) were also included in parallel sets and incubated under the same conditions. Following incubation, the media was gently aspirated and fresh DMEM containing MTT solution was added to the wells and further incubated for 4h at 37°C. The supernatant was then removed and the insoluble formazan product was solubilized in DMSO and its absorbance was measured in a microtitre plate reader (Infinite M200, TECAN, Switzerland) at 550 nm. MTT assay was performed in six sets for each sample. Data analysis and determination of standard deviation was performed with Microsoft Excel 2010 (Microsoft Corporation, USA). The absorbance obtained for solvent control samples (cells treated with DMSO alone) was considered to represent 100% cell viability, whereas the absorbance for **compound 1**-treated cells were compared to the solvent control cells to determine % cell viability.

Fluorescence microscope analysis was also pursued to ascertain the cytotoxic effect of **compound 1**. HeLa cells were seeded onto 96 well tissue culture plates (10<sup>4</sup> cells/well) and grown as mentioned before. The cells were then incubated with 24 µM of **compound 1** (2 x MIC against *S. aureus* MTCC 96) made in DMEM, for 24h under 5% CO<sub>2</sub>. Solvent control samples (cells treated with DMSO alone) were also incubated in parallel wells. In a separate set, cells were fixed with 4% paraformaldehyde for 10 min at room temperature followed by treatment with 0.1% Triton X-100 for 10 mins for complete permeabilization of cells. Cells belonging to all the experimental samples (solvent control cells, amphiphile-treated cells, and Triton X-100-treated cells) were washed with sterile PBS and separate sets of each sample were labelled with 50 µM cFDA-SE and 30 µM PI each for 15 min, respectively. The cells were washed thoroughly with sterile PBS and images of stained cells was recorded using a fluorescence microscope (Eclipse Ti-U, Nikon, USA) with a filter that allowed blue light excitation at 445-495 nm for cFDA-SE and green light excitation at 495-570 nm in case of PI stained cells.

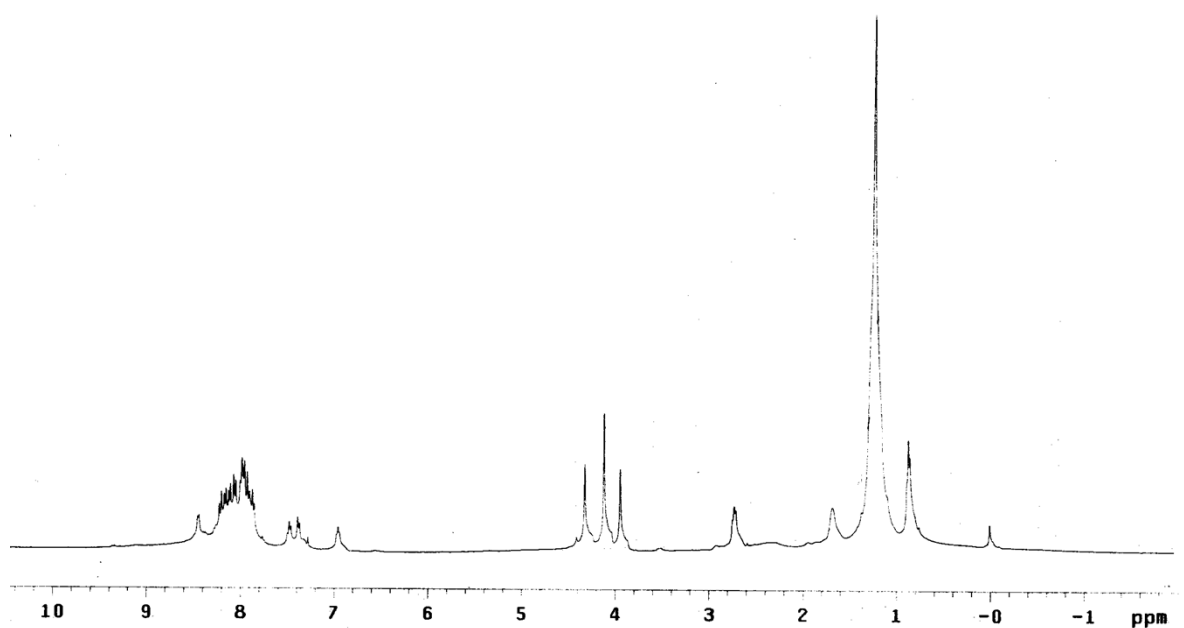
## 15.0 Reference

1. C. Kar, M. D. Adhikari, A. Ramesh, G. Das, *RSC Adv.* **2012**, 2, 9201-9206.
2. S. K. Vooturi, C. M. Cheung, M. J. Rybak, S. M. Firestine, *J. Med. Chem.* **2009**, 52, 5020-5031.
3. D.S. Raja, N. S. P. Bhuvanesh, K. Natarajan, *Inorg. Chem.* **2011**, 50, 12852-12866.
4. A. Basu, D. Thiagarajan, C. Kar, A. Ramesh, G. Das, *RSC Adv.* **2013**, 3, 14088-14098.
5. P. U. Maheswari, K. Lappalainen, M. Sfregola, S. Barends, P. Gamez, U. Turpeinen, I. Mutikainen, G. P. van Wezela, J. Reedijk, *Dalton Trans.* **2007**, 3676-3683.
6. C. T. Chung, S. L. Niemela, R. H. Miller, *Proc. Natl. Acad. Sci.* **1989**, 86, 2172-2175.

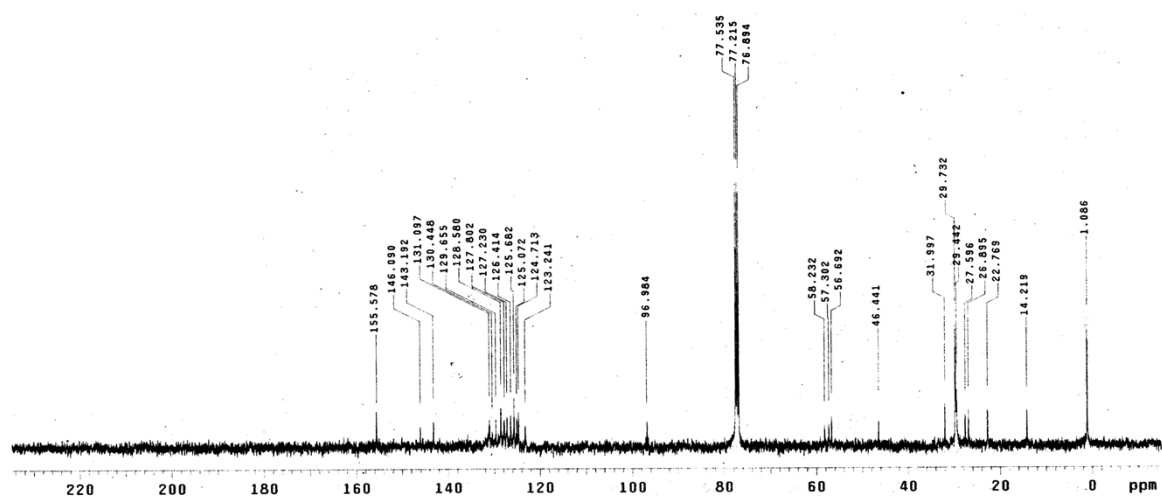
## RESULTS

### Characterization of Compound 1:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz): 0.87 ppm (t, 3H); 1.25 ppm (m, 18H); 1.69 ppm (broad multiplet, 2H); 2.73 ppm (t, 2H); 3.95 ppm (s, 2H); 4.12 ppm (s, 3H); 4.33 ppm (s, 2H); 6.96 ppm (t, 1H); 7.38 ppm (t, 1H); 7.47 ppm (t, 1H); 7.86-8.27 ppm (m, 9H); 8.45 ppm (d, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz): 155.58 ppm, 146.09 ppm, 143.19 ppm, 131.10 ppm, 130.45 ppm, 129.66 ppm, 128.58 ppm, 127.80 ppm, 127.23 ppm, 126.41 ppm, 125.68 ppm, 125.07 ppm, 124.71 ppm, 123.24 ppm, 96.98 ppm, 58.23 ppm, 57.30 ppm, 56.69 ppm, 46.44 ppm, 31.99 ppm, 29.73 ppm, 29.44 ppm, 27.60 ppm, 26.90 ppm, 22.77 ppm, 14.22 ppm, 1.086 ppm; MS (positive mode,  $m/z$ ): Calcd. for **Compound 1**: 505.357. Found 505.357.



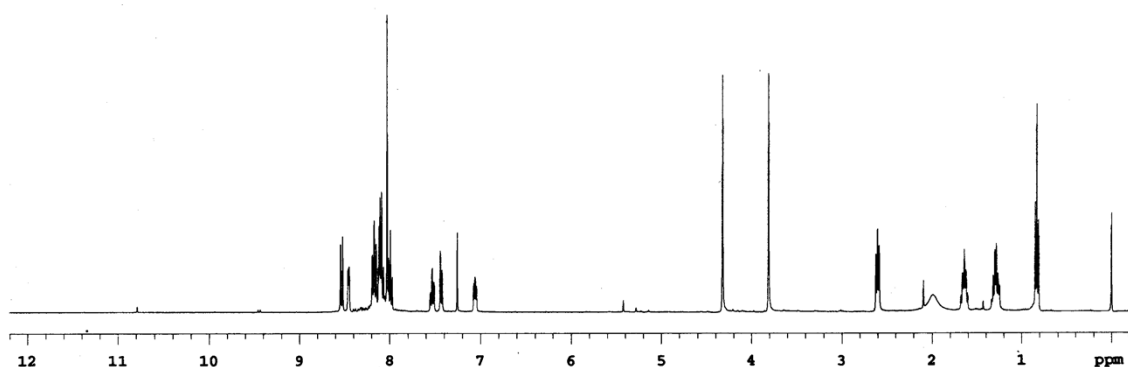
**Figure S2.**  $^1\text{H}$  NMR spectra of **compound 1** in  $\text{CDCl}_3$  solution.



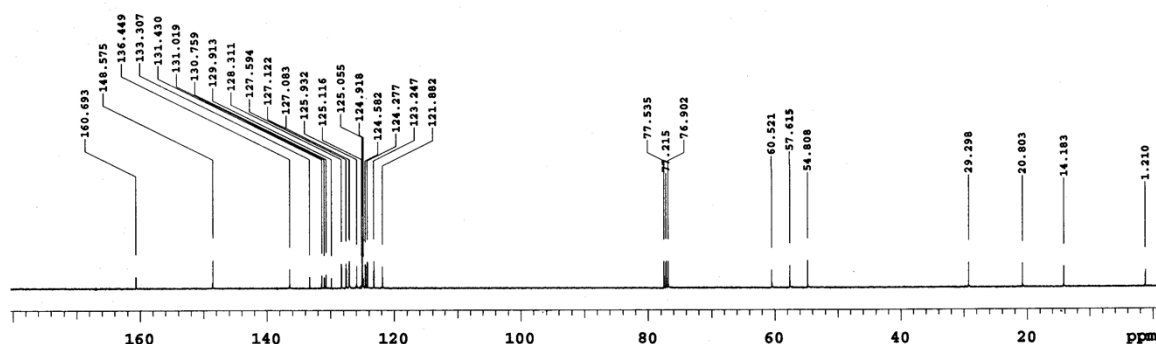
**Figure S3.**  $^{13}\text{C}$  NMR spectra of **compound 1** in  $\text{CDCl}_3$  solution.

#### Characterization of Compound 2:

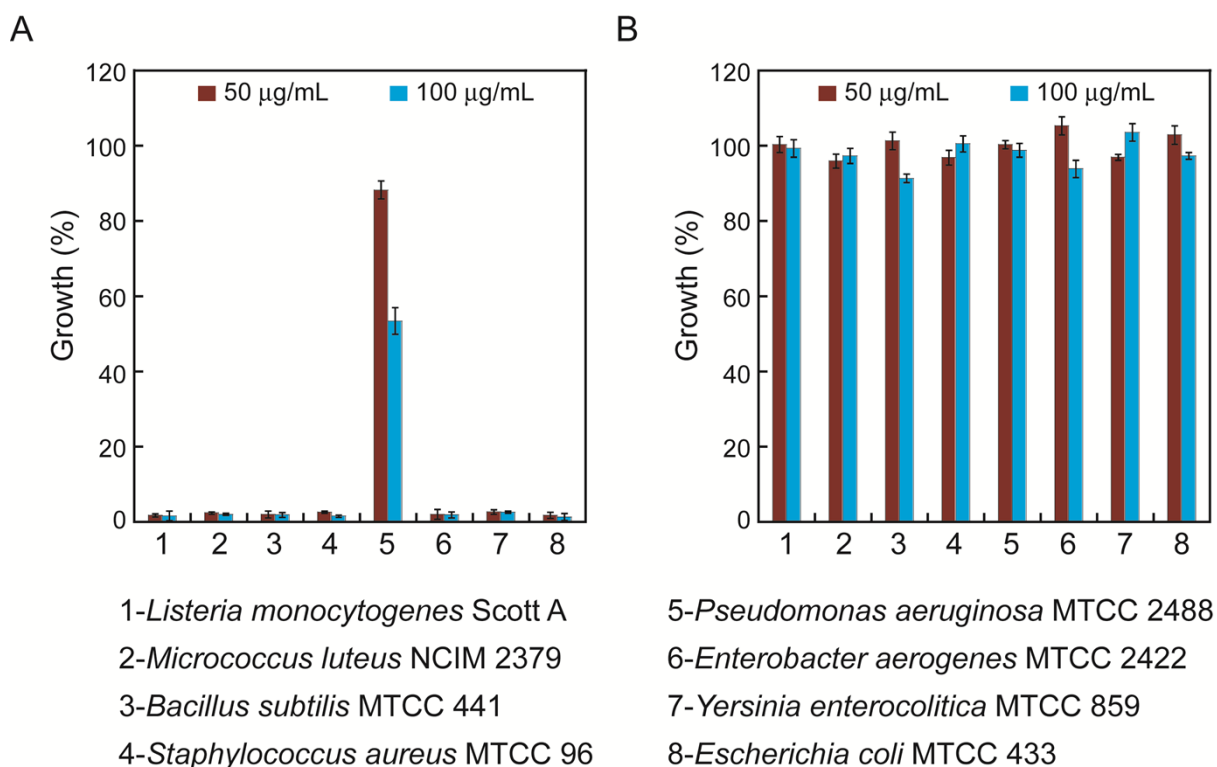
$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz): 0.83 ppm (t, 3H); 1.28 ppm (m, 2H); 1.64 ppm (m, 2H); 2.61 ppm (t, 2H); 3.81 ppm (s, 2H); 4.32 ppm (s, 2H); 7.06 ppm (t, 1H); 7.43 ppm (d, 1H); 7.53 ppm (t, 1H); 7.90-8.19 ppm (m, 8H); 8.45 ppm (d, 1H); 8.53 ppm (d, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz): 160.69 ppm, 148.57 ppm, 136.45 ppm, 133.31 ppm, 131.43 ppm, 131.02 ppm, 130.76 ppm, 129.91 ppm, 128.32 ppm, 127.59 ppm, 127.12 ppm, 127.08 ppm, 125.93 ppm, 125.12 ppm, 125.06 ppm, 124.92 ppm, 124.58 ppm, 124.28 ppm, 123.25 ppm, 121.88 ppm, 60.52 ppm, 57.62 ppm, 54.81 ppm, 29.30 ppm, 20.80 ppm, 14.18 ppm, 1.21 ppm, MS (positive mode,  $m/z$ ): Calcd. for **Compound 2+H**: 379.217. Found 378.218.



**Figure S4.**  $^1\text{H}$  NMR spectra of **compound 2** in  $\text{CDCl}_3$  solution.

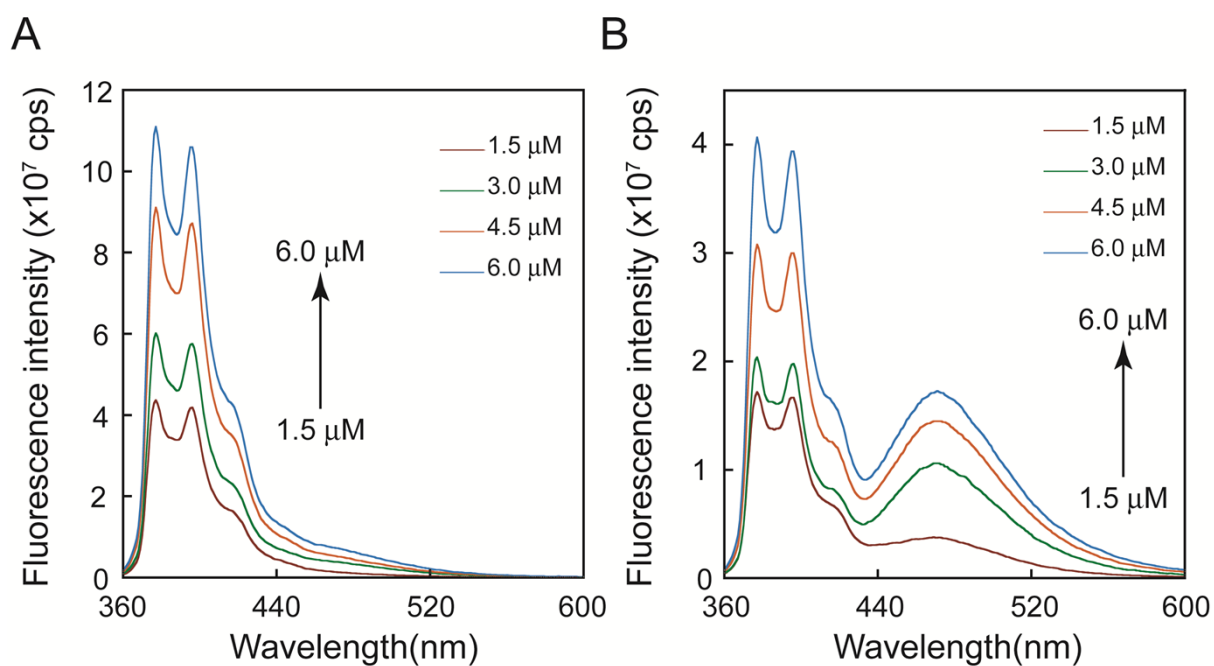


**Figure S5.**  $^{13}\text{C}$  NMR spectra of **compound 2** in  $\text{CDCl}_3$  solution.

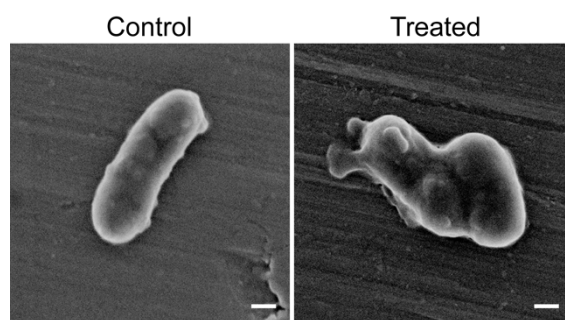


**Figure S6.** Antibacterial activity of (A) **compound 1** and (B) **compound 2** against pathogenic bacterial strains. The target pathogens were interacted with 50  $\mu\text{g/mL}$  and 100  $\mu\text{g/mL}$  amphiphile for 6 h.

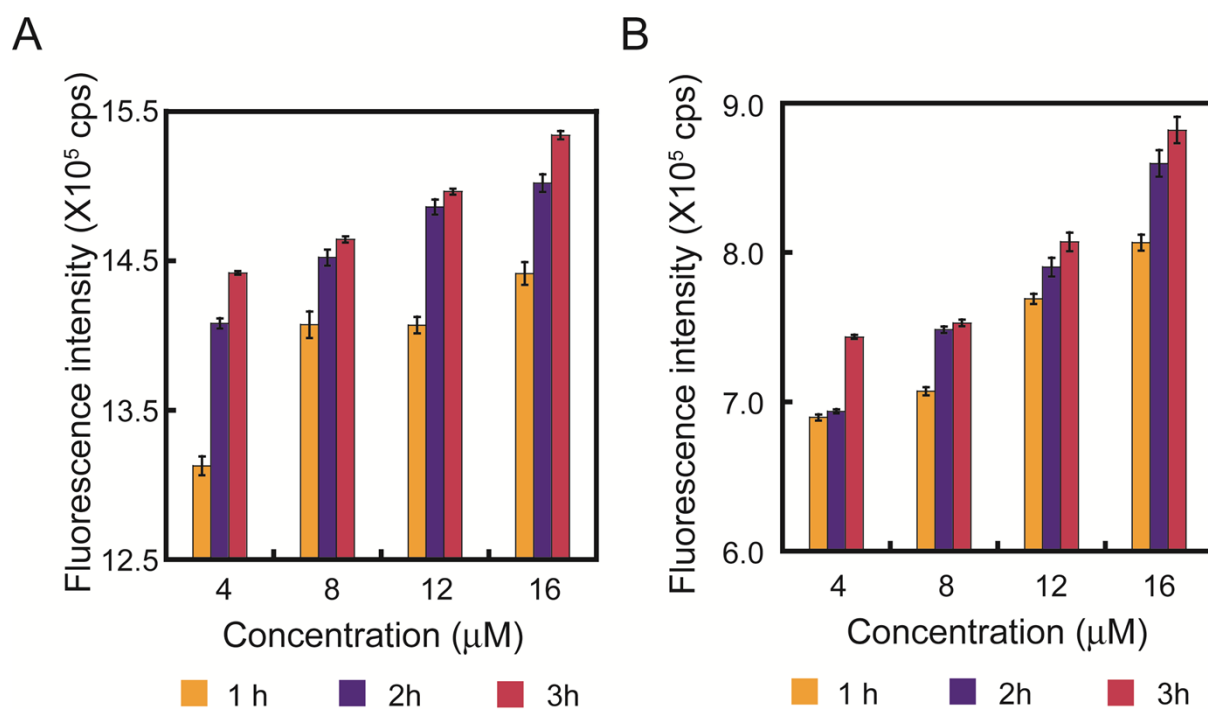




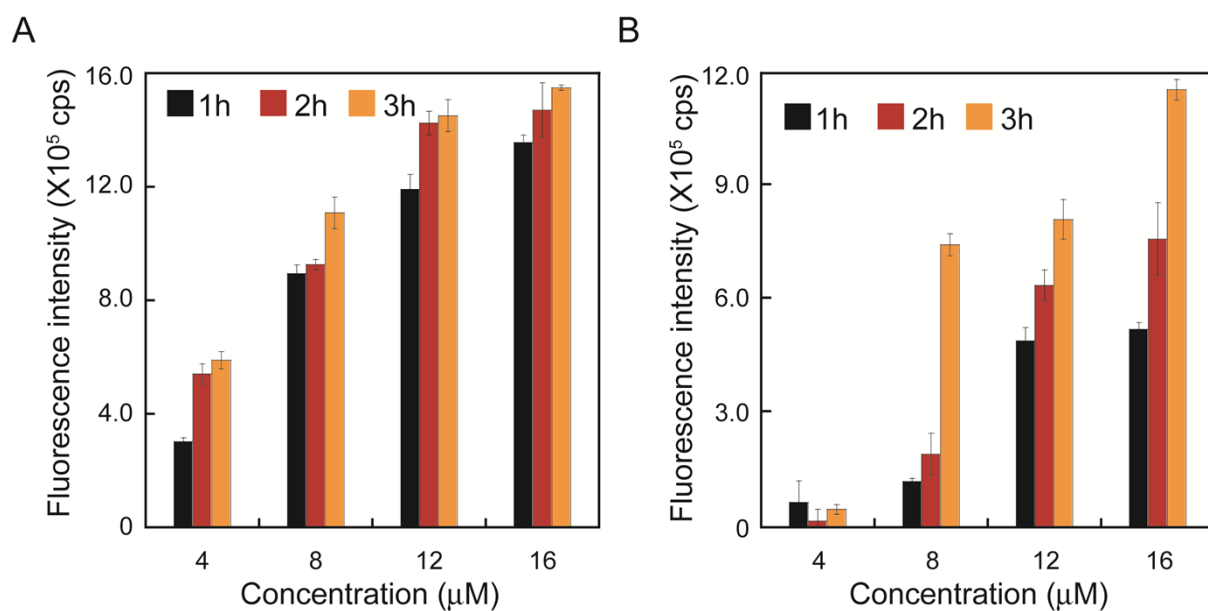
**Figure S7.** Fluorescence emission spectra of (A) **compound 1** and (B) *S. aureus* MTCC 96 bound-**compound 1**.



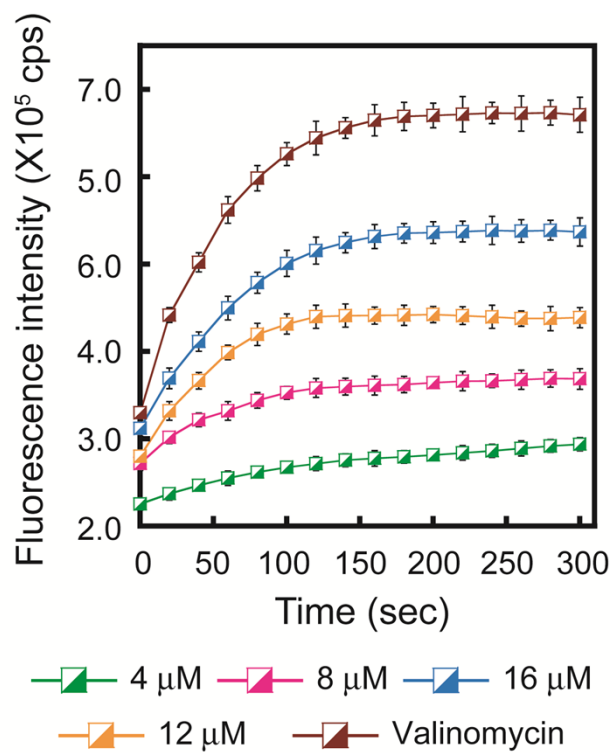
**Figure S8.** Field emission scanning electron microscope (FESEM) image of *E. coli* MTCC 433 treated with 40  $\mu\text{M}$  **compound 1**. Scale bar for the images is 300 nm.



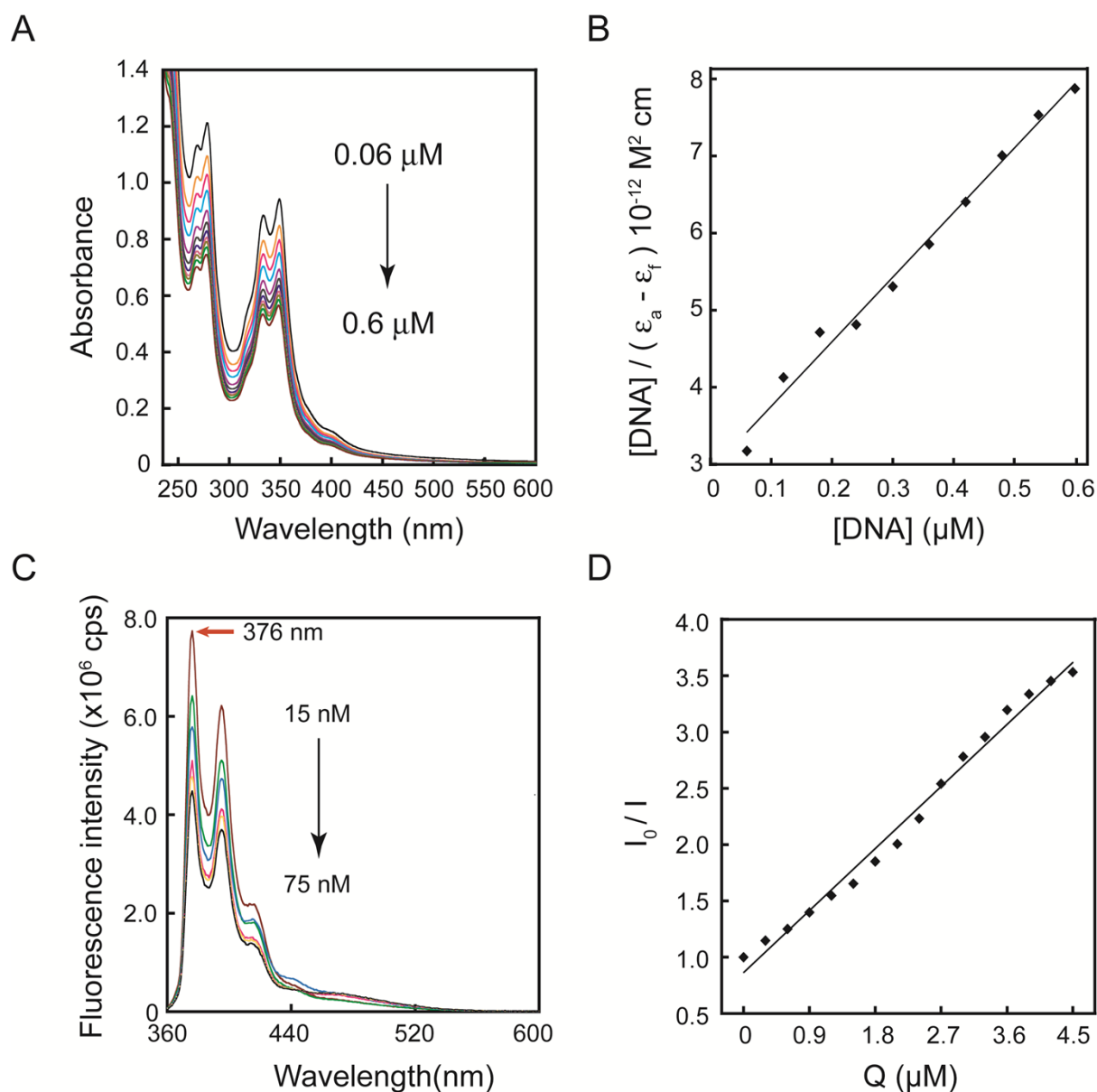
**Figure S9.** cFDA-SE leakage assay following interaction of **compound 1** with (A) *S. aureus* MTCC 96 and (B) *E. coli* MTCC 433.



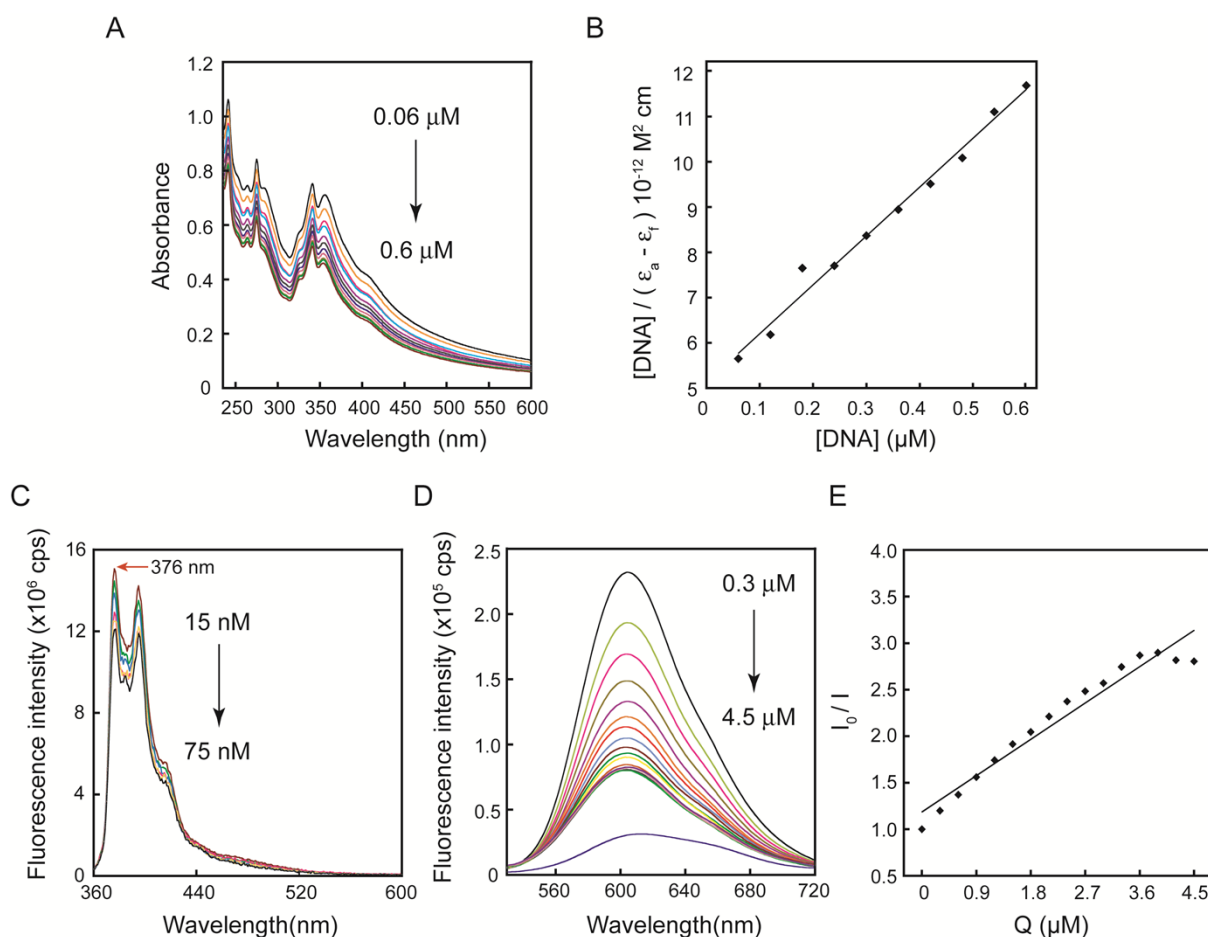
**Figure S10.** Propidium iodide uptake assay in (A) *S. aureus* MTCC 96 and (B) *E. coli* MTCC 433 treated with **compound 1**.



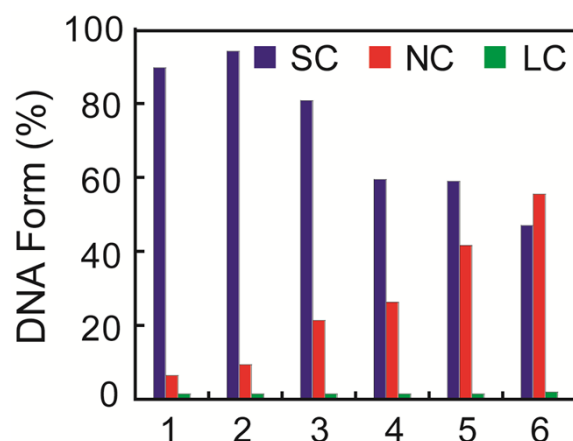
**Figure S11.** DiSC<sub>3</sub>5-based membrane depolarization assay following interaction of **compound 1** with *E. coli* MTCC 433.



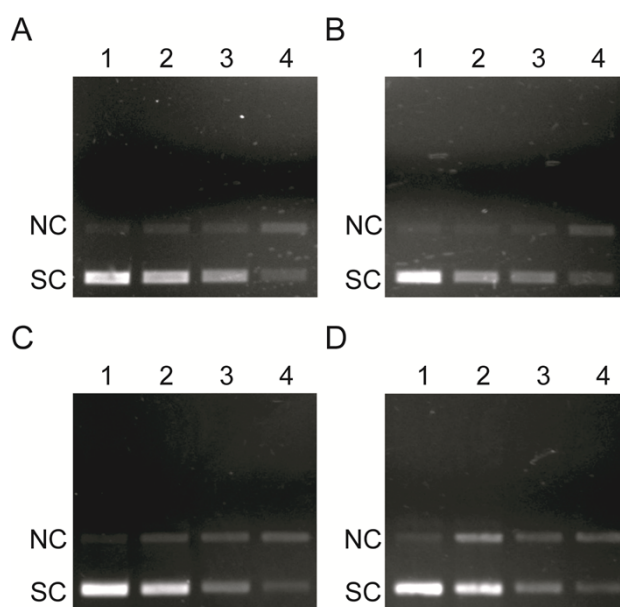
**Figure S12.** (A) UV-visible absorbance spectroscopy of **compound 1** (5.0  $\mu\text{M}$ ) upon addition of calf thymus DNA (0.06  $\mu\text{M}$  - 0.6  $\mu\text{M}$ ). (B) Binding isotherm of **compound 1** with calf thymus DNA determined by UV-visible absorbance titration spectroscopy. (C) Fluorescence emission spectroscopy of **compound 1** (150 nM) upon interaction with calf thymus DNA (15 nM - 75 nM). (D) Stern-Volmer plot for ethidium bromide displacement assay with **compound 1**.



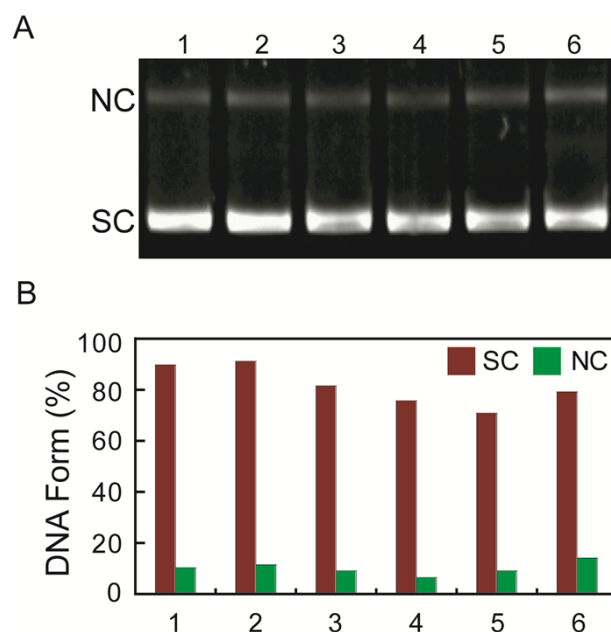
**Figure S13.** (A) UV-visible absorbance spectroscopy of **compound 2** (5.0  $\mu\text{M}$ ) upon addition of calf thymus DNA (0.06  $\mu\text{M}$  - 0.6  $\mu\text{M}$ ). (B) Binding isotherm of **compound 2** with calf thymus DNA determined by UV-visible absorbance titration spectroscopy. (C) Fluorescence emission spectroscopy of **compound 2** (150 nM) upon interaction with calf thymus DNA (15 nM - 75 nM). (D) Ethidium bromide displacement assay with **compound 2** (0.3  $\mu\text{M}$  - 4.5  $\mu\text{M}$ ). (E) Stern-Volmer plot for ethidium bromide displacement assay with **compound 2**.



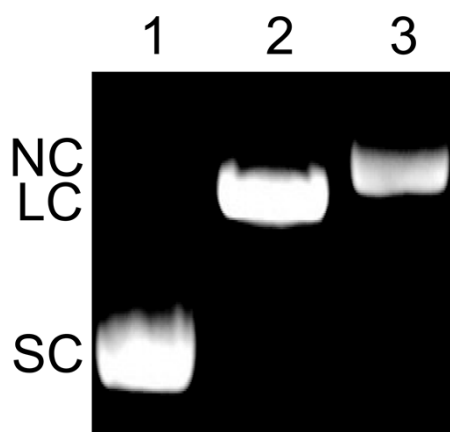
**Figure S14.** Quantification of band intensity of the topological forms of pUC18 plasmid DNA using ImageJ analysis software. 1: untreated plasmid DNA; 2-6: plasmid DNA treated with 6.0  $\mu\text{M}$ , 12  $\mu\text{M}$ , 18  $\mu\text{M}$ , 24  $\mu\text{M}$  and 30  $\mu\text{M}$  of **compound 1**, respectively. SC: supercoiled DNA, LC: linearised circular DNA, NC: nicked circular DNA.



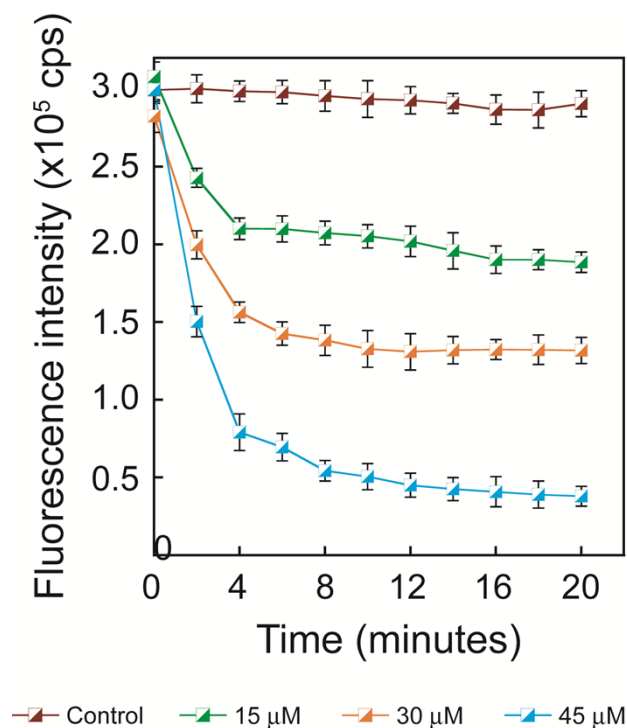
**Figure S15.** Agarose gel electrophoresis of pUC18 plasmid DNA treated with **compound 1** in (A) absence of  $\text{NaN}_3$ , (B) presence of  $\text{NaN}_3$ , (C) absence of DMSO and (D) presence of DMSO. Lanes 1: control (untreated plasmid DNA); 2-4: plasmid DNA treated with 6.0  $\mu\text{M}$ , 18  $\mu\text{M}$  and 30  $\mu\text{M}$  of **compound 1**, respectively. SC: supercoiled DNA, NC: nicked circular DNA.



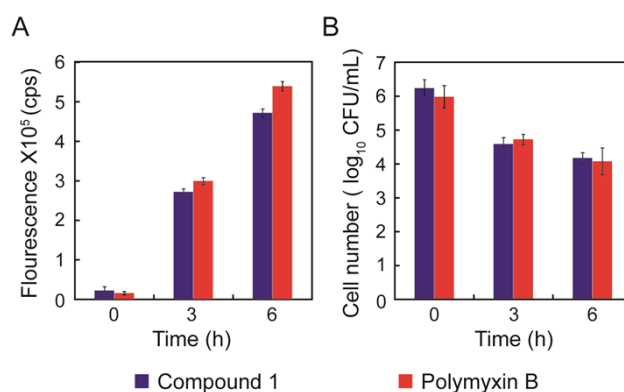
**Figure S16.** (A) Agarose gel electrophoresis of pUC18 plasmid DNA treated with **compound 2**. Lanes 1: control (untreated plasmid DNA); 2-6: plasmid DNA treated with 6.0  $\mu$ M, 12  $\mu$ M, 18  $\mu$ M, 24  $\mu$ M and 30  $\mu$ M of **compound 2**, respectively. (B) Quantification of band intensity of various topological forms of plasmid DNA obtained in (A) using ImageJ analysis software. SC: supercoiled DNA, NC: nicked circular DNA.



**Figure S17.** Agarose gel electrophoresis indicating various forms of pUC18 plasmid DNA used for transformation experiments. Lanes: (1) uncut CsCl-purified pUC18 plasmid DNA, (2) CsCl-purified pUC18 plasmid DNA treated with *Eco*RI, (3) CsCl-purified pUC18 plasmid DNA treated with **compound 1**. SC: supercoiled DNA, LC: linearised circular DNA, NC: nicked circular DNA.

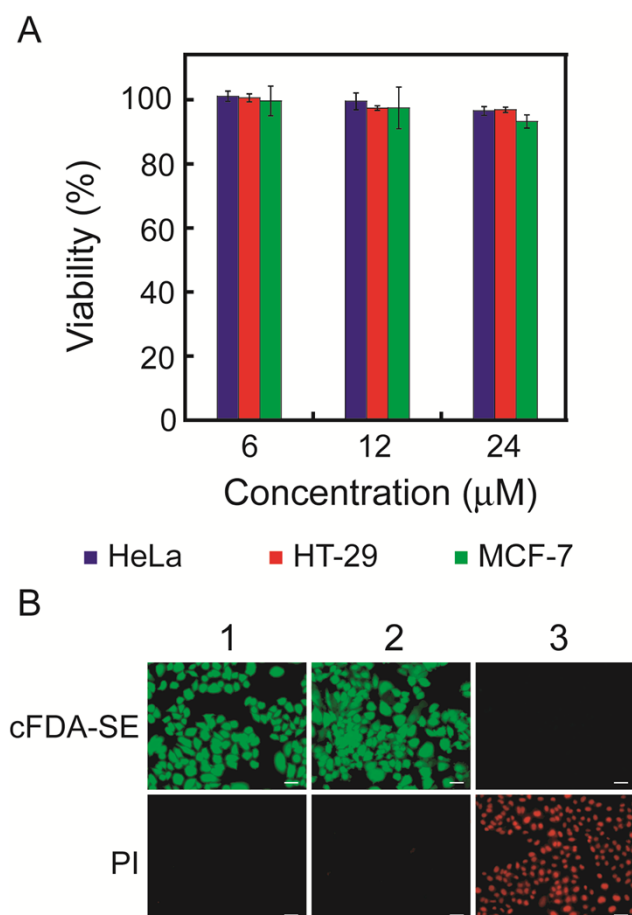


**Figure S18.** Uptake and intracellular DNA binding by **compound 1** in *E.coli* MTCC 433 cells. Cells were initially treated with 0.5  $\mu\text{g/mL}$  of polymyxin B and then stained with propidium iodide (PI). Fluorescence emission intensity of PI was measured following incremental addition of **compound 1** (15  $\mu\text{M}$ , 30  $\mu\text{M}$  and 45  $\mu\text{M}$ ).



**Figure S19.** (A) Fluorescence emission intensity of PI measured to ascertain membrane damage in *E.coli* DH5 $\alpha$  cells treated with either 45  $\mu\text{M}$  **compound 1** or 0.5  $\mu\text{g/mL}$  polymyxin B for 3h and 6h. (B) Loss in cell viability of *E.coli* DH5 $\alpha$  cells treated with either 45  $\mu\text{M}$  **compound 1** or 0.5  $\mu\text{g/mL}$  polymyxin B for 3h and 6h.





**Figure S20.** (A) MTT-based assay to ascertain *in vitro* cytotoxicity of **compound 1** on model human cell lines. (B) Fluorescence microscopic images of (1) untreated HeLa cells, (2) HeLa cells treated with 24 μM **compound 1** and (3) Triton X-100-treated HeLa cells. Scale bar for the images is 50 μm.

**Table S1.** MIC and MKC of **compound 1**

Amphiphile	<i>S. aureus</i> MTCC 96		<i>L. monocytogenes</i> Scott A		<i>E. aerogenes</i> MTCC 2822		<i>E. coli</i> MTCC 433	
	MIC ( $\mu\text{M}$ )	MKC ( $\mu\text{M}$ )	MIC ( $\mu\text{M}$ )	MKC ( $\mu\text{M}$ )	MIC ( $\mu\text{M}$ )	MKC ( $\mu\text{M}$ )	MIC ( $\mu\text{M}$ )	MKC ( $\mu\text{M}$ )
<b>Compound 1</b>	12.0	40.0	12.0	40.0	32.0	48.0	40.0	64.0

**Table S2.** Determination of binding constant ( $K_b$ ) and quenching constant ( $K_q$ ) of **compound 1** and **compound 2** following interaction with calf thymus DNA.

Amphiphile	Binding constant <sup>a</sup> ( $K_b$ )	Quenching constant <sup>b</sup> ( $K_q$ )
<b>Compound 1</b>	$2.87 \times 10^6 \text{ M}^{-1}$	$6.12 \times 10^5 \text{ M}^{-1}$
<b>Compound 2</b>	$2.11 \times 10^6 \text{ M}^{-1}$	$1.3 \times 10^5 \text{ M}^{-1}$

<sup>a</sup> Binding constant ( $K_b$ ) for **compound 1** and **compound 2** were determined from the binding isotherm (ESI†, Fig. S12B, Fig. S13B)

<sup>b</sup> Quenching constant ( $K_q$ ) for **compound 1** and **compound 2** were determined from Stern-Volmer plot (ESI†, Fig. S12D, Fig. S13E)