Cellular response to chirality and amplified chirality

Sarita Roy,^a Kaushik Bhattacharya,^b Chitra Mandal,^b and Anjan Kr. Dasgupta^{*a}

^aDepartment of Biochemistry, University of Calcutta, 35 Ballygunge Circular Road, Kolkata-700019, India. ^bCancer Biology and Inflammatory Disorder Division, Council of Scientific and Industrial Research-Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Jadavpur, Kolkata-700032, India.

*Corresponding Author Email: <u>adgcal@gmail.com</u> Telephone Number: +913324614849 Fax Number: +913324614849

FT-IR & TEM Characterization of citrate capped SNP

Functionalization of SNP with citrate is marked by the change in anti-symmetric & symmetric stretching IR frequency of the carboxylate group from 1582 & 1389 cm⁻¹ to 1646 & 1469 cm⁻¹ respectively. Signature frequency of hydroxyl group (3381 cm⁻¹) is also seen on SNP surface, a slight contribution at this frequency might also arise from the adsorbed water on nano surface.



Figure S1 (A) FT-IR study of SNP indicates the involvement of –COOH group. (B) TEM image of SNP reveals the spherical nature of particle. The scale bar is 10 nm.



Flow Cytometry based Annexin V staining assay to measure the cytotoxicity against drug (DPA, LPA), SNP & nano-conjugated drug (DPA-SNP, LPA-SNP)

Fig. S2 Histogram overlay representation of the annexinV binding efficacy revealed the higher sensitization of DPA-SNP than LPA-SNP at 250 μ M concentrations. Whereas at lower dose (150 μ M), apoptotic cell death was very less and both the chiral nano conjugates triggered parallel effect over U87MG cells after 72 hr of treatment. The sensitization of SNP at both the doses is approx. same or less than that of nano-conjugated forms as seen from the inset of the figure.

Flow Cytometry based PI uptake assay to measure the cytotoxicity against drug (DPA, LPA), SNP & nano-conjugated drug (DPA-SNP, LPA-SNP)



Fig. S3 Histogram overlay representation of the PI incorporation showed that at the dose of 250 μ M, sensitization of DPA in presence of SNP was much more pronounced than LPA-SNP. Whereas in the lower dose (150 μ M), PI⁺ cell was very less and both the chiral nano conjugates triggered parallel effect over U87MG cells. The PI⁺ cell against treatment of SNP at both the doses is approx. same or less than that of nano-conjugated forms as seen from the inset of the figure.

Effect of cell proliferation upon treatment of drug (DPA, LPA), SNP & nano-conjugated drug (DPA-SNP, LPA-SNP)



Figure S4 CFSE staining assay indicated that, DPA-SNP effectively suppressed cell division than its chiral counterpart LPA-SNP in U87MG cell after 72 hr treatment. Proliferation index value was evaluated by the division of 0th day CFSE fluorescence and 3rd day CFSE fluorescence in each set of treatment. Each value is the mean \pm SD of three independent experiments. *P < 0.05, significant difference between two test groups.





Figure S5 (A) Flow cytometric evaluation of cell cycle arrest in G2/M phase induced by both DPA-SNP and LPA-SNP at 250 μ M concentration after 72 hr treatment in U87MG cell. (B) Graphical representation of all three stages of cell cycle progression (G1, S and G2/M) indicated that DPA-SNP induced slightly enhanced cell cycle restriction than LPA-SNP. As cells were arrested in G2/M phase then G1 phase cells were also reduced prominently in the case of nano conjugated amino acids. Each value is the mean ± SD of three independent experiments. **P < 0.01 and *P < 0.05, significant difference between two test groups.