Supporting Information

Encapsulation of Ru(η^6-*p*-cymene) complex of the antibacterial drug trimethoprim into polydiacetylene-phospholipid assembly to enhance its In vitro anticancer and antibacterial activities

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General Information

Microanalysis (C, H, and N) was carried out with a Vario EL elemental analyzer. UV-Vis spectroscopy was recorded on a Specord 210 UV-Vis spectrophotometer using cuvettes of 1 cm path length. ^{1}H NMR spectra were recorded on a Bruker 500 MHz NMR spectrometer. Mass spectrometry was performed on a Shimadzu LC MS -2020 spectrometer. Emission intensity measurements were carried out using Fluoromax-4 spectrofluorometer. Transmission electron microscope (TEM) images were collected from HR-TEM (JEM-2100 Plus Electron Microscope, Japan). SEM analysis was performed using a high-resolution scanning electron microscope (FE-SEM, JEOL JSM-7100F) operating at 18 kV. Particle size distribution and zeta potential measurements were recorded on nanopartica SZ-100 (Horiba scientific, UK). The DNA cleavage activity was assessed using GELSTAN 1312 gel documentation system.

Since DMSO was used in cellular experiments, the stability of RATMP(C) was checked in DMSO-d^6 solvent using NMR spectroscopy. ^{1}H-NMR (500 MHz, DMSO - d^6): δ 1.201 (d, 6H, CH(CH_3)$_2$), 2.090 (s, 3H, CH_3), 2.861 (m, 1H, CH(CH$_3$)$_2$), 3.526 (s, 2H, CH_2), 3.614 (s, 3H, O-CH_3), 3.722 (s, 6H, O-CH_3), 5.748 (s, 2H, NH_2), 5.785 (d, 2H, η^6-C$_6H_4$), 5.827 (d, 2H, η^6-C$_6H_4$), 6.153 (s, 2H, NH_2), 6.555-7.513 (s, 3H, aromatic) (Figure S8).

DNA Binding and DNA cleavage Studies

Solutions of calf thymus (CT) DNA in 5 mM Tris HCl/50 mM NaCl buffer gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280}, of 1.9, indicating that the DNA was sufficiently free of protein.[1] Concentrated stock solutions of DNA (20.5 mM) were prepared in buffer and sonicated for 25 cycles, where each cycle consisted of 30 s with 1 min intervals. The concentration of DNA in nucleotide phosphate (NP) was determined by UV absorbance at 260 nm after 1:100 dilutions by taking the extinction coefficient, ε_{260nm}, as 6600 M^{-1} cm^{-1}. Stock solutions of DNA were stored at 4 °C and

used after no more than 4 days. Supercoiled plasmid pBR322 DNA was stored at -20 °C.

The DNA binding study was carried out by using the procedure reported already.[2] The concentrated stock solution of complex was prepared with CH_3CN solvent followed by dilution with 5 mM Tris-HCl/50mM NaCl buffer at pH 7.1 to prepare required concentration of solution. Prior to the absorption spectral titrations, the titration experiment was carried out by maintaining a constant concentration of the complex and varying the nucleic acid concentration. The effect of dilution has been taken care of suitably during UV-Vis absorption spectral titration. Prior to the absorption spectral titrations, the DNA solutions were pre-treated with the solutions of complex to ensure that no change in the concentration of the complex occurs during titration. Hence the spectral changes are not occurred by dilution factor. DNA was also added to the reference cuvette to correct for any absorbance or light scattering due to DNA itself. The solutions were thoroughly mixed using a micropipette and allowed to equilibrate for 10 min prior to measurements wherever required. The absorbance of solutions of the complex was recorded after successive addition of CT DNA. The intrinsic binding constant K_b was calculated using the equation,[3]

$$[DNA]/(\varepsilon_a\text{-}\varepsilon_f) = [DNA]/(\varepsilon_b\text{-}\varepsilon_f) + 1/K_b(\varepsilon_b\text{-}\varepsilon_f)$$

where [DNA] is the concentration of DNA in base-pairs, ε_a is the apparent extinction coefficient calculated as $A_{obs}/[complex]$, ε_f corresponds to the extinction coefficient of the complex in its free form and ε_b refers to the extinction coefficient of the complex in the bound form. Each set of data, when fitted into the above equation, gives a straight line with a slope of $1/(\varepsilon_b\text{-}\varepsilon_f)$ and an y-intercept of $1/K_b(\varepsilon_b\text{-}\varepsilon_f)$ and K_b is determined from the ratio of the slope to intercept.

The DNA cleavage study was carried out by using agarose gel electrophoresis technique. The super coiled pBR322 plasmid DNA (40 µM) was treated with desired concentration of complexes in 5 mM Tris-HCl/50 mM NaCl buffer at pH 7.1. The

samples were incubated for 1h at 37 ^{o}C and then a loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol (3 μL) was added and electrophoresis was performed at 60 V for 3 h in Tris-Acetate-EDTA (TAE) buffer (40 mM Tris-base, 20 mM, acetic acid, 1 mM EDTA) using 1% agarose gel containing 1.0 μg/mL ethidium bromide. The gels were viewed in a GELSTAN 1312 gel documentation system and photographed using a CCD camera.

References

1.   J. Marmur, *J. Mol. Biol*. **1961**, *3*, 208.

2.   Ganeshpandian, M.; Loganathan, R.; Suresh, E.; Riyasdeen, A.; Akbarsha, M. A.; Palaniandavar, M. *Dalton Trans.***2014,***43,* 1203-1219.

3.   M. T. Carter, M. Rodriguez and A. J. Bard, *J. Am. Chem. Soc.,* 1989, **111**, 8901-8911.
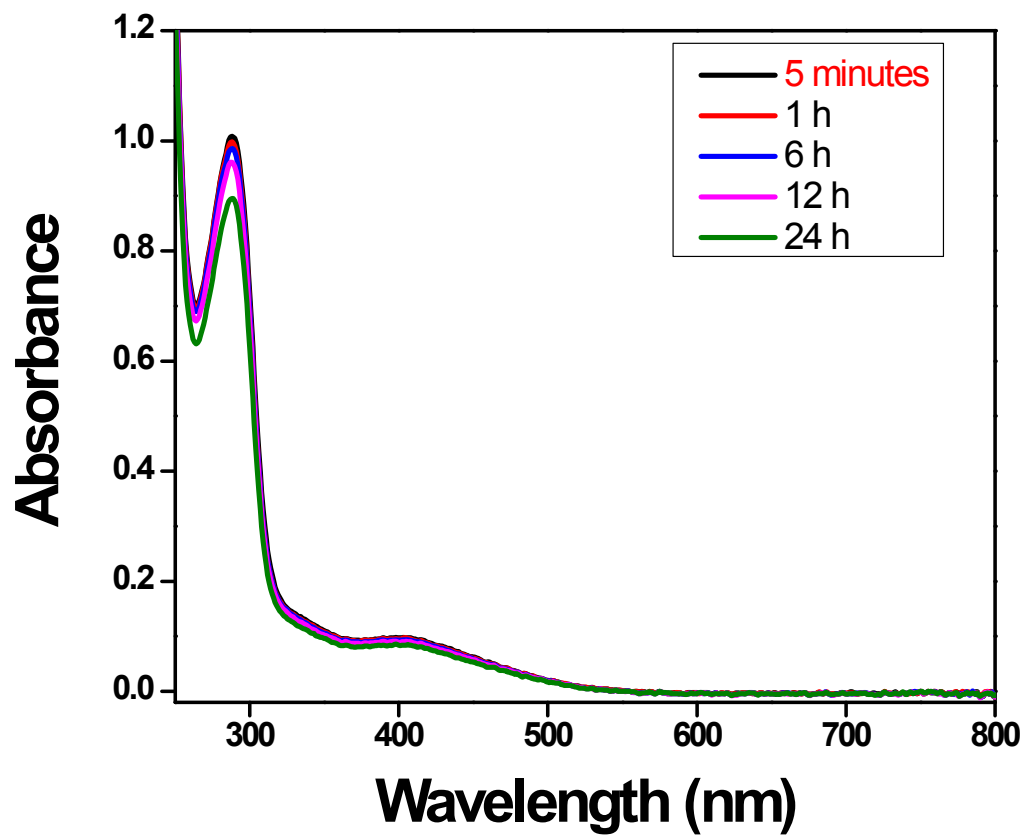
Figure S1. Stability studies of the RATMP(C) in 5% DMSO/10 mM PBS (pH = 7.2) solution at various time intervals up to 24 h.
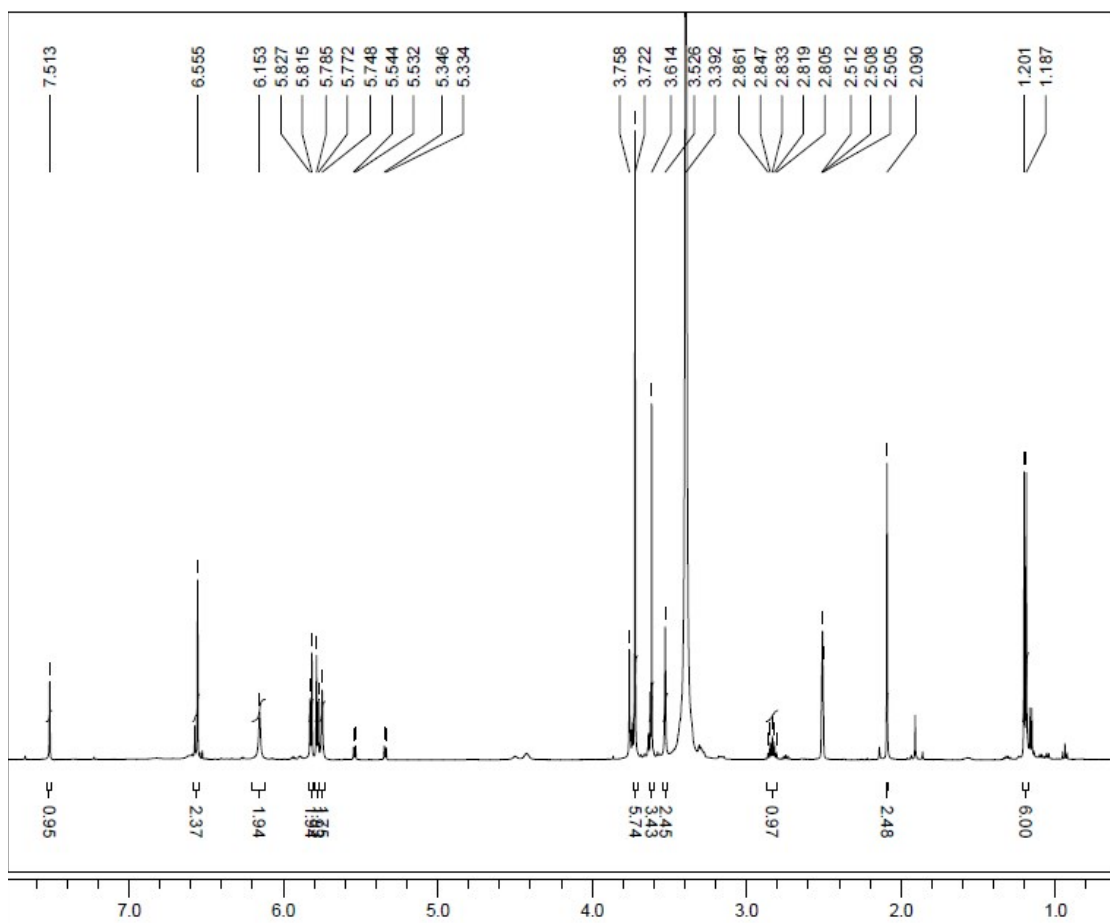
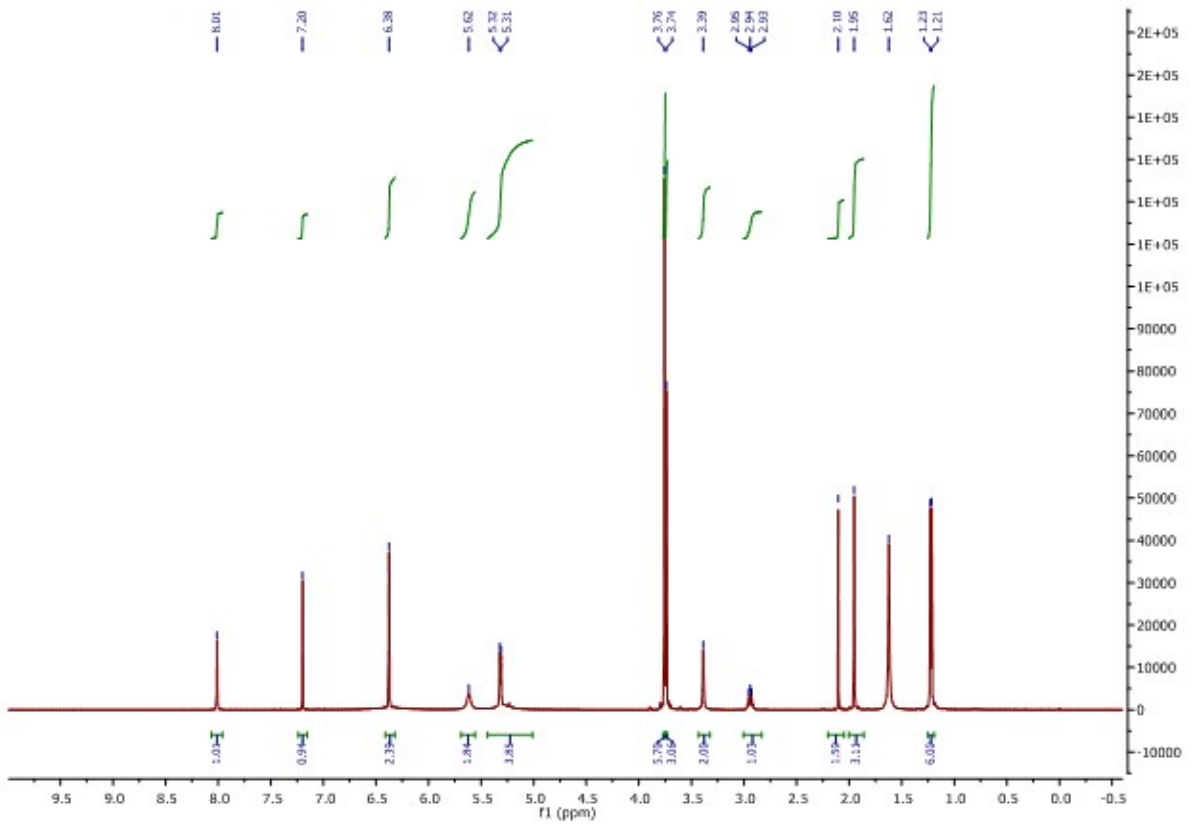**Figure S2**. ^{1}H-NMR spectrum of RATMP(C) in DMSO-d^6.

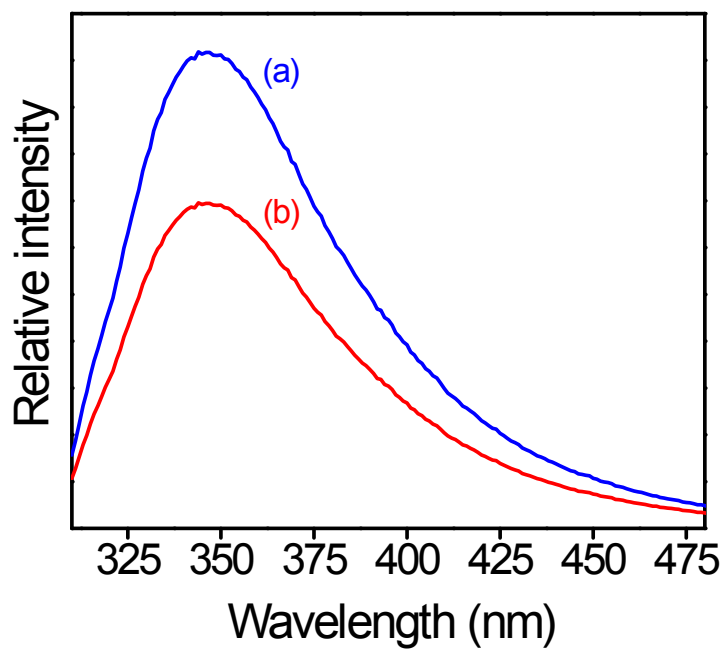**Figure S3**. ^{1}H-NMR spectrum of RATMP(C) in CDCl$_3$.

Figure S4. Steady state fluorescence spectra of RATMP(C) (1×10^{-4} M), in (a) 5% DMSO/PBS (b) in 5% DMSO/10 mM PBS (stock solution) with appropriate dilution by culture media (λ_{ext} =284 nm).

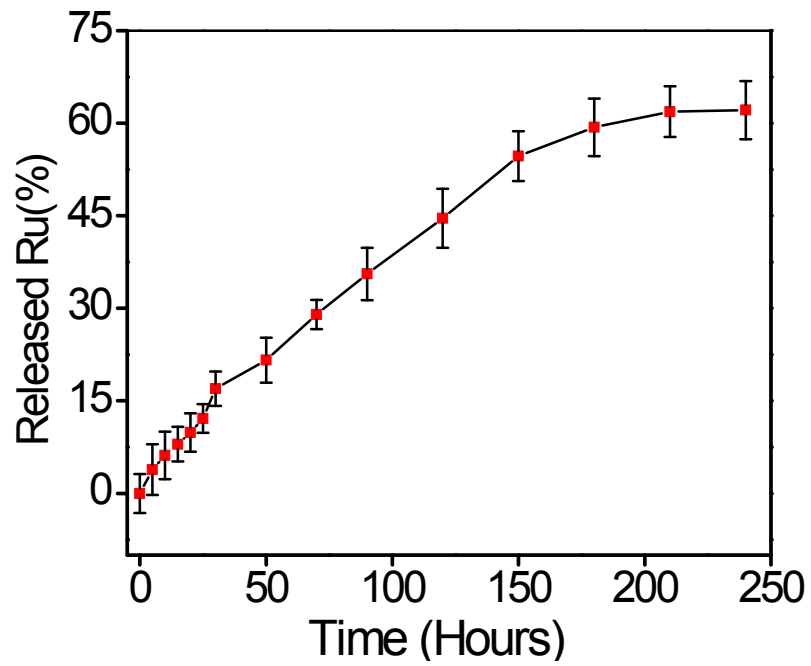**Figure S5**. In vitro release profile of Lip-RATMP(C) in 5% DMSO/10 mM PBS (stock solution) by the measured emission intensity of the cell culture media outside the dialysis bag (λ_{ext} = 284 nm, λ_{mon} = 346 nm).
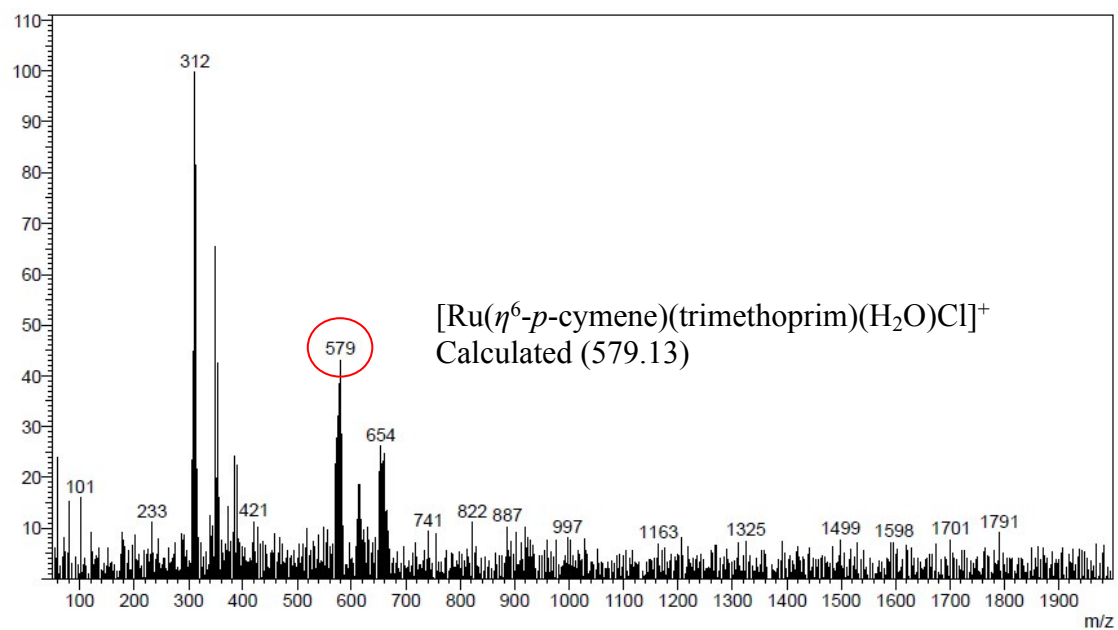
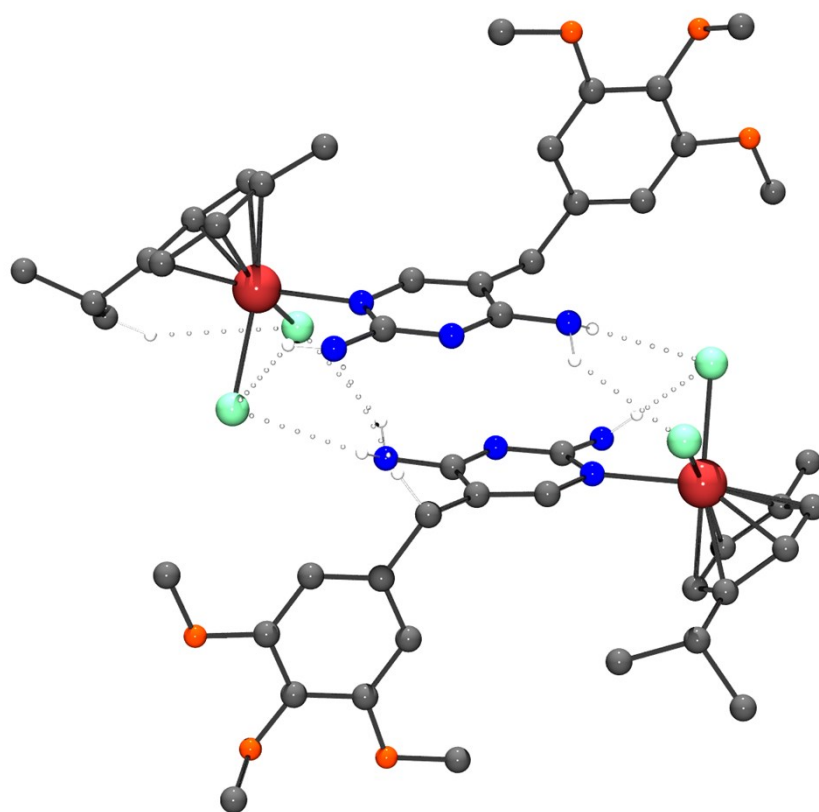**Figure S6**. ESI-Mass spectrum of RATMP(C) in acetonitrile.

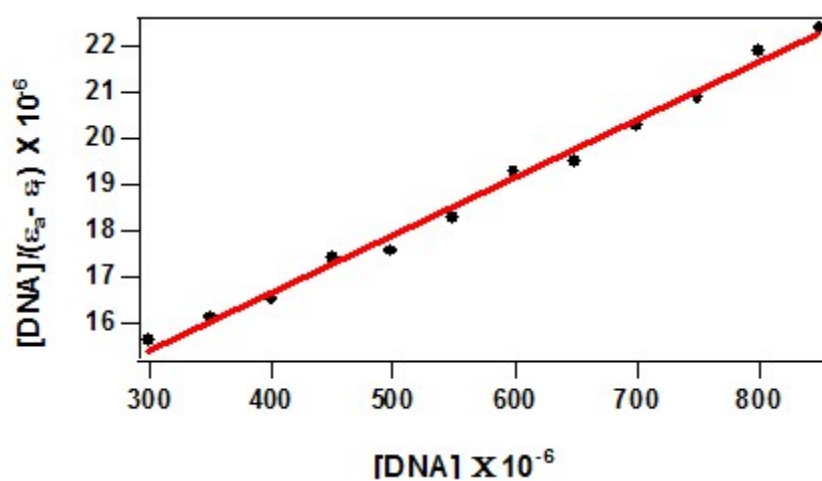**Figure S7**. The inter- and intramolecular hydrogen bonds present in RATMP(C)

Figure S8. The plot of [DNA] vs. [DNA]/(ε_a-ε_f) to calculate the DNA binding constant (K_b) of RATMP(C)
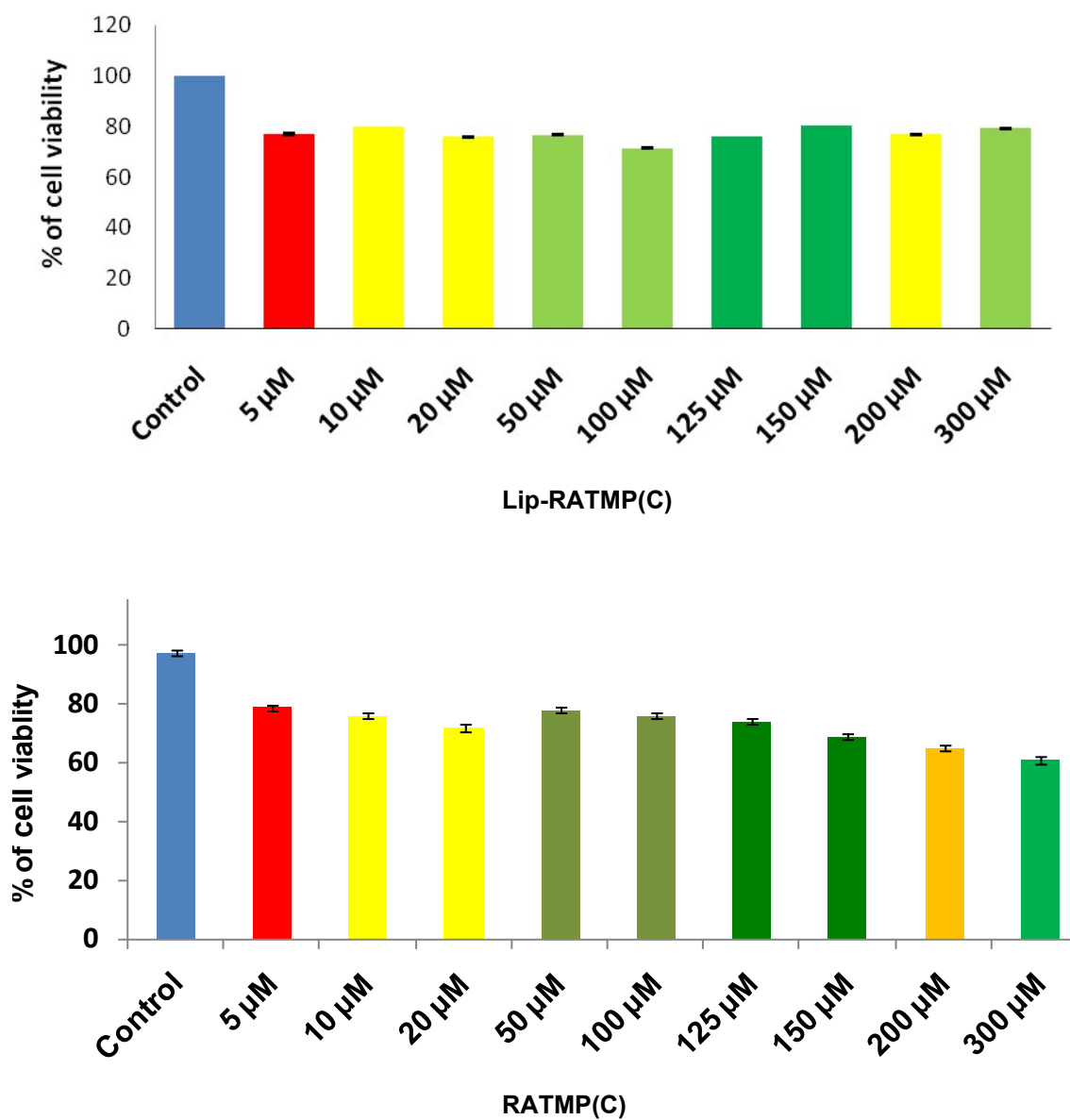
Figure S9. % of cell viability of human embryonic kidney (HEK-293) cells after treatment with varying concentration of Lip-RATMP(C) and RATMP(C).
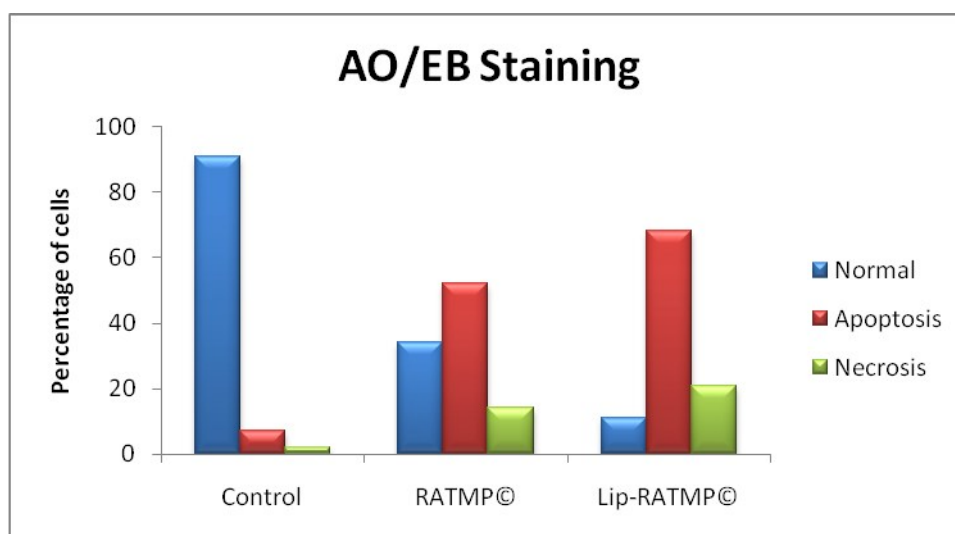
Figure S10. Percentage of the apoptotic and necrotic mode of cell death induced by RATMP(C) and Lip-RATMP(C).
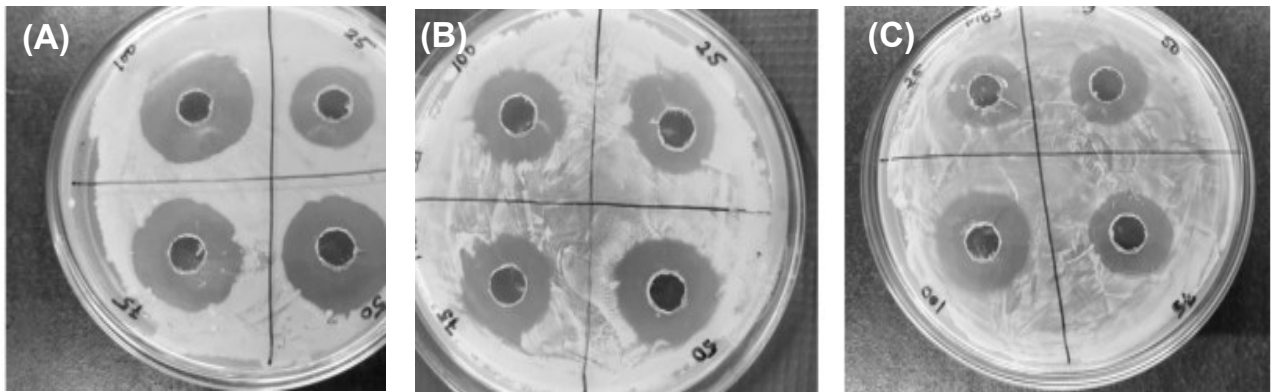
Figure S11. Panel A shows antibacterial effect of different concentrations (25, 50, 75 and 100 μM) of trimethoprim on *P. aeruginosa* while Panel B and C show antibacterial effect of different concentrations (25, 50, 75 and 100 μM) of RATMP(C) on *P. aeruginosa and S aureus* respectively.

Table S1. Size and distribution of bare liposomes and Lip-RATMP(C) measured by DLS.

	Size (nm)[a]	Zeta Potential (mV)
bare liposome	80.1 ± 4.4	-0.1
Lip-RATMP(C)	300.8 ± 24.8	-0.4

[a]The values are means ± standard deviations.

Table S2. Cleavage of pBR322 DNA (40 μM) by RATMP(C) in absence of an external agent in 5 mM TrisHCl/50 mM NaCl buffer at 37 ^{o}C.

Lane number	Reaction conditions	Form (%)	
		SC	NC
1	DNA	93.7	6.3
2	DNA+RATMP(C) (50 μM)	88.8	11.2
3	DNA+RATMP(C) (100 μM)	42.8	a
4	DNA+RATMP(C) (200 μM)	a	a

a-undetectable fragments

Table S3. Comparison of pBR322 DNA cleavage activity of RATMP(C) and Lip-RATMP(C).

Lane number	Reaction conditions	Form (%)	
		SC	NC
1	DNA (40 μM)	97.9	2.1
2	DNA+RATMP(C) (50 μM)	82.6	17.4
3	DNA+RATMP(C) (100 μM)	20.6	a
4	DNA+Lip-RATMP(C) (100 μM)	87.3	12.7
5	DNA+ RATMP(C) (200 μM)	17.6	a
6	DNA+ Lip-RATMP(C) (200 μM)	88.5	11.5
7	DNA+ TMP (200 μM)	90.6	9.4

a-undetectable fragments