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Nanofibrous matrixes with biologically active hydroxybenzophenazine pyrazolone compound for cancer theranostics

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ABSTRACT

The nanomaterial with the novel biologically active compounds has been actively investigated for application in cancer research. Substantial use of nanofibrous scaffold for cancer research with potentially bioactive compounds through electrospinning has not been fully explored. Here, we describe the series of fabrication of nanofibrous scaffold loaded with novel potential biologically active hydroxybenzo[*a*]phenazine pyrazol-5(4H)-one derivatives were designed, synthesized by a simple one-pot, two step four component condensation based on Michael type addition reaction of lawsone, benzene-1,2-diamine, aromatic aldehydes and 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one as the substrates. The heterogeneous solid state catalyst (Fe (III) Y-Zeolite) could effectively catalyze the reaction to obtain the product with high yield and short reaction time. The synthesized compounds (5a–5p) were analyzed by NMR, FTIR and HRMS analysis. Compound 5c was confirmed by single crystal XRD studies. All the compounds were biologically evaluated for their potential inhibitory effect on anticancer (MCF-7, Hep-2) and microbial (MRSA, MTCC 201 and FRCA) activities. Among the compounds 5i exhibited the highest levels of inhibitory activity against both MCF-7, Hep-2 cell lines. Furthermore, the compound 5i (BPP) was evaluated for DNA fragmentation, flow cytometry studies and cytotoxicity against MCF-7, Hep-2 and NIH 3T3 fibroblast cell lines. In addition, molecular docking (PDB ID: 1T46) studies were performed to predict the binding affinity of ligand with receptor. Moreover, the synthesized BPP compound was loaded in to the PHB-PCL nanofibrous scaffold to check the cytotoxicity against the MCF-7, Hep-2 and NIH 3T3 fibroblast cell lines. The *in vitro* apoptotic potential of the PHB-PCL-BPP nanofibrous scaffold was assessed against MCF-7, Hep-2 cancerous cells and fibroblast cells at 12, 24 and 48 h respectively. The nanofibrous scaffold with BPP can induce apoptosis and also suppress the proliferation of cancerous cells. We anticipate that our results can provide better potential research in nanomaterial based cancer research.

1. Introduction

The tissue engineering with the nanotechnology provides an alternate therapy for cancer treatments using the biologically active compound with enhanced effect and reduced systemic toxicity [1]. The surgical excision of the cancerous cells with extensive modalities to treat the cancer patients with improved care is needed [2]. The growth of materials science and nanotechnology in the field of drug delivery to the cancerous region has attracted the great attention in the use of nanofibrous matrix with the active compound [3]. The cancer cells were in close contact with the extra cellular matrix (ECM) for the non cellular physical functional support for cell survival and tissue integrity [4]. It is well recognized that nanofibrous scaffold with active compound or drug can restrict the growth of the cancerous cells. The nanofiberous matrix with the biologically active compound can be targeted over the removed cancerous tissues as a scaffold or implant. The nanofibrous matrix loaded with biologically active compound will protect the non cancerous tissues with the help of the intracellular drug release from the nanofibrous scaffolds [4,5].

The use of simple and more versatile electrospinning technique aids in the formation of nanofibrous scaffold has evolved for the fabrication of diverse nanostructure with wide range of polymers [6]. Among the various polymer used the use of highly biodegradable hydrophobic polymer is of great interest due to the unique property of the polymers

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in the formation of nanofibrous scaffold. The poly(e-caprolactone) (PCL) has been attracted for the easy use in the formation of nanofibers due to the better blending compatibility, good biocompatibility, biodegradable and low cost [7]. Similarly, the Poly(3-hydroxybutyric acid) (PHB) is a hydrophobic polymer with added medicinal interest due to the enhanced biocompatibility and biodegradability [8].

Among various heterocycles, benzo[*a*]phenazin [9–11] and Pyrazolone [12–14] derivatives represent an important class of nitrogen containing highly biologically active compounds. Interest in this substance has been intensified recently by synthesis of variety of nitrogen based complex molecules. The synthesis of complex molecules has been a crucial achievement of synthetic organic chemistry [15,16]. That was fascinate huge consideration in modern years due to wide spread application of medicinal chemistry [17,18]. The diversity oriented synthesis (DOS) [19–21] has revealed that assembly of heterocyclic organic molecules with various core structure, combination of different functional groups and essential for the generation by collection of regiochemically diverse molecules [22,23] Important methods for generating these novel bioactive molecules are by using multicomponent reactions (MCRs) for the advantage of increase molecular diversity and complexity which has low cost and time depended essential products.

This efficient method has been used successfully for the synthesis of biologically important complex molecules [24]. Pyrazolone and benzo[*a*]phenazin were described as important drug substances in pharmacological diversity [25]. For instances, NC-190, Clofazimine, Benzopyranophenazine, XR11576 and Benzo[*a*] phenazin-morpholine are most useful drugs [26] shown in Fig. 1. In addition benzophenazine and their derivatives have found application in a wide variety of therapeutics [27], which include antimicrobial [28–31], antibiotic [32–35], antimalarial [36], antiparasitic [37], antitumor, antimycobacterial [38, 39] and anticancer activities [40]. Therefore, the development of novel synthetic bioactive heterocycles has revealed drugs for neglected diseases.

Herein, we reported two-step hydroxynaphthoquinone based new sequence of a four-component reaction in the presence of highly active solid state catalyst (Fe-Y-Zeolite) for the regioselective synthesis. Synthesis of poly-substituted 4-((5-hydroxybenzo[*a*]phenazin-6-yl) (phenyl) methyl)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one derivative of all the synthesized compounds were evaluated for antimicrobial activity and anticancer activity cancer against Hep-2 cancer and MCF-7 cell lines. The IC_{50} value shows compound **5i** (BPP) was given lower value than the other tested compounds. In addition antimicrobial activity revealed against the Gram-positive bacterium, Methicillin resistant *Staphylococcus aureus* (*MRSA*), Gram-negative bacterium, *Pseudomonas aeruginosa* MTCC 201, *Escherichia coli*, *Salmonella typhi* and a yeast strain and *Fluconazole resistant Candida albicans* (*FRCA*). DNA Fragmentation assay for MCF-7 Cells and Hep-2 Cells has also determined the function of most important bioactive molecules. Furthermore, docking (PDB ID: 1T46 with the ligand) studies also supported more information to design bioactive molecule for further novel drug development.

In the present strategy, we first have fabricated the PHB-PCL nanofibrous scaffold loaded with novel hydroxybenzo[*a*]phenazin pyrazol-5(4H)-one (BPP) compound *via* four component reaction using the approach by using heterogeneous (Fe(III)-Y Zeolite. The morphology of the fabricated PHB-PCL-BPP nanofibrous scaffold and the *in vitro* cytotoxicity and apoptosis potential were evaluated against cancerous MCF-7, Hep-2 and normal NIH 3T3 fibroblast cell line.

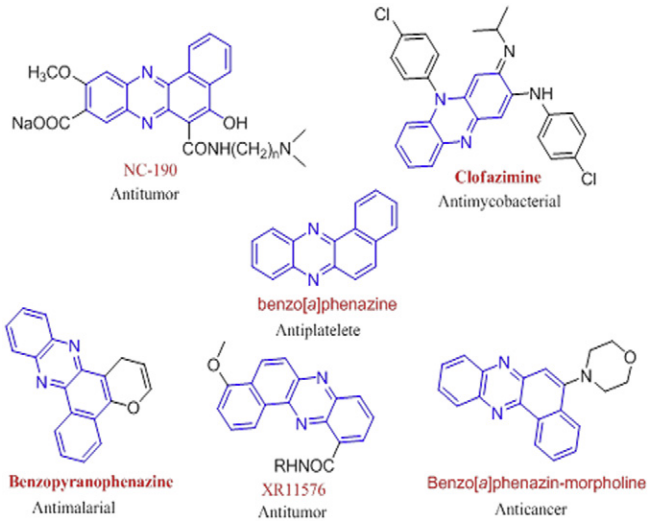

Fig. 1. Chemical structure some Benzo phenazin drugs.

2. Materials and methods

Poly(e-caprolactone) (PCL) (Average Mn ca. 60 kDa, Sigma), Poly(3-hydroxybutyric acid) (PHB), 1,1,1,3,3,3-hexa fluoro-2-propanol, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS), and supplementary antibiotics for tissue culture were purchased from Sigma Aldrich, India. Live/Dead Viability Kit (Life technology). The MCF-7, Hep-2 and NIH 3T3 fibroblast cell lines were obtained from the National Centre for Cell Science (NCCS), Pune, India. Other chemicals and culture wares were purchased from Sigma Aldrich, unless specified otherwise. All the chemicals were purchased from Sigma Aldrich. U.S.A. Analytical TLC was performed on precoated sheets of silica gel G/UV-254 of 0.2 mm thickness (Macherey-Nagel, Germany) using analytical grade solvents and visualized with iodine spray (10% (w/w) I_2 in silica gel) or UV light.

2.1. Synthesis of Fe (III) Y-Zeolite & Na-Y catalyst

Materials of Zeolite-Y (Si/Al = 2.7, Na_{52} $[(AlO_2)_{52}(SiO_2)_{140})]$, ferric chloride are obtained from Sigma-Aldrich. Ferric chloride 0.05 mmol was added to 50 mL aqueous solution of 0.5 g Na-Y Zeolite and stirred for 8 h at 90 °C. The obtained solids were filtered, washed with 100 mL of hot distilled water and dried under vacuum for 12 h at 80 °C. The resulting solid was soxhlet extracted with ethanol, water and finally the desired catalyst was obtained.

2.2. Synthesis of 4-((5-hydroxybenzo[a]phenazin-6 yl) (phenyl) methyl)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one derivatives (5a–5p)

First, we initiated 2-hydroxynaphthalene-1, 4-dione (0.174 g, 1 mmol), substituted benzene-1, 2-diamine (0.108 g, 1 mmol) and benzaldehydes (0.106 g, 1 mmol) and subsequently added 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (0.174 g, 1 mmol) and they were selected as substrate to the reaction. The above four component reaction was carried out by using 15 mg of Fe (III) Y-Zeolite solid state catalyst in the presence of EtOH: H_2O (1:1), stirred at room temperature. The reaction was completed within an hour due to the efficient contribution of the catalyst. Upon completion of the reaction, the mixture was filtered, washed with CH_3CN. Orange coloured solid product was obtained. The overall yield was 79–92%. Furthermore, the catalyst was recovered by nano filtration and washed with a mixture of water, dilute HCL, acetonitrile and methanol. The resulting orange coloured solid was dried at 80 °C for 6 h and collected.

2.3. Fabrication of PHB-PCL-BPP nanofibrous scaffold

To prepare the PHB-PCL nanofibrous scaffold, a 6 wt% concentration of polymer solution was prepared by dissolving 0.6 g of poly(3-hydroxybutyric acid) (PHB) and poly(e-caprolactone) (PCL) in 10 mL 1,1,1,3,3,3-hexafluoro-2-propanaol. The dissolved solution of PHB and