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LETTER

A Green Approach for the Synthesis of Bis (Substituted Sulfabenzamide) *para*-Benzoquinone Based on the Reaction of Sulfabenzamide with Electrochemically Generated *para*-Benzoquinone and its antibacterial evaluation†

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Electrochemical synthesis of a new bis (substituted sulfabenzamide) *para*-benzoquinone (**5**) was carried out via the electrochemical oxidation of hydroquinone (**1**) in the presence of sulfabenzamide (4-amino-*N*-benzoyl-benzenesulfonamide) (**2**). A plausible mechanism for the oxidation of **1** in the presence of **2** is presented. Hydroquinone (**1**) was converted into **5** via an *ECECE* mechanism. The electrochemical synthesis of **5** was successfully performed in one-pot, in water/ethanol mixture under green conditions without toxic reagents and solvents. Compound **5**, was evaluated for its in vitro antibacterial activity against *Escherichia coli* (*E. coli*) ATCC 35218 (Gram-negative) and *Staphylococcus aureus* (*S. aureus*) ATCC 6538 (Gram-positive). It was found that the tested compound was more active against gram-positive than gram-negative bacteria.

Sulfonamides are synthetic drugs that have various therapeutic potential uses which include anti-metalloprotease,[1] antibacterial,[2] anti-diabetic,[3] anti-carbonic anhydrase,[4] diuretic,[5] anti-inflammatory,[1] anti-thyroid,[6] and antiviral[1] activities. In addition it has also been found that sulfonamides can inhibit the growth of cancer cells.[7,8] In addition, quinones are of considerable interest because many drugs such as doxorubicin, daunurobicin and mitomycin C in cancer chemotherapy contain quinones.[9] Some of them also exhibit antitumor and antimalarial activities[10] and many of them are also involved in enzyme inhibition and DNA cross-linking.[11] Based on this information, we think that synthesis of an organic compound with both structures of sulfonamide and quinone would be useful from the point of view of pharmaceutical properties. This idea prompted us to investigate the electrochemical oxidation of hydroquinone (**1**) in the presence of sulfabenzamide (**2**) as a nucleophile. We describe a one-pot electrochemical method for the synthesis of a new bis (substituted sulfabenzamide) *para*-benzoquinone (**5**). This reaction is carried out in a single step with high atom economy under ambient conditions using a carbon anode.

The cyclic voltammogram of a solution of hydroquinone (**1**) (1.0 mM) in water (phosphate buffer, c = 0.2 M, pH = 8.0)/ethanol mixture (50/50, v/v) is shown in Figure 1. As can be seen, one anodic peak (A_1) at 0.06 V and the corresponding cathodic peak (C_1) at -0.06 V was obtained, which correspond to the transformation of **1** to *p*-benzoquinone (**1ox**) and vice versa within a quasi-reversible two electron process.[12,13] The electrochemical oxidation of 5.0 mM solution of sulfabenzamide (**2**) in the same conditions has also been performed using cyclic voltammetry (Fig. 1, curve b).

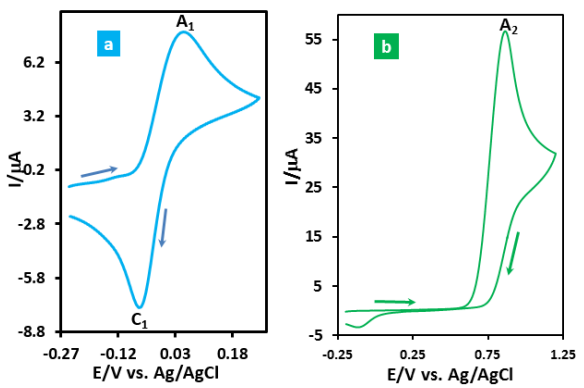
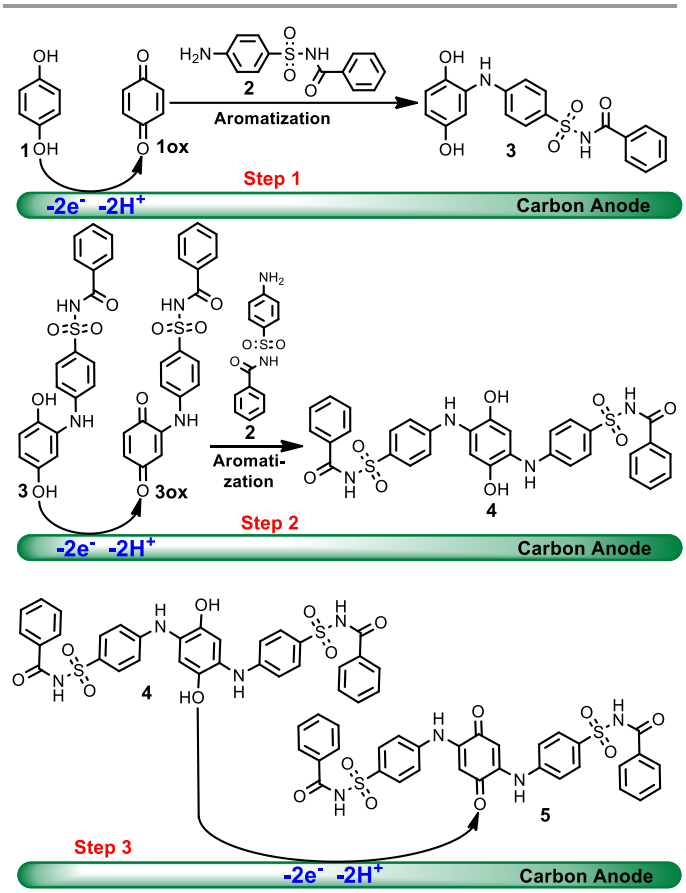


Fig. 1. a) Cyclic voltammogram of hydroquinone (1.0 mM). (b) Cyclic voltammogram of sulfabenzamide (5.0 mM). Scan rate: 50 mV s^{-1}. Working electrode: glassy carbon. Solvent: water (phosphate buffer, c = 0.2 M, pH = 8.0)/ethanol mixture (50/50, v/v). Temperature = 25±1 °C.

As shown in this figure, the cyclic voltammogram exhibits the quality of an irreversible electron-transfer process with an anodic peak (A_2) at 0.87 V vs. Ag/AgCl. Since, the oxidation of sulfabenzamide (**2**) occurs at the potentials much more positive than the oxidation of **1**, the selective electrochemical oxidation

of **1** in the presence of **2** using ordinary electrodes such as carbon electrode is possible.

Controlled potential coulometry can be useful to establish the actual electron consumption. Thus the coulometry was carried out in a cell containing **1** (0.25 mmol) and **2**(0.5 mmol) at 0.20 V versus Ag/AgCl. The measured consumption was 5.9 Faraday per mole of **1**. Diagnostic criteria of cyclic voltammetry and controlled potential coulometry on the one hand and spectroscopic data of final product (IR, ^{1}H NMR, ^{13}C NMR, and the molecular mass of 658) on the other hand allow us to propose the pathway in Scheme 1 for the electrochemical oxidation of **1** in the presence of **2**.



Scheme 1. Proposed mechanism for the electrochemical oxidation of hydroquinone (**1**) in the presence of sulfabenzamide (**2**).

According to the proposed mechanism, in the first step, the generation of *p*-benzoquinone (**1ox**) is followed by a Michael type addition reaction of **2** producing substituted hydroquinone **3**. The oxidation of substituted hydroquinone **3** is easier than the oxidation of **1** due to the presence of the electron-donating amine group. Therefore, the oxidation of **3** occurs during the electrolysis at the potential of 0.20 V vs. Ag/AgCl. Consequently in step 2, electrogenerated**3ox** would serve as a Michael acceptor in a reaction with **2** to form the di-substituted hydroquinone **4**.In the final step, the oxidation of **4** produces bis (substituted sulfabenzamide) *para*-benzoquinone **5** as a final product.

Substituted *p*-benzoquinone **3ox** is an asymmetric Michael acceptor and can be attacked by **2** to yield three types of products (Fig. 2). However, the presence of only one carbonyl peak at 187.7 ppm in the ^{13}C NMR spectrum of **5**, confirms that the two carbonyl groups located on quinone ring are equivalent and rejects the formation of ***m*-5**.

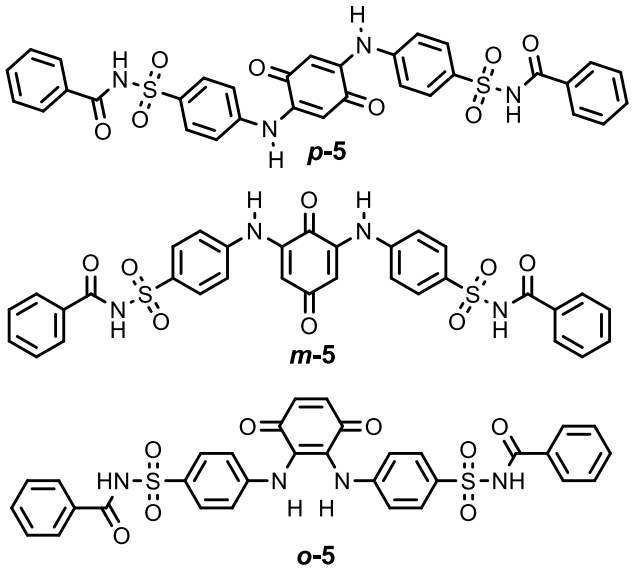


Fig. 2. Possible structures for bis (substituted sulfabenzamide) para-benzoquinone (5).

The steric energy has been calculated for compounds ***p*-5** and ***o*-5** using MM2 program after minimization of structures.[14] Results indicate that compound ***p*-5** is energetically more stable than the ***o*-5** with about 6.4 kcal/mol lower energy (Fig. 3). In conclusion, using ^{13}C NMR data and because of the less strict effect, we think that compound ***p*-5** is most probable compound in electrochemical oxidation of hydroquinone (**1**) in the presence of sulfabenzamide (**2**).

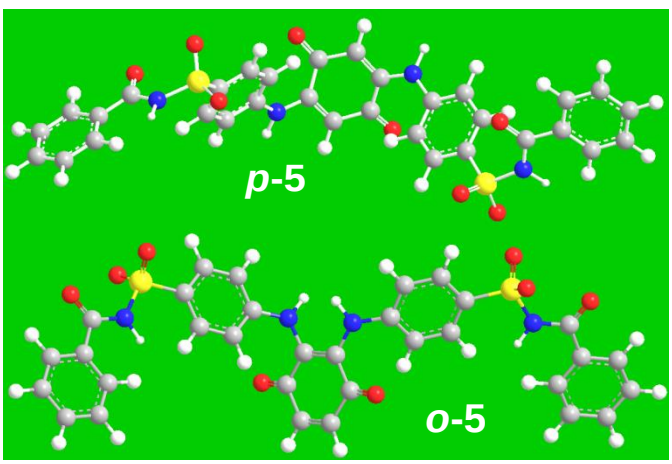


Fig. 3. The results of MM2 calculation for compounds ***p*-5** and ***o*-5**.

Figure 4, curve a, shows the cyclic voltammogram of a saturated solution of **5** in aqueous phosphate buffer (c = 0.2 M, pH = 8.0)/ethanol mixture (50/50, v/v). The cyclic voltammogram displays two anodic peaks (A_1 and A_2) on the forward and one cathodic peak (C_1) on the backward scan at 0.10, 0.82 and -0.09 V vs. Ag/AgCl, respectively. In addition, cyclic voltammogram of a solution containing both hydroquinone (**1**) and sulfabenzamide (**2**) under the same conditions is shown in Fig. 4, curve b. Comparison between these voltammograms also confirms the synthesis of substituted-benzoquinone. According to our results, the anodic peaks (A_1 and A_2) pertain to the oxidation hydroquinone and sulfabenzamide moieties in the structure of hydroquinone **4**, respectively. Obviously, the cathodic peak C_1 corresponds to the reduction of *p*-benzoquinone **5** into **4**. In addition, a comparison between the E_{pA1} in the voltammogram curves a and b ($E_{pA1(a)}<E_{pA1(b)}$) confirms the role of the presence electron-donating amine groups in the structure of **5**, in the decreasing oxidation potential of hydroquinone **4** compared to hydroquinone **1**.

The atom economy was calculated according to Eissen and coworkers (Eq. 1).[15]

$$\%\ \text{Atom Economy} = \frac{\text{Mass of atoms in desired product}}{\text{Mass of atoms in reactants}} \times 100 \quad \text{Eq. 1}$$

According to Scheme 1, the overall reaction mechanism for electrochemical synthesis of **5** is shown in Scheme 2. The calculated atom economy for the synthesis **5** is 99.09 %. The high atom economy indicates that all atoms (except six hydrogen atoms) from the starting materials are incorporated into the product.

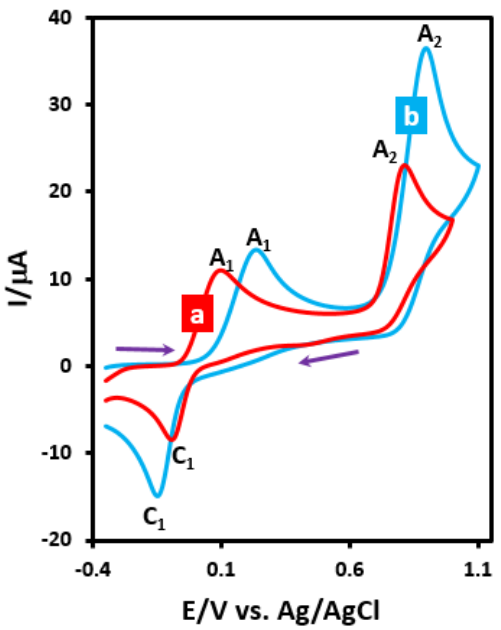
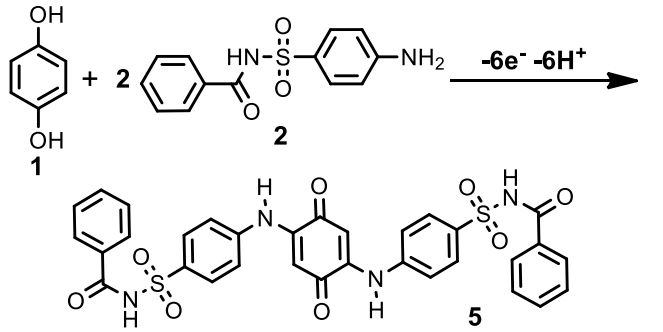


Figure 4. (a) Cyclic voltammogram of a saturated solution of product **5**. (b) Cyclic voltammogram of a solution containing both hydroquinone (1.0 mM) and sulfabenzamide (1.0 mM) at glassy carbon electrode in aqueous phosphate buffer (c = 0.2 M, pH = 8.0)/ethanol mixture (50/50, v/v); scan rate: 10 mV s^{-1}. Temperature = 25 ± 1 ºC. Note: In order to better compare the voltammogramsa and b, the currents shown in curve a have been amplified four times.

Compound **5** was tested to evaluate the antibacterial susceptibility by Agar-well diffusion method.[16] *Escherichia coli* (*E. coli*) ATCC 35218 (Gram-negative) and *Staphylococcus aureus* (*S. aureus*) ATCC 6538 (Gram-positive) were used as test organisms. The created well into the agar was filled with 3 mg of 5. The inhibition zone surrounding the wells (in millimeters) was measured to evaluate antibacterial activity. Compound **5** had effective inhibitory effect against the bacteria (Fig. 5). The results indicated that S. aureus (36 mm) was more sensitive to **5** than E.coli (21 mm). The permeable cell wall in Gram-positive bacteria (such as *S. aureus*) usually does not restrict the penetration of antimicrobials. While an outer membrane and a set of multi-drug resistance pumps in Gram-negative bacteria (such as *E. coli*) are quite effective barriers for antimicrobial compounds.[17-19]



Scheme 2. The overall reaction mechanism of the electrooxidation of hydroquinone (**1**) in the presence of sulfabenzamide (**2**).

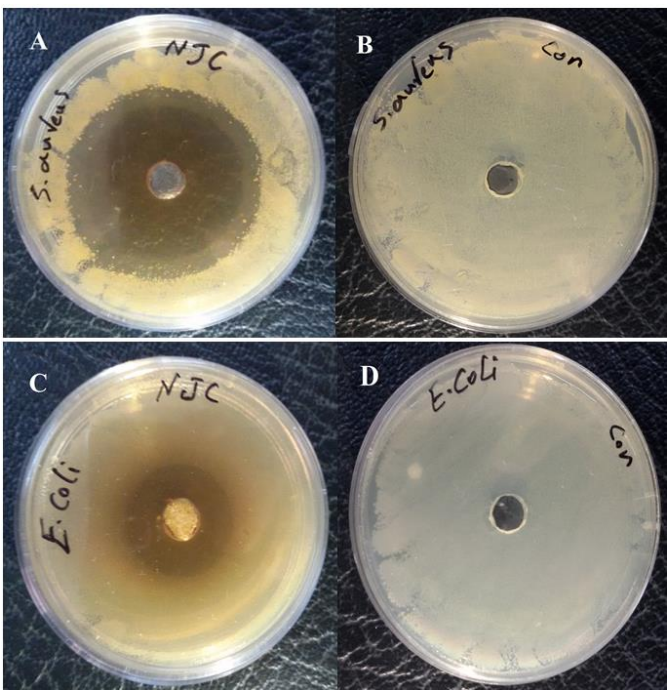


Figure 5. Inhibition zone (in mm) by NJC in concentration of 3 mg on the tested bacteria (A & D), negative control (B & D)

Finally, the pharmacological properties of sulfonamides and quinones encouraged us to synthesize a new compound containing both sulfonamide and *para*-benzoquinone moieties. These data show that electrochemically generated *p*-benzoquinone (**1ox**) is attacked by sulfabenzamide (**2**) twice. Final product is obtained via an *ECECE* mechanism after consumption of 6F/mol of **1**. The prominent features of this paper, the synthesis of a valuable compound in a water/ethanol mixture instead of toxic solvents, room-temperature conditions, high energy efficiency, high atom economy, and using the electrode as an electron source instead of toxic reagents, are in accord with the principle of green chemistry.[20-23]

Hyroquinone and sulfabenzamide were reagent-grade materials from Aldrich. These chemicals were used without further purification. Cyclic voltammetry and controlled potential coulometry were performed using an Autolab model PGSTAT 30 potentiostat/galvanostat. The working electrode used in the cyclic voltammetry experiments was a glassy carbon disc (1.8 mm diameter). All experiment were carried out at a temperature of 25±1 ºC. More details are described in our previous paper.[24]

A mixture of phosphate buffer solution (c = 0.2 M, pH = 8.0)/ethanol mixture (50/50, v/v) containing hydroquinone (0.25 mmol) and sulfabenzamide (0.5 mmol) was subjected to electrolysis in a divided cell at 0.20 V versus Ag/AgCl. The electrolysis was terminated when the current decayed to 5% of its original value. At the end of electrolysis the precipitated solid was collected by filtration and was washed with *n*-hexane. The precipitated solid was recrystallized from methanol to give the compound **5** (isolated yield, 74%) as an orange solid, m.p: 184-185 ºC.^{1}H NMR (300 MHz, DMSO-d_6)δ/ppm: 6.14 (br, 2H, NH, D_2O exchangeable), 6.61 (d, J = 8.7 Hz, 4H, aromatic), 6.86 (s, 2H, quinone), 7.46 (t, J = 7.6 Hz, 4H, aromatic), 7.60 (m, 6H, aromatic), 7.83 (d, J = 7.7 Hz, 4H, aromatic), 12.0 (br, ~2H, NH, D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm:112.2, 123.7, 128.2, 128.5, 130.1, 131.9, 132.9, 136.6, 153.7, 165, 187.7 (C=O). IR (KBr) v/cm^{-1}: 3459 (NH), 3357 (NH), 3243, 1678 (C=O), 1628, 1650, 1592, 1500, 1455, 1434, 1321, 1258, 1161, 1086, 876, 837, 786, 718, 683, 568, 548. MS (EI, 70 eV); *m/z* (relative intensity): 658 (M + 2H) (3), 505 (22), 503 (23), 428 (100), 426 (100), 347 (14), 276 (94).

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Notes and references

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*†Electronic supplementary information (ESI) available: FT-IR, ^{1}H NMR ^{13}C NMR and mass spectra of **5**. See DOI: 10.1039/c5nj00087d*

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