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Synthesis of novel benzodioxane midst piperazine moiety decorated chitosan silver nanoparticle against biohazard pathogens and as potential anti-inflammatory candidate: A molecular docking studies

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ABSTRACT

Nanoparticles (NPs) are currently being investigated along with the use of biodegradable polymer containing active agents in many areas of medicine for targeted applications. The present study was aimed to synthesize novel compound Benzodioxane midst piperazine (BP) and characterization of a BP decorated chitosan silver nanoparticles (BP*C@AgNPs) and shown effective against hazardous pathogens, and also having anti-inflammatory property. It was further evaluated for molecular docking proofs, and toxicity. The BP*C@AgNPs had spherical shape with size of 36.6 nm with wide biocidal activity against hazardous Gram-positive and Gram-negative bacteria with excellent inhibition at 100 μg/mL for *S. aureus* (10.08 ± 0.05 mm ZOI), and *E. coli* (10.03 ± 0.04 mm ZOI) compared to antibiotic Streptomycin. The anti-inflammatory activity exhibited IC_{50} value of 71.61 ± 1.05 μg/mL for BP*C@AgNPs compared to indomethacin (IC_{50} = 40.15 ± 1.21 μg/mL). Also, the docking study of BP showed excellent score for COX1 and DNA gyrase. This *in silico* study confirmed the achieved efficacy of BP, with less toxicity against normal PMBCs *in vitro* and *in vivo* studies. This study concludes that, the novel synthesized BP*C@AgNPs had excellent biocidal property and as anti-inflammatory candidate revealed by docking studies, it confirms BP*C@AgNPs for first-class therapeutic applications in the area of medicinal nanotechnology for the coming days.

1. Introduction

In recent years there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, greater attention has been paid to the screening of antimicrobial activity and its evaluation methods. The "Green chemistry" is a branch of chemistry that deals with new products with novel approach to minimize utilization and creation of harmful substances. The term "Green industry" is used to describe environmentally friendly practices, or those that produce eco-conscious products. Driven by increasing challenges to reach efficacy and safety for "difficult" drugs, such as proteins and peptides, the scope of drug delivery research has broadened considerably during the last decade or so, to include not only "traditional" drug delivery systems, such as lipids, surfactants, and polymers, but also a wide range of inorganic nanomaterials [1–4]. In addition, such nanomaterials are attracting considerable current interest as novel antimicrobial agents, not the least due to increasing resistance against conventional antibiotics, such as aminoglycoside, quinolone, β-lactam, macrolide, and tetracycline antibiotics [5,6].

Nowadays, antibacterial agents are widely used in treating infectious diseases caused by pathogenic bacteria. However, the

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wide use of antibiotics has led to a rise in microbial drug resistance, resulting in poor treatment efficacy and significant economic loss [7–9]. There is an urgent need to develop new antibacterial agents in place of antibiotics. In the past decade, considerable attention has been focused on applications towards environmental protection. The rapid development of science and technology leads to modernization which affected our natural environment. Natural chitosan has many advantageous properties for medical applications such as biocompatibility, biodegradability, and antibacterial activity. However, natural chitosan should be chemically modified for different purposes. Recently, many studies have examined the application of natural polymers as biomaterials. Natural polymers such as chitosan, gelatin, and hyaluronic acid have various biological properties such as anti-inflammatory, immune enhancement, and anti-coagulant effects [10–12].

Many cheaper natural antimicrobial polymers are developed from the polysaccharides chitin and chitosan [13], and their derivatives [14]. Chitosan is a deacetylated biopolymer from chitin in the exoskeletons of some crustacean shells [15]. Chitosan (CS) has many biological properties such as biocompatibility, nontoxicity, biodegradability, muco-adhesive, antimicrobial properties. In addition to being economically facile processed from chitin [16], chitosan has been widely used in many applications [17,18]. In addition, the antimicrobial activity of chitosan, gives it exceptional properties regarding the multi-tasks applications [19].

An increase in bacterial resistance to antibiotics and the lack of new antibiotics introduced into the market resulted in a need to find alternative strategies so as to cope with infections resulting from drug-resistant bacteria [20]. Development of alternatives for antibiotics and the discovery or development of adjuvant is amongst the potential strategies proposed. In the past few years, silver nanoparticles (AgNPs) have been considered potential antibacterial agents owing to their broad-spectrum antimicrobial activity [21,22]. It has been confirmed that the antibacterial properties of Ag NPs depend on several physicochemical properties of the particles, including their size, shape, chemistry, and surface coating [23,24]. Among these physicochemical properties, the surface coating plays a critical role in biocompatibility and the toxicity of silver nanoparticles [25]. The bacterial cell walls composed of a net negative charge. In Gram-positive bacteria, the teichoic acids are responsive for negative charge linked either to the peptidoglycan or to the plasma membrane. These teichoic acids are anionic owing to the presence of phosphates within their structure. The Gram-negative bacteria having lipopolysaccharides strongly confer a net negative charge on their cells surface [26]. Recently, the antibacterial activity of charged AgNPs has been extensively evaluated [27], and showed positively charged AgNPs had higher bactericidal activity than negative or neutral AgNPs [28,29]. This nonspecific electrostatic interaction between positive and negative charges the bactericidal effect. Therefore we suggest, the surface charge is important factor involved in antibacterial activity. Therefore, an urgent need for new synthetic alternative compounds with clear mechanism to solve microbial resistance problem and with greater biocidal efficacy. The development of efficient nano candidate having inherent biocidal property [30] and efficient for drug delivery with biocompatible natured nano-vehicles [31,32] attracted researchers in recent years to prevent diseases burden in effective doses *in vivo* [33].

Considering bacterial evolution and the current increase of antibiotic resistance, the discovery of new natural compounds that can be used to treat infections with lower secondary effects than existing antibiotics is becoming crucial, in order to guarantee the health of future generations. In this regard, in order to predict possible interactions between the synthesized compounds or agents against target proteins that would allow understanding and describing the mechanism of action. Therefore, along with *in vitro* evaluation an supporting *in silico* docking studies give much more evidence in order to evaluate their affinity to bacterial proteins that are known targets for tuning the synthesized candidate further application level in upcoming days.

With this background, for the first time we are developing a Benzodioxane midst piperazine (BP) nanoparticles using natural nontoxic polymers such as chitosan, has not been investigated as antiinfective and anti-inflammatory agent. In the present study, we evaluated novel benzodioxane midst piperazine decorated chitosan-silver nanoparticles (BP*C@AgNPs) against hazardous pathogens, as anti-inflammatory agent, and studied molecular docking to validate the experimental proofs for the first time.

2. Materials and methods

2.1. Reagents and bacterial strains

Nutrient agar, broth media and Chitosan were purchased from Hi-Media (Bangalore, India), Tris-buffer, $AgNO_3$ and HCl from Merck-Millipore (Bangalore, India), The all chemicals involved in experiments were procured from Sigma & Merck, India and Millipore water was used in all the experiments. All microorganisms obtained from Microbial Typing Culture Collection (MTCC), Chandigarh, India. *Salmonella typhimurium* (98), *Escherichia coli* (1610), *Staphylococcus aureus* (96), *Bacillus cereus* (430), and *Shigella flexneri* (1457) were cultured as per the protocol prescribed by MTCC.

2.2. Ssynthesis of (4-(4-methoxybenzoyl)piperazin-1-yl)(2,3-dihydrobenzo[b][1,4]dioxin-3-yl)methanone (benzodioxane midst piperazine)

The 1-(1,4-benzodioxane-2-carbonyl)piperazine (1) (1.0 eq) was dissolved in dichloromethane (DCM), then triethylamine (TEA) (3.0 eq) was added, stirred and cooled. After 10 min, 4-methoxy benzoyl chloride (3) (1.0 eq) were added and allowed to stir at room temperature for 6–7 h. Then, solvent was removed and residue was extracted with ethyl acetate. The organic layer was dried with anhydrous sodium sulphate and recrystalized from methanol

FT-IR (KBr, cm^{-1}): 1730 (C=O), 1524 (C=C), 1282 (C—N), 1064 (C—O), 771 (CH_2).^{1}H NMR (DMSO-d_6) δ ppm:3.46 (Piperazine-H, t, 4H), 3.50 (Piperazine-H, t, 4H), 3.78 (OCH_3, s, 3H), 4.68 (CH_2, d, 2H), 5.21 (CH, t, 1H), 6.65 (Aromatic-H, d, 2H), 6.74 (Aromatic-H, t, 2H), 6.91 (Aromatic-H, d, 2H), 7.84 (Aromatic-H, d, 2H). MS (ESI) *m*/*z*: 382.41. Anal. calcd for $C_{21}H_{22}N_2O_5$(in%): C 65.96, H 5.80, N 7.33;found C 65.86, H 5.72, N 7.22.

2.3. Benzodioxane midst piperazine loaded chitosan silver nanoparticles (BP*C@AgNPs)

The nanoparticles were developed according protocol of Manukumar et al. [34]. Briefly, a solution of chitosan (0.25 g) in 2% acetic acid and silver nitrate ($AgNO_3$, 0.25 g) in deionised water were mixed. Exactly above 5 mL of chitosan and silver nitrate solution were mixed in the boiling tube then kept in an autoclave at 120 °C for 1 h. Then cleared resulted yellow solution was mixed with 0.25 g of (4-(4-methoxybenzoyl)piperazin-1-yl)(2,3-dihydrobenzo[b][1,4]dioxin-3-yl)methanone and sonicated for 2 h at 30 °C. The resulting black colored solution indicates the formation of benzodioxane midst piperazine loaded chitosan-silver nanoparticles (BP*C@AgNPs).

2.4. The BP*C@AgNPs characterization

2.4.1. UV–vis spectral analysis

The synthesis of BP*C@AgNPs was studied using Beckman Coulter, (Shimadzu UV1800) scanning UV–vis spectrophotometer of