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The characterization of 1-(4-bromophenyl)-5-phenyl-1H-1,2,3-triazole on acute toxicity, antimicrobial activities, photophysical property, and binding to two globular proteins

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ABSTRACT

1-(4-Bromophenyl)-5-phenyl-1H-1,2,3-triazole (BPT) was a newly synthesized compound. The acute toxicities of BPT to mice by intragastric administration have been determined and the result indicates that the intragastric administration of BPT did not produce any significant toxic effect on Kunming strain mice. It is also evaluated for the antimicrobial activity of BPT against three kinds of plant mycoplasma, *Fusarium Wilt* (race 4), *Colletotrichum gloeosporioides* Penz. and *Xanthomonas oryzae* by different method in vitro. The compound exhibited distinct inhibitory activities against *Fusarium Wilt* (race 4) and *Colletotrichum gloeosporioides* Penz. by mycelium growth rate test and the values of EC_{50} were 29.34 and 12.53 μg/mL respectively. And BPT had also the most potent inhibitory activities against *Xanthomonas oryzae* when compared with that of control drugs by the agar well diffusion method. In addition, the structural and photophysical properties of BPT including ionization energy, electron affinities, and theoretical spectrum was studied by quantum-chemical methods. Then the interaction of BPT with two kinds of globular proteins, human immunoglobulin (HIg) and bovine hemoglobin (BHg) was investigated by using UV–vis absorption spectra, synchronous fluorescence, 3D fluorescence spectra, and fluorescence titration in combination with molecular modeling. UV–vis absorption, 3D and synchronous fluorescence measurements show that BPT has influence on the microenvironment surrounding HIg or BHg in aqueous solution and the fluorescence experiments show that BPT quenches the fluorescence intensity of HIg or BHg through a static mechanism. The binding parameters including the binding constants, the number of binding site and average binding distance between BPT and HIg or BHg at different temperatures were calculated. The thermodynamic parameters suggest that the hydrophobic interaction is the predominant intermolecular forces in stabilizing the BPT-HIg or BPT-BHg complex. Molecular docking was performed to reveal that the BPT moiety binds to the hydrophobic cavity of HIg or BHg and they are in good agreement with the spectroscopic measurements.

1. Introduction

l,2,3-triazoles are important heterocyclic compounds and have been known for long time. Although 1,2,3-triazole does not appear as natural, the synthetic molecules containing 1,2,3-triazole moieties and related heterocyclic compounds have been identified as bioactive molecules, and occupied a prominent place in medicinal chemistry because of their therapeutic properties and good metabolic stability. From a structural point of view, the 1,2,3-disubstituted-triazole unit presents two hydrogen bond acceptors (N2 and N3), a weak hydrogen bond donor (C—H) and an aromatic ring that can perform π-stacking interactions with aromatic amino acid side chains in the active site [1–3]. Thus, researchers took advantage of their drug-like properties and they have

Abbreviations: BPT, 1-(4-bromophenyl)-5-phenyl-1H-1,2,3-triazole; HIg, human immunoglobulin; BHg, bovine hemoglobin; HAS, human serum albumin; DMSO, dimethylsulfoxide; PDA, Potato Dextrose Agar; S.D., standard deviation; Tris, tris(hydroxymethyl) aminomethane; SPF, Specific Pathogen Free; HOMO, the highest occupied molecular orbital; LUMO, the lowest unoccupied molecular orbital; TD DFT, the time dependent density functional theory; CIS, the Configuration Interaction with Single excitations; *Xoo*, *Xanthomonas oryzae* pv. *oryzae*; FRET, Förster non-radiative energy transfer; *Fab*, fragment of antigen binding; 3D, three-dimensional; Phe, phenylalanine; Lys, lysine; Val, valine; Tyr, tyrosine; Leu, leucine; Trp, tryptophan; Ser, serine; Pro, proline; Ala, alanine; Ile, isoleucine; Glu, glutamic acid; Thr, threonine; Asp, aspartic acid; Gln, glutamine; Asn, asparagine; His, histidine.

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found much application in medicinal chemistry, such as anticancer [4], antifungal [5], antimicrobial [6], antioxidant [7], anti-HIV [8], anti-inflammatory activities [9], and cytotoxic activities [10]. Besides pharmaceutical applications, where this unit is important for biological activity, it is also important in materials science and in chemical biology [1], like the bicyclic triazole with interesting photoprotective properties prepared under metal-free microwave conditions [11] and a UV/blue-light-emitting fluorophore triazoles reported by Yan et al. [12]. In addition, triazole pestcides are one of the most important families of fungicides due to their great activity against a wide-spectrum of crop diseases. The derivatives of 1,2,3-triazole are applied as insecticides, fungicides, plant growth regulators [13]. 1,2,3-Triazoles have also widely used in industrial applications such as dyes, corrosion inhibition (of copper and copper alloys), photostabilizers, photographic materials, and agrochemicals [14].

In consideration of their chemical properties, synthetic triazoles and its derivatives are mainly distributed to the water compartments in the environment, and because of their wide use the potential effects on aquatic organisms are cause of concern. However, the search for potent, safe, and environmentally friendly l,2,3-triazoles with satisfactory therapeutic effect is also a crucial aspect of modern medicinal research. Most of the world's population relies on synthetic chemical drugs for their healthcare needs. Therefore, to develop safe chemicals, preliminary toxicity studies are necessary to evaluate possible risks such as undesirable side effects and to determine appropriate dosage levels and regimens to avoid overdosing or poisoning of patients. Nitrogen heterocycles with triazole ring have recently been well studied due to their less toxic effects and numerous pharmacological properties [15]. For example, a series of novel 2H–chromen-2-one derivatives decorated with 1,2,3-triazole moiety were designed and identified as lead compounds for future investigation due to their lower toxicity [16]. 4-Phenyl-1-(phenylselanylmethyl)-1,2,3-triazole did not cause the acute toxicity in biochemical markers hepatic and renal investigated [17]. In view of the biological importance of the l,2,3-triazoles, this coupled with our interest in studying on the interaction between bio-macromolecule and small drug molecules. This study focused on the biochemical evaluation of a novel synthesized triazole compound, 1-(4-bromophenyl)-5-phenyl-1H-1,2,3-triazole (BPT, Fig. 1) [18] which was then subjected through acute toxicity, antimicrobial activities and binding to two kinds of globular proteins, Human Immunoglobulin (HIg) and bovine hemoglobin (BHg) by different spectroscopic and chemoinformatics methods.

Since 1,2,3-triazole compounds have good metabolic stability but have lower aqueous solubility. Recent studies have revealed that the hydrogen bonding and dipole interactions of the triazole core can favour their binding to biomolecular targets and hence results in improving their solubility [19]. A number of biochemical and molecular biological investigations have demonstrated that proteins are the 'targets' for therapeutically active drugs of both natural and synthetic origins. Binding of small molecules to proteins is particularly an essential biochemical and biological process and is used as a basis for drug design [20]. Any knowledge of interaction mechanisms between small molecules and proteins is very important for us to understand the pharmacokinetics and pharmacodynamics of them. In general, these experiments are generally performed using readily available model proteins such as albumins and lysozyme. In this work, and in continuation of our previous work to study the interaction between human serum albumin (HSA) and BPT [21], we aimed to further investigate how BPT binds to other proteins. As cell surface receptor of B lymphocyte, immunoglobulin also plays critical roles in human immune response and many cell actions. Especially, human immune gammaglobulin is present itself in the blood of adults at 9.5–12.5 mg/mL, and as one of human plasma proteins, it is capable of binding an extraordinarily diverse range of metabolites, drugs, organic compounds and relevant antigens [22]. The globular protein human immunoglobulin (HIg), which consists of 670 amino acids in two identical polypeptide chain groups with molecular weight of 150 kDa, is commonly used as a model protein in the study of such interactions [23–25]. Another model protein is bovine hemoglobin (BHg), which is economical, easily available, well-characterized, and has been used in numerous studies [26]. Hemoglobin (Mr = 64.5 kDa) is an oxygen-carrying transport protein in the vascular system of animals with a diameter of approximately 5 nm. It also helps the transport of carbon dioxide and regulates the pH of blood. It exists as a tetramer comprising two identical α-chains of 141 amino acids each and two identical β-globin chains of 146 amino acids each which are bound to each other by hydrogen bonding, salt bridges and hydrophobic interactions. As a kind of intracellular protein, the concentration of hemoglobin is 330 mg/mL, by contrast, bigger than the serum albumin 40 mg/mL. It is very similar that hemoglobin can also reversibly bind with many kinds of small bioactive molecules [27], such as tannic acid [28], phenylurea herbicide [29], analogs of biphenyldicarboxylate [30] and surfactant [31], and so on. Therefore, the binding of drugs to HIg or BHg has an important role in therapeutic drug monitoring as the binding may be influenced by a number of drug and patient-associated factors, resulting in altered free drug concentration and thus drug efficacy and toxicity may be altered.

In the present work, we report on the experimental results of acute toxicity, antimicrobial activities of 1-(4-bromophenyl)-5-phenyl-1H-1,2,3-triazole on the basis of related methods, and then photochemical properties and the binding of BPT to HIg or BHg by a variety of spectroscopic methods in combination with quantum chemistry and molecular modeling under simulated physiological conditions. These are the first spectroscopic and bioactivity results on BPT at the molecular level, which can illustrate the nature of BPT information in vitro and in vivo.

2. Materials and methods

2.1. Materials

2.1.1. The characterization of BPT

1-(4-Bromophenyl)-5-phenyl-1H-1,2,3-triazole (BPT, ≥99.9%) was provided by the State Key Laboratory of Applied Organic Chemistry, Hainan Normal University. The basic physical and chemical properties: pale yellow solid; mp 135–136 °C (lit.[3] 100–102 °C); ^{1}H NMR ($CDCl_3$,

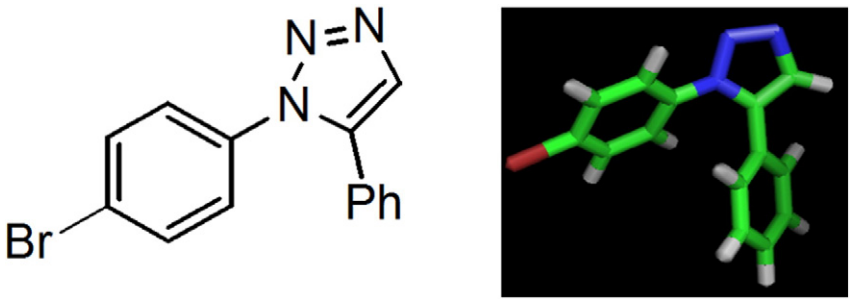

Fig. 1. Chemical structure of BPT (left) and the optimized structure of BPT (right).