Electronic Supplementary Information

Chemicals

N-isopropylacrylamide (NIPAAM) was purchased from Across Organics (USA) and re-crystallized with N-hexane (Distilled) at 40^{o} C and dried under vacuum, stored at 4^{o}C. N-vinyl 2-pyrrolidone (VP) and Acrylic acid (AA) were purchased from Across Organics (USA) and freshly distilled before use. Malondialdehyde (MDA) was procured from Sigma (USA) and 3-(4, 5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, USA). Dulbecco Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), antibiotic Streptomycin (100X), Trypsin (with 0.5% EDTA), sodium chloride, potassium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate,ammonium per sulphate (APS) was bought from Hi-media, USA and ferrous ammonium sulphate (FAS) from Qualigens (India). MCF-7, MDA-MB 231 and Human Embryonic Kidney cells 293 (HEK-293) cell lines were procured from National Centre for Cell Science (NCCS), Pune, India.

Preparation of plant extract

The dried plant samples were powdered and 5 gm of the each sample was mixed with 20 mL ethanol and kept for 24 hour in an incubator shaker for maceration at 28 ± 2°C with gentle shaking at 100 rpm. This procedure was repeated 3 times consecutively. All there day filtrate fractions were collected in one flask and then solvent was subjected to the reflux at 40-45°C and when it remained up to 5 mL it was filtered and stored at 4°C until use in further experiments.

Table S1 Phytochemical screening of root extract of *I. turpethum*

Chemical constituents	Name of test	Root ethanolic extract
Alkaloids	Dragendroff's test	++
Steriods	Ferric chloride test	++
Triterpenes	Vanillin-sulphuric acid test	++
Tanin	Ferric chloride test	+
Glycoside	Keller-Killiani test	+++
Carbohydrate	Molisch test	++
Flavonoids	Alkaline Reagent test	+
Saponins	Lead acetate test	+++
Proteins	Ninhydrin test	-

+++ Strong positive; ++ Low positive; +Weak positive; - negative test.

Preparation and Characterization of polymeric nanoparticles

In the synthesis of nanoparticles the monomers NIPAAM, VP and AA (water soluble) were used in the molar ratio of 90:10: 5. The reaction was initiated and activated by APS and FAS respectively. The MDA was used as a cross-linker in the reaction. In detailed protocol we used 180 mg NIPAAM, 20 µL VP (freshly distilled) and 10 µL AA (freshly distilled) in 20 mL of double distilled water. The crosslinking of the monomers were achieved by adding the 150 µL of MDA (0.049 g/ml) and to remove the dissolved oxygen from the reaction nitrogen gas was passed for 30 min. To start the polymerization in the reaction 50 µL of FAS (5mg/L) and 50 µL of APS (saturated) were added. The polymerization of the reaction was achieved at the 32^{o}C for 24 hour under the nitrogen gas atmosphere. Once the reaction was completed the total solution was dialyzed for 24 hour by via cellulose dialyzing membrane that having the cut off of 12kDa. After that the complete dialyzed solution was lyophilized to dry for later use. Now, the characterization of synthesized nanoparticles was take place.

The DLS spectroscopy (Malvern Zeta sizer, Malvern, UK) was used to calculate the average size distribution as well as polydispersity of the synthesized particles at 25^{o}C in the solution. The size and the morphology of the synthesized particles were also confirmed by the help of transmission electron microscopy (Tecnai G2, 20 S–twin, FEI Electron Optics, Holland) equipped digital imaging and 35 mm photography system) at All India Institutes of Medical Sciences (AIIMS), New Delhi, India. The nanoparticles solution was diluted in the double distilled water for the TEM analysis and then applied on the carbon grid.

Drug entrapment

The physical entrapment method was used for loading the drug (extract of plant) onto the characterized nanoparticles. For this, 40 mg of the lyophilized nanoparticles were dissolved in the 20 mL double distilled water. The drug solution of 5mg/mL (plant extract) was prepared in ethanol and for the loading on to the nanoparticles it was added slowly in the solution with vortexing and minor sonication. The haziness and the formation of the precipitate in the solution determined the completion of the loading. After the completion of the loading the solution of drug loaded nanoparticles was lyophilized for the future use.

MTT assay to determine the cell viability

For this the cells were seeded on the 96 well plates and incubated for 24 hour. After that the cells were treated with the plant extracts, void nanoparticle and plant extract loaded nanoparticles respectively in different concentrations. The treated cells were then incubated for 24 hour and then MTT (Sigma Aldrich, Mumbai, India) solution (20 µL/ well of 5 mg/mL in PBS) was added and plates were incubated for 4 hour more. Then, the MTT was removed and produced formazan crystals were dissolved in 100 µL DMSO and gradually shaken. The absorbance was taken at

550 nm by the microplate reader (Spectra Max 340 PC, Molecular devices, USA). All these experiments were performed in triplicate and results were expressed in term of percentage viability of the cells with respect to the tested drugs.

Cellular uptake study

For this study, MCF-7 and MDA-MB-231 cells (10,000 cells/ mL) were cultured with the Rhodamine-B loaded nanoparticles and incubated for 3-4 hour and then washed 2-3 times with PBS. The cells were fixed with the 4% aqueous solution of paraformaldehyde, washed with PBS and visualized under the confocal microscopy (Leica DMRE) equipped with a confocal head TCS-SPE (Leica, Wetzlar, Germany)

CFSE cell proliferation assay

Cell proliferation was investigated with the BD Horizon™ CFSE (carboxy fluoresceindiacetate succinimidyl ester), which has 490nm and 520 nm excitation maximum and emission maximum respectively. CFSE covalently binds with the amino group of the protein and can be used for cytometric monitoring of the cell division. To perform this assay the cells were transferred into the 15 ml polypropylene tubes and washed 2-3 times with 1X DPBS. After that the CFSE dye (5μM) was added to the single cell suspension and incubated at 37 C for 15 min on water bath. Then, 9× the original volume of 1× DPBS was added to the cells and centrifuged at 1000 rpm for 5min to pellet the cells. The supernatant was discarded and pellet was gently mixed. To the pellet, 10 mL of the complete media (with 10% FBS) was added and the suspension was centrifuged and supernatant was decanted. The pellet was gentle mixed and re-suspended in complete media and then the CFSE tagged cells were seeded on the six well plate and then the cells were treated with the drug loaded nanoparticles and incubated for 24 hours. After that, the

cells were trypsenized, washed with PBS and cytomeric analysis was done by the BD-LSR II flow cytometer.

Annexin-V-FITC/PI assay to investigate apoptosis

For this study, cells were seeded on the 25 cm^2 flask and treated with the IC_{50} value of plant extract loaded nanoparticles (NVA-IT). Then, these treated as well as the control cells ($1x10^6$ cells/mL) were firstly re-suspended in Annexin V binding buffer and then incubated in dark for 15 min in 100 µL cell suspension with Annexin V-FITC. PI then spiked into 400 µL Annexin V binding buffer and added immediately to the cell suspension and subsequent analyzed using BD™ LSR II Flow Cytometer.

Cell cycle study by PI

MCF-7 and MDA-MB231 cells (1000 cells/mL) were cultured with plant extract loaded nanoparticles (NVA-IT) and incubated for 24hrs. After that the cells were trypsinized and re-suspended in 1mL 0.1 % sodium citrate congaing 0.05 mg PI and 50 µg RNase (Merck, Germany) and incubated for 30 min at room temp in the dark. Flow cytometric analysis was performed with the BD™ LSR II Flow Cytometer and BD FACS Diva 8.0.1 software.

DAPI nuclear staining

For this, the control and the treated cells were firstly fixed with the methanol and after that DAPI (1 µg/ mL) was added to the cells and incubated for 15min at 37 C. The morphological changes of the nucleus were examined and imaged with the confocal microscopy.

LCMS/MS analysis

For this, the protein was isolated by guanidium chloride method and quantified by Bradford's method (M. Bradford, 1976). Briefly, 25 µg of the sample were first reduced with 5 mM TCEP and further alkylated with 50 mM iodoacetamide and digested with trypsin (1:50, trypsin: lysate ratio) for 16 hours at 37°C. These digests were further cleaned up using C18 silica cartridge and dried using speed vac. The dried pellet was resuspended in Buffer- A (5 % acetonitrile / 0.1% formic acid).

1µg of the peptide mixture was loaded on precolumn and resolved using 15 cm Pico-Frit filled with 1.8 µm C18-resin (Dr. Maeisch). The peptides were loaded with Buffer A and eluted with a 0-40% gradient of Buffer-B (95% acetonitrile/0.1% Formic acid) at a flow rate of 300nl/min for 90 minutes. The QExactive was operated using the Top10 HCD data-dependent acquisition mode with a full scan resolution of 70,000 at m/z 400. MS/MS scans were acquired at a resolution of 17500 at m/z 400. Lock mass option was enabled for polydimethylcyclosiloxane (PCM) ions (m/z = 445.120025) for internal recalibration during the run. All the experiments were performed using EASY-nLC 1000 system (Thermo Fisher Scientific) coupled to QExactive mass spectrometer (Thermo Fisher Scientific) equipped with nano-electrospray ion source.

MS data was acquired using a data-dependent top10 method dynamically choosing the most abundant precursor ions from the survey scan.

All four raw files were analyzed with Proteome Discoverer 2.2 against the Uniprot Human reference proteome database. For Sequest HT and MS Amanda 2.0 search, the precursor and fragment mass tolerances were set at 10 ppm and 0.5 Da, respectively. The protease used to generate peptides, i.e. enzyme specificity was set for trypsin/P (cleavage at the C terminus of "K/R: unless followed by "P") along with maximum missed cleavages value of two.

Carbamidomethyl on cysteine as fixed modification and oxidation of methionine and N-terminal acetylation were considered as variable modifications for database search. Both peptide spectrum match and protein false discovery rate were set to 0.01 FDR and determined using percolator node. Relative protein quantification of the proteins were performed using Minora feature detector node of Proteome Discoverer 2.2 with default settings and considering only high PSM (peptide spectrum matches) confidence. Based on Uniprot accession number Pfam, KEGG pathways and GO annotations were assigned for the list of identified proteins. Heatmap was generated among C3-D3 and C4-D4, using Perseus software for visualization.

Table S2 List of some important significantly differentially expressed proteins in NVA-IT treated MCF-7 and MDA MB-231 cells with respect to the control cells; p value < 0.05.

Cell line	**Types of differentially expressed proteins**			
	Proliferation	**Vesicular trafficking**	**Apoptosis**	**Tumor suppressor**
MCF-7	L-lactate dehydrogenase B chain (LDHB)	Nuclear transport factor 2 (NUTF2)	Nucleolysin TIAR (TIAL1)	Endophilin-B1 (SH3GLB1)
	Receptor-type tyrosine-protein kinase FLT3 (FLT3)	Synaptosomal-associated protein 23 (SNAP23)	Survival of motor neuron-related-splicing factor 30 (SMNDC1)	Growth/differentiation factor 8 (MSTN)
	Actin-related protein 3C (ACT3C)	Mitogen-activated protein kinase 3 (MAPK3)	Serine/threonine-protein phosphatase PP1-gamma catalytic subunit (PPP1CC)	
	Platelet-derived growth factor receptor alpha (PDGFRA)	Mitogen-activated protein kinase 15 (MAPK15)		
	Platelet-derived growth factor receptor beta (PDGFRB)	Vacuolar protein sorting-associated protein 29 (VPS29)		
	Vascular endothelial growth factor receptor 3 (FLT4)	Serine/threonine-protein kinase NLK (NLK)		

	Coatomer subunit zeta-1 (COPZ1)	Exportin-1 (XPO1)		
	Vascular endothelial growth factor receptor 2 (KDR)			
	Vascular endothelial growth factor receptor 1 (FLT1)			
	Mast/stem cell growth factor receptor Kit (KIT PE)			
	Glutamine--tRNA ligase (QARS)			
	Probable RNA-binding protein 23 (RBM 23)			
	NADH-cytochrome b5 reductase 3(CYB5R3)			
	UDP-N-acetylhexosamine pyrophosphorylase-like protein 1(UAP1)			
	4F2 cell-surface antigen heavy chain (SLC3A2)			
	Aldo-keto reductase family 1 member B15 (AKR1B15)			
	Ribonucleoside-diphosphate reductase large subunit (RRM1)			
	RNA polymerase II subunit A C-terminal domain phosphatase SSU72 (SSU72)			
	Histone H1.5 (HIST1H1B)			
	UDP-N-acetylhexosamine pyrophosphorylase (UAP1)			
	Histone H2B type 1-A (HIST1H2BA)			
	Hemoglobin subunit beta (HBB)			
MDA MB-	Probable RNA-binding protein 23 (RBM 23)	Nuclear transport factor 2 (NUTF2)	Deoxyuridine 5'-triphosphate	Deoxyuridine 5'-triphosphate

231			nucleotidohydrolase, mitochondrial (DUT)	nucleotidohydrolase, mitochondrial (MSTN)
	Annexin A4 (ANXA4)	Protein transport protein Sec61 subunit alpha isoform 2 (SEC61A2)	Acidic leucine-rich nuclear phosphoprotein 32 family member E (ANP32E)	Cornifin-A (SPRR1A)
	UDP-N-acetylhexosamine pyrophosphorylase-like protein 1 (UAP1L1)	Protein transport protein Sec61 subunit alpha isoform 1(SEC61A1)	Twisted gastrulation protein homolog 1 (TWSG1)	
	SHC-transforming protein 1 (SHC)		Serine/threonine-protein phosphatase PP1-gamma catalytic subunit (PPP1CC)	
	Putative hexokinase HKDC1 (HKDC1)		Ubiquitin-60S ribosomal protein L40 (UBA52)	
	Superoxide dismutase [Mn], mitochondrial (SOD2)			
	General transcription factor IIF subunit 1 (GTF2F1)			
	Hexokinase-1 (HK1)			
	Guanine nucleotide-binding protein subunit beta-4 (GNB4)			
	Ferritin heavy chain (FTH1)			

Statistical analysis

Out of total protein groups and peptides groups identified in Thermo Proteome Discoverer against Human database, 1488 proteins were filtered out based on Abundance counts (=1). Differential analysis was carried out further using these 1488 proteins. To get an overview of the

overall data, Box and whisker plot was plotted. In differential analysis, fold change was observed among: C3-D3 and C4-D4. Corresponding heatmaps were plotted for the same based on z-score normalized Abundance values using Perseus software (v1.6.0).GO annotations among the comparisons, were represented using Pie charts for Biological process, Cellular Component and Molecular Function using Thermo Proteome Discoverer software.

Real time analysis

For this analysis, firstly we isolated the total RNA from the control cells (untreated) as well as from treated cells of both cancer cell lines by RNA isolation kit (Nucleopore, Genetix Biotech Asia). The quantification of the RNA samples was done by NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific) and the A260/A280 ratio was used for purity valuation. The corresponding cDNAs were synthesized by using 1500 ng normalized RNA samples with the help of RevertAidTM H Minus first strand cDNA synthesis kit (Thermo Fisher Scientific). The integrity of the cDNA was insured by performing PCR with the GAPDH specific primers (Table 7). The PCR was performed by using the reaction; 1 µl cDNA, 1X Taq buffer, 1U Taq polymerase, 1.5 mM MgCl2, 0.4 mM dNTPs, 0.4 µM each primers; with the conditions (i) 95 ºC for 3 min (initial denaturation) (ii) 40 cycles at 95 ºC for 30 sec, 58 ºC for 30 sec, 72 ºC for 1 min (iii) final extension (72 ºC for 5 min). The real-time PCR was carried out with the help of SYBR green dye chemistry on the Roche LC 480 light cycler (Roche, USA). The 10µl reaction of the real-time PCR contained 5µL of Maxima SYBR Green qPCR Master Mix (Thermo Fisher Scientific), 0.4 µM of each primer (Table 7), 1 µl of tenfold diluted cDNA, and nuclease-free water. The conditions used for the thermal cycler were (i) 95 ºC for 5 min (initial denaturation), (ii) 45 cycles at 95 ºC for 30 s, 62 ºC for 30 s, and 72 ºC for 30 s, followed by (iii) melt curve analysis at temperature range of 55 ºC to 95 ºC. The Ct values were calculated via constant

threshold value for all the genes studied. The comparative ΔCt (2- ΔΔCT) method was used for the quantification of comparative gene expression and *GAPDH* was used as the normalizer gene.

Table S3 List of the primers used for the real-time PCR analysis

S. No.	Name	Sequence	Tm	GC Content
1.	LDHB	GACGTGCGGGGTAGTACTTG	60.46	60.00
		TTTTGCACAAGGACAAGTAGGG	59.04	45.45
2.	GNB4	GAGTTGGCTGCTGGGAGG	60.05	66.67
		TGAAAACAGCTGGAGGGAGC	60.25	55.00
3.	PPP1CC	TGTAGAGCCCATCAGGTGGT	60.25	55.00
		TGCTTGCTTTGTGATCATACCC	59.25	45.45
4.	GAPDH	CAAGGTCATCCATGACAACTTTG	58.20	43.48
		GTCCACCACCCTGTTGCTGTAG	63.08	59.09

BLI (Bio-layer interferometry) analysis

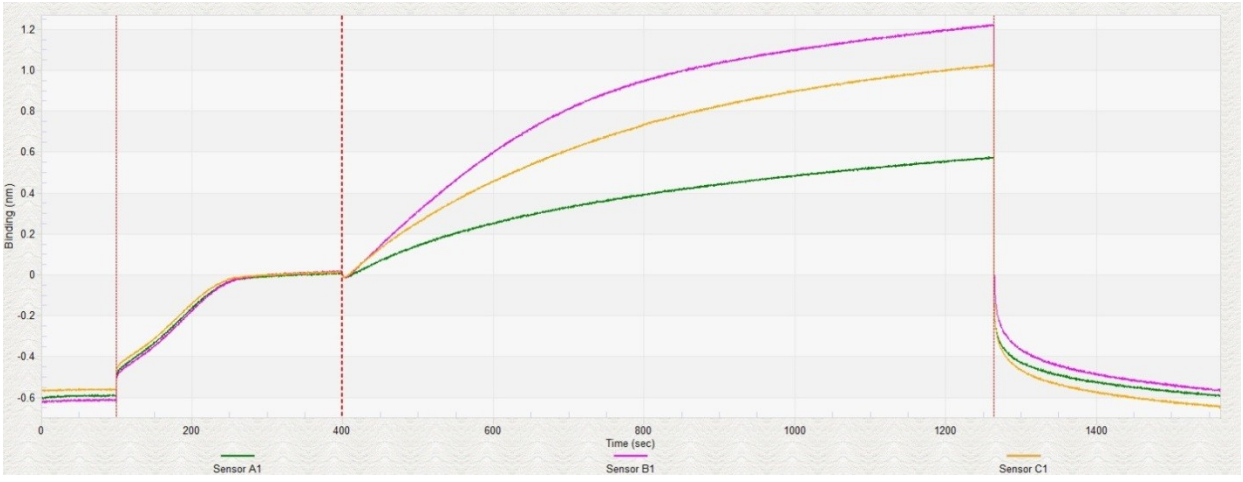

Figure S1 Schematic representation of the BLI experiment for the three biosensors (β-actin, TIAL1 and UBA52).The sensor A1, B1 and C1 represent β-actin, TIAL1 and UBA52 respectively. The association and dissociation steps are shown.