Synthesis, Spectral and Antioxidant Studies of Indole-2,3-dione derivatives

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Abstract— **The changes in lifestyle, food habits and climate have significantly influenced the biology of human beings. Stress has become one of the major illnesses in today's world. Oxidative stress leads to the formation of free radicals in the body which becomes a major cause for diseases. Hence, over the last decade, there is an increased interest to find effective free radical scavengers that could address this issue. Compounds that have potential antioxidant activity from plant sources are on being explored. 1H-Indole-2,3-dione derivatives are one such class of compounds that show potential antioxidant activities. In the present work new potent molecules having indolin moiety with enhanced free radical scavenging properties have been synthesized. The synthesized indole-2,3-dione derivatives were characterized by Fourier transform infrared, ^{1}H nuclear magnetic resonance, ^{13}C nuclear magnetic resonance and mass spectroscopy techniques. The in vitro antioxidant activity was evaluated using hydrogen peroxide scavenging activity and total antioxidant capacity by a phosphomolybdenum assay and their reducing power ability. The results show that the synthesized indole-2,3-dione derivatives have remarkable antioxidant activity and are suitable candidates for development into drug for oxidative stress and other diseases.**

Keywords: indole-2,3-dione, antioxidant, free radical scavengers

I. INTRODUCTION

Antioxidants play a significant role in several important biological processes such as immunity, protection against tissue damage, reproduction and growth or development. They reduce damage to cells and biochemicals caused by free radicals, which are normal products of metabolism[1]. Isatin and its derivatives are among an extensive diversity of heterocycles that have been explored for developing pharmaceutically important lead compounds. They are biologically active and have significant importance in medicinal chemistry. Several isatin derivatives are in the development phase as potential new drugs for various diseases[2] which includes antifungal, antihistaminics, antiemetics, neuroleptics, tranquilizers[3], analgesic, anticonvulsant[4], anti-inflammatory[5], antiplasmoidal[6], anti HIV[7], anthelmintic[8], antidepressant, antimicrobial[9], anticancer[10], antiviral[11], antitubercular[12], antibacterial[13], especially with antioxidant activity[14].

The presence of the keto groups at carbonyl positions 2 and 3 can either enter into addition reaction at the C-O bond or form condensation products with release of water. Through the NH group, compounds of the isatin series are capable of entering into N-alkylation and N-acylation[15]. In present work, we have synthesized isatin derivatives, exploiting the above possibilities. The two isatin derivatives namely, 1-benzoyl indole-2, 3-dione (S1) and 1-benzoyl-3-(4-bromophenylimino)indolin-2-one (S3) were studied for their potential antioxidant activity and reducing power ability.

II. MATERIALS AND METHODS

A. *Materials and physical measurements*

All the chemicals and solvents were used as such without any further purification. Infrared spectra were recorded on a Perkin-Elmer FT-IR Spectrometer in the range of 4000-500 cm^{-1}, using KBr pellets. The proton and carbon-13 NMR spectra were recorded using Bruker ^{1}H NMR having a frequency of 500 MHz and ^{13}C NMR having frequency of 125 MHz. The solvents used were $CDCl_3$ and C_6D_6 with reference TMS. Mass spectra were recorded by Thermoscientific LT2 X2 model ESI of m/z range 100-1000. The solvent used was chloroform. The electronic absorption for various antioxidant studies were recorded on a Systronics UV-Visible

spectrophotometer 118. The spectra were recorded in the region 250-800 nm at 25 ˚C using a matched pair of Teflon stoppered quartz cell of path length 1cm. The melting points (m.p.) were determined in open capillaries, using an instrument purchased from Raagaa industries, Madras, expressed in ˚C.

B. Synthesis of isatin derivatives

1) Synthesis of 1-benzoyl indole-2, 3-dione (S1)

To a solution of isatin (0.01 mol) and benzoyl chloride (0.01 mol) in 20 mL of ethanol, a solution of sodium carbonate (0.025 mol) in 5 mL of water was added and refluxed for 2-3 hrs. The reaction mixture was cooled and the precipitate was filtered. The solid mass separated was dried and recrystallized with benzene[16]. The synthetic route is shown in Fig. 1. Mol.formula: $C_{15}H_9O_3$, Mol.wt: 251, Cream crystals, m.p: 165 °C, yield: 65%

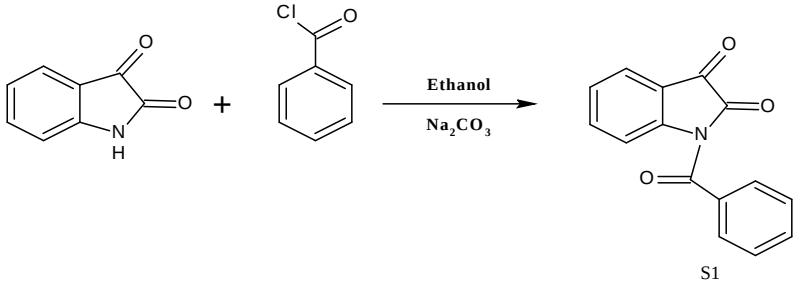

Fig 1. Schematic representation for the synthesis of compound S1

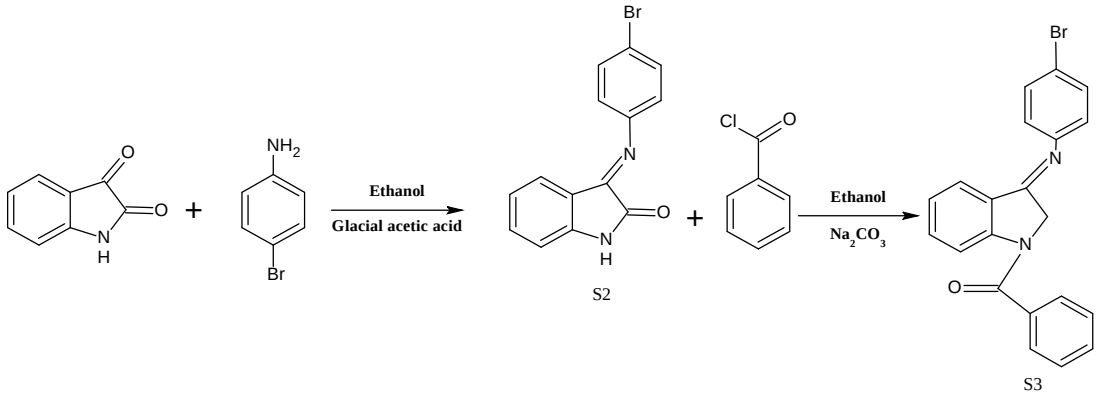

Fig 2. Schematic representation for the synthesis of compound S3

2) Synthesis of 1-benzoyl-3-(4-bromophenylimino)indolin-2-one (S3)

Equimolar quantities of isatin (0.005 mol) and p- bromo aniline (0.005 mol) were added into a 20 mL of absolute ethanol containing a few drops of glacial acetic acid in a 250 mL round bottom flask. The reaction mixture was refluxed for 2 hrs. The solvent was stripped off and the product (S2) was recrystallized from ethanol[15]. To a solution of intermediate obtained(S2), (0.01 mol) and benzoyl chloride (0.01 mol) in 20 mL of ethanol, a solution of sodium carbonate (0.025 mol) in 5 mL of water was added and refluxed for 2-3 hrs. The product was cooled and filtered. The solid mass separated was dried[16]. The schematic representation is shown in Fig. 2. Mol. formula: $C_{21}H_{13}BrN_2O_2$, Mol.wt: 405, Pale yellow crystals, m.p: 185 °C, yield: 68%

C. Antioxidant activity

1) Hydrogen peroxide scavenging activity

The ability of test compounds to scavenge hydrogen peroxide was determined by using the method of Sanchez (2001) and Famey et al. (1998). The solution of hydrogen peroxide (40mM) was prepared

in phosphate buffered saline (pH 7.4). Various concentrations of 1 mL of test compounds and standards were added to 2 mL of H_2O_2. Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against the blank. Ascorbic acid was used as a reference standard[16].

2) Total antioxidant capacity by phosphomolybdenum assay

The principle of this assay is based on the reduction of Mo(VI) to Mo(V) by test compounds and subsequent formation of a green phosphate/ Mo(V) complex at acid pH. An aliquot of 0.1 mL of the test solution in ethanol (chloroform, in the case of S3) was mixed with 1 mL of a reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated at 55 °C for 90 min. The samples were cooled at room temperature and then absorbance was measured at 695 nm against the blank. The blank solution was containing 1 mL of the reagent solution and an appropriate volume of the same solvent used in the test compound.

The total antioxidant capacity of the tested compounds was calculated according to the equation,

$$TAC\ (\%) = [(A_o - A_t)/ A_o] \times 100$$

Where, A_t is the absorbance value of the test compound and A_o is the absorbance of the blank sample. The reference standard is ascorbic acid[16].

3) Reducing power ability

The reducing power of the test compounds were determined according to the method previously described by Oyaizu (Oyaizu, 1986). Different concentrations of the test compounds (100-1000 mg) in 1 mL distilled water were mixed with phosphate buffer (2.5 mL, 0.2M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50 ˚C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. A portion of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was taken as the standard. Phosphate buffer (pH 6.6) was used as blank solution. The absorbance of the final reaction mixture was taken[17].

III. RESULTS AND DISCUSSION

A. Spectral characterization

1) 1-benzoyl indole-2, 3-dione (S1)

FT-IR spectrum: 3100 -3500 cm^{-1} (N-H), 1789 cm^{-1} (C=O), 1693, 1424 cm^{-1} (aromatic C=C), 1326 cm^{-1} (C-N). ^{1}H NMR spectrum: δ 7.08-7.2 (Ar-**H** of indole group), δ 8.20-8.22 (Ar-**H** of benzoyl group). ^{13}C NMR spectrum: δ 129-130 (**C** in indole group), δ 133-134 (**C** in benzene ring of the benzoyl group), δ 172 (**C**=O). Mass spectrum: m/z 274 (M+Na) peak.

2) 1-benzoyl-3-(4-bromophenylimino)indolin-2-one (S3)

FT-IR spectrum: 3069 and 3007 cm^{-1} (C-H), 2838 and 2560 cm^{-1} (C-H str), 1687 cm^{-1} (C=O), 1602 cm^{-1} (C=N), 1581 cm^{-1} (C=C), 1326 cm^{-1} (C-N str), 1292cm^{-1} (C-O-C). ^{1}H NMR spectrum: δ 7.5-7.6 (Ar-**H** in the indole and C=N- group), δ 8.2 (Ar-**H** of benzoyl group). ^{13}C NMR spectrum: δ 128-133 (Ar-**C**), δ 172 (**C**=O). Mass spectrum: m/z 405 (M^+) peak.

B. In vitro antioxidant activity

1) Hydrogen peroxide scavenging activity

The radical scavenging effect of the synthesized compounds against H_2O_2 was measured spectrophotometrically. The graph for the % inhibition of the tested compounds and that of the reference standard is shown in Fig. 3

All the compounds produced a concentration-dependent scavenging of free radical. As seen, compound S3 was found to have potent antioxidant activity than compound S1. But both the compounds were comparable and possess greater antioxidant activity than ascorbic acid. It was observed that compound S3 with electron withdrawing group (bromine) on the aromatic ring favors antioxidant activity [18].

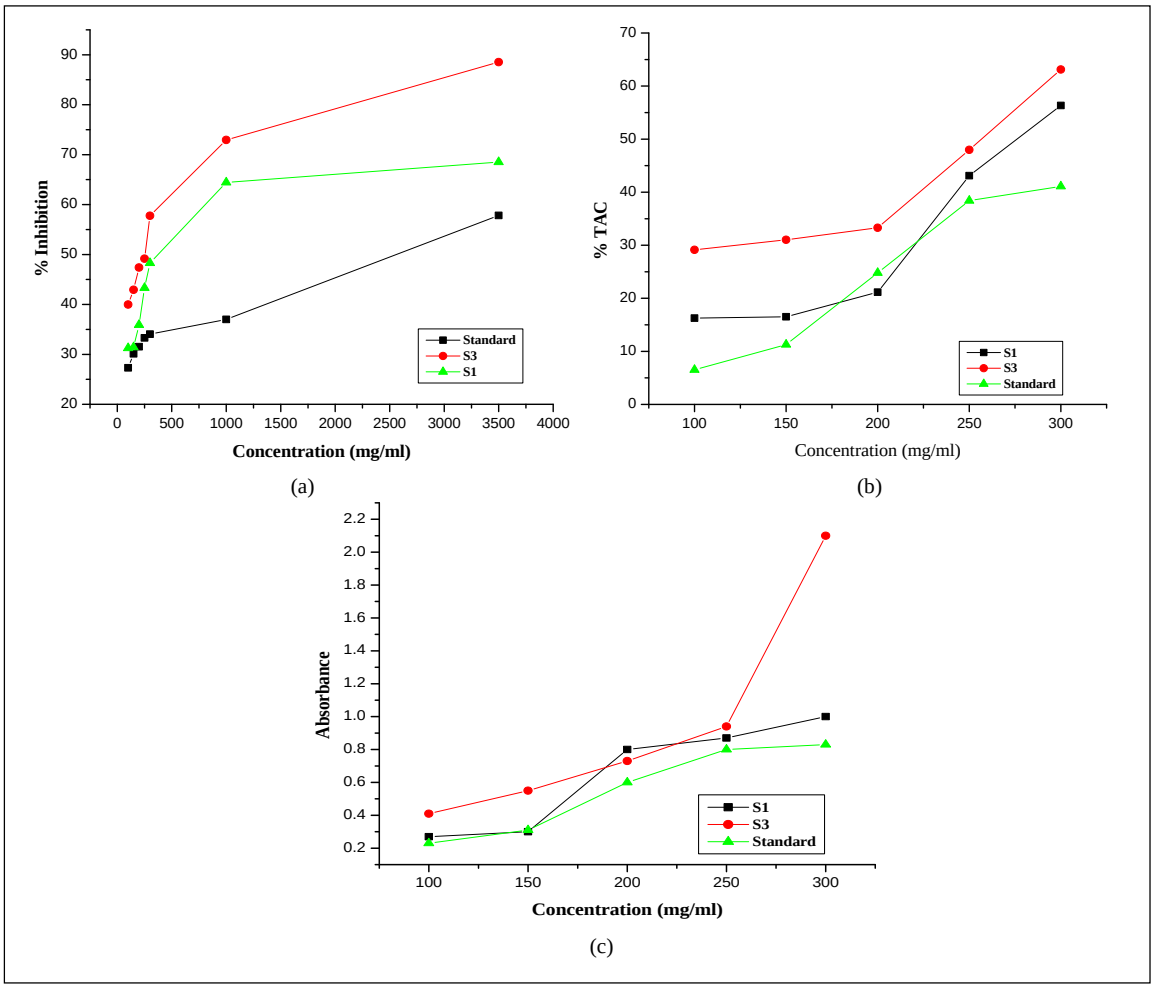

Fig 3. Comparative graphs of: (a) hydrogen peroxide scavenging activity, (b) total antioxidant capacity and (c) reducing power ability

2) Total antioxidant capacity

The results are expressed as % TAC. The higher the TAC value, the more effective is the antioxidant activity [18]. The graph for the % TAC of the tested compounds and that of the reference standard is shown in Fig. 3. The total antioxidant activity of the synthesized compounds was determined by phosphomolybdenum assay. The absorbance decreased with the increasing concentrations of the compounds, which indicates that the compounds could effectively decrease the amount of formed peroxides. The % TAC, thereby increases with increase in concentration of the compounds. The compounds showed good antioxidant activity but at higher concentrations. Both the compounds are comparable with the standard but compound S3 has more % TAC than compound S1.

3) Reducing power ability

The graph for the reducing power of the synthesized compounds and that of the reference standard is shown in Fig. 3. The reducing power of the synthesized compounds was remarkable and it was found to increase as the concentration increases. The absorbance increases as the concentration increases.

IV. CONCLUSION

The present study shows the significance of newly synthesized derivatives, which have appreciable antioxidant activities than that of the reference standard, ascorbic acid. Between these two compounds, 1-benzoyl-3-(4-bromophenylimino)indolin-2-one (S3) had much better antioxidant activity than 1-benzoyl indole-2, 3-dione (S1) which could be attributed to the presence of the electron withdrawing group, bromine, in it. Thus, these two compounds are potential candidates for development into drugs with antioxidant capacity.

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