Journal of Materials Science and Nanomaterials

Aliyev, J Mater Sci Nanomater 2022, 6:6

Review Article Open Access

N-Rich Phytochemicals Corrugated Aromatic Polymer

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Abstract

When designing effective biomaterials or drug carriers for cancer treatment, N-rich organic materials with nanoscale porosities and polyphenolic moieties in their building networks pose a significant challenge.TrzTFPPOP, a novel polyphenolic porous organic polymer based on triazine, was used to investigate the possibility of in vitro anticancer activity on the human colorectal carcinoma (HCT 116) cell line.The functionalized organic material (-OH, -NH, and -C=N) had a BET surface area of 2140 m2 g1 and hierarchical porosity (micropores and mesopores). It also caused apoptosis, which made it possible for the DNA damage pathway, which is controlled by p53, to kill colon cancer cells with high efficiency.The MTT assay yielded results of 1.24, 3.25, and 5.25 g/mL, respectively, for the IC_{30}, IC_{50}, and IC_{70}.

Keywords: Porous polymer; Polyphenolic amine linked; High surface area; DNA damage; Anticancer activity

Introduction

Preventing this fatal illness from killing people is a significant challenge, and the word "cancer" connotes ominousness.The scientific community has focused a lot of attention on this important issue over the years.However, the objective is still a long way off, and ongoing research is being carried out to locate cancer in its earliest stages and halfway through treatment [1]. Cancer is caused by uncontrolled cell growth in various body parts.One in every six deaths worldwide is caused by cancer. Despite the global COVID-19 pandemic, the World Health Organization (WHO) reported in 2020 that 10.1 million new cases of common cancers and 10 million deaths from cancer occurred. Breast, colon, oral, skin, prostate, ovarian, bone marrow, and blood cancers are just a few of the types of cancer that can affect both men and women.935 thousand patients will die from colon and rectum cancer in 2020.The following symptoms can be used to diagnose colon cancer:polyps in the colon, unusual weight loss, colon bleeding that is not explained, changes in bowel habits, etc [2]. Colorectal cancer is commonly treated with a variety of well-known treatments, including medical, surgical, biological, chemotherapeutic, and radiation therapy, as well as cold atmospheric plasma (CAP) treatment. However, the majority of cancer treatments involve the destruction of cancerous cells through chemo- and radiotherapy, which has the serious potential to disturb cellular equilibrium, harm the nervous system, and result in dysfunction of multiple organs [3].

Results and Discussion

The Schiff base polycondensation reaction between trialdehyde and tetra-amine resulted in the formation of the phenolic-OH and N-rich TrzTFPPOP [4]. The material's FTIR spectrum, which measured the transmittances of synthesized monomers and POP materials between 4000 and 400 cm1, was first used to look for different bonding connectivities. An aldehydic >C=O stretching band at 1666 cm^{-1} and a -NH2 deformation peak between 1650 and 1657 cm^{-1} attenuate the condensation reaction between the aldehyde and amine monomer units. The characteristic peak at 1543 cm^{-1} seen in both the SL-1 ligand and TrzTFPPOP indicates the presence of a triazine unit. The presence of -OH, aminal (-CH-NH-), and imine (-The FTIR spectra of TrzTFPPOP tests remained unchanged after seven days of treatment with corrosive 2(M), base 2(M), and deionized (DI) water, indicating the primary honesty of the polymer organization [5].

To affirm the polymerization cycle further, we did a strong state 13C CP MAS NMR examination on the TrzTFPPOP material.The downfield NMR signals at 171, 168, and 165 ppm showed that carbon atoms were present in the triazine ring and the imine bond that was close to the phenyl ring.However, [6]. the presence of distinct aromatic carbon atoms is demonstrated by the NMR peaks at 110, 148, and 138 ppm.The various aromatic carbon atoms located adjacent to the phenolic-OH, triazine-N, and aliphatic-CH2 groups are denoted by NMR peaks at 148, 138, and 110 ppm, respectively.Aliphatic carbon and carbon atoms in the phenyl ring caused NMR signals at 53 and 129 ppm.The permeable natural polymer TrzTFPPOP was formed by the arrangement of both essential and optional aminal holding joins, as confirmed by the strong state 13C-NMR range examination previously mentioned [7].

The material's amorphous nature was confirmed by the broad peak between two values of 20 to 30 degrees, which is typical of an amorphous material PXRD pattern. The N2 adsorption/desorption isotherm of TrzTFPPOP can be described as a combination of two isotherms using the IUPAC nomenclature. In the P/P0 region, a steady increase in N2 uptake is preceded by a type I isotherm. From 4 to 40 degrees of 2, the powder X-ray diffraction pattern of the TrzTFPPOP material was collected [8]. An abrupt rise in the isotherms' N2 uptake at low pressure indicates the presence of microporosity in the polymer network, as revealed by porosity and surface region examination [9].

As a result of interparticle void spaces, the gradual rise in N2 uptake suggested mesopores.The pore size conveyance (PSD) of the TrzTFPPOP material was determined by applying non-neighborhood thickness useful hypothesis (NLDFT) to the N2 adsorption-desorption isotherms [10]. The BET surface region of the TrzTFPPOP material was 2140 m2 g-1, and the absolute pore volume was 1.91 cc g-1. The polymer organization's progressive miniature and mesoporosity was proposed by the material's noticed pore widths of 1.16, 2.73, and 4.5 nm, individually. For the purpose of morphological analysis,

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Received: 01-Nov-2022, Manuscript No: JMSN-22-80932; **Editor assigned:** 04-Nov-2022, Pre-QC No: JMSN-22-80932 (PQ); **Reviewed:** 18-Nov-2022, QC No: JMSN-22-80932; **Revised:** 25-Nov-2022, Manuscript No: JMSN-22-80932 (R); **Published:** 30-Nov-2022, DOI: 10.4172/jmsn.100055

Citation: Aliyev RZH (2022) N-Rich Phytochemicals Corrugated Aromatic Polymer. J Mater Sci Nanomater 6: 055.

HR-TEM images of the powder samples prior to and following cell treatment are shown.Due to its wide porosity, high BET surface area, and organic functionality (phenolic -OH, secondary amine, and imine), the TrzTFPPOP material may be a potential candidate for combating the affected colorectal cancer cells [11]. The HRTEM images suggested nanowire-like self-gathered particles with typical widths of 8-10 nm.After treatment, TrzTFPPOP was extracted from the cells, and HRTEM images were taken.These images, which are very similar to the material as it was synthesized, show the material's structural and morphological integrity.The TGA profile diagram demonstrated that the organic polymer network of TrzTFPPOP is thermally stable up to 308°C; TG analysis was carried out in air at a 10°C/min ramp at temperatures ranging from 25 to 800°C, and we also observed temperature structural degradation.Up to around 100°C, the underlying weight reduction might have been brought about by the vanishing of surface-consumed dampness.Similar thermal stability was also demonstrated during the synthesis of the chiral cationic POP material, which is essential for figuring out how to use them in harsh environments [12]. The material's strong state UV-Vis range confirmed its apparent ability to retain light.The current focus of intense research into anticancer nanomaterials is nanoparticles smaller than 100 nm, which are able to easily enter cells and selectively target cancer cells without affecting other parts of the body.Nanoconjugates and submicron particles are increasingly being utilized as drug carriers bound to cells.The majority of nanoparticles that enter the cell through endocytosis represent the initial step in this process.As a result, we used the MTT assay to measure TrzTFPPOP's cytotoxicity in the human colorectal carcinoma (HCT 116) cell line [13].

A characteristic of apoptosis, also known as cell death, is the externalization of phosphatidylserine.When phosphatidylserine is present, Annexin V-FITC binds to the outer leaflet of the plasma membrane.Using stream cytometry, the population of live (Q3), early apoptotic (Q4), late apoptotic (Q2), and necrotic (Q1) cells was examined.After 12 hours of treatment with 3 g/mL TrzTFPPOP, the cell populations of Q3, Q4, Q2, and Q1 increase, while the Q3 population decreases after 24 hours and the populations of late apoptotic cells and necrotic cells increase. The cell populations of Q3, Q4, Q2, and Q1 shift to 39.1, 23.2, 17.4, and 20.2, as well as 15.7, 0.7, 25.6, and 58, respectively.According to the flow cytometric results, apoptosis is the hallmark of cell death.In order to comprehend the cell-cycle arrest, the percentage of DNA in each phase of the cell cycle was measured following confirmation of apoptosis. On a time-dependent basis, the treatment with 3 g/mL TrzTFPPOP was carried out.We are confident that G2/M arrest initiates apoptosis thanks to the flow cytometric data. The DNA content increased over time at the G2/M phase in comparison to the control DNA, as measured by the percentage of DNA [14].

Through the p53/p21 pathways, DNA damage frequently results in cell cycle arrest. The model of BD LSRFortessaTM software version 10 provided the flow cytometric data.DNA damage and DNA double-strand breaks (DSBs) are primarily characterized by histone H2AX phosphorylation on serine 139 (-H2AX).We used immunofluorescence to locate the phosphorylation of -H2AX that was underlying the formation of foci in the nucleus in order to evaluate the role that TrzTFPPOP plays in the onset of DNA damage.A significant number of - H2AX foci were observed when the cells were given 3 g/mL of TrzTFPPOP. Over time, there were more foci. Based on these findings, it appears possible that TrzTFPPOP's phosphorylation of -H2AX during G2/M arrest is associated with the apoptosis mechanism. Graphpad Prism Software was also used to plot the statistical mean fluorescence intensity [15].

Materials and Methods

Cell Lines and Chemicals

Our requirements for terephthalonitrile and dicyandiamide were met by Sigma-Aldrich in Balgalore, India.We received trifluoroacetic acid, hexamine (hexamethylenetetramine), and phloroglucinol from Spectrochem in Mumbai, India.KOH was bought from Merck, which has its headquarters in Bangalore, India. Spectrochem, India's dimethylsulfoxide (DMSO), 2-methoxyethanol, THF, MeOH, and acetone were among the organic solvents we used.components of cell culture media, including an antibiotic cocktail known as Penicillin–Streptomycin–Neomycin (PSN), Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), ethylenediaminetetraacetic acid (EDTA), and trypsin from Gibco, USA. Sigma-Aldrich in the United States and SRL in Mumbai, India provided us with additional raw chemicals and mandatory fines. We obtained human epithelial kidney (HEK293) and colorectal carcinoma (HCT 116) cell lines from India's National Centre for Cell Sciences (NCCS). Cell Signaling Technology (CST) and eBioscience, both based in the United States, supplied us with antibodies.For the additional reagents, we used the best commercially available reagents and carried out all reactions without purifying them further.

Merits of the Material

Using a spectrophotometer from Boston, MA's PerkinElmer,FTIR spectra of the monomers and TrzTFPPOP as they were synthesized and treated with various solvents were gathered.USA). A 500 MHz Bruker Advance III spectrometer from Karlsruhe, Germany, with a 10 kHz MAS frequency was used to capture the solid-state 13C CP MAS NMR spectrum of POP material.We gathered the wide-angle PXRD patterns of TrzTFPPOP from the Bruker D-8 Advance SWAX diffractometer in Karlsruhe, Germany, using Cu K radiation (= 0.15406 nm).Before being activated at 150 °C under high vacuum to produce guest-free material for sorption analysis, the powder was thoroughly washed with DI water and a variety of organic solvents, then dried for 12 hours. The Quantachrome Autosorb 1-C surface region analyzer, fabricated in Boynton Ocean side, Florida,USA) to get N2 adsorption/desorption isotherms at 77 K. To ascertain the initiated material's pore size conveyance (PSD), we applied non-nearby thickness utilitarian hypothesis (NLDFT) to the N2 adsorption/desorption isotherms. We examined the morphology with a JEOL JEM 2010 transmission electron microscope from Tokyo, Japan.We used a thermogravimetric analyzer (TGA) made by TA Instruments, New Castle, DE, USA, with an air scanning rate of 10°C/min to generate the thermal analysis profile diagram.We analyzed the solid-state UV–Vis spectrum with the Shimadzu UV-2401PC from Tokyo, Japan. We gathered 1H and 13C NMR spectra with a Bruker AVANCE II spectrometer from Karlsruhe, Germany, operating at 400 MHz. We tested the absorbance of various solutions with an ELISA reader (Model:Using flow cytometry (BD LSRFortessaTM, San Jose, CA, USA), we determined the proportions of necrotic, apoptotic, and live cells (Emax, Agilent-Thermo Fisher, Tokyo, Japan, at 595 nm). We examined the double immunofluorescence staining images with a confocal laser scanning microscope (FV 10i, Olympus, Tokyo, Japan).

Triformylphloroglucinol synthesis (TFP)

Anhydrous phloroglucinol (6.014 g, 49 mmol), hexamethylenetetramine (15.098 g, 108 mmol), and 90 mL of trifluoroacetic acid were added to a 250 mL two-neck RB with a magnetic stir bar in order to maintain an inert atmosphere. From the literature,

we made few modifications to the standard procedure.After heating the mixture for three hours at 100 °C, we added 150 mL of 3MHCl.Under the same conditions, the reaction continued for another 1.5 hours. Celite was used to filter the reaction mixture after it had been brought to room temperature.Using 450 mL of dichloromethane for solvent extraction, we gathered the desired product (TFP).The mixture was then dried using a filter and anhydrous Na2SO4.We then used a rotary evaporator to dry the filtrate, resulting in pure triformylphloroglucinol (TFP) of 1.48 grams (7.0 mmol, 14% yield).The product was described using 1H and 13C NMR: 14.12.

Transcription of TrzTFPPOP

We poured tetraamine ligand SL-1 and tri-outfitted aldehyde TFP into a 30 mL Schlenk tube containing 10 mL of DMSO dissolvable and an attractive mix bar in a straightforward Schiff base polycondensation response.After that, the reaction mixture was stirred for 10 to 15 minutes at 120 °C to dissolve both monomers. The reaction mixture in the Schlenk tube was then heated continuously for three days.The dark orange-colored precipitate was obtained by filtering it through Whatmann 40 filter paper.To produce pure TrzTFPPOP (70 percent yield), the materials were washed successively with hot DMSO, a lot of water, THF, and MeOH, and then dried overnight at 100 °C in a hot air oven and a vacuum.

Cytotoxicity of cells

The cell's viability was assessed using the MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.After plating and placing 4 103 cells in each well of a 96-well culture plate, we treated the cells with TrzTFPPOP at concentrations ranging from 0 to 10 g/mL for 12 hours prior to adding 20 L of 5 g/mL MTT stock solution to each well.The intracellular formazan crystals were dissolved in acidic isopropanol after four hours at 37 °C.

Microscopy with Confocal

According to the most recent report, confocal microscopy was used to estimate the expression of p53, p21, and Gamma H2AX.DNA damage is significantly affected by these proteins.The slides were blocked for an hour before being incubated overnight at 4°C with the appropriate primary antibody (Gamma H2AX, Phospho-p53Ser46, and p21Waf1/Cip1). The secondary antibody was PE-labeled FITC for phospho-p53Ser46 and p21Waf1/Cip1.Trz was applied to the cover slips containing HCT 116 cells. After mounting the slides with the prolong anti-fade reagent (Molecular Probe, Eugene, OR, USA) and counterstaining them for ten minutes with 6-diamidino-2-phenylindole (DAPI), we diluted the secondary antibodies 1:100 in the blocking solution and incubated them for two hours.

Statistical Analysis

The data were presented using the standard error of the mean (SEM).FlowJo, a statistical data analysis software, was used to analyze our flow cytometry results. Using OriginPro 8.0 software (San Diego, CA, USA), we evaluated measurable importance and contrasts among the groups using one-way analysis of variation (ANOVA). We considered the data to be statistically significant when p values were less than 0.05.To appreciate the cycles' consistency, we did each analysis multiple times.

Conclusion

TrzTFPPOP, a secondary amine and imine-linked polyphenolic porous organic polymer with hierarchical nanoscale porosity and a high BET surface area of 2140 m2 g-1, was synthesized as shown here.Through the DNA damage pathway via the p53 axis, this highly porous organic polymer material was successfully utilized as an effective anticancer agent against in vitro colon cancer cells.The phosphorylation of -H2AX during G2/M arrest in this porous organic polymer, as demonstrated by our experiments, may play a role in the apoptosis pathway.As a result, TrzTFPPOP has a bright future as a potent anticancer agent for colon cancer treatment.

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