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Full Length Article

Study of binding interaction between anthelmintic 2, 3-dihydroquinazolin-4-ones with bovine serum albumin by spectroscopic methods

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ABSTRACT

A new series of brominated derivatives of 2, 3-dihydroquinazolin-4(1H)-one were synthesized and their structures were confirmed using IR, NMR and mass spectra. The synthesized derivatives were screened for their in vitro anthelmintic activity. The investigations on interaction of the bioactive compound, **2i** with bovine serum albumin (BSA) were evaluated. The quenching mechanism of the compound, **2i** was deduced based on the results of Stern–Volmer equation. The number of binding site, prediction of binding site region and the changes in the secondary structure of protein were predicted using various spectroscopic studies.

1. Introduction

In the drug discovery pipeline, high attrition rate of the drug may be due to inappropriate pharmacokinetic (absorption, distribution, metabolism, excretion) behavior of the drug. Drug distribution has been one of the important components of drug discovery. It has been carried out by the plasma proteins [1]. Reversible binding of drug with plasma protein determines both the free concentration and storage form of the drug. Human Serum Albumin [HSA] is the predominant protein in plasma and it has excellent ligand binding properties [2]. BSA is the most common model protein for HSA because of its low cost, stability, and ready availability [3]. Various literatures related to protein-drug interactions have been reported [4]. Helminth infections cause a huge burden on developing countries and it requires low-cost and effective therapeutic intervention to improve the public health [5]. In 1961, the first member of benzimidazoles and thiabendazole were discovered which was followed by a group of other benzimidazoles [6]. In spite of the synthesis of various moieties, there's still a requirement for the necessity of effective medications. There is a loss in efficiency of the treatments in due course owing to resistance [7]. This can be true just in case of the infectious diseases and anthelmintic drugs also not an exception to the current issue [8]. Underdosing and overdosing on the drug was found to be the foremost reason for the drug resistance. A molecule of high utility should be designed to develop an effective agent. Halogenation could be a very important parameter for enhancing hydrophobicity, membrane permeability and halogen bonding interactions [9]. It might be as a result of the improvement of the binding affinity and binding property. Halogens are small atoms which can access the hydrophobic pockets in the protein [10] and they are known to be involved in highest affinity towards the receptor [11]. The enhancement in the anthelmintic activity of the organic compound was also reported due the presence of halogen [12,13]. Among the halogens, brominated derivatives are effective comparatively towards the biological receptors [14]. This can be understood from the diversified actions of bromine containing drugs existing in the market [Fig. 1]. In the chemistry era, the various forms of catalysts [15–18] and numerous diseases [19–25] have been discussed. Even though the synthesis of novel moieties in quinazolinones were reported already [26] the explorations of new synthetic methodologies [27–29] have created this nucleus still a budding one. In addition, the importance of quinazolinones with wide variety of substitutents was also proven with potent anthelmintic activity [30–32]. Even though brominated compounds have variety of applications in industrial purposes, it is less frequently used as medicine. Therefore, anthelmintic activity [33] of the brominated series of 2, 3-dihydroquinazolin-4(1H)-ones were carried out in this study. Also the interaction of the bioactive compound among the synthesized derivatives **2(a–i)** with BSA were studied by fluorescence quenching, excitation emission spectra (EEM) spectra and circular dichroism (CD) spectroscopy.

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2. Experimental section

2.1. Materials and methods

All commercially available reagents were used for synthesis without any further purification. Column chromatography was performed on Silica Gel (60–120 mesh, E. Merck). ^{1}H and ^{13}C NMR were obtained using a Bruker Avance 400 MHz spectrometer in $CDCl_3$ and DMSO-d_6 solution with TMS as an internal standard. Open capillary tube was used to measure the melting points which was measured on Elchem Microprocessor based DT apparatus and standard benzoic acid was used for correction. The mass spectrum were recorded using ESI-MS Thermo Fleet instrument. Bovine serum albumin was obtained from Himedia, stored at 4 °C and was used without further purification. Sodium phosphate buffer (pH 7.4) containing Na_2HPO_4 and NaH_2PO_4 were prepared using double distilled water. Fluorescence spectrum was recorded from Hitachi F-7000 spectrofluorometer. Circular dichroism (CD) spectrum was recorded on JASCO J-715 chiroptical spectrometer (JASCO corp.,).

2.2. General procedure for the synthesis of 6-bromo-2, 3-dihydroquinazolin-4(1H)-one, *2(a–i)*

About 1 mmol of the respective 2, 3-dihydroquinazolin-4(1H)-ones, **1(a–g)** were dissolved in 5 mL of DMSO solution. An equimolar amount of N-bromosuccinimide was added to it. Then reaction mixture was stirred at room temperature for 1 h. After confirming the completion of the reaction by TLC, the reaction mixture was poured into the crushed ice. The precipitated product was filtered off, dried and used for further reaction without purification. The compound series, **1(a–g)** were prepared as per the reported procedure (Scheme 1a) [34]. The compounds **2(h** and **i)** were prepared from 1.2 equivalent and the other derivatives **2(a–g)** were prepared from 1 equivalent of N-bromosuccinimide (Scheme 1b and c).

2.3. Anthelmintic evaluation

All the synthesized derivatives **2(a–i)** were tested for anthelmintic activity as per the reported protocols [35]. *Pheretima posthuma* of length (8 ± 1 cm) were randomly selected for the present study. The organisms were obtained from the vermicompost in Vellore manufacturing farm. The worms were made to adapt the laboratory conditions before carrying out the experiments. The earthworms were split into two groups of each containing six earthworms. Albendazole was diluted with normal saline to get 1.5 and 3% w/v served as standard and were poured into petri dishes. The synthesized compounds **2(a–i)** were dissolved in minimum quantity of DMSO (less than 5%) and diluted to arrange the concentration of 1.5 and 3% w/v with normal saline. Normal saline was used as diluents for preparing the solutions. The time taken for the complete paralysis and death of the earthworm was recorded. The mean paralysis time and mean lethal time were calculated for each compound. The time taken for worms to become motionless was noted as paralysis time. To determine death, every worm was frequently subjected to external stimuli that stimulate and induce movement in earthworms, if alive.

2.4. Computational analysis

The synthesized molecules **2(a–i)** were built and processed by VEGA ZZ molecular modeling package and geometrically optimized using Molecular Orbital PACKage (MOPAC) [36] with Austin Model 1 (AM1) Hamiltonian. Theoretically modeled structure of BSA (PDB ID: **4OR0**) was taken from Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). All the docking simulations were calculated out using Auto Dock 4.0 tool. As per Lamarckian genetic algorithm, gasteiger charges were assigned and the non-polar hydrogen atoms were merged. The grid maps were calculated in a grid box of 78 Å × 76 Å × 60 Å. The lowest energy conformer was selected and visualized using Discovery Studio 2016 Client. The log P values are calculated using the molinspiration tool.

2.5. Procedures for interaction study with biomacromolecules

A fixed concentration of BSA (1 μM) were titrated with successive addition of 1 μM stock solution of compound, **2i**. The protein was excited at 280 nm and the emission was recorded in the wavelength range of 300–500 nm at 298 K. The binding location of compound, **2i** was predicted in the presence of various concentrations of (1–6 μM) two site-specific probes (warfarin, ibuprofen). CD spectra were recorded from 200–260 nm with three scans averaged for CD spectra. The EEM spectra or three-dimensional fluorescence spectra were measured under the following conditions: the emission wavelength scan was recorded between 200–800 nm at an interval of 10 nm whereas excitation

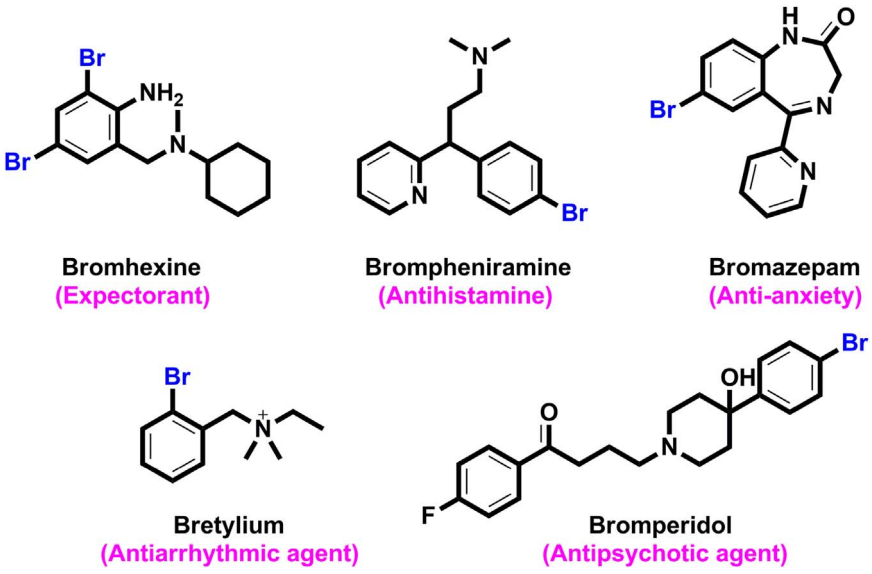

Fig. 1. List of marketed drugs containing bromine.