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Synthesis, characterization and antioxidant evaluation of 2-(2-substituted) naphthalene-1,4-dione derivatives

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ABSTRACT

Background: 1,4- naphthoquinone (Lawsone) are widely distributed in nature and have been used since ancient times in traditional medicine. Lawsone has been used as a dye, and both its natural form and synthetic derivatives exhibit antifungal, antibacterial, antitumor, antimalarial, molluscicidal and antioxidant activity. **Methods:** 1,4- naphthoquinone (Lawsone) was isolated from the leaves of *lawsonia inermis* by using pH gradient method. A convenient synthesis of 2-substituted amino naphthalene-1,4-dione **(3a-e)** has been achieved by reaction of isolated 1,4- naphthoquinone with substituted aniline in the presence of ethanol and evaluated for *in- vitro* antioxidant activity using DPPH model. The structure of the final analogues has been confirmed on the basis of elemental analysis, FTIR, ^{1}H NMR and mass spectra. All the values of elemental analysis, FTIR, ^{1}H NMR and mass spectra were found to be prominent. **Results and discussion:** The results indicate that synthesized compound **3d** having IC_{50} 75.39 ± 4.12 mg/ml showed potent antioxidant activity comparable to standard ascorbic acid (IC_{50} 45.54 ± 3.06 mg/ml). **Conclusion:** This study suggests that leaves of *Lawsonia inermis* have bioactive compounds for a new antioxidant drug development.

KEY WORDS: *Lawsonia inermis,* Naphthoquinone, *In-vitro* antioxidant activity, leaves extract, DPPH

1. INTRODUCTION

Naphthoquinone derivatives have pulled in proceeding with enthusiasm throughout the years due to the utilization of its ring framework as vital center structure in numerous drug substances and reported to cover broad range of pharmacological applications[1,2]. Plant-derived natural products like phenolic compounds (flavonoids), steroids, terpenoids, saponins, volatile oils, glycosides, etc. have received significant attention in recent years due to their various pharmacological properties including antioxidant activity. Furthermore, especially particularly 1,4 naphthoquinones are widely distributed phenolic compounds in nature such naphthoquinones are reported to exhibit diverse pharmacological properties like antibacterial[3], antifungal, antiviral, anti-inflammatory antipyretic properties and anticancer activity[4]. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infection and degenerative diseases[5]. Although clinical trials and experiments involving whole animals are important in natural product screening however significance of in vitro screening is picking up prevalence because of money related, moral and time requirements. The ability to quickly recognize active compound in complex mixture of natural product extract, lead optimization as well as lead characterization are important factors in the natural product screening. Numerous reports of natural antioxidants of plant origin have been published and their importance in food, health and protective medicine has been well recognized[6-10]. The current investigation presents the report on comparative analysis of *in-vitro* antioxidant activity of different naphthoquinone derivatives, which could help in finding novel antioxidant compound (s). In view of the above mention and as a part of our continuous efforts towards the development of more potent antioxidant agents[11], it was thought of interest to combine the above mentioned boilable rings together in a molecular framework to investigate the additive effect of these rings towards antioxidant activities.

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2. MATERIALS AND METHODS

2.1. Collection of Plant Material

Fresh leaves were collected from the *Lawsonia inermis* plant growing in the medicinal garden of Hygia Institute of Pharmaceutical Education and Research, Lucknow, U.P., India in the month of March and April (2013) and identified by Dr. G. D. Bagchi an expert taxonomist in Department of Taxonomy & Pharmacognosy, Central Institute of Medicinal and Aromatic plants, Lucknow. The plant specimens were authenticated (Ref. No. LI-1) and the leaves were washed in running water and air-dried.

2.2. Chemicals

Purchases were as follows. Organic solvents: acetone, chloroform, *n*-hexane, ethyl acetate, ethanol from Qualigens® Fine Chemicals (Mumbai); petroleum ether, diethyl ether, DMSO from Central Drug House (P) Ltd. (New Delhi) and ethanol from Sd Fine-chem Limited (Mumbai) (all analytical grade). 1,1-Diphenyl-2-picrylhydazyl radical (DPPH) from Sigma-Aldrich (New Delhi). Other reagents used were of analytical grade and obtained from different commercial sources.

2.3. Experimental

The melting points were determined in open capillary tubes and were uncorrected. The purity of all the synthesized compounds were checked by TLC on precoated silica gel-G aluminum sheets (Type 60 GF_{254}, Merck) and the spots were detected by exposure to iodine vapors. The infrared (FT-IR) spectra were recorded on 470-Shimadzu infrared spectrophotometer using the KBr disc prepared by pressed pellet technique and υ_{max} is expressed in cm^{-1}. NMR spectra were measured in DMSO-d_6 as solvent at 300 MHz (^{1}H NMR) on a BRUKER AVANCE-300 spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are given in parts per million (ppm). Spin multiplicities are given as s (singlet), d (doublet), dd (double doublet) and m (multiplet). Mass spectra were obtained on Shimadzu 2010A LC-MS spectrometer. Elemental analysis was carried on Elemental Vario EL III Carlo Erba 1108 and the values were within ±0.04% of the theoretical values. All the solvents were distilled and dried with usual desiccant.

2.4. Isolation and Purification of Lawsone

Shade dried and 200 g powered henna are extracted by agitation for 2 h with 20% sodium bi carbonate solution (800 ml). The extract was filtered, marc is re-extracted with 400 ml of same solution for 1hr, filtered and the alkaline extract was pooled together. The extract was acidified with dil. sulphuric acid and crude product obtained on standing was re-extracted with sufficient quantity of ammonium hydroxide and again acidified with dil. hydrochloric acid. The product is finally extracted with two successive quantities of benzene (160 ml) and filtered[12]. The filtrate was distilled to yield yellow brown colour crystal of lawsone and the purity was checked by TLC .The red spot obtained, had R_f value 0.07 and its melting point was obtained as 196 ºC.

2.5. Synthetic Procedure

2.5.1. General procedure for the synthesis of 2-(2-substituted) naphthalene-1,4-dione (3a-e)

Naphthoquinone (**1**) (300 mg, 1.89 mmol) in 20 mL absolute ethanol was stirred at 5-10 ºC until solid completely dissolved. To this naphthoquinone solution was added dropwise a solution of the corresponding amine (**2**) (0.95 mmol) in 10 mL of absolute ethanol at 5-10 ºC. The reaction mixture was allowed to reach room temperature and was stirred for another 12–18 h. The completion of the reaction was monitored by TLC. The solid product thus obtained was filtered, washed with water and recrystallized from acetone to give compounds (**3a-e**)[13].

2.5.2. *2-(2-methoxyphenylamino)naphthalene-1, 4-dione (3a)*

Yield 45%; Red solid; mp 149 ºC; eluent-*n*-hexane/ethyl acetate 70:30 v/v, R_f= 0.67; FTIR (KBr, υ cm^{-1}): 3100 (Ar C-H), 1685, 1676 (C=O, α,β unsaturated), 1285 (C-N), 3403 (N-H), 1570 (C=C), 1204 (C-O-C asym.), 1033 (C-O-C sym); ^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm): 3.74 (s, 3H, OCH_3), 4.17 (s, 1H, H-N, D_2O exchangeable), 6.31-7.02 (m, 4H, Ar-H), 7.92-8.15 (d, 2H, Ar-H), 8.19-8.22 (dd, 2H, Ar-H), 8.43 (s, 1H, Ar-H); EIMS (m/z): 279.29 $[M]^+$, 280.90. $[M+1]^+$; Anal. Calcd. For $C_{17}H_{13}NO_3$: C, 72.17; H, 4.5; N, 5.26 Found: C, 72.14; H, 4.2; N, 5.20 %.

2.5.3. *2-(4-methoxyphenylamino)naphthalene-1, 4-dione (3b)*

Yield 48%; Red solid; mp 152 ºC; eluent-*n*-hexane/ethyl acetate 70:30 v/v, R_f= 0.71; FTIR (KBr, υ cm^{-1}): 3055 (Ar C-H), 1655, 1678 (C=O, α,β unsaturated), 1277 (C-N), 3409 (N-H), 1590 (C=C), 1230 (C-O-C asym.), 1028 (C-O-C sym); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 3.72 (s, 3H, OCH_3), 4.05 (s, 1H, NH, D_2O exchangeable), 6.88-6.92 (m, 4H, Ar-H), 7.66-7.90 (d, 2H, Ar-H), 8.06-8.11 (dd, 2H, Ar-H), 8.23 (s, 1H, Ar-H); EIMS (m/z): 279.29 $[M]^+$, 280.90. $[M+1]^+$; Anal. Calcd. For $C_{17}H_{13}NO_3$: C, 72.17; H, 4.5; N, 5.26 Found: C, 72.15; H, 4.3; N, 5.23 %.

2.5.4. ***2-(4-hydroxyphenylamino)naphthalene-1, 4-dione (3c)*** Yield 66 %; Brown solid; mp 248 ºC; eluent-*n*-hexane/ethyl acetate 70:30 v/v, R_f= 0.63; FTIR (KBr, υ cm^{-1}): 3107 (Ar C-H), 1645, 1654 (C=O, α,β unsaturated), 1256 (C-N), 3402 (N-H), 1509 (C=C), 1102 (C-O), 3599 (O-H); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 4.73 (s, 1H, NH, D_2O exchangeable), 5.02 (s, 1H, OH, D_2O exchangeable), 6.02-6.95 (m, 4H, Ar-H), 7.12-7.89 (d, 2H, Ar-H), 7.99-8.43 (dd, 2H, Ar-H); EIMS (m/z): 252.24$[M]^+$, 253.90. $[M+1]^+$. Anal. Calcd. For $C_{15}H_{10}NO_3$: C, 71.42; H, 4.0; N, 5.55 Found: C, 72.43; H, 4.2; N, 5.53 %.

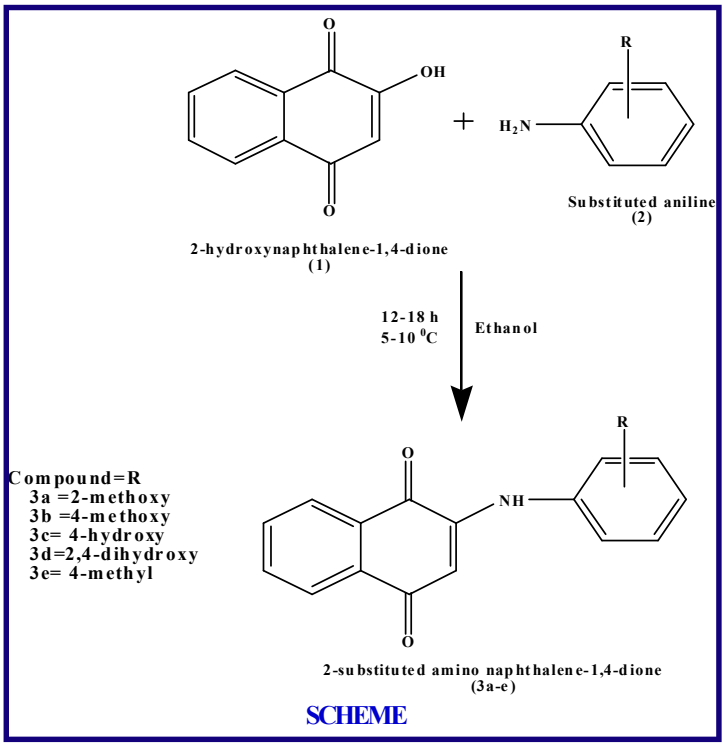

Figure 1: Synthetic protocol of compounds **(3a-e)**

2.5.5. ***2-(2, 4-dihydoxyphenylamino)naphthalene-1, 4-dione (3d)*** Yield 73 %; Brown solid; mp 263 ºC ; eluent-*n*-hexane/ethyl acetate 70:30 v/v, R_f= 0.68; FTIR (KBr, υ cm^{-1}): 3102 (Ar C-H), 1633, 1687 (C=O, α,β unsaturated),), 1282 (C-N), 3412 (N-H), 1554 (C=C), 1209 (C-O), 3599 (O-H); ^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm): 4.36 (s, 1H, NH, D_2O exchangeable), 5.02 (s, 2H, OH, D_2O exchangeable), 6.85-6.97 (m, 6H, Ar-H), 7.88-7.92 (d, 2H, Ar-H); EIMS (m/z): 281.26[M]$^+$, 282.90. [M+1]$^+$; Anal. Calcd. For $C_{16}H_{11}NO_4$: C, 68.32; H, 3.94; N, 4.98 Found: C, 68.44; H, 3.93; N, 4.96 %.

2.5.6. ***2-(-p-tolylamino)naphthalene-1, 4-dione (3e)*** Yield 57 %; Yellow crystal;mp 227 ºC; eluent-*n*-hexane/ethyl acetate 70:30 v/v, R_f= 0.73; FTIR (KBr, υ cm^{-1}): 3035 (Ar C-H), 1635, 1640 (C=O, α,β unsaturated), 1272 (C-N), 3490 (N-H), 1535 (C=C), 1289 (C-O), 3578 (O-H); ^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm): 2.32 (s, 3H, CH_3), 4.28 (s, 1H, NH, D_2O exchangeable), 6.22-6.91 (m, 4H, Ar-H), 7.19-7.27 (d, 2H, Ar-H), 7.60-7.74 (dd, 2H, Ar-H), 7.96 (s, 1H, Ar-H); EIMS (m/z): 263.29 [M]$^+$, 264.89. [M+1]$^+$; Anal. Calcd. For $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N, 5.32 Found: C, 77.53; H, 4.97; N, 5.33 %.

2.6. Biological activity

2.6.1. In-vitro antioxidant activity

DPPH (1,1-diphenyl-2-picryl-hydrazyl) method is the most excellent, easiest and commonly used method for testing preliminary free radical-scavenging activity[14,15]. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. DPPH is known to abstract labile hydrogen[16,17]. DPPH-radical scavenging activity of synthesized compounds (**3a-e**) was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. Solution of DPPH was prepared and was added to all the synthesized compounds (**3a-e**) at different concentrations (1-1000 mg/ml). Thirty minutes later, the absorbance was measured at 517 nm. All the analysis was made with the use of UV-Visible Spectrophotometer (Shimadzu 1700). Absorbance of various concentrations was taken and percentage inhibition was calculated. Lower absorbance of the reaction mixture indicates higher free radical-scavenging activity. Ascorbic acid was used as a standard antioxidant. IC_{50} (Inhibitory Concentration 50%) value denotes the concentration of sample required to scavenge 50% of the DPPH free radical. IC_{50} of all synthesized compounds (**3a-e**) was determined from % Inhibition v/s concentration graph (Figure 1). The percentage discoloration was calculated as follows: DPPH radical scavenging activity (%) = [AC517 – AE517 / AC517] x 100. Where; AC517 is absorbance of a DPPH solution without fraction; AE517 is the absorbance of the tested compounds with DPPH.

Table 1: Comparison of IC_{50} values of Compounds (3a-e) at various concentration

Compounds	IC_{50} (µg/ml)
Std	45.54
3a	118.95
3b	95.75
3c	90.14
3d	75.39
3e	137.01

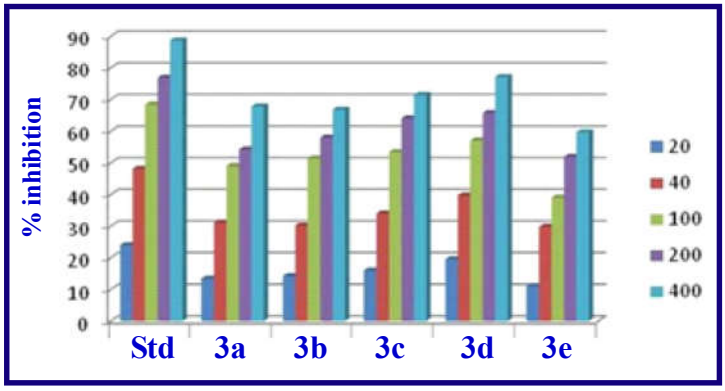

Figure 2: DPPH Scavenging assay of synthesized compounds compared with standard ascorbic acid (% of Inhibition *vs* Concentration)

2.7. Statistical analysis

The results of the experiment were expressed as mean ± SEM. For group comparison, analysis of variance followed by Tukey's HSD multiple comparison test with SPSS version 10 was used. The

difference among means considered statistically significant when *p*-value was less than 0.05.

3. RESULTS

IR spectra of all final naphthoquinone analogues **(3a-e)** showed a strong, characteristic band in the region 3402-3490 cm^{-1} due to the N-H stretching vibration. The ^1H NMR spectra of products (**3a–e)** show a singlet at δ 4.05–4.73 due to the N-H (D_2O exchangeable) of ring and which confirm the conversion of substrates into the expected products. All the other aromatic and aliphatic protons were observed at expected regions. In the mass spectra of all compounds (**3a-e**), the $[M+1]^+$ peak was observed**.** All compounds gave satisfactory elemental analysis.

3.1. DPPH-scavenging activity

Figure 2 illustrates a significant decrease in the concentration of DPPH radical due to scavenging ability of the compounds. Ascorbic acid was used as a reference to antioxidant compounds. The compounds tested with this method exhibited marked DPPH free radical scavenging activity in a concentration-dependent manner. Figure 2 illustrate a decrease in the concentration of DPPH radical due to the scavenging ability of the compounds. These results indicate that the derivative **3d** exhibited more antioxidant activity than the other. It may be due to the presence of two OH groups, which enhance the radical scavenging activity by hydrogen donation. The results indicate that compound **3d** having IC_{50} 75.39 ±4.12 mg/ml showed potent antioxidant activity comparable to standard ascorbic acid (IC_{50} 45.54 ± 3.06 mg/ml) as shown in table 1. Data of percentage inhibition showed that amongst all the test compounds having hydroxyl groups as substituent showed highest activity as indicated by % inhibition in antioxidant activity. This may be due to the available OH group present in **3d** & **3c** and OCH_3 present in **3a & 3b**. The synthesized compounds have shown good antioxidant effect, amongst **3d** has shown excellent activity. Rest of the compounds (**3a-c** & **3e**) showed mild-to-moderate antioxidant effect.

4. CONCLUSION

A novel series of naphthoquinone analogues **(3a-e)** from 2-hydroxy naphthalene-1,4-dione isolated from the leaves of *lawsonia inermis* were synthesized and evaluated for *in-vitro* antioxidant activity and the better efficacious compound found out. Physical and analytical parameters of the newly synthesized naphthoquinone derivatives were confirmed by TLC, IR, ^{1}HNMR, MASS and elemental analysis. Subsequently, in biological screening, the compound 2-(2, 4-dihydoxyphenylamino)naphthalene-1,4-dione **(3d)** showed potent antioxidant agent as compared to other derivatives. Further research on naphthoquinone core is needed for the discovery of a potent antioxidant agent. Thus we observed that there is enough scope for further study in developing such compounds as a good lead molecule with better pharmacological profile.

Conflict of interest

None of the author has any conflict of interest in the context of this work.

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