Supporting Information

A new triazine based π-conjugated mesoporous 2D covalent organic framework: It's in vitro anticancer activities

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Section S1

Experimental Section

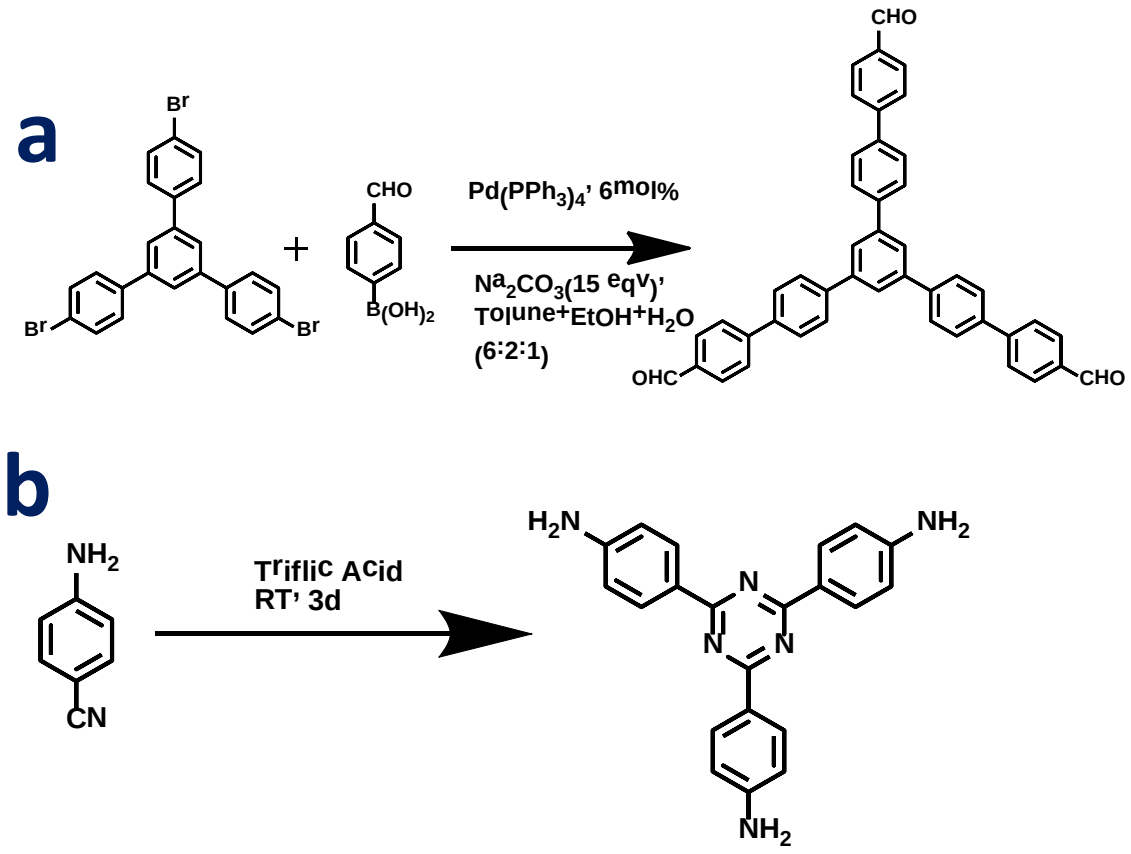
Cell Lines and Chemicals:

Human colorectal carcinoma (HCT 116) cell line was obtained from National Centre for Cell Sciences (NCCS), Pune, India. Constituents of cell culture media viz. Dulbecco's Modified Eagle Medium (DMEM), Fetal bovine serum (FBS), Penicillin-Streptomycin-neomycin (PSN) antibiotic cocktail, trypsin and ethylenediaminetetraacetic acid (EDTA) were procured from Gibco (Grand Island, NY, USA). Other necessary raw and fine chemicals were procured from Sisco Research Laboratories (SRL), Mumbai, India, and Sigma-Aldrich, St. Louis, MO, USA, respectively. Antibodies were obtained from Santa Cruz Biotechnology, Dallas, Texas, USA and eBioscience, San Diego, USA. 2,4,6-tris(4-aminophenyl)-1,3,5-triazine (TAPT) and 1,3,5-tri(4-formylbiphenyl) benzene (TFBPB*)* were synthesized by previously reported procedure.[1,2] Tetrakis (triphenylphosphine) palladium(0), was purchased from Sigma-Aldrich. 4-fromyl phenyl boronic acid, dimethyl acetamide, *o*-dichlorobenzene were received from Spectrochem, India. Other solvents, acid, bases were supplied from the local commercial source. All reagents were supplied from best available commercial sources and were used without further purification.

Instrumentation:

The PXRD patterns of the TrzCOF material was collected from an X'Pert PRO of PAN analytical diffractometer using Cu Kα (= 0.15406 nm) radiation. FTIR spectra of the as synthesized material as well as the materials after analysis were taken by using Perkin-Elmer Spectrum 100 spectrophotometer. A 500 MHz Bruker Advance III spectrometer was used to record solid state ^{13}C CP MAS NMR spectrum of TrzCOF at a MAS

frequency of 10 kHz. To measure surface area and porosity of the COF material, Soxhlet extraction of the sample was done using methanol as solvent. Then the sample has been activated at 150 ^{0}C under high vacc to remove the solvent molecule from the materials surface for the generation of guest free material for sorption analysis. N_2 adsorption/desorption isotherms was recorded using an Autosorb iQ-MP (Quantachrome Instruments, USA) at 77 K. The sample was first kept in oven for overnight at 120 ^{o}C. Prior to the experiment the powdered sample was degassed at 140 °C for 5 h. From the N_2 adsorption/desorption isotherms, Non-Local Density Functional Theory (NLDFT) was applied to calculate pore size distribution (PSD) of the porous COF material. Morphological studies have been carried out by using both scanning electron microscopic and transmission electron microscopic images using a JEOL JEM 6700F and a JEOL JEM 2010 transmission electron microscope, respectively. X-ray photoelectron spectroscopy (XPS) experiment was performed using an Omicron nanotech operated at 15 kV and 20 mA. Thermal stability of TrzCOF was investigated with TA-SDT Q-600 of TA instruments by heating the powder material at heating ramp of 10°C/min under N_2 atmosphere from 25 to 800 ^{o}C. The ^{1}H and ^{13}C NMR spectra were collected using a 400 MHz Bruker AVANCE II spectrometer.



Scheme S1: Synthesis scheme of a) 1,3,5-tri(4-formylbiphenyl)benzene (TFBPB) and b) 2,4,6-tris(4-aminophenyl)-1,3,5-triazine (TAPT).

Synthesis of 2,4,6-tris(4-aminophenyl)-1,3,5-triazine (TAPT):

TAPT was synthesized following a reported procedure with a little variation (Scheme S1).[1] A 50 mL round bottom flask was equipped with an ice bath and 4-Amino benzonitrile (2 gm) was taken into it. 2 mL of triflic acid was added to the flask drop-wise and the solution was then allowed to stir for 48h at room temperature. The reaction mixture was poured into 200 mL of ice cold water and neutralized with 2 (M) NaOH solution. With the progress of addition of NaOH solution clear deep yellow coloured solution appeared and after the neutral point immediate deep yellow precipitation occurred. The precipitate was collected by filtration and washed with plenty of water followed by vacuum dry to obtained constant weight of the pure compound. Finally, 1.8 g of pure compound was collected. The ^{1}H and ^{13}C NMR spectra with

NMR data of 2,4,6-tris(4-aminophenyl)-1,3,5-triazine (TAPT) are represented in the Supporting Information (Figure S1 and S2).

^{1}H NMR (DMSO-d^{6}, 500 MHz, ppm): δ 8.36 (d, *J* = 8.8 Hz, 6H), 6.70 (d, *J* = 8.4 Hz, 6H), 5.89(s,6H).

^{13}C NMR (DMSO d^{6}, 125 MHz, ppm): δ 169.5, 152.9, 130.6, 130.1, 122.9 and 113.

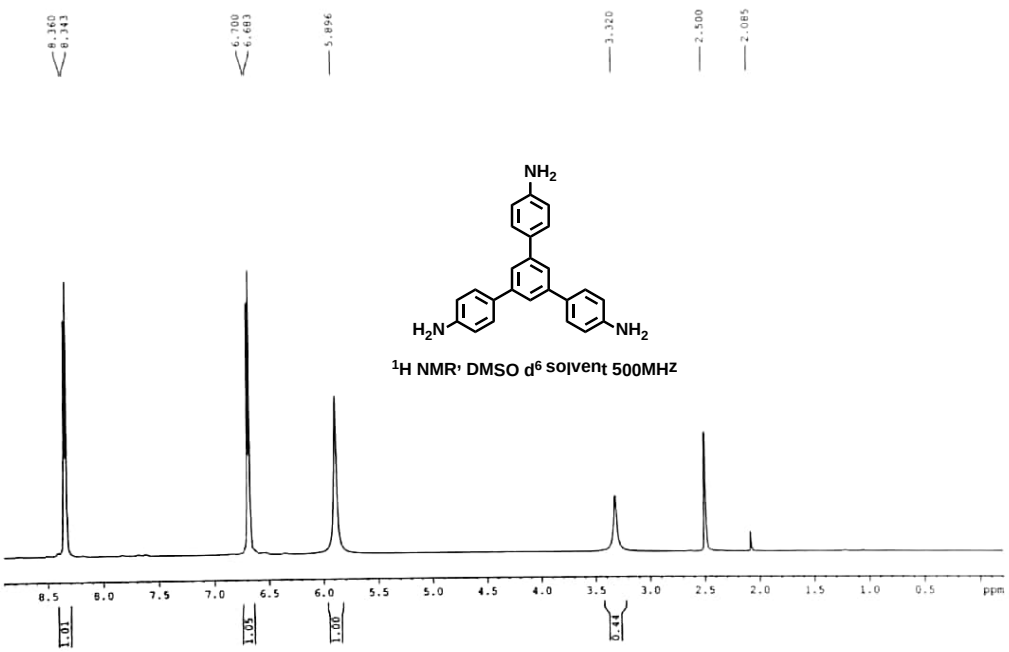


Figure S1: ^{1}H NMR spectra of 2,4,6-tris(4-aminophenyl)-1,3,5-triazine (TAPT).

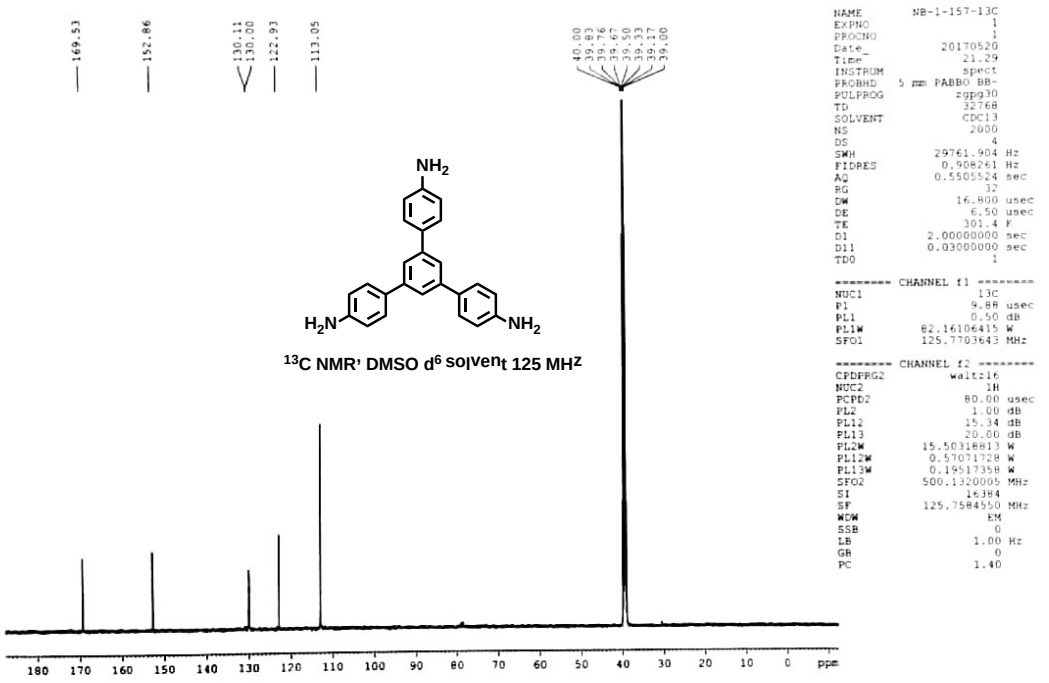


Figure S2: ^{13}C NMR spectra of 2,4,6-tris(4-aminophenyl)-1,3,5-triazine (TAPT).

Synthesis of 1,3,5-tris(4-bromophenyl)benzene:

Following a literature reported procedure,[2] to a stirred solution of *p*-bromoacetophenone (4.5gm, 20.5 mmol) in dry ethanol (7ml), 2.75 ml (37.5 mmol) of thionyl chloride was added dropwise. The mixture was refluxed for 2.5 hour and then cooled to room temperature. The solid obtained was purified by filtration with plenty of water, ether followed by recrystallisation from anhydrous ethanol. The amount of the analytically pure product was 3.8 gm (84%). The ^{1}H and ^{13}C NMR spectra with NMR data of 1,3,5-Tris(4-bromophenyl)benzene were provided in the Supporting Information (Figure S3 and S4).

^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ 7.69 (s, 3H); 7.60 (d, J = 8.8 Hz, 6H); 7.53 (d, J = 8.4 Hz, 6H).

^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ 141.7, 139.8, 132.2, 129.0, 125.1, 122.3.

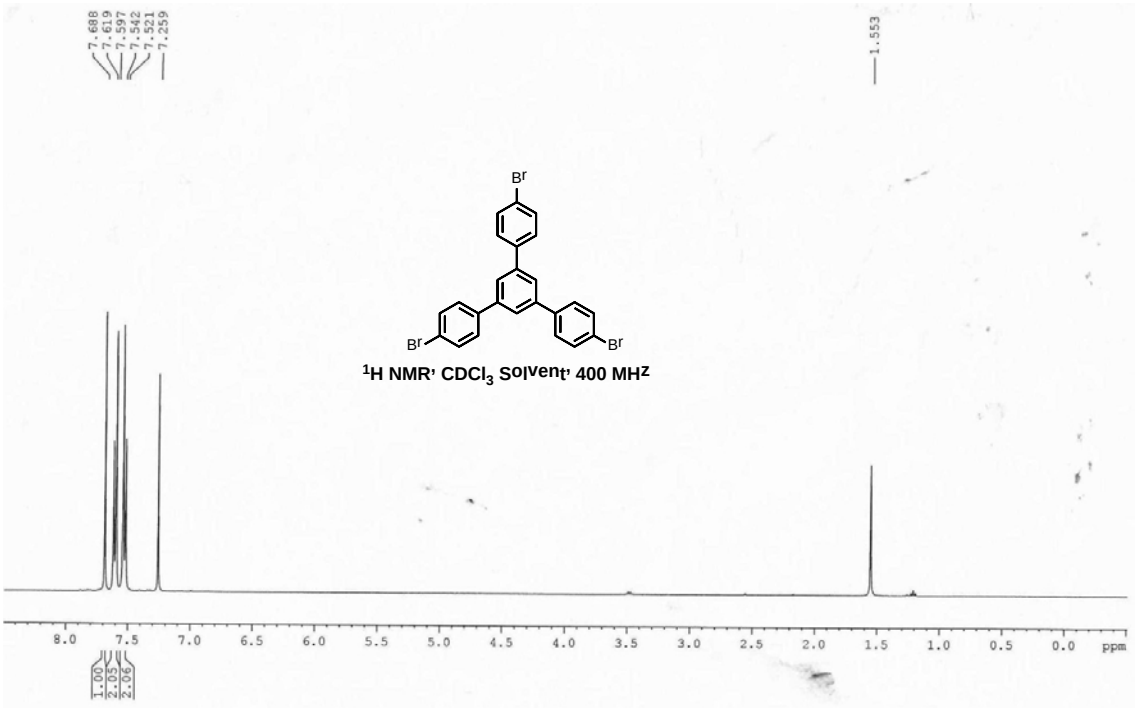


Figure S3: ^{1}H NMR spectra of 1,3,5-Tris(4-bromophenyl)benzene.

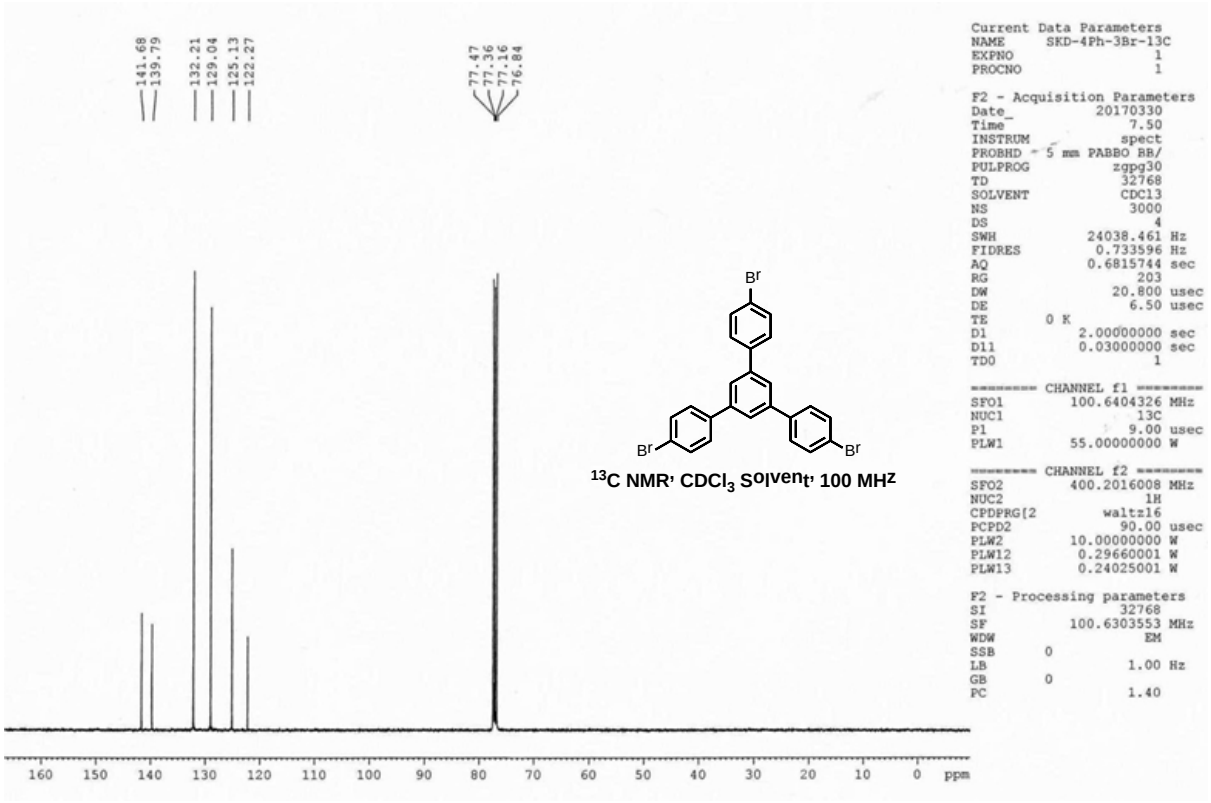


Figure S4: ^{13}C NMR spectra of 1,3,5-Tris(4-bromophenyl)benzene.

Synthesis of 1,3,5-tri(4-formylbiphenyl)benzene (TFBPB):

TFBPB was synthesized following a reported procedure with slight modification Scheme S1.[2] 1,3,5-Tris(4-bromophenyl)benzene (1g, 1.84 mmol), (4-formylphenyl) boronic acid (994 mg, 6.62mmol) and Na_2CO_3 (320 mg, 15.9 mmol) were dissolved in

a mixture of degassed toluene (20 mL), water (3.3 mL) and ethanol (6.6 mL) taken in a 100 mL Schlenck tube. Then the catalyst, $Pd(PPh_3)_4$ (138 mg, 0.119 mmol) was added under Argon flow and the mixture was degassed three times again. The resulting solution was then heated at 90 °C for 72 h. The combined layer was evaporated under reduced pressure. The crude product was purified directly without any workup by chromatography on silica gel using n-hexane/ EtOAc/acetone (7.5:1.5:1) as the eluent to give the title 1,3,5-Tris(4-bromophenyl)benzene as an off-white solid (960 mg, 84%). The ^{1}H and ^{13}C NMR spectra with NMR data of 7Ph3CHO were provided in the Supporting Information (Figure S5 and S6).

^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 10.09 (s, 3H); 8.0 (d, J = 8.0Hz, 6H); 7.92 (s, 3H); 7.87–7.84 (m, 12H); 7.8 (d, J = 8.4Hz, 6H).

^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 192.0, 146.7, 142.0, 141.2, 139.3, 135.6, 130.5, 128.1, 128.1, 127.8, 125.5.

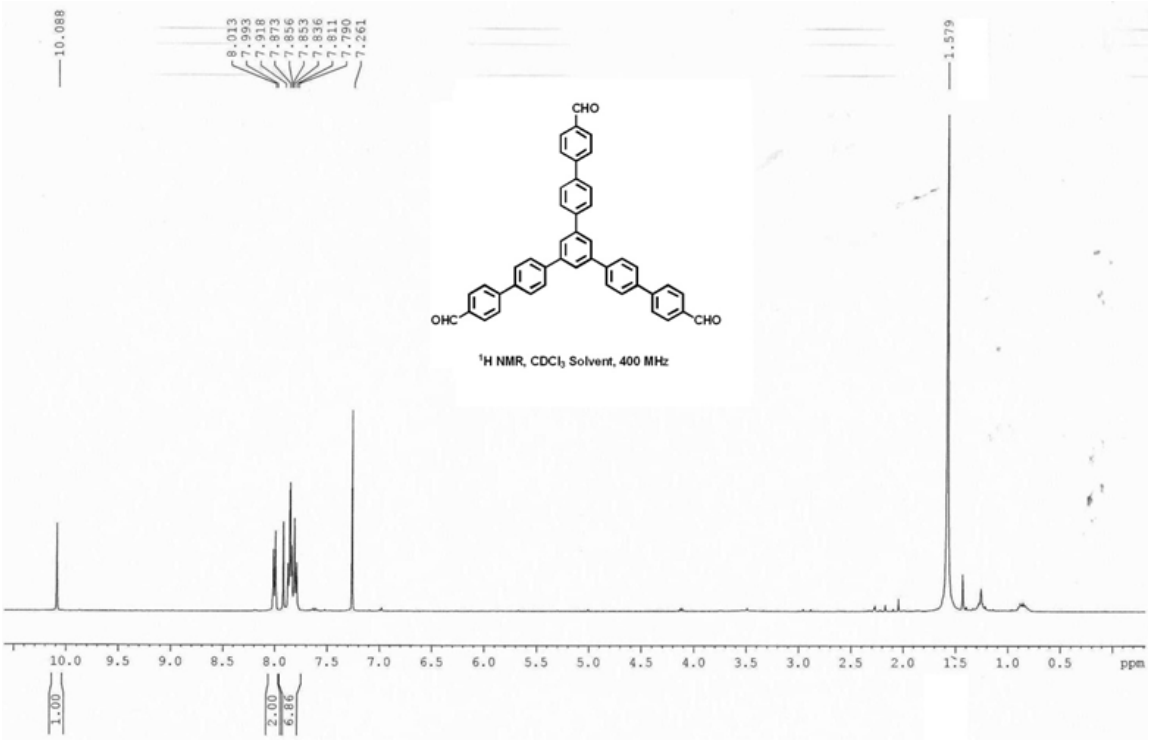


Figure S5: ^{1}H NMR spectra of 1,3,5-tri(4-formylbiphenyl)benzene (TFBPB).

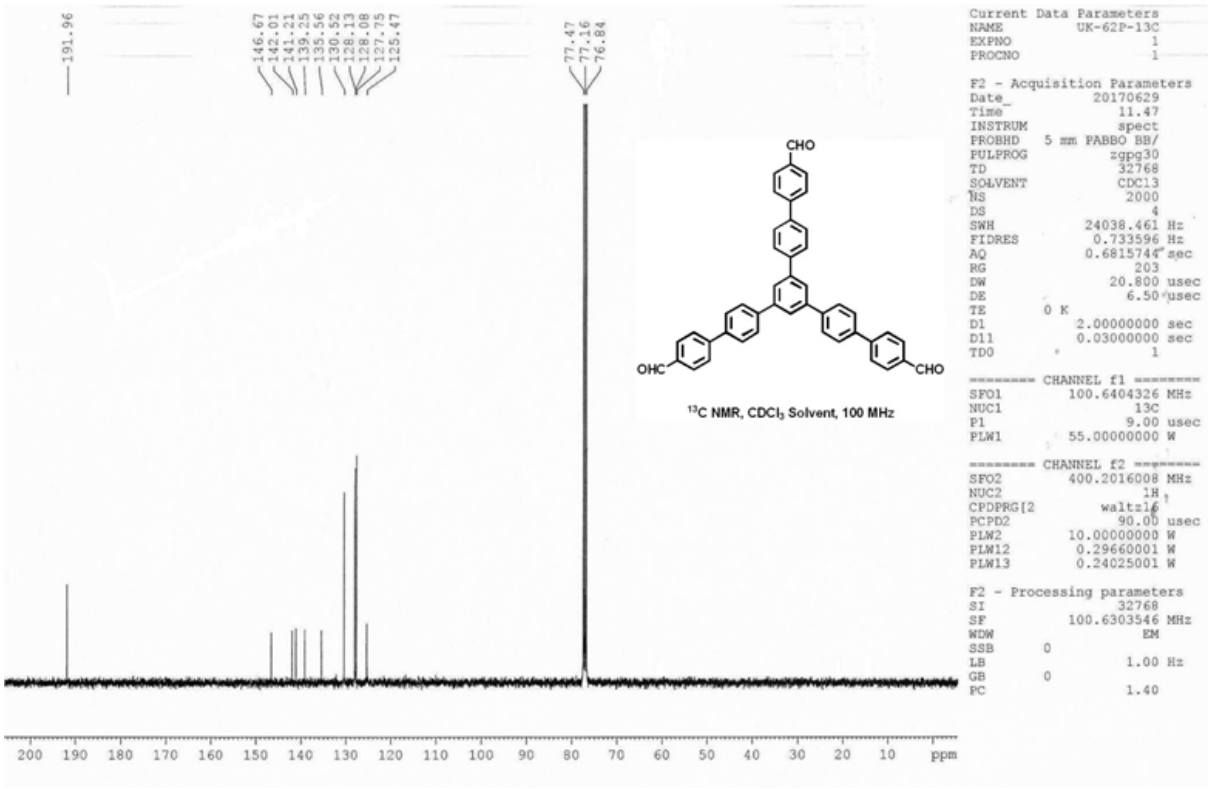


Figure S6: ^{13}C NMR spectra of 1,3,5-tri(4-formylbiphenyl)benzene (TFBPB).

Synthesis of TrzCOF:

185.62 mg (0.3 mmol) of TFBPB and 106.32 mg (0.3 mmol) of TAPT were taken in a schlenk tube (15 mL). 3 ml of a 1:1 mixture of *o*-dichlorobenzene (*o*-DCB) : ethanol (1:1) mixture with 0.2 mL of acetic acid (6M) were added to the amine and alhedyde mixture followed by 20 min sonication (Scheme 1). Then the reaction mixture was degassed via freeze-pump-thaw cycles for three times. The tube was then heated statically at 120 °C for 4 days. The yellow precipitate was obtained through centrifugation, washed several times with ethanol (EtOH), methanol (MeOH), tetrahydrofuran (THF) and acetone. The yellow coloured material remains insoluble in different common organic solvents. Soxhlet extraction has been done with MeOH :THF

(1:1) mixture for two days to remove the trapped guest molecules. The powder was finally washed with dry acetone and dried at 120 °C under vacuum for 20h to produce guest free porous TrzCOF (isolated yield 88 %).

Cell culture:

HCT 116 cells were cultured in DMEM complemented with 10% fetal bovine serum (FBS) and 1% antibiotic (PSN) at 37°C in a humidified atmosphere under 5% CO2. After getting 75–80% confluence, cells were harvested with 0.25% trypsin and 0.52 mM EDTA in phosphate buffered saline (PBS) and seeded at the essential density to allow them to re-equilibrate for 24 h before starting the experiment.

Cell Viability:

MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was used to evaluate cell viability as previously described. In each well of a 96-well culture plate $4x10^3$ cells kept in an incubator followed by treatment with different concentrations of TrzCOF(With or without NAC) at concentrations ranging from 0-80 µg/ml for 12 h and 24 h. After the respective treatment time, 20 µl of 5 mg/ml MTT stock solution was added in each well. After additional 4 h incubation at 37 °C, the resulting intracellular formazan crystals were solubilized with acidic isopropanol and the absorbance of the solution was measured at 595 nm using an ELISA reader (Model: Emax, Molecular device, USA). Absorbance (O.D.) of medium containing with the different concentrations of the TrzCOF (0-80 µg/ml) and MTT reagents was subtracted from the respective experimental sets and then viability percentage was calculated. After getting the half maximal inhibitory concentration (IC50) of 8 µg/ml from the initial MTT assay, we have again checked the cell viability with the constant dose of 8 µg/ml of TrzCOF and pretreated with 2 mM of ROS scavenger (NAC) and siRNAp53 and the viability percentage was calculated.

Determination of Intracellular ROS (iROS):

Time dependent ROS generation was evaluated by DCF-DA. After treatment with 8 µg/ml of TrzCOF, the cells were incubated with 10 mM of DCF-DA at 37°C for 15 min. Then the samples were analyzed at an excitation wave length of 480 nm and an emission wave length of 525 nm by a flow cytometer (BD LSRFortessaTM TM San Jose, CA, USA). In this experiment, 2 mM NAC (N-acetyl-cysteine) was also used before 1 h of TrzCOF treatment to confirm the ROS-mediated cell death mechanism, where it served as a negative control.

7-AAD Staining for Determining Live and Dead Cells:

7-AAD (7-amino-actinomycin D) has a high DNA binding constant and is efficiently excluded by intact cells. It is useful for DNA analysis and dead cell discrimination during flow cytometric analysis. 1 µL 7-AAD stock solution from 1mg/ml solution of 7-AAD has been added after treatment with 8 µg/ml of TrzCOF time dependently. Then the samples were analyzed by BD LSRFortessaTM. 2 mM NAC was also used before 1 h of TrzCOF as a negative control.

Quantification of Apoptotic Cell Death:

Apoptotic and necrotic cell death was estimated by using the Annexin-V FITC/DAPI apoptosis detection kit (Calbiochem, CA, USA). After treatment of TrzCOF, HCT116 cells were washed and stained with DAPI and Annexin-V-FITC as per manufacturer's instructions. The percentages of live, apoptotic (early and late) and necrotic cells were calculated using a flow cytometer (BD LSRFortessa TM San Jose, CA, USA). NAC has been used as a negative control.

Measurement of Mitochondrial Membrane Potential:

TrzCOF treated HCT116 cells were incubated with the cationic dye, JC-1(5,5′,6,6′-tetrachloro-1,1′,3,3′ tetraethyl benzimidazolyl carbocyanine iodide) followed by flow

cytometric (BD LSRFortessaTM TM San Jose, CA, USA) analysis according to the manufacturer protocol. JC-1 display potential dependent accretion in mitochondria and establish a fluorescence emission shift from green (525 nm) to red (590 nm). Therefore the change of fluorescence intensity indicates the percentage of depolarized and hyperpolarized mitochondria on the basis of the resultant fluorescence of aggregation of JC-1 monomer.

Analysis of Protein Expression by Flow Cytometry:

Briefly, the TrzCOF treated cells were fixed with 4% paraformaldehyde in PBS (pH 7.4) for 15-20 min at room temperature. After that the cells were permeabilized with 0.1% Triton X-100 in PBS along with 0.1% FBS for 5min. After permeabilization the cells gone through the blocking step for 30 min. Then permeabilized cells were incubated with primary antibody (Gamma H2AX, pATM, Phospho-p53^{Ser46}) overnight at 4°C. The cells were then incubated with respective fluorescence-conjugated goat anti-rabbit IgG as a secondary antibody for 2 h at room temperature and washed in PBS before performing flow cytometry. The mean fluorescence intensities (MFI) were calculated using Flow Jo (Version 10.0) software.

Western Blot Analysis:

Immunoblotting is a extensively used analytical technique used to detect specific proteins. Briefly, the cell lysate was separated by SDS-PAGE (10-15%) followed by an optimized transfer to PVDF membranes (Millipore, Bedford, MA). The membranes were then blocked using 5% bovine serum album (BSA) for 2 h and incubated overnight with respective anti rabbit/mouse primary antibodies (Bcl_2 , Bak and β-Actin) at 4°C. B-Actin was used as a loading control. Further, the membrane was washed with alkaline phosphatise conjugated secondary antibodies before the incubation for 2 h at room temperature. At last, the resultant protein expression was visualized using chromogenic

substrates, NBT-BCIP. The intensities of each immunoblot were measured using ImageJ software and represented as mean relative intensities of each protein.

Measurement of Caspase 3 and Caspase 9 Activity:

Caspase 3/9 activities were measured according to the manufacturer's instructions with commercially existing caspase 3 and caspase 9 colorimetric assay kits (Bio Vision Research Products, Mountain View, CA) correspondingly. Caspase activities were determined at 405 nm by an ELISA reader.

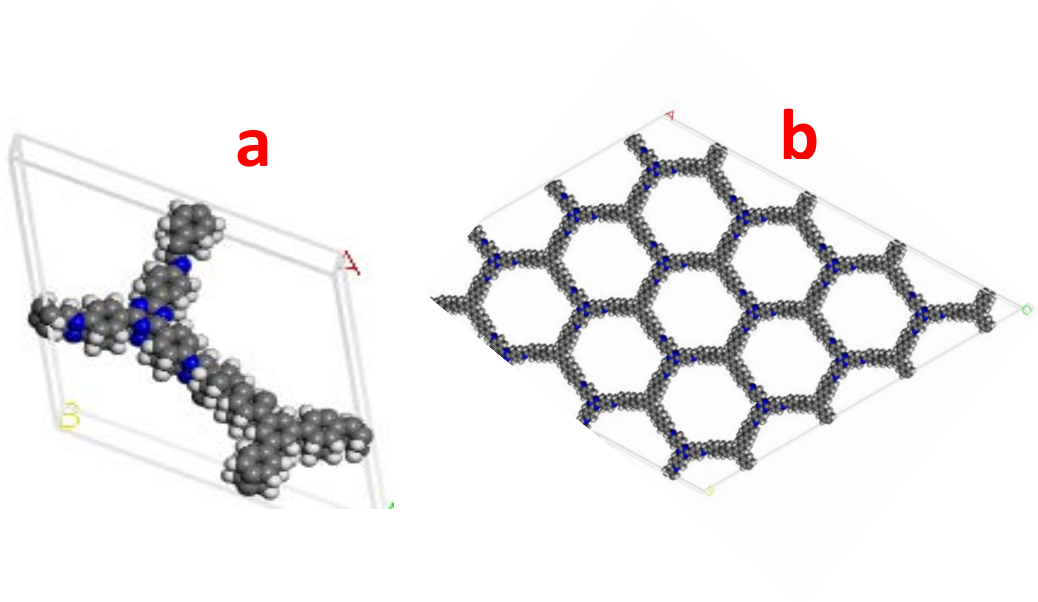


Figure S7: Asymmetric unit and hexagonal polymer framework of TrzCOF.

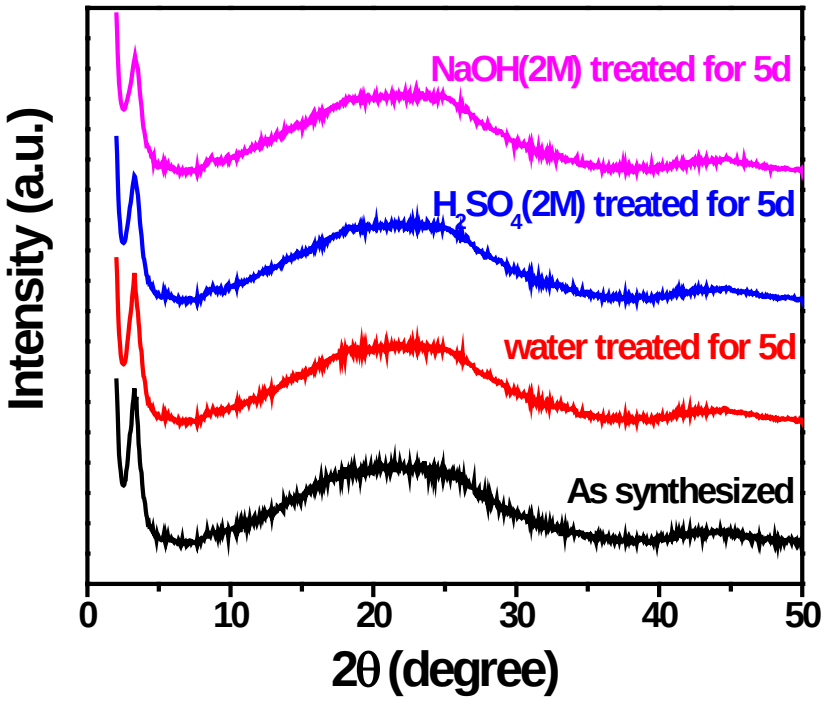


Figure S8: PXRD of TrzCOF before and after water, NaOH (2M) and H_2SO_4 (2M) solution treatment for 5 days.

Section S2: Unit cell analysis of TrzCOF

Unit cell of the hexagonal TrzCOF was generated by using Material Studio 5.0.[3] The structure of the COF was optimised by using density-functional tight-binding (DFTB+) program including Lennard-Jones (LJ) dispersion. Standard DFTB parameters were employed from the mio set, where the space group was selected as P_1.[4] The lattice parameters were optimized simultaneously with the geometry. We performed Pawley refinement by taking DFTB optimized structure to evaluate the lattice parameters, until the RWP value converges. The functions for whole pseudo-Voigt profile fitting and instrument geometry as Bragg-Brentano were used during the refinement processes.

Table S1. Fractional atomistic coordinates of the COF after Pawley refinement

Space group	P_1		
Unit cell	a = 33.391Å, b = 34.527Å, c = 6.977Å, α = 89.937°, β = 89.953°, γ = 59.126°		
Atom	x	y	z
N	0.54676	0.67452	0.00525
N	0.47413	0.67669	0.0057
N	0.54555	0.60606	0.00542
C	0.47223	0.60685	0.00592
C	0.49685	0.55901	0.00639

C	0.42277	0.63069	0.00573
C	0.47311	0.5354	0.00666
H	0.53551	0.54031	0.00661
C	0.39875	0.60729	0.00592
H	0.40326	0.66808	0.00545
C	0.42337	0.55927	0.00631
H	0.49388	0.49808	0.00726
H	0.36013	0.62613	0.00571
C	0.41464	0.49519	0.00476
H	0.45366	0.47085	0.00235
N	0.39612	0.53874	0.00673
C	0.38631	0.47374	0.00538
C	0.40906	0.42594	0.0044
C	0.33677	0.49866	0.00673
C	0.38363	0.40399	0.00455
H	0.44772	0.40553	0.00353
C	0.31143	0.47653	0.00684

H	0.31803	0.53601	0.00757
C	0.33397	0.42854	0.00563
H	0.40357	0.36667	0.00369
H	0.27288	0.49769	0.00786
C	0.30731	0.40469	0.00547
C	0.25774	0.42825	0.00499
C	0.33019	0.35678	0.00577
C	0.23299	0.40553	0.00492
H	0.23713	0.46559	0.00465
C	0.30544	0.33409	0.00564
H	0.36872	0.33577	0.00621
C	0.25587	0.35762	0.00524
H	0.19446	0.42658	0.00459
H	0.32614	0.29677	0.0059
C	0.22936	0.33345	0.00512
C	0.25251	0.28567	0.00519
C	0.17994	0.35781	0.0048

C	0.22776	0.26236	0.00507
H	0.29103	0.26603	0.00528
C	0.15369	0.33604	0.00464
H	0.16132	0.39501	0.00459
C	0.17834	0.28825	0.0049
H	0.15847	0.27071	0.00496
C	0.25299	0.21197	0.00502
C	0.22926	0.18757	0.00465
C	0.30249	0.18568	0.00527
C	0.25332	0.14027	0.00462
H	0.19068	0.20544	0.00439
C	0.3265	0.1385	0.00528
H	0.32375	0.20186	0.00552
C	0.30287	0.11403	0.005
H	0.23227	0.12382	0.00431
H	0.36503	0.12085	0.0056
C	0.32929	0.06377	0.00513

C	0.37894	0.0393	0.00436
C	0.30697	0.03795	0.00601
C	0.40464	0.99217	0.00423
H	0.39865	0.05702	0.00375
C	0.33266	0.99064	0.00597
H	0.26842	0.05493	0.00671
C	0.3822	0.96669	0.00495
H	0.44328	0.97449	0.00359
H	0.31412	0.9717	0.00657
C	0.62047	0.60156	0.00456
C	0.64331	0.55378	0.00449
C	0.64801	0.622	0.00405
C	0.69205	0.52723	0.00388
H	0.62192	0.53767	0.005
C	0.69681	0.59567	0.00348
H	0.63061	0.6593	0.00418
C	0.71979	0.54763	0.00325

H	0.70943	0.48997	0.0038
H	0.71691	0.61306	0.00338
C	0.47333	0.74741	0.00558
C	0.42384	0.77178	0.00517
C	0.49689	0.77182	0.00616
C	0.39869	0.819	0.00529
H	0.40526	0.75286	0.0048
C	0.47199	0.81906	0.00637
H	0.53555	0.75309	0.00654
C	0.42223	0.8436	0.00583
H	0.36007	0.83764	0.00493
H	0.49188	0.83665	0.00709
C	0.79772	0.5324	0.99973
H	0.78605	0.5695	0.99627
C	0.41131	0.91717	0.00434
H	0.45017	0.90364	0.00198
N	0.76861	0.51801	0.00312

N	0.39385	0.89111	0.00625
C	0.1016	0.36295	0.00398
C	0.07715	0.41085	0.00491
C	0.07374	0.34271	0.00225
C	0.02843	0.43668	0.00404
H	0.09603	0.4294	0.00653
C	0.02484	0.36862	0.00123
H	0.09007	0.30555	0.00156
C	4.60E-4	0.41657	0.00198
H	0.01246	0.47375	0.00524
H	0.00561	0.35036	0.99964
C	0.9486	0.44497	8.90E-4
C	0.91914	0.42635	0.00309
C	0.92628	0.49286	0.99769
C	0.87039	0.45389	0.00259
H	0.93432	0.38932	0.00548
C	0.87772	0.52042	0.99745

H	0.94687	0.50969	0.99516
C	0.8486	0.50165	5.00E-5
H	0.84856	0.43827	0.00438
H	0.86183	0.55757	0.99515
C	0.22839	0.33281	0.50533
C	0.15796	0.33499	0.50472
C	0.2273	0.26641	0.50514
N	0.17993	0.28927	0.50478
N	0.25255	0.2871	0.5054
N	0.18114	0.35772	0.50504
C	0.25446	0.35693	0.5054
C	0.22984	0.40477	0.50489
C	0.30392	0.3331	0.50567
C	0.25358	0.42838	0.5046
H	0.19117	0.42347	0.50459
C	0.32794	0.3565	0.50548
H	0.32343	0.29571	0.50589

C	0.30332	0.40451	0.50507
H	0.23281	0.46571	0.50379
H	0.36655	0.33765	0.50571
C	0.31205	0.46859	0.50792
H	0.27304	0.49292	0.51223
N	0.33057	0.42504	0.50438
C	0.34038	0.49004	0.50695
C	0.31763	0.53784	0.50898
C	0.38991	0.46513	0.50453
C	0.34306	0.55979	0.50867
H	0.27897	0.55825	0.51084
C	0.41526	0.48725	0.50424
H	0.40865	0.42778	0.50293
C	0.39272	0.53525	0.50633
H	0.32313	0.59711	0.51049
H	0.4538	0.4661	0.50223
C	0.41938	0.5591	0.50618

C	0.46895	0.53554	0.50641
C	0.39649	0.607	0.50584
C	0.4937	0.55826	0.50626
H	0.48956	0.49819	0.50678
C	0.42125	0.62969	0.50578
H	0.35796	0.62801	0.5055
C	0.47081	0.60616	0.50595
H	0.53223	0.5372	0.50641
H	0.40055	0.66702	0.50554
C	0.49733	0.63033	0.50576
C	0.47417	0.67812	0.50587
C	0.54675	0.60598	0.5054
C	0.49893	0.70142	0.50565
H	0.43566	0.69775	0.50616
C	0.573	0.62775	0.50518
H	0.56536	0.56877	0.50523
C	0.54834	0.67554	0.50534

H	0.56822	0.69307	0.5052
C	0.4737	0.75182	0.50566
C	0.49743	0.77621	0.50609
C	0.4242	0.77811	0.50515
C	0.47337	0.82352	0.50611
H	0.53601	0.75835	0.50646
C	0.40018	0.82528	0.50509
H	0.40294	0.76193	0.50473
C	0.42382	0.84975	0.50562
H	0.49441	0.83996	0.50657
H	0.36165	0.84293	0.50447
C	0.3974	0.90002	0.50568
C	0.34775	0.92448	0.50761
C	0.41971	0.92583	0.50389
C	0.32205	0.97162	0.50775
H	0.32804	0.90676	0.50926
C	0.39403	0.97315	0.50406

H	0.45827	0.90886	0.50227
C	0.34449	0.9971	0.50602
H	0.28341	0.9893	0.50929
H	0.41257	0.99208	0.50271
C	0.10622	0.36222	0.50404
C	0.08338	0.41001	0.50457
C	0.07868	0.34178	0.50243
C	0.03464	0.43656	0.50354
H	0.10477	0.42612	0.50569
C	0.02988	0.36811	0.5013
H	0.09608	0.30448	0.50195
C	0.0069	0.41616	0.50196
H	0.01726	0.47381	0.50397
H	0.00978	0.35072	0.4997
C	0.25336	0.21637	0.50508
C	0.30285	0.19201	0.50528
C	0.2298	0.19196	0.50455

C	0.328	0.14478	0.50502
H	0.32143	0.21093	0.50554
C	0.2547	0.14473	0.50421
H	0.19113	0.2107	0.50431
C	0.30446	0.12019	0.50458
H	0.36662	0.12614	0.50518
H	0.23481	0.12713	0.50346
C	0.92897	0.43138	0.50238
H	0.94063	0.39428	0.50517
C	0.31538	0.04661	0.50681
H	0.27652	0.06014	0.51038
N	0.95808	0.44578	0.50069
N	0.33284	0.07267	0.50392
C	0.62508	0.60084	0.5046
C	0.64954	0.55293	0.5048
C	0.65295	0.62107	0.50366
C	0.69825	0.5271	0.50388

H	0.63066	0.53439	0.50574
C	0.70184	0.59516	0.50273
H	0.63662	0.65823	0.50356
C	0.72623	0.54721	0.50265
H	0.71422	0.49003	0.50413
H	0.72108	0.61342	0.50193
C	0.77809	0.51881	0.50154
C	0.80754	0.53744	0.50088
C	0.80041	0.47092	0.50126
C	0.8563	0.50989	0.50062
H	0.79236	0.57447	0.50077
C	0.84897	0.44336	0.5013
H	0.77983	0.45408	0.50116
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H	0.86487	0.4062	0.50133

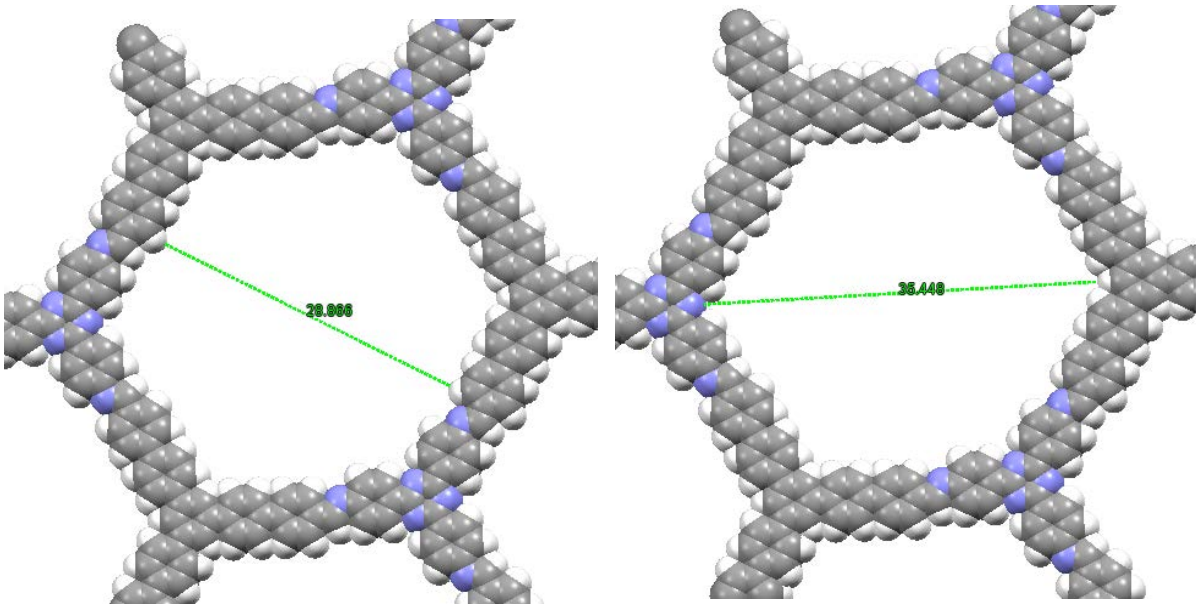

Figure S9: Pore Width obtained from simulated crystal structure of TrzCOF.

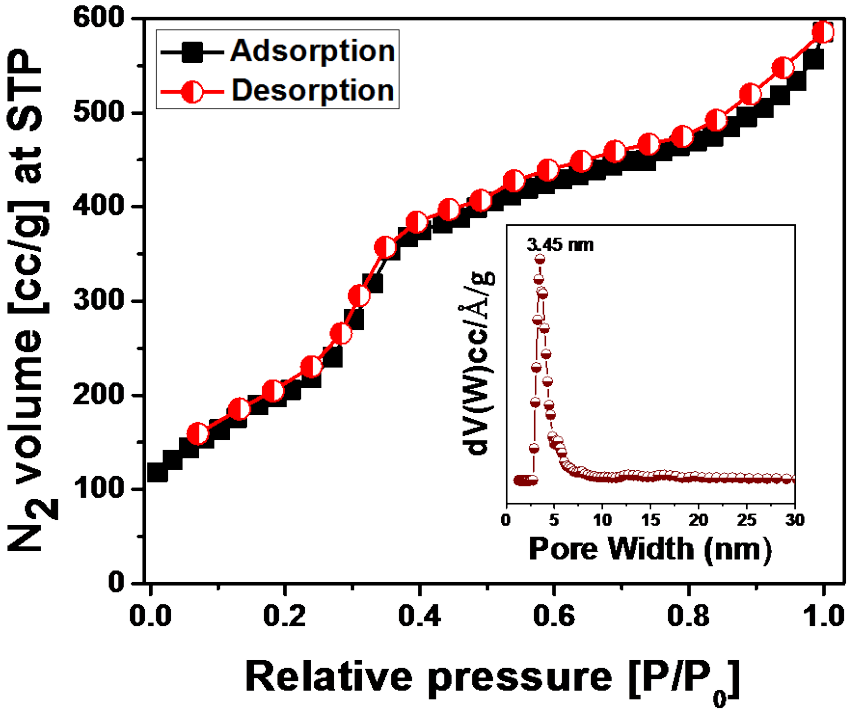

Figure S10: After pH-5.5 treatment (48h) of TrzCOF, N_2 adsorption/desorption isotherm.

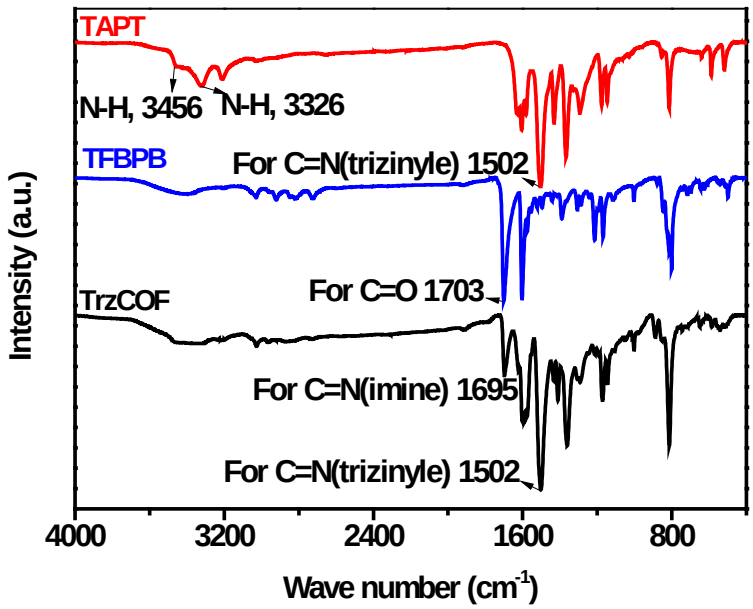

Figure S11. FTIR plot of TrzCOF and two monomers.

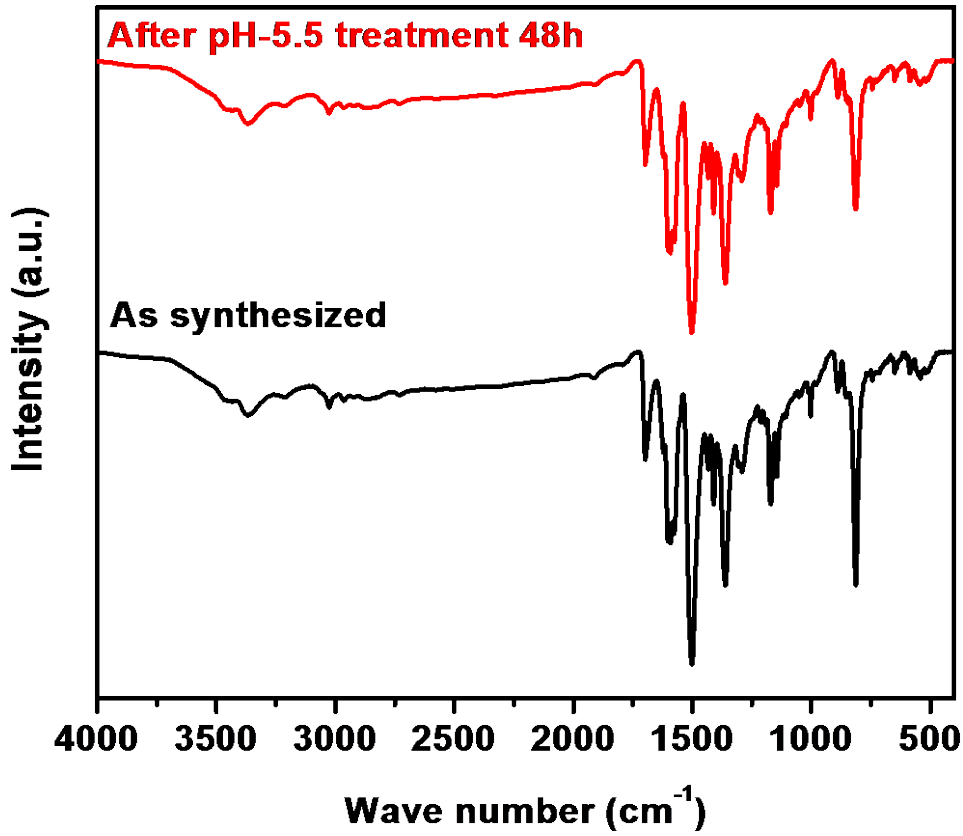

Figure S12: After pH-5.5 treatment and as synthesized FTIR spectra of TrzCOF.

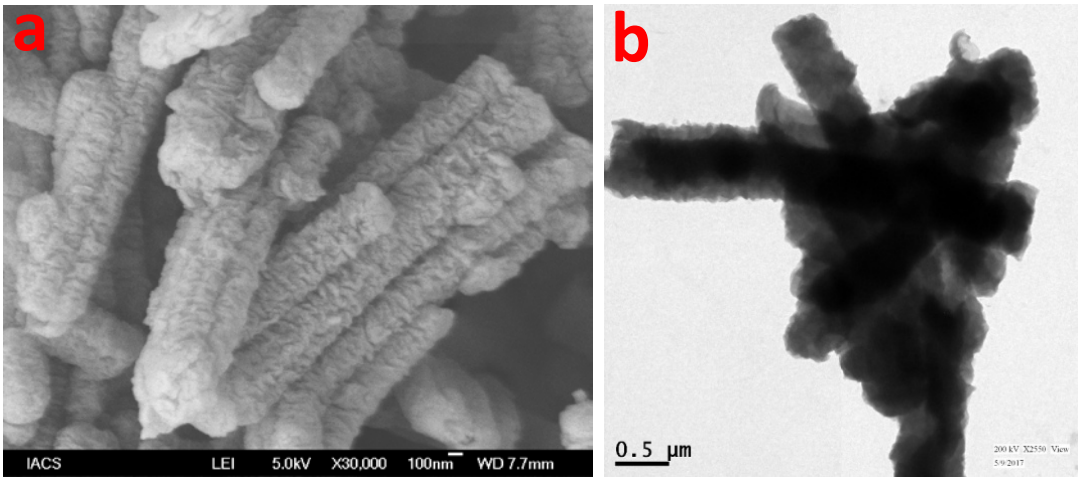

Figure S13: FESEM image (a) and HRTEM image (b) of TrzCOF.

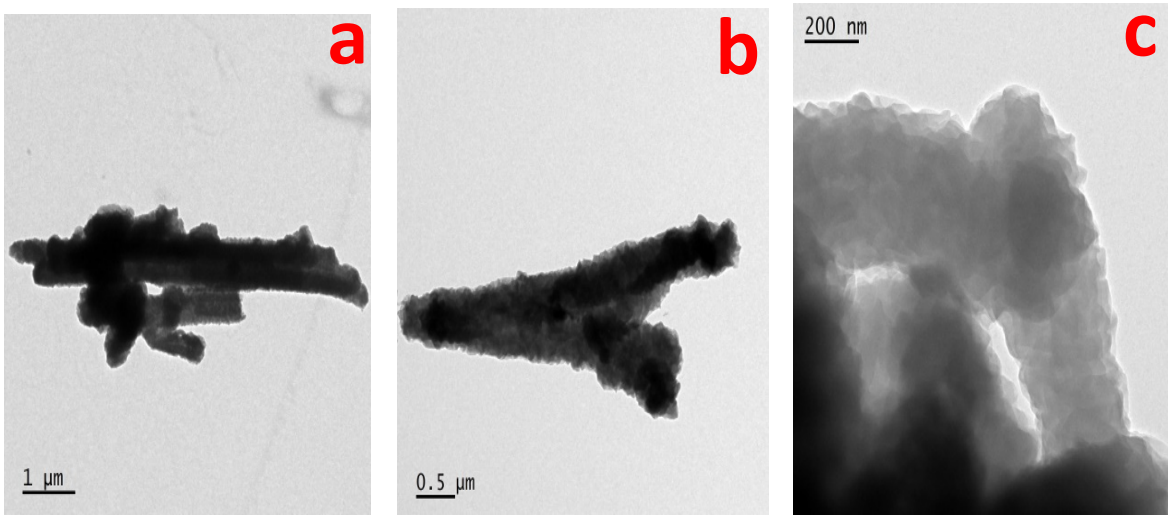

Figure S14: HRTEM images of TrzCOF after treatments at (a) pH 5.5 (acidic) for 48 h, (b) pH 7.4 (PBS buffer) for 48 h (c) pH 7.0 (neutral) for 48 h.

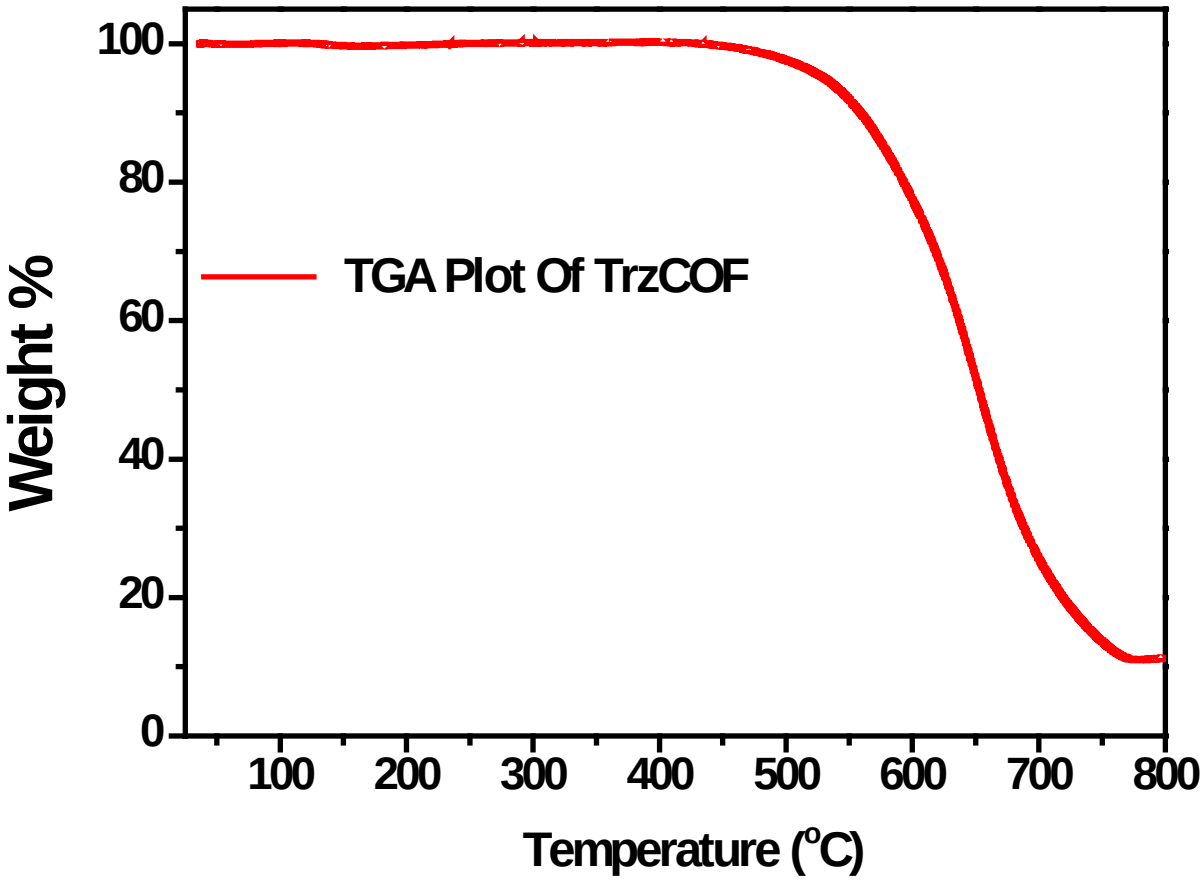

Figure S15: TGA profile diagram of TrzCOF.

Table S2: Time dependent inhibitory concentrations (IC_{30}, IC_{50} and IC_{70}) of TrzCOF against HCT116.

Inhibitory Concentrations	After 12 h (μg/ml)	After 24 h (μg/ml)
IC_{30}	13.24±1.51	3.25±0.03
IC_{50}	42.01±4.21	8.05±0.09
IC_{70}	72.27±8.45	22.14±3.05

Table S2: Determination of cytotoxicity underlying the evaluation of IC_{50}

Cell line	**IC_{50} (**μg/ml)
B16F10	10.47±2.63
HepG2	9.55±2.13
HCT116	8.31±1.67
HEK293	14.39±2.25

*Values are presented as Mean±SEM (n=3).

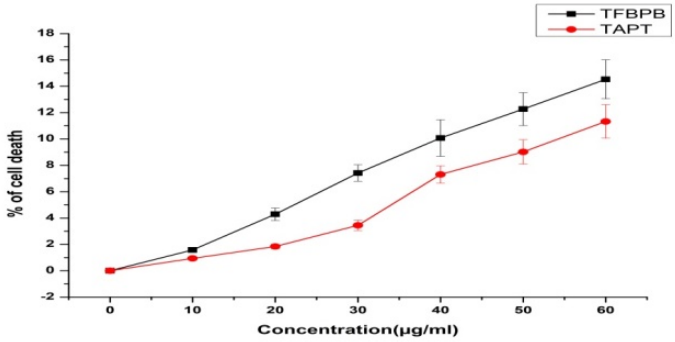

Figure S16: Cytotoxicity of TFBPB and TAPT in HCT116.

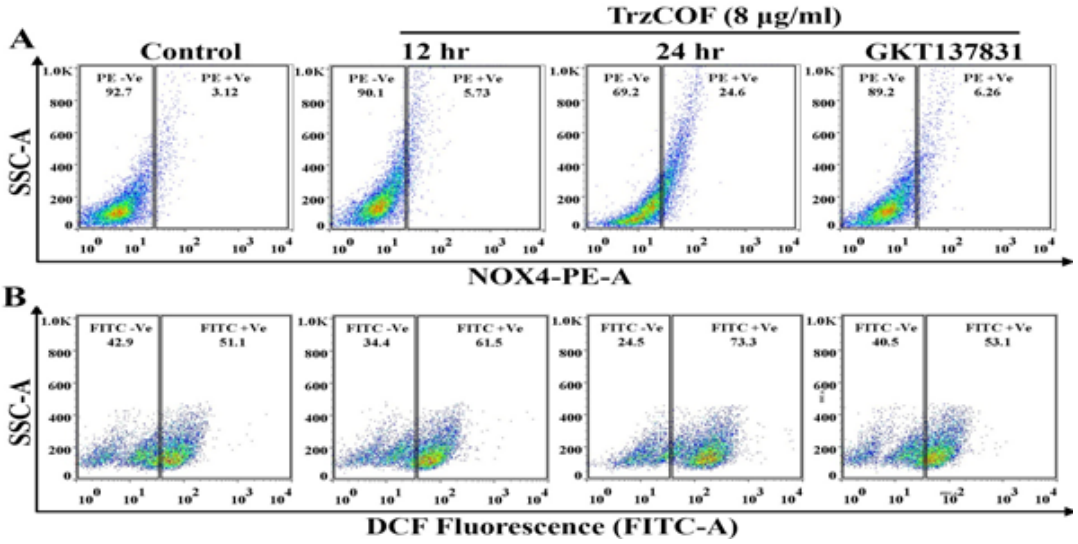

Figure S17: Flow cytometric Assessment of A. NOX4 B. ROS of TrzCOF treated cells in time dependent manner with and without NOX4 inhibitor.

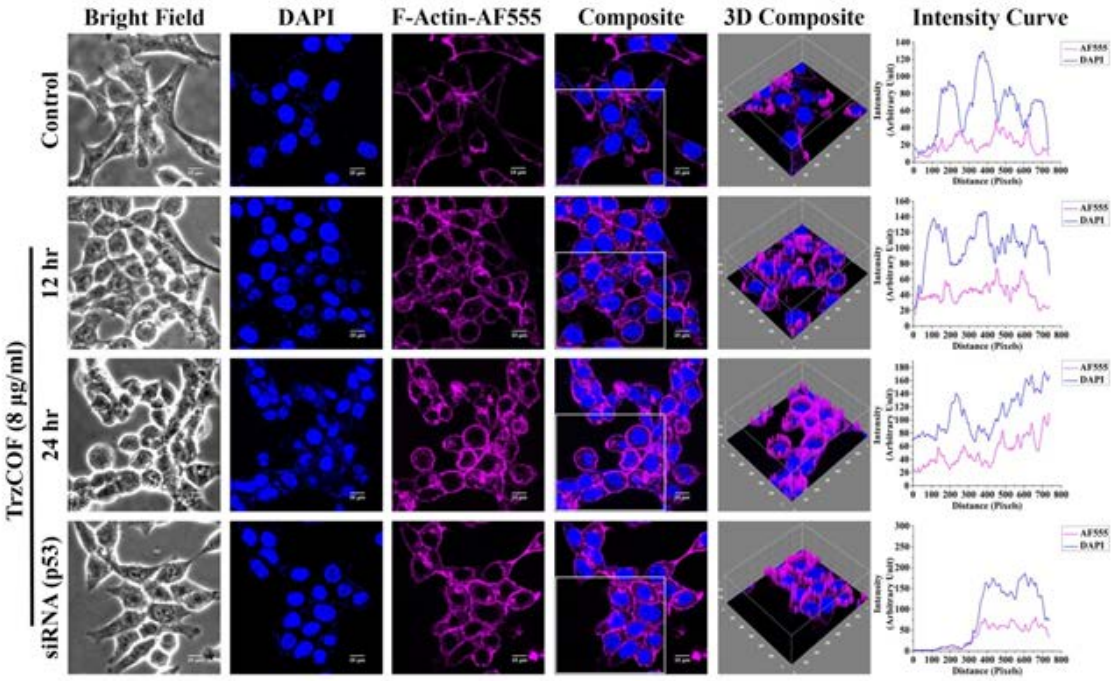

Figure S18: Immunofluorescene images showing qualitative expression of F-Actin. Nucleus was counterstained with DAPI (Magnification: 60×). Intensity analysis of relative fluorescence of F-actin-AF555 and DAPI were done using Image J software from the respective immunofluorescence micrographs.

Section S3:

Figure caption S3. (A) Assessment of cytotoxicity in HCT-116 cells upon the treatment of different concentration of TrzCOF (1, 5, 10, 20, 40, and 80 µg/ml) on 12 h and 24 h. Values are presented as mean ± SEM (n=5). $P<0.05$ was considered as significant. Statistical comparison: *Control vs. TrzCOF (5 µg/ml); ** Control vs. TrzCOF (10 µg/ml); #Control vs. TrzCOF (20 µg/ml), ##Control vs. TrzCOF (40 µg/ml), @Control vs. TrzCOF (80 µg/ml), and NS=Non significant. (B) Bar graph representing the quantitative evaluation of cell viability

with or without treatment of TrzCOF (IC_{50}), siRNA specific for p53 and NAC on 24 h. Values are presented as mean ± SEM (n=5). P<0.05 was considered as significant. Statistical comparison: *Control vs. TrzCOF (8 μg/ml), **TrzCOF (8 μg/ml) vs. TrzCOF (8 μg/ml) + NAC, and # TrzCOF (8 μg/ml) vs. TrzCOF (8 μg/ml) + siRNA (p53). (C) Determination of iROS upon the treatment of TrzCOF (8 μg/ml) along with/without of NAC on 12 and 24 h. Representive flow cytometric dot plot and gating hierarchy used to define the DCF +Ve cells and DCF –Ve cells. (D) Confirmative assessment of cell viability using 7-AAD. Representive flow cytometric dot plot and gating hierarchy used to define the viable cells and dead cells. (E) Determination of apoptosis and necrosis in HCT-116 cells upon the treatment of TrzCOF (8 μg/ml) along with/without of NAC on 12 hr and 24 hr. Representive flow cytometric dot plot and gating hierarchy used to define the viable cells (Q4), early apoptotic cells (Q3), late apoptotic cells (Q2), and necrotic cells (Q1).

Figure caption S4: Representive flow cytometric dot plot and gating hierarchy used to define the (A) γ-H2AX-APC +Ve cells and γ-H2AX-APC –Ve cells, (B) p-ATM-FITC +Ve cells and p-ATM-FITC –Ve cells, (C) p-p53-APC +Ve cells and p-APC-APC –Ve cells.

Figure caption S5: (A) Representive immunoblot of Bax, Bak, and Bcl2. B-Actin was used as a internal control. (B) Densitometric analysis of relative protein expression of Bax, Bak, and Bcl2. (C) Bar graph representing caspase 3 and caspase 9 activity. (D) Representive flow cytometric histogram and gating hierarchy used to define the cell population with high Ψm and low Ψm. Values are presented as mean ± SEM (n=5). P<0.05 was considered as significant. Statistical comparison: *Control vs. TrzCOF (8 μg/ml) on 12 h, **Control vs. TrzCOF (8 μg/ml) on 24 h, and #TrzCOF (8 μg/ml) on 24 hr vs. TrzCOF (8 μg/ml) + siRNA (p53) on 24 h

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