SYNTHESIS, CHARACTERIZATION AND STUDY OF ANTIMICROBIAL ACTIVITY OF 2,6-DITERTIARY BUTYL-1,4-BENZOQUINONE HYDRAZONES

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ABSTRACT

Four 2,6-di-tert-butyl-1,4-benzoquinone hydrazones have been synthesized viz. A1, A2, A3 and A4. The purity was checked using HPLC. These compounds were characterized by FTIR and ^1H -NMR and they show a prominent absorption of $-(\text{C}=\text{N})-$ in FTIR. It also shows a common peak of olefinic proton at 6.50 Hz. These hydrazones were tested for their antimicrobial activity against Gram +ve and Gram -ve bacteria. A3 was found to be active at a minimum concentration of $50 \mu\text{g} \cdot \text{mL}^{-1}$ where as A2 was inactive.

Keyword: Hydrazones, 2,6-Di-tert-butyl-1,4-benzoquinone, antimicrobial activity, Hydrazone derivatives.

INTRODUCTION

Benzoquinones are the compounds with important biological functions in oxidative phosphorylation and electron transfer processes¹, bioenergetic transport^{2,3}. They possess diverse pharmacological properties such as anti-inflammatory⁴, antimicrobial^{5,6}, antiviral activities⁷ and anticancer⁸⁻¹¹. The compounds are used in the design and synthesis of various heterocyclic drug molecules as potential synthetic building blocks. The importance of research on these compounds in recent years is provided by the discovery of chemo preventive and therapeutic properties observed for a naturally occurring benzoquinone compound, viz. Thymoquinone, which is found in the Black Cumin (*Nigella sativa*) seed oil¹². The black cumin seeds are being used as a spice in the Mediterranean region and in Western Asian countries including India. This herb is known by many different names such as 'Kalonji' in India 'Panacea' in Latin, 'Habbah Sawda' in Arabic, or 'Hak Jung Chou' in Chinese. The extracts of this plant have indicated potent antitumor activities without serious toxic effects suggesting that compounds having benzoquinone nucleus may be developed into useful therapeutic agents¹³. Most of the investigations on the biogenesis of benzoquinones have been reported with moulds such as *Aspergillus fumigatus* and *Penicillium patulum*¹⁴. Benzoquinones are the simplest structural quinines which are widely distributed in plants, animals and bacteria. Majority of the fungal benzoquinones are formed by the acetatemononate pathway yielding ubiquinones having polyprenyl side chains¹⁵. These compounds are found in most aerobic organisms from bacteria to higher plants and animals and are involved in mitochondrial electron transport chains. The *Streptomyces* bacteria also produce large number of nitrogen containing heterocyclic quinones¹⁶. Radio-labelled precursor studies have indicated two biosynthetic routes for the formation of benzoquinone compounds which serve as important toxic defense for many insects and arthropods such as methylbenzoquinone, 2, 3-dimethyl and 2, 5-dimethylbenzoquinone. Comparatively less is known about the biogenesis of benzoquinones in higher plants¹⁷. The methods employed to isolate naturally occurring benzoquinones are common to natural products chemistry

and involve sequential extraction with solvents of increasing polarity while the appropriate fractions are purified by column or thin layer or preparative thin layer chromatography. Ultrasonic or critical fluid extractions carried out in recent years offer advantages in terms of avoiding residual solvent levels¹⁸. Chemically 1, 4-benzoquinone is a multi-functional moiety exhibiting properties of a ketone, oxidant and an alkene undergoing addition reactions. It is a simple six-membered, non-aromatic compound which is the oxidised derivative of 1, 4-dihydroquinone. It can be easily reduced to 1, 4-benzenediol. Equimolar mixture of 1, 4-benzoquinone and 1, 4-benzenediol which is used as a standard electrode material is known as quinhydrone¹⁹. Several features make it an attractive motif for building supramolecular assemblies which are associated with the quinonoidal structure. Similarly, the quinonoidal carbonyl groups are capable of evolving hydrogen bonded assemblies yielding inclusion compounds^{20,21}. The electron deficient benzoquinone system also yields charge transfer complexes very easily²²⁻²⁷. The facile redox cycling associated with benzoquinones makes them excellent oxidizing agents²⁸. They are known to react with acetic anhydride and sulphuric acid to give triacetate of 1, 3, 4-trihydroxybenzene and the reaction is known as Thiele reaction²⁹. They react with large number of primary and secondary aliphatic or aromatic amines yielding mostly disubstituted compounds. The benzoquinones easily undergo nucleophilic addition reactions and serve as an excellent dienophiles in the Diels- Alder reaction. However, single substitution is observed with secondary amines under selected conditions. During such substitutions one or two moles of reduced benzoquinone molecules are also formed as shown in Figure 1³⁰. Harley-Mason and Laird³¹ have reported on the formation of 1, 2, 3, 4-tetrahydro-1, 4, 5, 8-tetraazaanthracene after reacting 2, 5-dihydroxy-p-benzoquinone with ethylenediamine in presence of air in aqueous media. A similar reaction with 2,3-dimethoxy-p-benzoquinone and ethylenediamine yields 2, 5-bis(2-hydroxyethylamino)-3, 6-dichloro-1, 4-benzoquinone which on further heating for several hours gives tetra-aza-anthracene Figure 2³². Aromatic amines also undergo nucleophilic substitution at 2 and 5

position of p-benzoquinone. For example, Aseed and Omer have synthesised 2, 5-Diamino-3, 6-dibromo-1, 4-benzoquinone by reacting various amines with 2, 3, 5, 6-tetrabromo-1, 4-benzoquinone Figure 3 which exhibit antibacterial activity against *Aspergillus niger* and *Candida albicans* respectively³³. In present paper we have reported the synthesis and antimicrobial or antibacterial activity of four hydrazones of 2,6-Di-tert-butyl-1,4-benzoquinone. The compounds are tested for their antimicrobial activity against Gram +ve (*Staphylococcus aureus*) and Gram -ve (*Escherichia coli*).

MATERIALS AND METHODS

Solvents for synthesis were reagent grade and dried by standard procedures²³. The starting materials such as 2,6-ditertiary butyl-1,4-benzoquinone, thiosemicarbazide, 4-hydroxy benzoic acid hydrazide, 3-hydroxy naphthoic acid hydrazide, Indol 3-acetic hydrazide, were obtained from

Sigma-Aldrich chemicals and acetone, methanol, ethanol and dichloromethane, which were obtained from SRL Chemical Limited, India. All compounds were routinely checked by TLC on silica gel G plates using petroleum ether / ethyl acetate (7 : 3; 6 : 4; 5 : 5 by V / V) as solvent system and the developed plates were visualized by UV light and iodine vapours. The detailed synthesis has been shown in Scheme 1. Melting points of as synthesized compounds were determined with open capillary tube on a VEEGO melting point apparatus. The $^1\text{H-NMR}$ and Liquid chromatography mass spectra (LCMS) were obtained from NCL, Pune, India and purity was checked by "HPLC—Systronics". IR spectra were recorded by "FT-IR Jasco" spectrometer at the centre. Nutrient broth was used for growth of the organisms. The media was prepared and autoclaved for 15 minutes at 121°C, 15 lb pressure. The autoclaved media were inoculated with seed culture and incubated at 37°C for 24 hours.

Table 1: Antimicrobial Activity Results in Comparison with Standard

Compound ($\mu\text{g}\cdot\text{mL}^{-1}$)	<i>Staphylococcus aureus</i> (G+)			<i>E. coli</i> (G-)		
	50	100	150	50	100	150
A1	-	3	5	1	4	5
A2	-	-	-	-	-	-
A3	1	2	4	1	1	2
A4	-	1	2	2	2	3
DMSO	-	-	-	-	-	-
Ciprofloxacin	5	6	9	6	6	8

Figure 1: Nucleophilic Substitution Reactions of Benzoquinone

Fig (2). Preparation of tetra-aza-anthracene from reaction of 2,5-dihydroxy-1,4-benzoquinone with ethylene diamine (EDA).

Fig (3). Reaction of tetrabromo-substituted 1,4-benzoquinone with amines.

Where-

Scheme 1: General Scheme of Synthesis of Hydrazones

RESULTS AND DISCUSSIONS

Procedure for the Synthesis of A1: N'-(3,5-di-tert-butyl-4-oxocyclohexa-2,5-dienylidene)-2-(1H-indol-2-yl)acetohydrazide

200 mg of 2,6-ditertiarybutyl-1,4-benzoquinone and 172 mg Indol-3-acetic acid hydrazide were dissolved in the 15 ml methanol and refluxed for 2-3 hours with constant stirring. Monitor the reaction by TLC. The reaction mixture was poured in ice to get orange coloured solid. Colour: Orange. Yield: 69 %, M. P.: 198°C. Purity (HPLC): 99.6 %, Mass (LCMS): 391, FTIR (KBr, cm^{-1}): 3265.86 (-NH), 2958.27 (-CH), 1660.41 (-C=O), 1647.56 (-C=N), 1057.03 (-N-N). $^1\text{H-NMR}$ (CDCl_3) (δ , ppm.): 1.29 (s, 18H, -C(CH₃)₃), 3.56 (s, 2H, -CH₂-), 6.49 (s, 2H, olefinic H), 7.24 (s, 1H, -NH), 7.36 (s, 4H), 6.66 (s, 1H, indolic H), 10.4 (s, 1H, indolic NH).

Procedure for the Synthesis of A2: (1-(3,5-di-tert-butyl-4-oxocyclohexa-2,5-dienylidene) thiosemicarbazide

200 mg of 2,6-ditertiarybutyl-1,4-benzoquinone and 82.72 mg thiosemicarbazide were dissolved in the 15 ml methanol and refluxed for 2-3 hours with constant stirring. Monitor the reaction by TLC. The reaction mixture was poured in ice to get yellow coloured solid. Colour: Yellow. Yield: 85 %, M. P.: 66°C. Purity (HPLC): 98.7 %, Mass (LCMS): 293, FTIR (KBr, cm^{-1}): 3385.42 (-NH), 2963.09 (-CH), 1671.02 (-C=O), 1525.42 (-C=N), 1139.72 (-N-N). $^1\text{H-NMR}$ (CDCl_3) (δ , ppm.): 1.28 (s, 18H, -C(CH₃)₃), 2.5 (s, 2H, -NH₂), 6.50 (s, 2H, olefinic H), 7.24 (s, 1H, -NH).

Procedure for the Synthesis of A3: N'-(3,5-di-tert-butyl-4-oxocyclohexa-2,5-dienylidene)-4-hydroxybenzohydrazide

200 mg of 2,6-ditertiarybutyl-1,4-benzoquinone and 138 mg 4-hydroxy benzoic acid hydrazide were dissolved in the 15 ml methanol and refluxed for 2-3 hours with constant stirring. Monitor the reaction by TLC. The reaction mixture was poured in ice to get yellow coloured solid. Colour: Yellow. Yield: 89 %, M. P.: 164°C. Purity (HPLC): 99.8 %, Mass (LCMS): 354, FTIR (KBr, cm^{-1}): 3370 (-NH), 3026.20 (-OH), 2958.27 (-CH-), 1618.95 (-C=O), 1551.45 (-C=N), 1225.21 (-N-N). $^1\text{H-NMR}$ (CDCl_3) (δ , ppm.): 1.28 (s, 18H, -C(CH₃)₃), 3.56 (s, 2H, -CH₂-), 6.50 (s, 2H, olefinic H), 7.24 (s, 1H, -NH), 7.84 (dd, J = 8 Hz, 2H), 7.68 (dd, J = 8 Hz, 2H), 9.75 (s, 1H, exchangeable -OH).

Procedure for the Synthesis of A4: N'-(3,5-di-tert-butyl-4-oxocyclohexa-2,5-dienylidene)-3-hydroxynaphthalene-2-carbohydrazide

200 mg of 2,6-ditertiarybutyl-1,4-benzoquinone and 183.63 mg 3-hydroxy 2-naphthoic hydrazide were dissolved in the 15 ml methanol and refluxed for 2-3 hours with constant stirring. Monitor the reaction by TLC. The reaction mixture was poured in ice to get yellow coloured solid. Colour: Yellow. Yield: 85 %, M. P.: 202°C. Purity (HPLC): 99.6 %, Mass (LCMS): 404, FTIR (KBr, cm^{-1}): 3426.89 (-NH), 3291.89 (-OH), 2968.87 (-CH), 1589.70 (-C=O), 1504.20 (-C=N), 1057.03 (-N-N). $^1\text{H-NMR}$ (CDCl_3) (δ , ppm.): 1.28 (s, 18H, -C(CH₃)₃), 6.50 (s, 2H, olefinic H), 7.24 (s, 1H, -NH), 7.66 (m, 6H, naphthoic Hs), 9.73 (s, 1H, exchangeable -OH).

Antimicrobial Activity

The anti-microbial results showed that some of the compounds were active against both Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria. Among the tested compounds the compounds A1, A3 and A4 showed good antibacterial activity against the test

organisms. The compound A2 had no anti-bacterial activity against. Maximum antibacterial activity was shown by compounds containing the heterocyclic rings in the structure. On the other hand, compared to the standard antibacterial drugs namely, Ciprofloxacin was showed moderate activity against test organisms. The obtained results of antibacterial activity have been summarized in Table 1.

CONCLUSION

Out of four hydrazones synthesized only three shows effective antibacterial activity. A1 is more active than A3 and A4 where as A2 is non-active. All the three hydrazones are moderately active when compared with ciprofloxacin. The Minimum Inhibitory Concentration (MIC) of A1 is 100 $\mu\text{g}\cdot\text{mL}^{-1}$ for Gram +ve and 50 $\mu\text{g}\cdot\text{mL}^{-1}$ for Gram -ve bacteria; whereas the MICs for A3 and A4 are 50 and 100 $\mu\text{g}\cdot\text{mL}^{-1}$ respectively for Gram +ve and 50 $\mu\text{g}\cdot\text{mL}^{-1}$ each for Gram -ve bacteria. The shown activity can be accounted for the presence of either a heterocyclic or an aromatic moiety in the respective hydrazide. As the synthesized hydrazones are biologically active, study of their anticancer activity is of our further interest.

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