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Pharmacological investigation of 2-aminobenzothiazolium-4-methylbenzenesulphonate: Synthesis, spectral characterization and structural elucidation

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ABSTRACT

An organic charge transfer complex, 2-aminobenzothiazolium-4-methylbenzenesulphonate (ABPTS) was synthesized and single crystals grown by slow solvent evaporation solution growth technique at ambient temperature. The single crystal X-ray diffraction analysis was carried out to establish the molecular structure of the title crystal. FT-IR spectral study was carried out to identify the various functional groups present in the crystal. The ^{1}H and ^{13}C spectra were recorded to further confirm the molecular structure of the CT complex. The TG/DTA analyses were carried out to establish the thermal stability of the complex. The antibacterial and antifungal activities of synthesized complex were examined against various bacteria and fungi strains, to identify the antibacterial and antifungal activity. The DNA binding profile of the complex has been investigated through absorption spectroscopy and complex has intrinsic binding constant $3.6 \times 10^{4}\ M^{-1}$. A gel electrophoresis assay demonstrated the ability of the complex to cleave the pBR322 DNA. The free radical scavenging activity of the complex has been determined against DPPH, OH and ABTS radicals.

1. Introduction

The proton transfer or charge transfer interaction between various electron donor and acceptor molecules is of enormous interest and emerged significantly in recent years [1–3]. The study of charge transfer complex play a highly prominent role in biological systems, viz., analysis of some drugs, pharmaceutical preparations and also significant physical properties of the charge transfer products, such as, electrical conductivities and their applications in the field of photonic and solar cells [4–7]. Moreover, CT complexs play a very important task in many biological systems, such as DNA-binding, antibacterial, antifungal, insecticides as well as take part a vital role in the field of drug receptor binding mechanism due to the presence of aromatic electron donors and electron acceptors containing nitrogen, oxygen or sulphur atoms [8–11]. Benzothiazole is an important heterocyclic compound having both nitrogen and sulphur. Hence, it is used in the field of molecular design, optical, liquid and electronic properties [12]. Benzothiazole and its derivatives have been of great interest in the last three decades, because of its highly pronounced biological applications, such as, anti-tumour [13], anti-inflammatory [14], analgesic [15], anti-microbial [16], anti-leishmanial [17], anti-convulsant [18], anti-malarial [19], potential anti-HIV agents [20] and selective inhibition of HIV Type 1 reverse transcriptase [21]. They are broadly found in bioorganic and medicinal chemistry with applications in drug discovery and development for the treatment of diabetes [22–25]. The interaction of small molecules with DNA is a dynamic, thriving field which has drawn ever escalating research interests in recent decades [26–28]. Furthermore, understanding the interactions of small molecules binding to DNA will potentially be useful in the design of such new compounds, which can recognize specific sites or conformations of DNA [29,30].

Although several classes of antibacterial agents are presently available, resistance to most of the pathogenic bacteria to these drugs constantly emerges. In order to prevent this serious medical problem, the elaboration of new types of antibacterial agents or the expansion of bioactivity of the previous drugs is a very important task. Taking into consideration the above described beneficial effects of the benzothiazole derivatives, we realized that it would be of interest to focus the development of new biologically active compound with novel target so as to surpass the problem of acquired resistance. Hence we herein report the Pharmacological Investigation of 2-aminobenzothiazolium-4-methylbenzenesulphonate: Synthesis, Spectral Characterization and Structural Elucidation.

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2. Experimental details

2.1. Materials and instrumentation

All the chemicals used were chemically pure and AR grade. Commercially available samples of 4-methylbenzenesulphonic acid and 2-aminobenzothiazole were used to synthesize ABPTS crystal. Solvents were purified and dried according to the standard procedure [31]. CT-DNA and pBR322 DNA were purchased from Bangalore Genei, Bangalore, India. Tetracycline, Nystatin and Ager were purchased from Hi-media, Mumbai.

The electronic absorption spectrum was recorded in methanol employing SHIMADZU 1601 UV–Vis spectrophotometer in the range 200–800 nm. In order to confirm the functional groups, the crystal was subjected to FT-IR spectral analysis by employing Perkin Elmer FT-IR 8000 spectrophotometer in the range of 4000–400 cm^{-1} using the KBr pellet technique. ^{1}H and ^{13}C NMR spectra were recorded using Bruker AV III 500 MHZ spectrometer instrument using TMS as an internal reference standard. Thermal stability of was established by employing thermogravimetric analysis (TGA) and differential thermal analysis (DTA) simultaneously. This analysis was carried out between 25 °C and 600 °C in a nitrogen atmosphere at a heating rate of 10 °C min^{-1} using NETZSCH STA 409 C/CD TG/DTA instrument. The DNA cleavage study was carried out using Gelstan–Gel documentation system. The antioxidant study was carried out at the Kovai Medical Centre and Hospital Pharmacy College, Coimbatore, Tamil Nadu, India.

2.2. Single crystal X-ray diffraction studies

Single crystal X-ray diffraction data of ABPTS crystal was collected at room temperature on a Bruker AXS KAPPA APEX2 CCD diffractometer equipped with a fine focused sealed tube. The unit cell parameters were determined and the data collections of ABPTS was performed using a graphite-mono chromate Mo Kα (λ = 0.71073 Å) radiation by φ and ω scans. The structure of the complex was solved by direct methods [32] using SHELXS-97, which revealed the position of all non-hydrogen atoms, and was refined by full-matrix least squares on F^2 (SHELXL-97) [33]. All non-hydrogen atoms were refined anisotropically, while the hydrogen atoms were placed in calculated positions and refined as riding atoms.

2.3. Synthesis and growth of single crystal

2-Aminobenzothiazole and 4-methylbenzenesulphonic acid were used to synthesize ABPTS crystals. Equimolar solutions of the reactants were dissolved separately in methanol and millipore water, mixed together and stirred well for about 30 min at an ambient temperature to yield a homogenous mixture of solution, when a crystalline salt was precipitated. The crystalline salt was filtered off, dried and repeatedly recrystallized to enhance the purity of the product. The formation of the product, 2-aminobenzothiazolum-4-methylbenzenesulphonate is indicated in Scheme 1.

A saturated solution of ABPTS in dry methanol was prepared by stirring well for about 2 h followed by slight heating. Suspended impurities were removed using Whatman 41 grade filter paper. The clear filtrate so obtained was kept aside undisturbed in a dust-free room for the growth of single crystals. Well-defined and transparent crystals were collected at the end of the eighth day.

3. Biological evaluation

3.1. Antibacterial activity

The antibacterial activity of the newly synthesized complex was tested *in vitro* against two Gram-positive bacteria *Staphylococcus aureus*, and *Bacillus subtilis* and five Gram-negative bacteria *Proteus* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas* sp. and *Klebsiella pneumoniae*. DMSO solvent was used and set up as a control. The discs measuring 5 mm in diameter were prepared from Whatman No. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile discs were soaked previously in concentrated test solution and were placed in the nutrient agar medium. The Petri plates were invested and kept in an incubator for 24 h at 37 °C and growth was monitored visually. The screening was performed at 100 μg/mL concentration of test complex with antibiotic disc. Tetracycline (30 mg/disc) was used as a control. Logarithmic serially two fold diluted amount of test complex and controls was inoculated within the range 10^{-4}–10^{-5} cfu/mL. To obtain the diameter of the zone, 0.1 mL volume was taken each and spread on agar plates. The number of colony forming units (cfu) was counted after 24 h of incubation at 35 °C. After incubation the zone of inhibition was measured and expressed as mm in diameter [34,35].

3.2. Antifungal activity

The newly synthesized complex was also screened for its antifungal property against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Penicillium* sp., in DMSO solvent by using standard agar disc diffusion method. The synthesized complex was dissolved in DMSO solvent and media with DMSO was set up as a control. All cultures were routinely maintained on Sabouraud Dextrose Agar (SDA) and incubated at 28 °C. Spore formation of filamentous fungi was formed from seven days old culture on sterile normal solution, which was diluted to approximately 10^5 cfu/mL. The culture was centrifuged at 1000 rpm, pellets were resuspended and diluted in sterile Normal Saline Solution (NSS) to obtain a viable count 10^5 cfu/mL. With the help of spreader, 0.1 mL volume of approximately diluted fungal culture suspension was taken and spread on agar plates. The fungal activity of the complex was compared with Nystatin (30 g/disc) which is used as standard drug. The cultures were incubated for 48 h at 37 °C and the growth was monitored. Antifungal activity was determined by measuring the diameters of the zone (mm) in triplicate sets.

3.3. DNA binding – Titration experiments

The binding affinities with the CT-DNA of the complex was carried out in double distilled water with tris(hydroxymethyl)-amino methane (Tris, 5 mM) and sodium chloride (50 mM) and adjusted to pH 7.2 with hydrochloric acid. A solution of CT-DNA in the buffer gave a ratio of UV absorbance of about 1.8–1.9 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar extinction coefficient value of 6600 dm^3 - mol^{-1} cm^{-1} at 260 nm. The complex was dissolved in a mixed solvent of 5% DMSO and 95% Tris HCl buffer for all the experiments [36]. Stock solutions were stored at 4 °C and used within 4 days. Adsorption titration experiment was performed with the fixed concentration of the complex (25 μM) with varying concentration of DNA (0–50 μM). While measuring the absorption spectra, an equal amount of DNA was added to the all test solutions and the reference solution to eliminate the absorbance of DNA itself.