Supporting Information

A short GC rich DNA derived from microbial origin targets tubulin/microtubule and induces apoptotic death of cancer cell

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Experimental Section

Materials:

Chemicals: Graphite powder (<60 μm) was purchased from Loba Chemie. Sulphuric acid, Sodium nitrate, Sodium Chloride, Trifluoroacetic acid (TFA), Sodium hydroxide, Sodium bicarbonate, di-Sodium hydrogen phosphate dihydrate, Potassium hydroxide, Potassium dihydrogen phosphate, Hydrochloric acid, Magnesium chloride hexahydrate, $NiCl_2$ Hexahydrate, Hydrogen peroxide (30%) and N, N′-dimethyl formamide (DMF) were purchased from Merck. Dimethyl sulphoxide was purchased from Spectrochem. Potassium permanganate, Ethylenediamine Tetraacetic Acid Disodium Salt Dihydrate (EDTA), Tris(hydroxymethyl) aminomethane (Tris) and Potassium Chloride were purchased from Fisher Scientific. Methanol was purchased from Finar. Diamino-polyethylene glycol with MW 3000 Da (NH_2-PEG_{3000}-NH_2) was purchased from Rapp Polymer. Triton-X-100 was purchased from SRL.*β*-Mercaptoethanol, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), Kanamycin sulfate, N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), 3-(4, 5-dimethylthiazol-2-yl)-2, Dulbecco's Modified Eagle's Medium **(**DMEM) medium, Guanosine 5′-triphosphate sodium salt hydrate (GTP), 4, 6-diamidino-2-phenylindole (DAPI), Trypsin-EDTA solution, PIPES, Ethylene glyol-bi(2-aminoethylether)-N, N, N′, N′-tetraacetic acid (EGTA), *β*-casein, Cell cultured DMSO, Paclitaxel, Colchicine and formaldehyde were purchased from Sigma Aldrich. 2-[4-(2-hydroxyethyl) piperazin-1-yl] ethanesulfonic acid (HEPES) was purchased from Himedia. Diisopropyl carbodiimide (DIC) was purchased from Fluka. O^{t}Bu protected Tris-(nitrilo Tris-acetic acid) [(O^{t}Bu)Tris-NTA] was received as kind gift from Dr. Thomas Surrey's

laboratory in EMBL, Heidelberg Germany. Penicillin-Streptomycin and fetal bovine serum were purchased from Invitrogen. Rabbit monoclonal anti-alpha Tubulin IgG (EP1332Y) antibody, Goat pab to Rb IgG (Cy3.5 ®) and Streptavidin protein (hexa His tag) were purchased from Abcam. Bisbenzimide H 33258 (hoechst) and RNase A were purchased from Calbiochem. Propidium iodide, p53 (F-8) mouse monoclonal IgG, p21 (F-5) mouse monoclonal IgG and annexin V apoptosis detection kit were purchased from Santa Cruz Biotechnology. Goat Anti-Mouse IgG (H+L) Fluorescein conjugate (Green) was purchased from Merck Millipore. HPLC purified Primers 5'-GCGCATGCTACGCG-3', 5'-CGCGTAGCATGCGC-3', 5'-biotin-GCGCATGCTACGCG-3', 5'-rhodamine-CGCGTAGCATGCGC-3', 5'-ATATATGCTATATA-3', 5'-TATATAGCATATAT-3', 5'-biotin- ATATATGCTATATA-3', 5'-GCATGCATCGTACG-3', 5'-CGTACGATGCATGC-3' and 5'-biotin- GCATGCATCGTACG-3' were purchased from Eurofins Genomics. All compounds were used without further purification. Cover glass bottom dishes were purchased from SPL.

Protein biochemistry: Tubulin was isolated from pig brain. The purification of tubulin from pig brain was performed as described in the literature.[1]

Cell culture: MCF-7 cell line (human breast cancer cell line), A549 cell line (human lung cancer cell line), HeLa cell line (human cervical cancer cell line) and WI38 cell line (human lung fibroblust cell line) were purchased from NCCS, pune (India) and cultured in Dulbecco Modified Eagle medium (DMEM) containing 10% (v/v) heat-inactivated fetal bovine serum at 37 ^{o}C and 5% CO_2 atmosphere in our lab.

Methods:

Annealing the Oligonucleotides for the preparation of double stranded DNA: First both the complementary oligos were dissolved in annealing buffer containing 10 mM Tris, 50 mM NaCl and 1 mM EDTA, pH 7.5-8.0 at the same molar concentration. Equal volumes of both the complementary oligos were mixed in a microcentrifuge tube and placed in a water-bath at 90-95 ^{o}C for 3-5 minutes and cooled slowly to room temperature for overnight. The sample was stored at 4 ^{o}C.

Tubulin Polymerization Assay/Tubulin turbidity assay: Turbidity experiment was carried out with different concentration of tubulin (5, 10 and 20 μM) tubulin, 4 mM GTP, 10% dimethyl sulfoxide and the short DNA. We performed the tubulin turbidity assay with different concentration of GCD [2 μg/mL (0.2 μM), 5 μg/mL, 50 μg/mL, 100 μg/mL], ATD [5 μg/mL (0.5 μM), 50 μg/mL, 100 μg/mL] and GAD [5 μg/mL (0.5 μM), 50 μg/mL, 100 μg/mL] differently. 100 μL of tubulin solution in Brinkley Reassembly Buffer 80 (BRB 80; 80 mM PIPES, 1 mM EGTA, 1 mM $MgCl_2$, pH 6.9) containing 4 mM GTP, 10% dimethyl sulfoxide and the short DNA was prepared. All the components were mixed in ice and injected into 37 °C heated quartz cuvettes having path length 10 mm and the polymerization of tubulin was performed at 37 °C and the turbidity was measured by measuring absorbance at 350 nm for 40

min in the UV-Vis Spectrophotometer (G6860A Cary 60 UV-Vis Spectrophotometer, Agilent Technologies). DMSO was used for initiating the polymerization.[2] There was no DNA in the control experiment. Critical concentration of the tubulin was determined by performing the turbidity assay in presence of different concentration of tubulin. Then the linear plot was obtained by plotting the maximum polymerization vs tubulin concentration and extra plotting the linear curve to X-axis, we obtained the critical concentration of tubulin, required for polymerization, which was found to be 4 μM.[3]

Microtubule depolymerisation assay: Tubulin was polymerized in presence or absence of 20 μM paclitaxel and the turbidity was measured for 20 min as described before to study the effect of GCD on microtubule depolymerisation.[3] Then, 100 μg/mL (10 μM) of GCD was added and the turbidity was measured at 350 nm wavelength for approximately 40 min. In the control experiment, no GCD was added.

Regrowth assay: Tubulin was incubated with different concentration of GCD (50 and 100 μg/mL) for 30 min in BRB80 buffer (without GTP) at room temperature to examine the reversibility of the inhibitory effect of GCD.[3] Then, the samples were diluted 10-fold with cold BRB80 and then concentrated 10 fold by ultrafiltration with 10,000 cut-off filters in centrifuge. The samples were diluted to their original volume and 4 mM GTP was added after measuring the volume of the sample. Then, the sample was warmed to 37 °C, and polymerization was monitored as described above.

Microtubule assembly assay: Fluorescence intensity of DAPI solution increases on binding with microtubule.[4] Therefore, fluorescence intensity of DAPI solution with time was measured to compare the amount of microtubule formation in absence or in presence of GCD. A mixture of 10 μM DAPI in BRB80 buffer containing 100 μM tubulin, 10 mM GTP and different concentration of GCD [5 μg/mL (0.5 μM), 12.5 μg/mL and 50 μg/mL) was prepared and the emission spectra were recorded in region from 400 nm to 600 nm wavelength exciting the solution at 355 nm wavelength at 37 ^{o}C for 60 min in five min time interval using Quanta Master Spectrofluorometer (QM-40), which is equipped with peltier for controlling the temperature during experiment. Control experiment was carried out under same condition in absence of GCD. Another control was performed without DAPI to ensure the fluorescent signals come from the binding of DAPI to assembled microtubules, not from the aggregation of tubulin. The data was calculated in origin Pro 8.5 software.

Determination of binding affinity of GCD and TGO-bGCD by fluorescence intensity quenching study of intrinsic Tryptophan residue of tubulin: Intrinsic Tryptophan fluorescence intensity of tubulin is quenched in presence of a drugs, which binds with the tubulin.[5] So, we measured intrinsic Tryptophan fluorescence intensity of tubulin in presence of different concentration of GCD and TGO-bGCD. From that, binding constant was calculated using a modified Stern-Volmer equation.[2] 10 μM of tubulin was mixed with different concentration of GCD and TGO-bGCD differently in BRB80 buffer in ice and the fluorescence

emission spectra was recorded from 310 to 450 nm exciting the sample at 295 nm at 4 °C using Quanta Master Spectrofluorometer (QM- 40) equipped with peltier for controlling the temperature.

Docking studies: We have performed docking study using HEX server.[6] We have used PDB structures of GCD (PDB ID: 2M2C)[7] and tubulin heterodimer (as a receptor; PDB ID: 1JFF)[8] for docking study from RCSB Protein Data Bank having the aforesaid PDB ID. The parameter use for docking study are –

- Correlation type – Shape only
- FFT mode – 3D fast lite
- Grid dimension – 0.6
- Receptor range angle – 180
- Ligand range angle – 180
- Docking receptor step size – 7.5
- Docking ligand step size – 7.5

Docking study of GCD with tubulin heterodimer reveals that the GCD binds close (~3.4 Å) to the non-exchangeable GTP site of α tubulin but most of it binds to β-tubulin in α/β tubulin heterodimer interface with lowest energy value (E-value -690.33).

Colchicine binding assay: Colchicine binding assay was performed to check whether GCD binds to the colchicine binding site of the tubulin or not.[9] For that, two solutions (1) 10 μM solution of tubulin and 5 μM solution of GCD in BRB80 buffer and (2) 10 μM solution of tubulin and 10 μM solution of colchicine were incubated differently at 37 °C for 60 min and the emission spectra was recorded from 375 to 650 nm exciting at 355 nm. After that 10 μM solution of colchicine was added to solution 1 and 5 μM solution of GCD was added to solution 2 and the mixture was incubated for another 60 minutes and then the emission spectra was recorded. 10 μM solution of colchicine, 10 μM solution of ctubulin and 5 μM solution of GCD was incubated for 60 min at 37 °C and the emission spectra was recorded as a control.

Detailed method of preparation of TGO: Synthesis of TGO was carried out as described in our previous manuscript.[10] Detailed method is given below. Graphene oxide was synthesized from graphite powder (<60 μm) by a modified Hummers method.[11] Milli-Q water solution of graphene oxide (concentration ~4 mg/mL) was ultra-sonicated for an hour. 3M NaOH solution was added to it and it was ultra-sonicated for another 3 hour. HCl was added to neutralise (pH 7.0) the solution and then the solution was filtered through Whatmann 40 paper. After washing several times with Milli-Q water, Milli-Q water was added to make the concentration of GO-COOH solution ~1 mg/mL. 10 mL GO-COOH solution (concentration ~1 mg/mL) was ultra-sonicated for 5 min after addition of 100 mg of H_2N-PEG_{3000}-NH_2. Then N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) was added into solution mixture to reach 5 mM followed by bath sonication for 30 min. After addingenough EDC in order to reach 20 mM concentration, the solution was stirred at room temperature for overnight. Finally, 50 μL of *β*-

Mercaptoethanol was added to terminate the reaction and the solution was lyophilised to get dry PEGylated GO. Next, dry DMF solution of 100 mg of PEGylated GO was sonicated for 15-30 minutes under N_2 atmosphere. After addition of 100 mg O^tBu protected Tris-NTA and 30 µL DIC as a coupling reagent the solution was stirred under N_2 atmosphere for overnight. Reaction mixture was centrifuged at 12000 rpm for 15 min followed by washingthe residue 3-4 times with DMF by resuspension and centrifugation process. TFA was added for 6 hour under N_2at room temperature for deprotection of tertiary butyl group of Tris-NTA after evaporating the DMF. Excess TFA was removed and milli-Q water was added to the residue followed by sonication for 10 min after removing the excess TFA. Mixture was centrifuged at 12000 rpm for 10 min and the residue was washed with Milli-Q water for 3-4 times. Finally TGO was stored in Milli-Q water at 4 ^oC.

Loading of biotin-GCD-rhodamine on TGO and fluorescence microscopic imaging: For the preparation of TGO-biotin-GCD-rhodamine, TGO (concentration- 3 mg/mL) was equilibrated with 10 mM $NiCl_2$ solution in a mechanical shaker for 15 min.The solution was centrifuged at 10000 rpm for 10 minutes followed by washing the residue with Milli-Q water for 3 times with intermittent sonication and centrifugation. Next, Ni^{+2} loaded TGO was equilibrated with Brinkley Reassembly Buffer 80 (BRB80) at 4 ^oC. Then Ni^{+2} loaded TGO was incubated with *β*-casein (concentration-1mg/mL) by gentle shaking for 15 min at 4 ^oC followed by the removal of excess *β*-casein by centrifugation at 10000 rpm for 10 min at 4 ^oC.The *β*-casein loaded TGO was washed with BRB80 for 2-3 times through sonication and centrifugation method at 4 ^oC.Streptavidin protein (hexa His tag, concentration 10 µg/mL) was loaded to *β*-casein loaded TGO and the solution was incubatedfor 15 min by gentle shaking at 4 ^oC followed by the removal of excess streptavidin protein (hexa His tag) by centrifugation at 10000 rpm for 10 min at 4 ^oC. The residue was washed with BRB80 through sonication and centrifugation method at 4 ^oC. Then streptavidin protein (hexa His tag) loaded TGO was incubated with biotin-GCD-rhodamine solution (concentration-10 µg/mL) by gentle shaking for 15 min at 4 ^oC followed by the removal of excess biotin-GCD-rhodamine by centrifugation. The TGO-biotin-GCD-rhodamine was washed with BRB80. Finally, 20 µL of TGO-biotin-GCD-rhodamine suspension in BRB80 was loaded on ethanol cleaned microscopic glass slide and on top of that solution a 22 mm/22 mm coverslip was placed for making a sandwich. Sandwich was sealed and imaged using 561 nm laser through Nikon Eclipse Ti-U inverted fluorescence microscope.

Control Experiment: We performed the control experiment with PEGylated GO. The binding of biotin-GCD-rhodamine to PEGylated GO was carried out following the same procedure described previously (similar method as described in Loading of biotin-GCD-rhodamine on TGO and fluorescence microscopic imaging section) and also the imaging was done as previously described. No fluorescent signal was observed at 561 nm laser light, which clearly indicates that biotin-GCD-rhodamine binds to the TGO only through functionalization.

Cellular uptake studies: Cellular uptake of TGO-biotin-GCD-rhodamine and GCD-rhodamine was studied MCF-7 cell line. The cells were seeded at a density of 20000 cells per cover glass

bottom dish one day prior to treatment. Binding of biotin-GCD-rhodamine to TGO was performed as described previously. Then the cells were treated with TGO-biotin-GCD-rhodamine (biotin-GCD-rhodamine concentration was 2 μg/mL and the concentration of the conjugate was 50 μg/mL) and GCD-rhodamine (2 μg/mL) in DMEM medium containing 10% FBS for 24 hour differently. After that the cells were washed with colourless serum free medium and observed under an NIKON inverted microscope (Model Ti-U).

Preparation of biotin-GCD and TGO (TGO-bGCD) conjugate: The TGO-bGCD conjugate was prepared following the same procedure as described in the**"Loading of biotin-GCD-rhodamine on TGO and fluorescence microscopic imaging"** section. Only biotin-GCD was used instead of biotin-GCD-rhodamine here.

Preparation of biotin-ATD and TGO (TGO-bATD) conjugate: The TGO-bATD conjugate was prepared following the same procedure as described in the**"Loading of biotin-GCD-rhodamine on TGO and fluorescence microscopic imaging"** section. Only biotin-ATD was used instead of biotin-GCD-rhodamine here.

Preparation of biotin-GAD and TGO (TGO-bGAD) conjugate: The TGO-bGAD conjugate was prepared following the same procedure as described in the**"Loading of biotin-GCD-rhodamine on TGO and fluorescence microscopic imaging"** section. Only biotin-GAD was used instead of biotin-GCD-rhodamine here.

Cytotoxicity study: Cytotoxicity of GCD, TGO-bGCD, ATD, TGO-bATD, GAD, TGO-bGAD, biotin-GCD and TGO towards MCF-7 cancer cells and GCD, TGO-bGCD towards A549, HeLa and WI38 cells was evaluated by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction. The cells were seeded at a density of 10000 cells per well in a 96-well plate one day prior to the traetment. Then MCF-7 cells were treated with GCD, TGO-bGCD, ATD, TGO-bATD, GAD, TGO-bGAD, biotin-GCD and TGO and A549, HeLa and WI38 cells were treated with GCD and TGO-bGCD differently in DMEM medium containing 10% FBS for 24 h in different 96-well plate. Following the termination of experiment, cells were washed and promptly assayed for viability using MTT. Results were expressed as percent viability = [(A550 (treated cells)-background)/(A550 (untreated cells)-background)] x 100.

Study the effect of TGO-biotin-GCD (TGO-bGCD), GCD, biotin-GCD and TGO on the microtubule network of MCF-7 cell line: Effect on the microtubule network of MCF-7 cell line after treatment with TGO-biotin-GCD (TGO-bGCD), GCD, biotin-GCD and TGO was studied by following method. The cells were seeded at a density of 20000 cells per cover glass bottom dish for 18-24 hours before the compounds' treatment. After that the cells were treated with TGO-bGCD (GCD concentration was 2 μg/mL and the concentration of the TGO-bGCD was 50 μg/mL), GCD (2 μg/mL), biotin-GCD (2 μg/mL) and TGO (50 μg/mL) differently in DMEM medium containing 10% FBS for 24 h. In case of control experiment there was no treatment. Then the cells were fixed with 4% paraformaldehyde solution after washing with 1X

Phosphate Buffer Saline (PBS) and permealized by treatment with 0.1% Triton-X-100 for another 30 min. Then the fix cells were treated with primary antibody Rabbit monoclonal anti-alpha Tubulin IgG (EP1332Y) antibody; 1:300) for two hour. Then it was treated with secondary antibody (Goat pab to Rb IgG (Cy3.5 ®); 1:500) for another two hour followed by Bisbenzimide H 33258 (hoechst)(1 μg/mL) treatment for 30 minutes. Following the completion of the experiment, the cells were washed with 1X PBS and observed in the inverted microscope (Model Nikon Eclipse Ti-U) in DIC mode, 405 and 561 nm fluorescence channels.

p53 assay: MCF-7 cells were seeded at a density of 20000 cells per cover glass bottom dish one day prior to the treatment. After that the cells were treated with TGO-bGCD (GCD concentration was 2 μg/mL and the concentration of the TGO-bGCD was 50 μg/mL), GCD (2 μg/mL)in DMEM medium containing 10% FBS for 24 h. In case of control experiment there was no treatment. Then, cells were treated with 4% paraformaldehyde solution for 30 minutes for cell fixing after washing with PBS and permealized by treatment with 0.1% Triton-X-100 for another 30 min. Then mouse monoclonal IgG P53 (F-8) antibody with dilution 1:300 was added to the cell after washing with PBS for 2 hours. After that cells were washed with PBS and incubated with secondary antibody Goat Anti-Mouse IgG (H+L) Fluorescein conjugate (Green) with dilution 1:600 for another 2 hours followed by incubation with Hoechst 33258 (1 μg/mL) for 30 minutes. Fixed cells were imaged through a Nikon Ti-U eclipse fluorescence microscope with a 40X objective in bright field, 405 and 488 nm wavelength laser lights.

FACS analysis for apoptosis study: Fluorescence activated cell sorting (FACS) experiment was carried out for understanding the type of cell death. MCF-7 cells were seeded at a density of ~5 X 10^5 cells per well in a 6-well plate one day prior to the treatment. After that the cells were treated with TGO-bGCD (GCD concentration was 2 μg/mL and the concentration of the TGO-bGCD was 50 μg/mL), GCD (2 μg/mL) in DMEM medium containing 10% FBS for 24 h. In case of control experiment there was no treatment. Then the cells were trypsinized and collected by centrifugation at 3000 rpm for 3 minutes.Then the cells were kept in dark at 37 ^oC for 15 minutes adding 100 μL solution of assay buffer containing 10 μL of Propidium iodide (PI) (stock concentration was 50 μg/mL) and 2.5 μL of annexin V (stock concentration was 200 μg/mL) after washing with PBS by centrifugation. After that another 400 μL of assay buffer was added to the cells and analyzed by FACS in BD LSRFORTESA flow cytometer using emission filters at 530 and 610 nm. Data was analyzed using FACS DIVA software.

p21 assay: In case of p21 assay, all the procedure up to treatment with Triton-X-100 was same as in the p53 assay. After that the cells were incubated with mouse monoclonal IgG P21 (F-5) antibody with dilution 1:300 for 2 hours after washing with PBS. Then the cells were washed with PBS and secondary antibody (Anti-Rabbit IgG (H+L), Fluorescein conjugate (goat polyclonal IgG)) with dilution 1:600 was added for another two hours. Then it was incubated with Hoechst 33258 (1 μg/mL) for 30 minutes. Fixed cells were imaged through a Nikon Ti-U eclipse fluorescence microscope with a 40X objective in bright field, 405 and 488 nm wavelength laser lights.

Cell cycle analysis by flow cytometry: MCF-7 cells were seeded at a density of 5 X 10^5 cells per well in a 6 well plate one day prior to the treatment. After that the cells were treated with TGO-bGCD (GCD concentration was 2 μg/mL and the concentration of the conjugate was 50 μg/mL), GCD (concentration was 2 μg/mL) and TGO (concentration was 50 μg/mL) in DMEM medium containing 10% FBS for 24 h. In case of control experiment there was no treatment. Then the cell was trypsinizd and collected by centrifugation at 3000 rpm for 3 minutes. All cell pellets were taken in phosphate buffer saline (PBS, pH 7.4) and fixed by adding cold ethanol slowly and kept at 4 ^oC overnight. The final concentration of ethanol was 70% (v/v). After that the cells were washed with PBS (pH 7.4) through centrifugation to remove ethanol. Propidium iodide (final concentration 100 μg/mL) and RNase A (final concentration 10 μg/mL) was added to the cell suspension in PBS for 45 minutes at room temperature. Cell suspensions were homogenated and transferred into FACS tube and analysed by BD LSRFORTESA flow cytometer.

Data Analysis: Microscopic images were analysed using Image J software.

References:

1. A. Hyman, D. Drechsel, D. Kellogg, S. Salser, K. Sawin, P. Steffen, L. Wordeman and T. Mitchison, *Methods in Enzymology*,1991,**196**, 478.

2. S. Chakraborti, L. Das, A. N. Kapoor, V. Dwivedi, A. Poddar, G. Chakraborti, M. Janik, G. Basu, D. Panda, P. Chakrabarti, A. Surolia and B. Bhattacharyya, *J. Med. Chem.* 2011, **54**, 6183.

3. C. D. Smith and X. Zhang, *J. Bio. Chem.,* 1996, **271**, 6192.

4. (a) D. Bonne, C. Heusele, C. Simon and D. Pantaloni, *J. Biol. Chem.* 1985, **260**, 2819; (b) J. G. Ghosh, S. A. Houck and J. I. Clark, *PLoS ONE*, 2007, **2**, e498.

5. K. Gupta and D. Panda, *Biochemistry*, 2002, **41**, 13029.

6. (a) G. Macindoe, L. Mavridis, V. Venkatraman, M. D. Devignes and D.W. Ritchie, *Nucleic Acids Res.*, 2010, **38**, W445. (b) HEX server (http://hexserver.loria.fr/.)

7. A. Ghosh, R. K. Kar, J. Jana, A. Saha, B. Jana, J. Krishnamoorthy, D. Kumar, S. Ghosh, S. Chatterjee and A. Bhunia, *ChemMedChem.*, 2014, **9,** 2052.

8. J. Löwe, H. Li, K. H. Downing and E. Nogales, *J. Mol. Bio*., 2001, **313**, 1045.

9. A. Saha, S. Mohapatra, P. Kurkute, B. Jana, P. Mondal, D. Bhunia, S. Ghosh and S. Ghosh, *Chem. Commun.,* 2015, **51**, 2249.

10. B. Jana, A. Biswas, S. Mohapatra, A. Saha and S. Ghosh, *Chem. Commun.,* 2014, **50,** 11595.

11. W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc.,* 1958, **80**, 133.

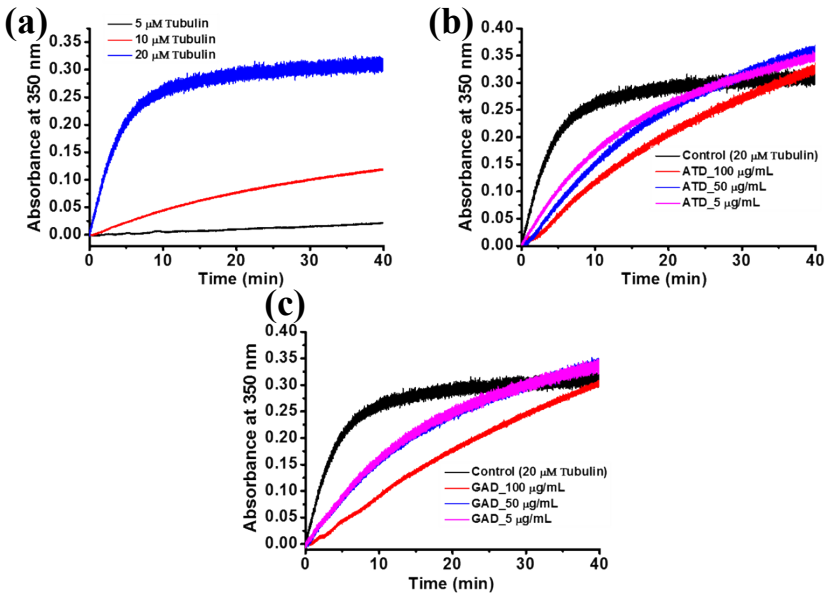

Figure S1: (a) Tubulin turbidity assay in presence of different concentration of (a) Tubulin, (b) ATD and (c) GAD. The results indicate that ATD and GAD have no effect on the inhibition of tubulin polymerization.

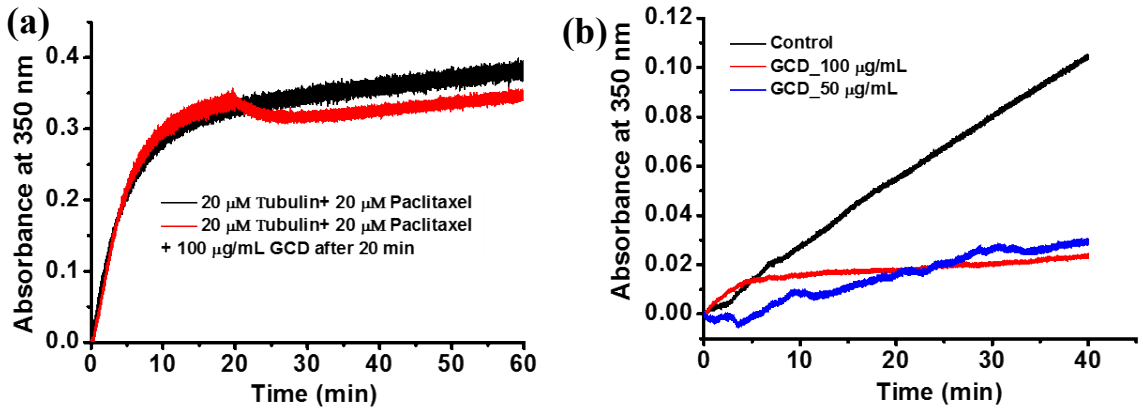

Figure S2: (a) Effect of GCD on microtubule depolymerisation. (b) Effect of GCD on regrowth of microtubule.

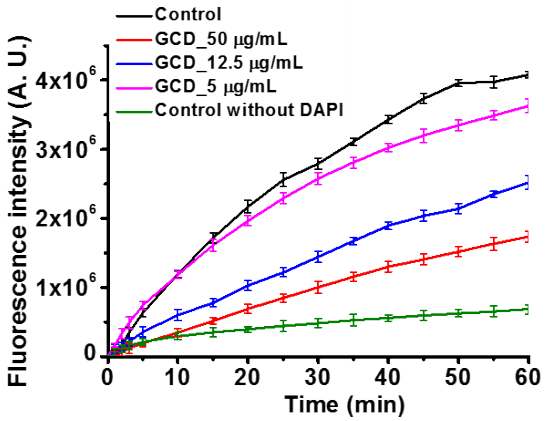

Figure S3: Rate of enhancement of DAPI fluorescence in tubulin polymerization decreases in presence of GCD due to inhibition of polymerization.

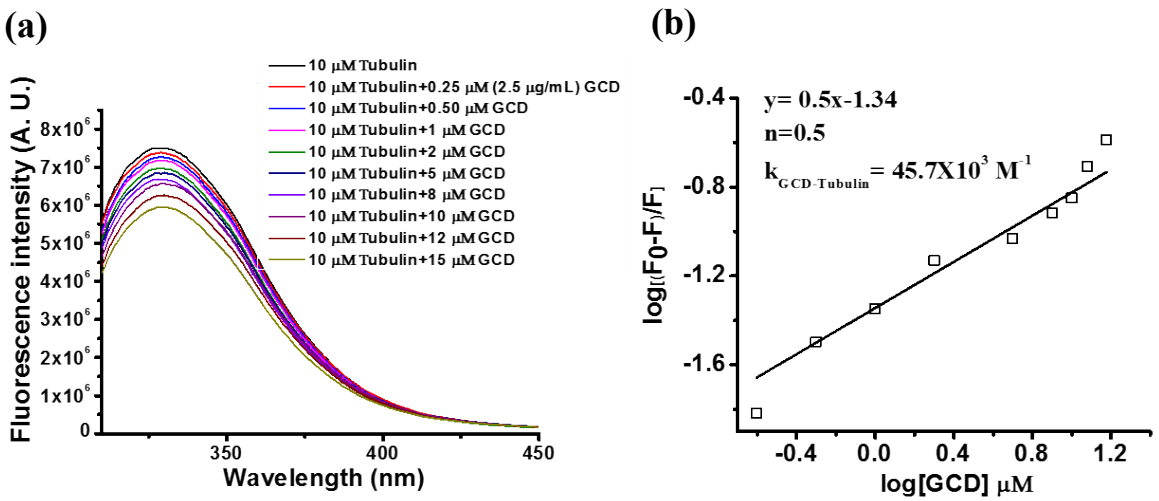

Figure S4: (a) Quenching of Tryptophan fluorescence of tubulin in presence of different concentration of GCD and (b) binding constant curve of tubulin and GCD.

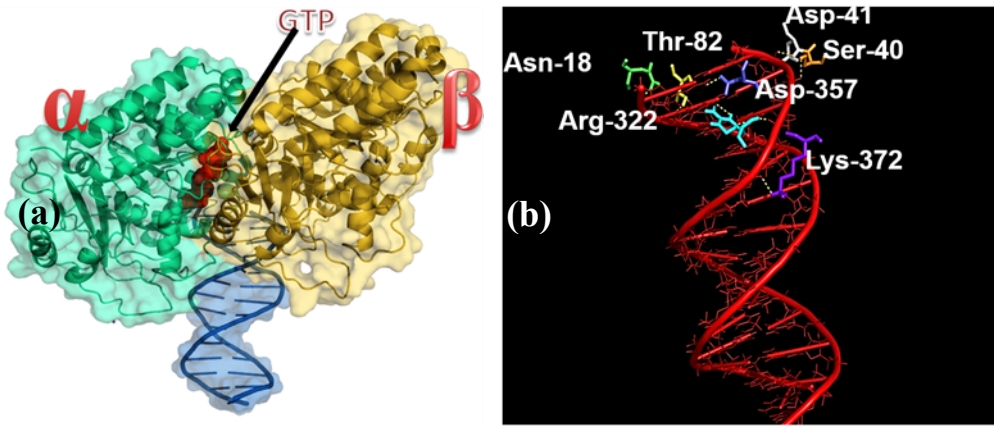

Figure S5: (a) Docking image shows that GCD binds with the tubulin close to GTP binding site and (b) amino acids of tubulin, involved in this interaction.

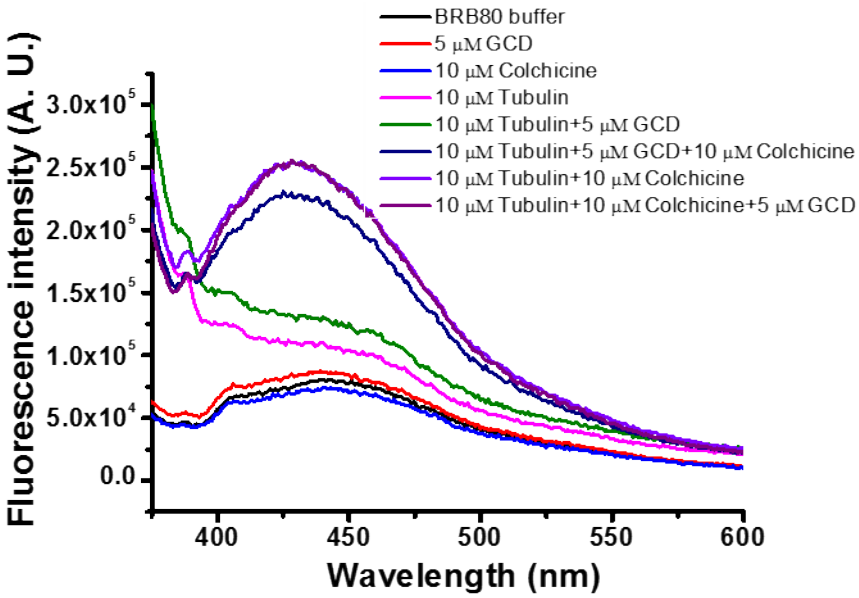

Figure S6: Colchicine binding assay indicates that GCD does not bind to colchicine binding site of tubulin.

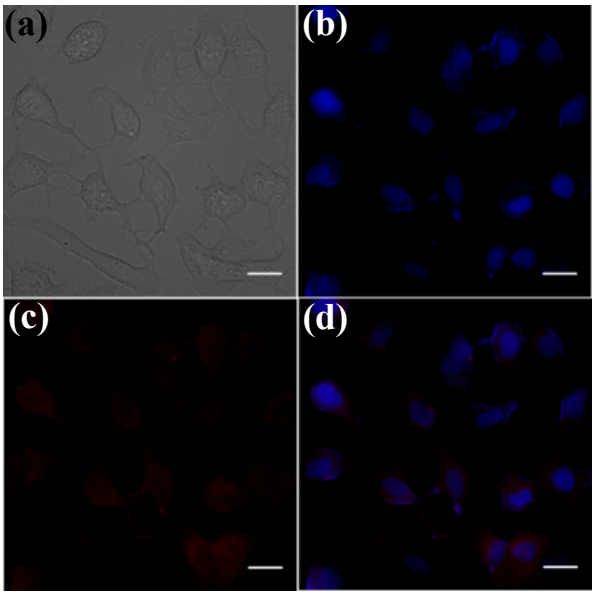

Figure S7: Cellular uptake of GCD-rhodamine in MCF-7 cell line. (a) DIC images of MCF-7 cell lines after treatment with GCD-rhodamine. Images at (b) 405 nm channel is due to nucleus staining by Hoechst and at (c) 561 nm channel is due to rhodamine bound to GCD. (d) Merged image. It clearly indicates weak uptake of GCD-rhodamine by MCF-7 cells. Scale bar corresponds to 30 µm**.**

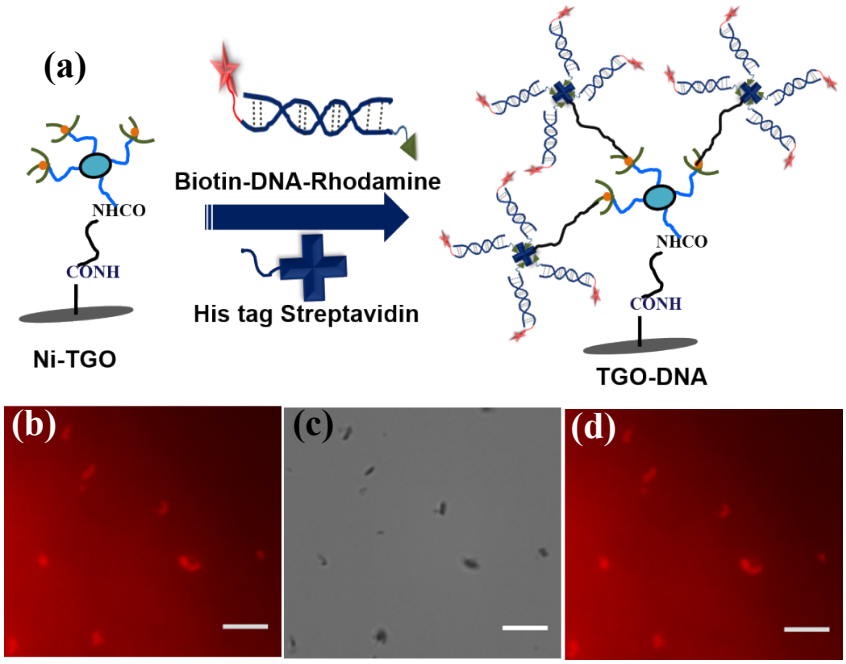

Figure S8: (a) Cartoon diagram of biotin-GCD-rhodamine loaded TGO. **Fluorescence microscopic** images of TGO-biotin-GCD-rhodamine in (b) 561 nm channel and (c) DIC mode. (d) Merged image, which clearly indicates the binding of biotin-GCD-rhodamine on TGO. Scale bar corresponds to 10 µm.

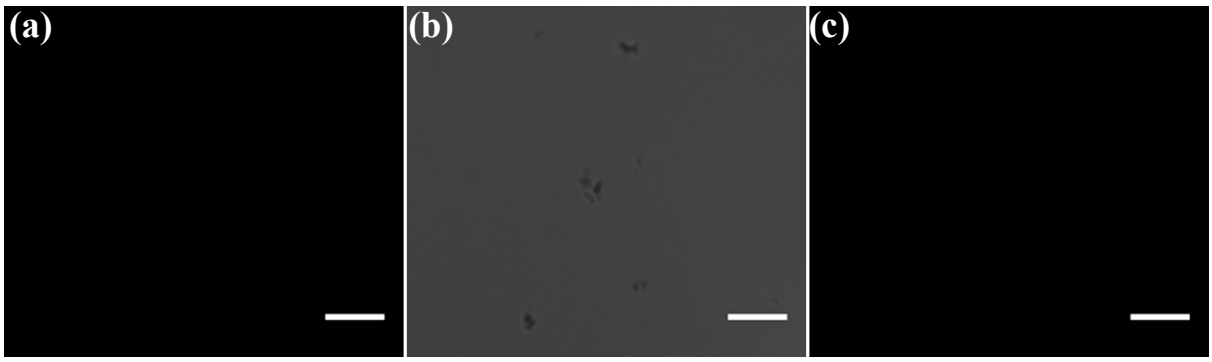

Figure S9: Fluorescence microscopic images of biotin-GCD-rhodamine loaded PEGylated GO in (a) 561 nm channel, (b) DIC mode and (c) merged image. No red signals in 561 nm channel clearly indicates that no binding of biotin-GCD-rhodamine on PEGylated GO. Scale bar corresponds to 10 μm.

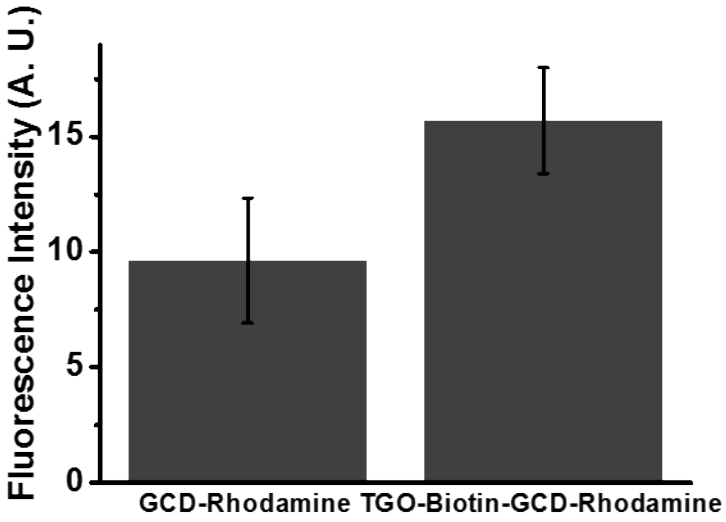

Figure S10: Quantitative analysis reveals fluorescence intensity of rhodamine attached to GCD and TGO-biotin-GCD inside GCD-rhodamine and TGO-biotin-GCD-rhodamine treated MCF- 7 cells.

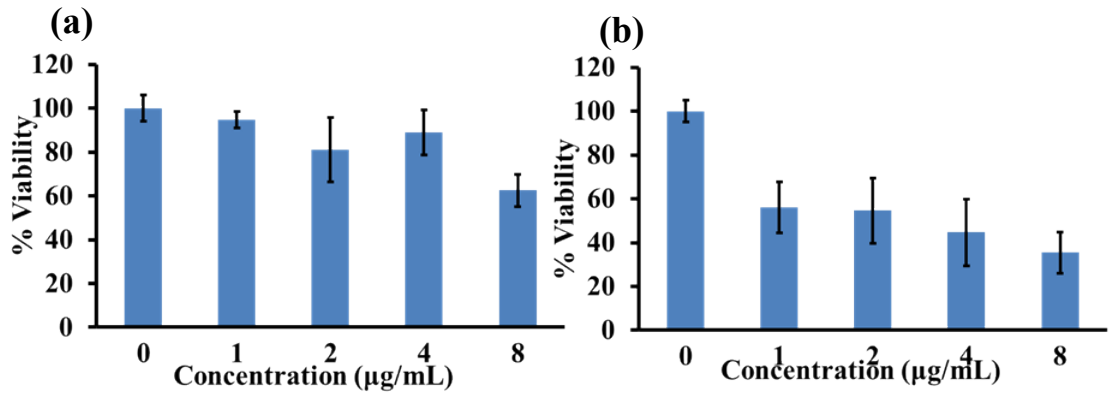

Figure S11: Survival of MCF-7 cells was assessed by MTT assay after treatment with (a) GCD and (b) TGO-bGCD conjugate at higher concentration for 24 hours.

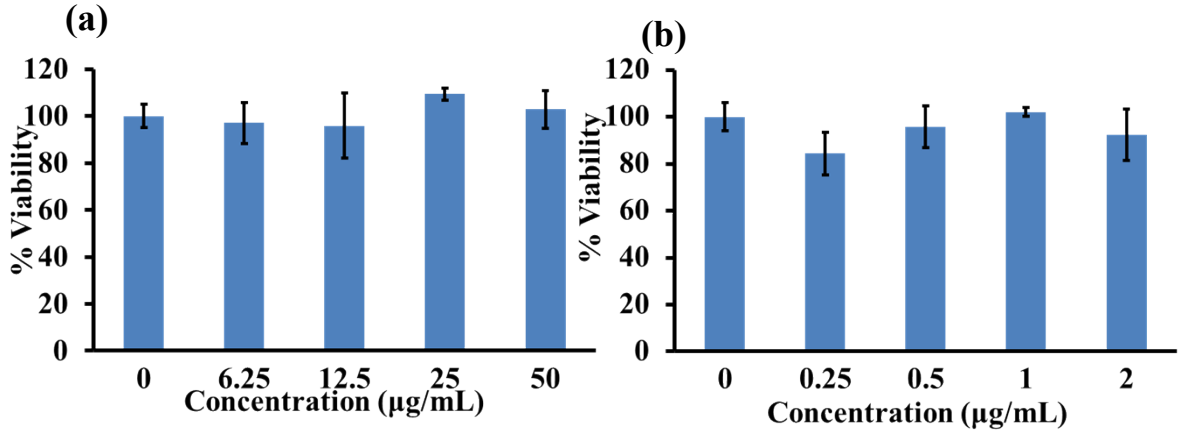

Figure S12: Survival of MCF-7 cells was assessed by MTT assay after treatment with (a) TGO and (b) biotin-GCD conjugate for 24 hours.

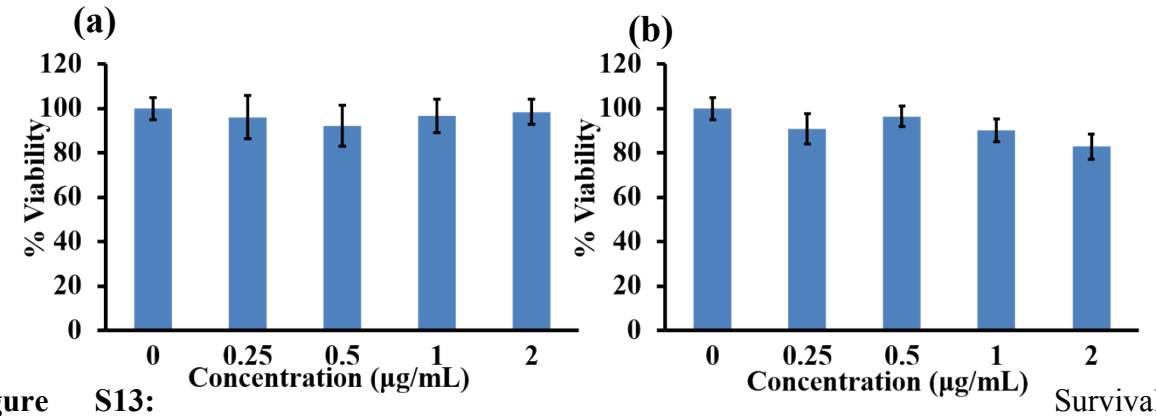

Figure S13: Survival of MCF-7 cells was assessed by MTT assay after treatment with (a) ATD and (b) TGO-bATD conjugate for 24 hours.

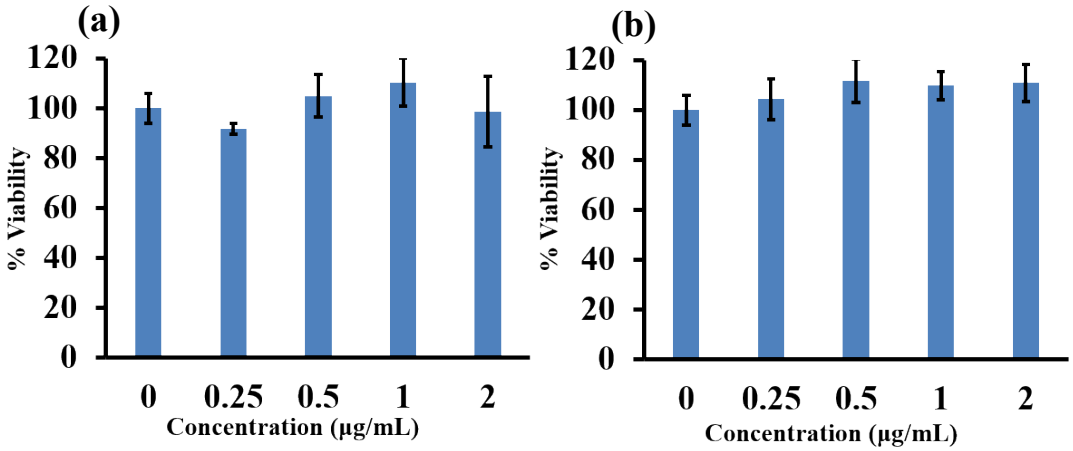

Figure S14: Survival of MCF-7 cells was assessed by MTT assay after treatment with (a) GAD and (b) TGO-bGAD conjugate for 24 hours.

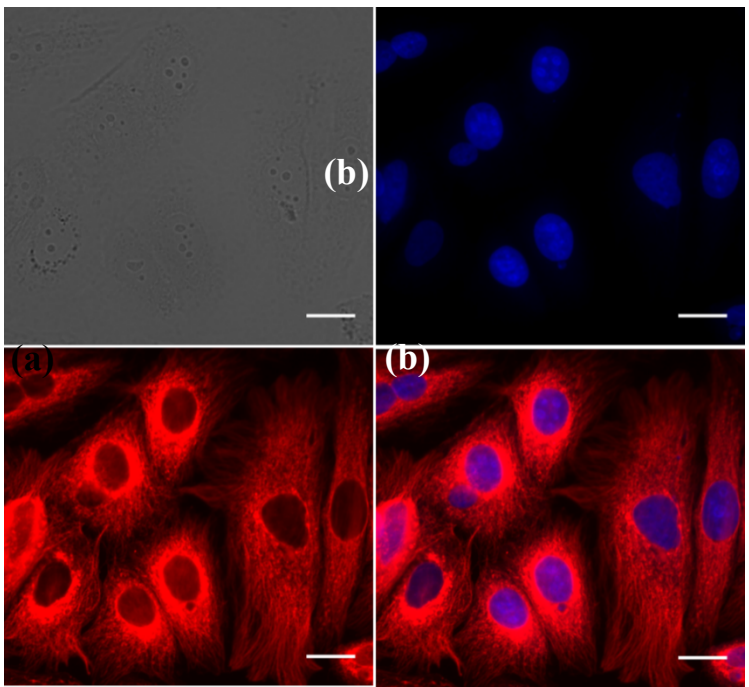

Figure S15: Microtubules network of MCF-7 cells. Fluorescence microscopic images of MCF-7 cells in (a) DIC mode, (b) 405, (c) 561 nm channel after antibody and hochest treatment and (d) merged image. Scale bar corresponds to 30 µm.

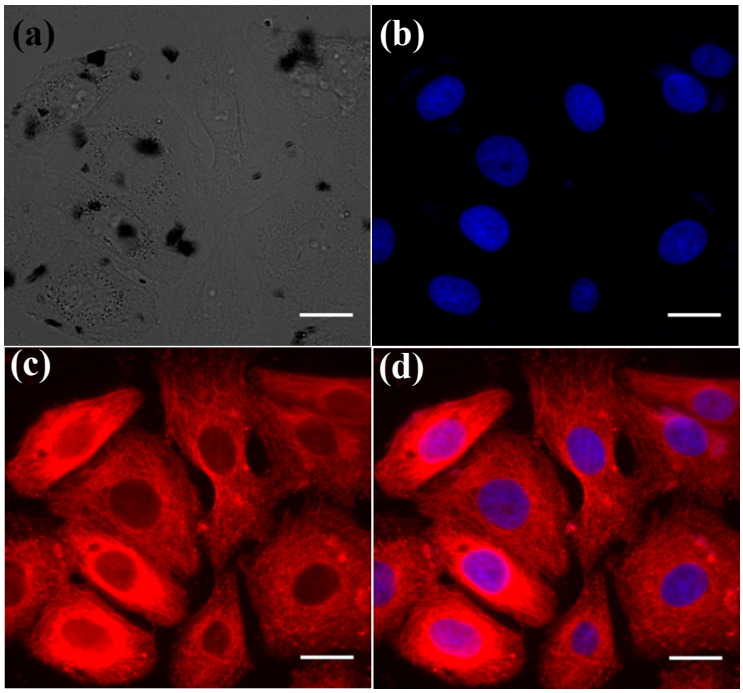

Figure S16: Microtubules network of MCF-7 cells after TGO treatment. Fluorescence microscopic images of MCF-7 cells in (a) DIC mode, (b) 405, (c) 561 nm channel after antibody and hochest treatment and (d) merged image. Scale bar corresponds to 30 µm.

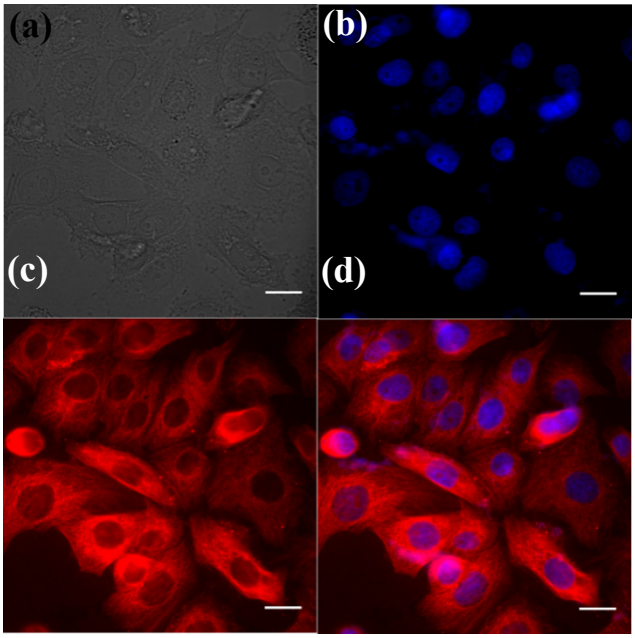

Figure S17: Microtubules network of MCF-7 cells after GCD treatment. Fluorescence microscopic images of MCF-7 cells in (a) DIC mode, (b) 405, (c) 561 nm channel after antibody and hochest treatment and (d) merged image. Scale bar corresponds to 30 µm.

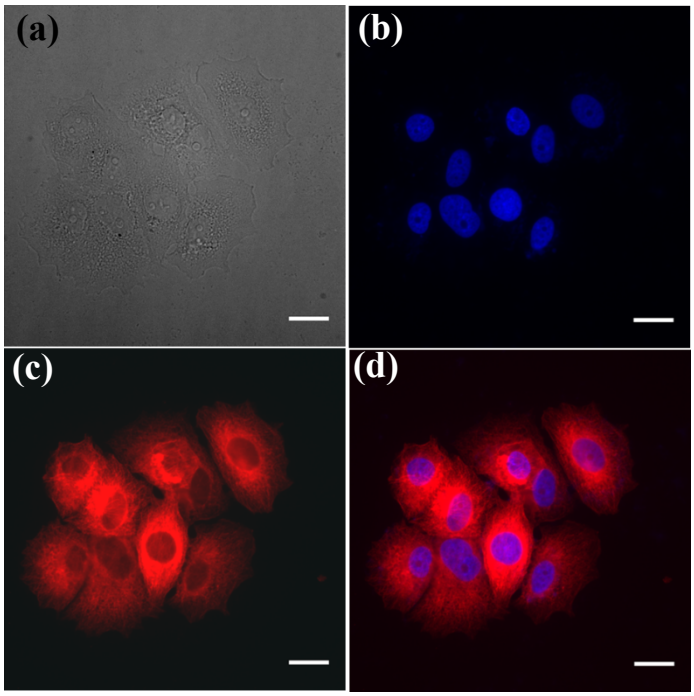

Figure S18: Microtubules network of MCF-7 cells after biotin-GCD treatment. Fluorescence microscopic images of MCF-7 cells in (a) DIC mode, (b) 405, (c) 561 nm channel after antibody and hochest treatment and (d) merged image. Scale bar corresponds to 30 µm.

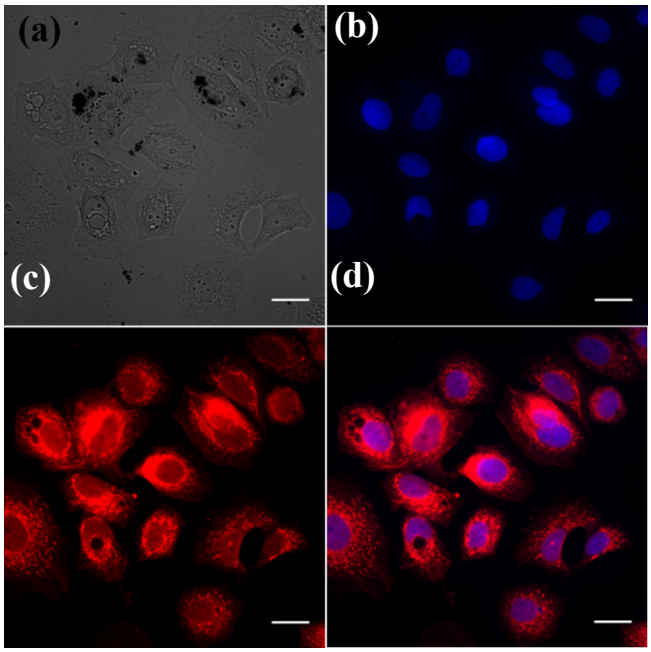

Figure S19: Microtubules network of MCF-7 cells after TGO-bGCD treatment. Fluorescence microscopic images of MCF-7 cells in (a) DIC mode, (b) 405, (c) 561 nm channel after antibody and hochest treatment and (d) merged image. Scale bar corresponds to 30 µm.

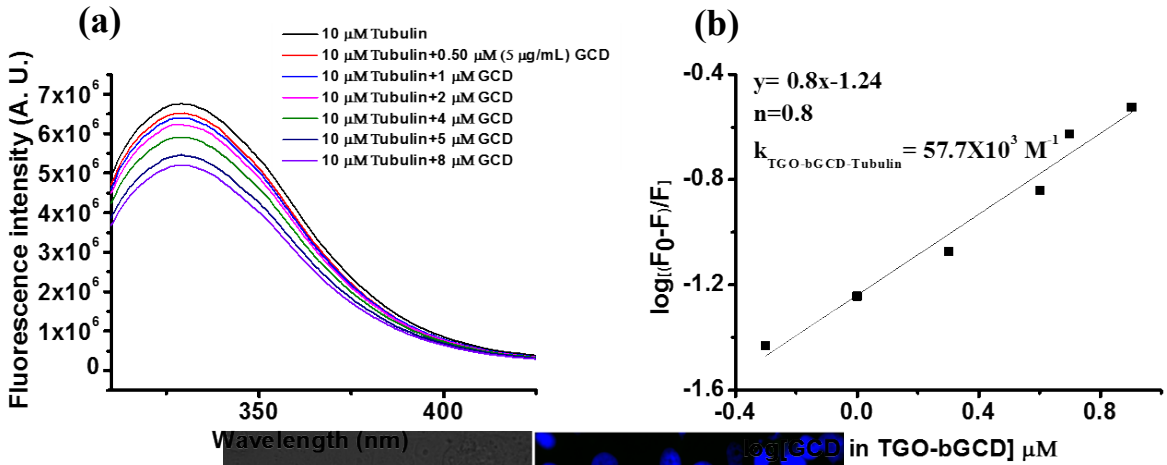

Figure S20: (a) Tryptophan Quenching of fluorescence of tubulin in presence of different concentration of GCD in TGO-bGCD and (b) binding constant curve of tubulin and TGO-bGCD.

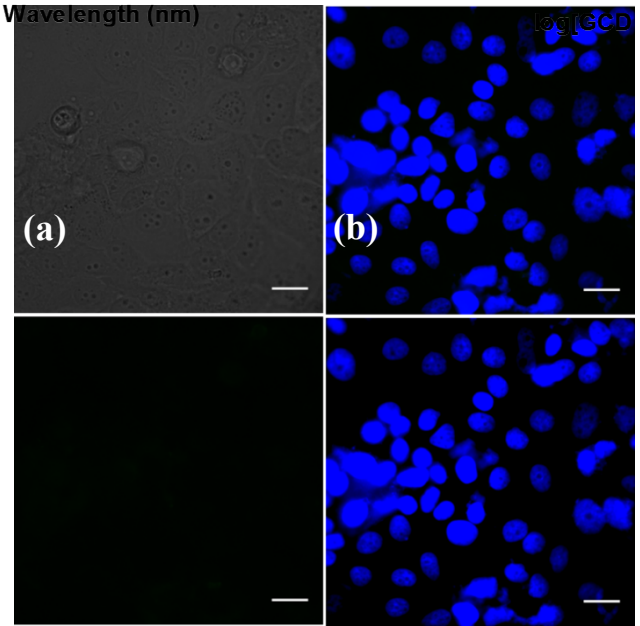

Figure S21: Estimation of p53 protein activation in MCF-7 cells. Nucleus was stained with Hoechst (blue). Fluorescence microscopic images in (a) DIC mode, (b) 405 nm channel (c) 488 nm channel and (d) merged image. Scale bar corresponds to 30 μm.

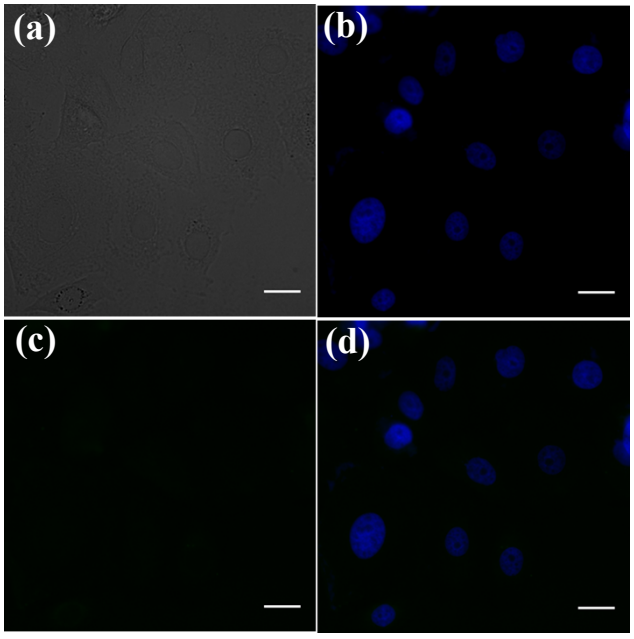

Figure S22: Estimation of p53 protein activation in MCF-7 cells after GCD treatment. Nucleus was stained with Hoechst (blue). Fluorescence microscopic images in (a) DIC mode, (b) 405 nm channel (c) 488 nm channel and (d) merged image. Scale bar corresponds to 30 μm

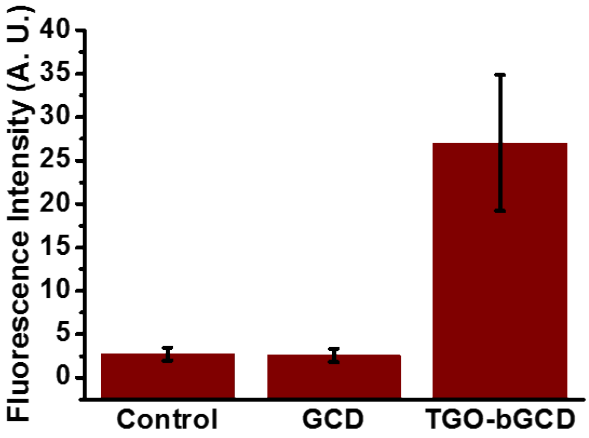

Figure S23: Quantitative analysis revealsfluorescence intensity inside the MCF- 7 cells in 488 nm channel due to p53 activation after GCD and TGO-bGCD treatment along with control.

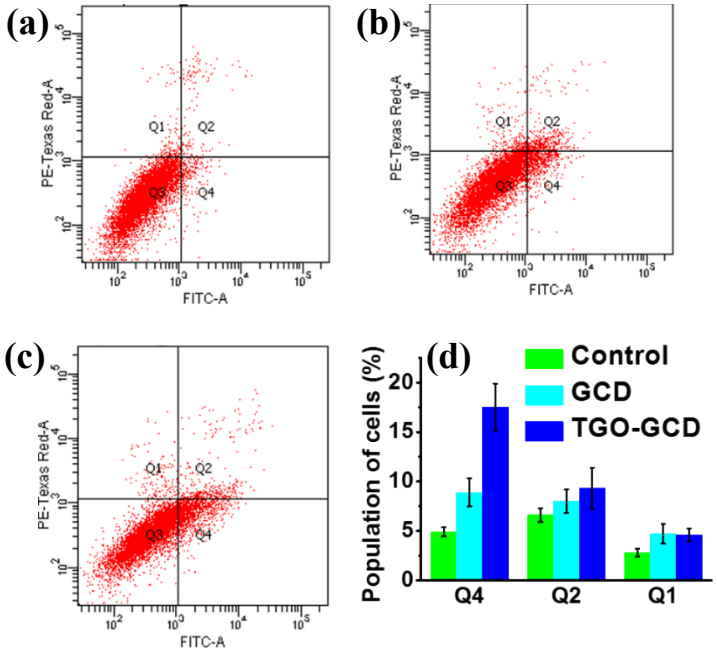

Figure S24: FACS analysis in MCF-7 cells by flow cytometer (a) control and after treatment with (b) GCD, (c) TGO-bGCD. (d) Comparative bar diagram representing % of cell population in early apoptotic, late apoptotic and necrotic death after treatment with GCD and TGO-bGCD along with control. The result shows % of early apoptotic death in TGO-bGCD treated cells is higher than GCD treated cells as well as control.

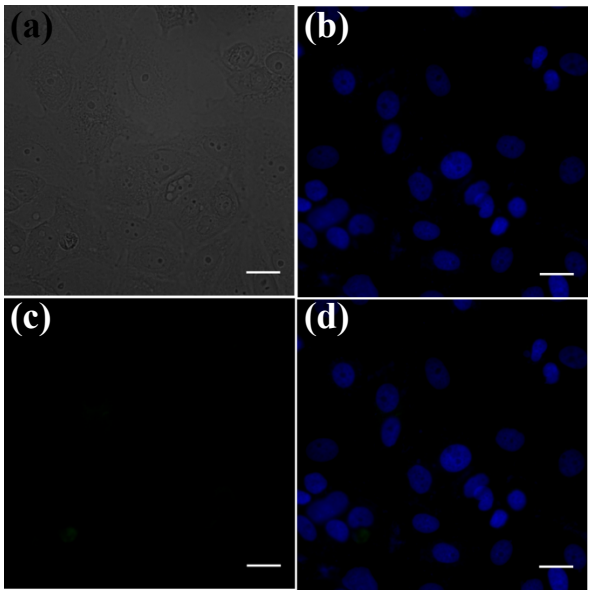

Figure S25: Estimation of p21 protein activation in MCF-7 cells. Nucleus was stained with Hoechst (blue). Fluorescence microscopic images in (a) DIC mode, (b) 405 nm channel (c) 488 nm channel and (d) merged image. Scale bar corresponds to 30 μm.

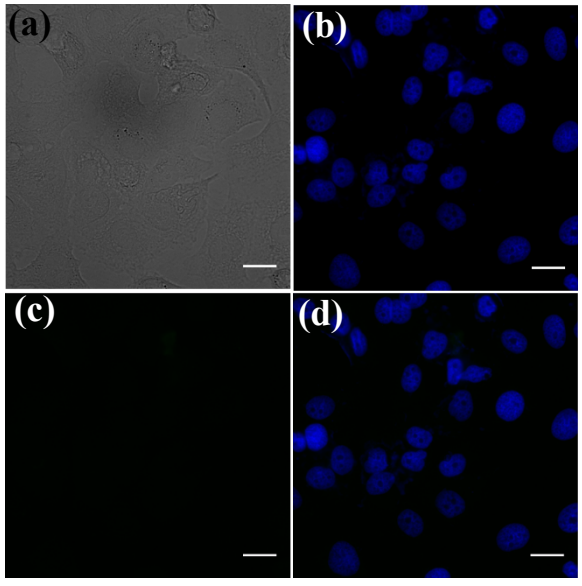

Figure S26: Estimation of p21 protein activation in MCF-7 cells after GCD treatment. Nucleus was stained with Hoechst (blue). Fluorescence microscopic images in (a) DIC mode, (b) 405 nm channel (c) 488 nm channel and (d) merged image. Scale bar corresponds to 30 μm.

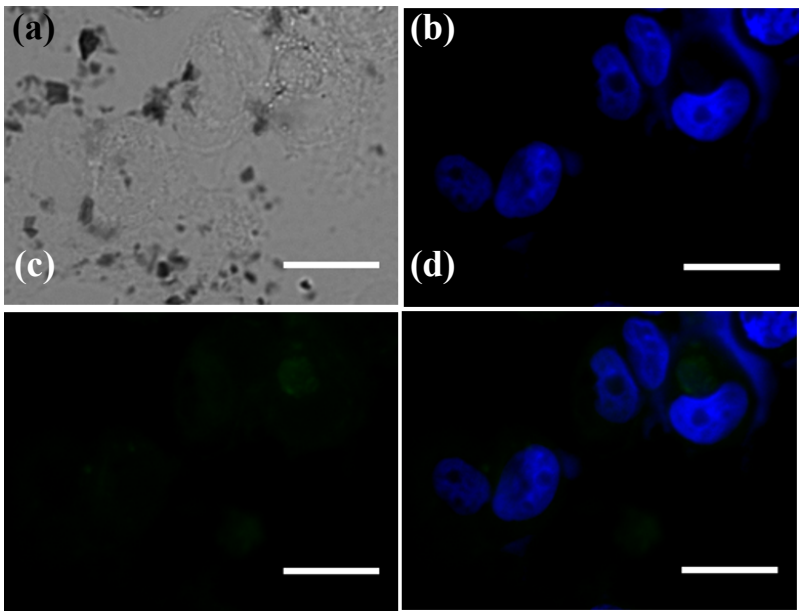

Figure S27: Estimation of p21 protein activation in MCF-7 cells after TGO-bGCD treatment. Nucleus was stained with Hoechst (blue). Fluorescence microscopic images in (a) DIC mode, (b) 405 nm channel (c) 488 nm channel and (d) merged image. Scale bar corresponds to 30 µm.

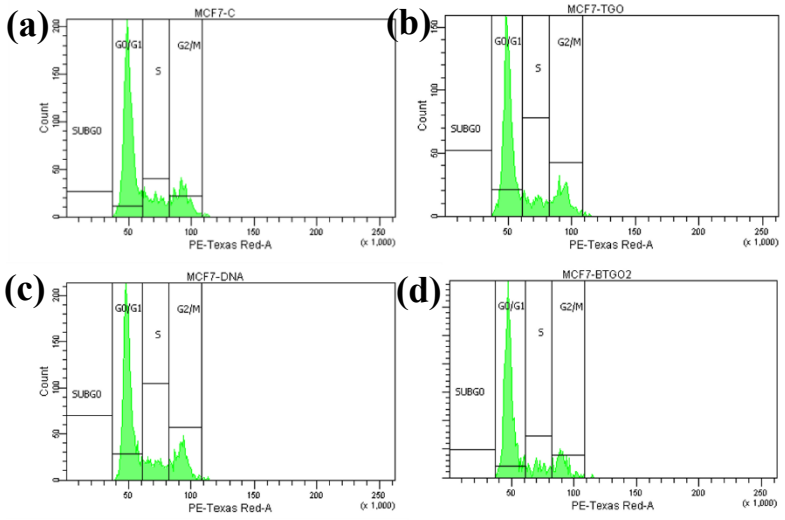

Figure S28. Cell cycle analysis in MCF-7 cells after (b) TGO, (c) GCD and (d) TGO-bGCD treatment along with (a) control.

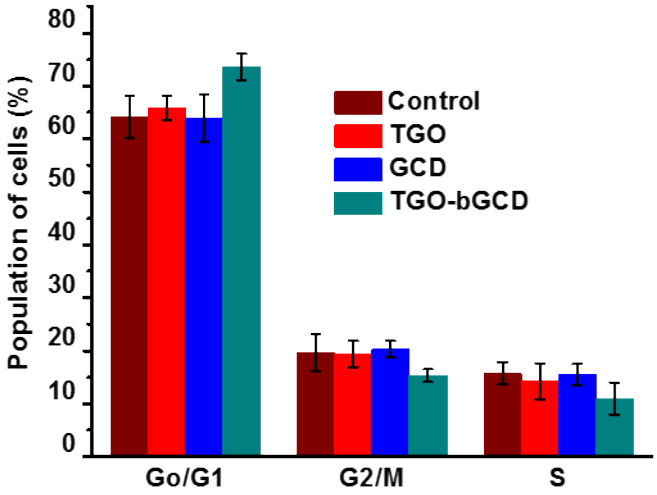

Figure S29. Quantitative analysis of cell cycle analysis in MCF-7 cells.