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Selective binding of naphthoquinone derivatives to serum albumin proteins and their effects on cytotoxicity

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ABSTRACT

Naphthoquinone derivatives such as lapachol, plumbagin, dichloroallyl lawsone show anticancer activity and generally cytotoxicity measurements are carried out in presence of bovine serum albumin; so understanding on the ability of serum albumin binding with such derivatives are essential. We have investigated cytotoxicity and serum albumin binding of a series of structurally related naphthoquinone derivatives. Substrate dependency and high selectivity in binding of naphthoquinone tethered carboxylic acids or pyridines with bovine serum albumin (BSA) and human serum albumin (HSA) are observed. For example, the binding constant of BSA with 3-(1,4-dihydro-2-methyl-1,4-dioxonaphthalen-3yl-thio)propanoic acid is ~594 times higher than 3-(1,4-dioxo-1,4-dihydronaphthalen-2-yl-amino)benzoic acid; whereas 4-(1,4-dioxo-1,4-dihydronaphthalen-2-yl-amino)benzoic acid shows ~367 times higher binding constant than the latter compound. The BSA weakly bind to pyridine tethered naphthoquinones, whereas HSA does not binds with them. The binding constant of HSA with 2-(1,4-dihydro-2-methyl-1,4-dioxonaphthalene-3-ylthio)benzoic acid is 134 times higher than the HSA binding constant with 2,2′-(1,4-dihydro-1,4-dioxo-naphthalen-2,3-diylthio)dipropanoic acid. Among the naphthoquinone carboxylic acids, the 3-(1,4-dioxo-1,4-dihydronaphthalen-2-yl-amino)benzoic acid binds selectively to BSA, but it does not bind to HSA. The 2-hydroxybenzoic acid or 4-mercaptobenzoic acid strongly binds to BSA. The binding of BSA with 4-hydroxybenzoic acid or 2-mercaptobenzoic acid are insignificant. We have not observed clear relationships of structure of naphthoquinone derivatives versus serum albumin binding, but could identify the compound having the best IC_{50} values of cytotoxicity among the twelve naphthoquinone compounds. The compound 3-(1,2-dihydro-1,2-dioxonaphthalen-4-yl-thio)propanoic acid in four cancer cell lines has IC_{50} values in the range 2.7–7.6 μM. This compound also has optimum binding constant with BSA (35.042×10^3 L mol^{-1}) or HSA (21.427×10^3 L mol^{-1}). The cytotoxicity values of the compounds were influenced by concentration of BSA.

1. Introduction

Serum albumin is the most abundant protein in the circulatory system of human; it plays important role in the transport and deposition of endogenous and exogenous compounds in human body [1,2]. The human serum albumin (HSA) and bovine serum albumin (BSA) are indispensible proteins in cytotoxicity measurements. Thus, prior understanding on substrate binding with HSA and BSA would help to ascertain the cytotoxic action of a drug. Generally the naphthoquinone derivatives are cytotoxic [3–17]. Several of naphthoquinone derivatives are used as anti-cancer drugs. The β-lapachone is a 1,2-naphthoquinone derivative is a inhibitor for DNA topoisomerase I and II [18,19] and it causes apoptotic cell death [20–22]. Lapachol is a naphthoquinone derivative which exhibits antitumor activity [23]. Dichloroallyl lawsone is a 1,4-naphthoquinone derivative inhibits dihydroorotate dehydrogenase metabolism [24,25]. Juglone induces apoptosis and it also prevents mitotic exit and plumbagin inhibits cell proliferation and induces apoptosis [26]; plumbagin has shown enhancement of autophagy in breast cancer cell lines [27]. The cell proliferation caused by 5-hydroxy-2-methyl-1,4 naphthoquinone was found to be concentration dependent [28], this makes avenue to systematically study the cytotoxicity of various naphthoquinone compounds and their actions in the presence of proteins, as the latter can bind and affect cytotoxicity. In practice, the effect of protein binding with naphthoquinone derivatives on cytotoxicity has not been studied [29–35]. Hence, we have taken up a study on the

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interactions of naphthoquinone derivatives with serum albumins and to determine a correlation between their binding to naphthoquinone substrate with cytotoxicity. A series of naphthoquinone tethered pyridines or carboxylic acids (1–12) shown in the Fig. 1 were chosen to make a distinction of the supramolecular binding of naphthoquinone tethered carboxylic acids versus naphthoquinone tethered pyridines, as they differ in their acid base properties. These functional groups are very often come across in biology. While studying the interactions of serum albumins with naphthoquinone derivatives, we found recognition of certain naphthoquinone derivatives by HSA and BSA.

2. Materials and methods

2.1. General

Infrared spectra were obtained using Perkin–Elmer FT-IR spectrophotometer (KBr, 4000–400 cm^{-1}). The UV–visible spectra were recorded using Perkin–Elmer Lambda 750 UV–Visible spectrophotometer. The fluorescence emissions were measured in a Perkin–Elmer LS-55 spectrofluorimeter. The fluorescence life times were measured by a picosecond time-resolved cum steady state luminescence spectrometer of Eddinburg instruments, model: FSP920 and LifeSpecII. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in Varian-AS400 (Oxford) multi probe 400 MHz NMR spectrometer. ESI-mass spectra were recorded on a liquid chromatography Water Q-TOF premier mass spectrometer attached to aquity HPLC. The chemicals used for synthesis were commercially procured from Aldrich Co. (USA) and used without purification. BSA (assay ⩾ 97% and with ⩽0.05% IgG) and HSA (assay ⩾ 97%) were purchased from Sigma Aldrich Chemicals, USA and used. For measurements of UV–visible absorption and fluorescence emission, solutions of the proteins in Tris-buffer solution at appropriate concentration were prepared and 3 mL of solution in each case was taken for study. The synthesis of the naphthoquinone compounds and cytotoxicity study are described in Supporting information.

2.2. Preparation of solution of BSA protein in buffer

Phosphate buffer (10 mM) of pH 7.4 was prepared in double-distilled water for all the protein binding experiments. A stock solution of BSA (14 μM) was prepared in buffer (20 mM Tris, 160 mM NaCl, 50 μM $ZnSO_4$, pH 7.4), while stock solutions of naphthoquinone compounds were prepared in DMSO because of their lower solubility in water.

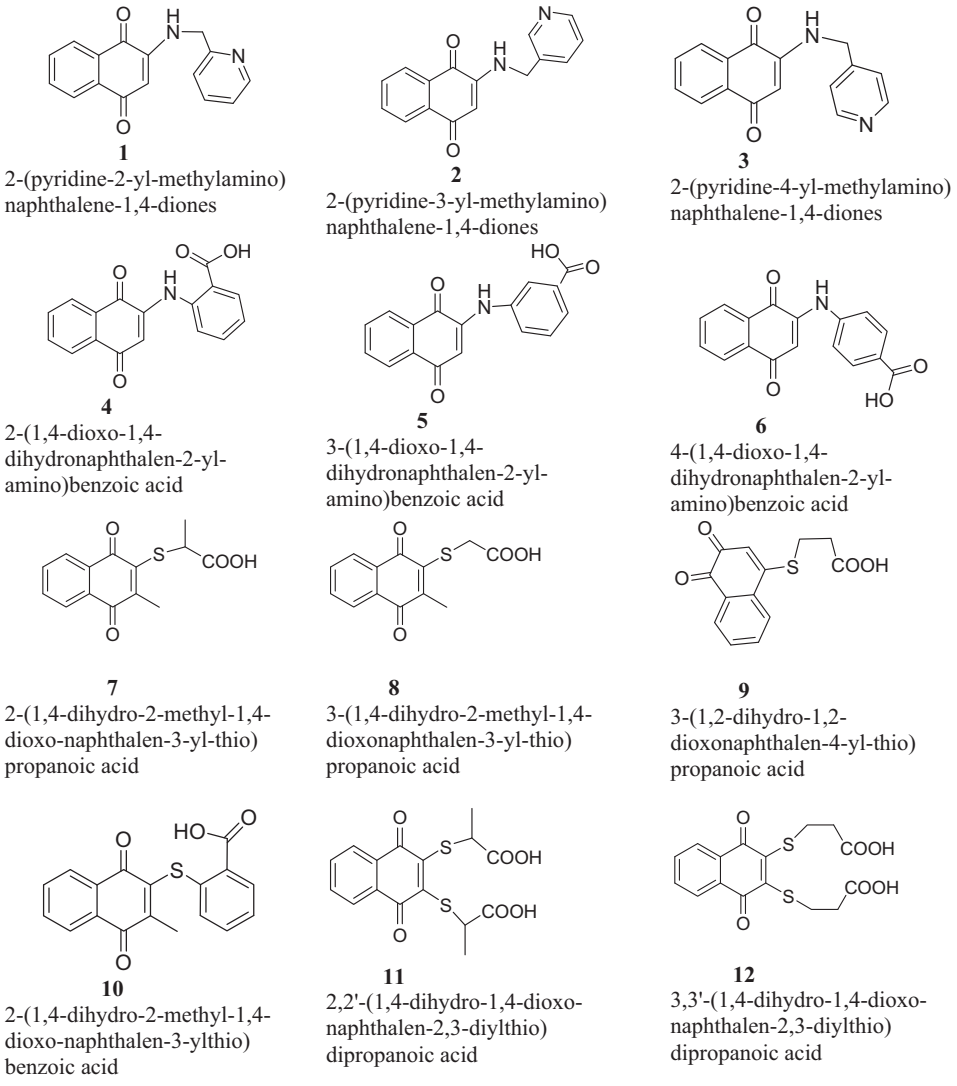

Fig. 1. The structures of the naphthoquinone derivatives.