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(THIO)-SUBSTITUTED-1,4-BENZOQUINONES: SYNTHESIS, ELECTROCHEMISTRY, DFT CALCULATIONS, AND POTENTIAL ANTIPROLIFERATIVE EFFECT AGAINST MDA-MB-231 CELLS

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This study explores the synthesis, electrochemical properties, and computational aspects of mono-, bis-, tris-, and tetrakis(thio)-substituted 1,4-benzoquinones. The electrochemical properties of the compounds were analyzed using staircase cyclic voltammetry (CV). Ground-state energies were determined through time-dependent density functional theory (TD-DFT), utilizing the B3LYP functional and the 6-311G(d,p) basis set. Additionally, the in vitro anticancer efficacy of 2-(*tert*-butylthio)-3,5,6-trichlorocyclohexa-2,5-diene-1,4-dione **(**compound **5)** was evaluated using the triple-negative breast cancer cell line (MDA-MB-231). Notably, compound **5** demonstrated significant potency, with values of 50 μM 50 % cytotoxic concentration (IC_{50}).

Key words: benzoquinones; thiols; *p*-chloranil; time-dependent density functional theory

(ТИО)-СУПСТИТУИРАНИ-1,4-БЕНЗОХИНОНИ: СИНТЕЗА, ЕЛЕКТРОХЕМИЈА, DFT ПРЕСМЕТКИ И ПОТЕНЦИЈАЛЕН АНТИПРОЛИФЕРАТИВЕН ЕФЕКТ ПРОТИВ КЛЕТКИ НА MDA-MB-231

Во овој труд е претставена синтезата, електрохемиските својства и теоретските пресметки на електронската густина на моно-, бис-, трис- и тетракис(тио)-супституирани 1,4-бензохинони. Електрохемиските својства на испитуваните соединенија беа анализирани со примена на циклична скалеста волтаметрија. Енергиите на основната состојба на сите испитувани соединенија беа определени со примена на функционална временски зависна теорија на електронската густина (TD-DFT), со употреба на функционален пакет B3LYP и основен сет 6-311G(d,p), што се дизајнирани за ваков тип пресметки. Покрај тоа, *in vitro* антиканцерогениот потенцијал на соединението 2-(*tert*-бутилтио)-3,5,6-трихлорциклохекса-2,5-диен-1,4-дион (**5**) беше евалуиран користејќи ја клеточната линија за рак на дојка, со употреба на негативен трианјонски рецептор (MDA-MB-231). Важно е да се напомени дека соединението **(5)** покажа значителна ефикасност, со вредности од 50 μM и 50 % цитотоксична концентрација (IC_{50}).

Клучни зборови: бензохинони; тиоли; *p*-хлоранил; временски зависна теорија на функционалот на електронската густина

1. INTRODUCTION

Quinones are naturally occurring compounds found in plants, animals, and microorganisms.[1,2] They exhibit various biological activities, including antiproliferative, antibacterial, antifungal, and antiviral properties.[2–5] The biological activity of quinones is related to their redox proper-

ties,[1,2] which are influenced by the electron-withdrawing or electron-donating substituents on the quinoid skeleton.[6] In addition, the introduction of groups such as OCH_3, OH, and SH into the quinone structure enhances their biological activities, including antibacterial[4] and cytotoxic effects.[7,8]

Among quinones, *p*-chloranil belongs to the halo-substituted 1,4-benzoquinones. Numerous studies have reported on its reactions with nucleophiles, including the following: reactions of *p*-chloranil with 4-aminobenzoic acid in ethanol (EtOH) at room temperature;[9] azomethines in ethylacetate;[10] ethylmercaptan in the presence of sodium metal in dry ice/acetone;[11] thiols and dithiols in EtOH with sodium carbonate under room/reflux temperature;[12,13] aniline in dioxane at 15 °C;[14] monosubstituted anilines under solid-phase microwave irradiation conditions;[15] 2-aminonitrophenols/arylamines in EtOH with sodium acetate;[16,17] arylthiols in water;[3] and potassium alkyl/aryl mercaptides in benzene/ethanol.[8] Among these, Tandon et al.[3] showed that the reactions of chloranil with arylthiols in water to yield bis-, tris-, and tetrakis(thio)-substituted *p*-benzoquinones. Notably, the compound 2,3,5,6-tetrakis(phenylthio) cyclohexa-2,5-diene-1,4-dione exhibited superior antifungal properties than antifungal drugs fluconazole and flucytosine.[3]

Additionally, extensive research from our laboratories[19–23] has focused on reactions between quinones and nucleophiles, such as amines and thiols.

Cancer, being the second leading cause of death globally,[24,25] has driven continued interest in developing treatments. Quinones hold promise as anticancer agents, with certain quinoid compounds already utilized as anticancer drugs, such as doxorubicin, idarubicin, mitomycin-C, and streptonigrin.[1] Several 1,4-quinone derivatives have demonstrated antiproliferative activity against cancer cell lines, including MDA-MB-231[20,26] and MCF-7 breast cancer cells.[27]

Considering the significant role of quinones in medicinal and chemical research, this study reports the synthesis, electrochemical behavior, and computational studies of benzoquinones derived from reactions between *p*-chloranil and thiols, including sodium-2-methyl-2-propanethiolate (**2**), 4-*tert*-butylbenzenethiol (**3**), or sodium 2-propanethiolate (**4**). Additionally, 2-(tert-butylthio)-3,5,6-trichlorocyclohexa-2,5-diene-1,4-dione (**5**) was evaluated for its antiproliferative activity against MDA-MB-231 cancer cells.

2. MATERIALS AND METHODS

2.1. *Chemistry*

Mass spectra were recorded using a Thermo Finnigan LCQ Advantage MAX (USA) system in both positive and negative ion modes with an electrospray ionization (ESI) source. $^1H/^{13}C$ NMR spectrums were obtained using a Varian Unity Inova spectrometer by using tetramethylsilane (TMS) as an internal standard and $CDCl_3$ as the solvent. UV-Vis spectra were measured on a Shimadzu 1800 (Japan) UV-spectrophotometer. Column chromatography was performed on silica gel (Merck Kieselgel 60, 70 – 230 mesh).

2.2. *Synthesis of quinoid compounds*

Synthesis of 2-(*tert*-butylthio)-3,5,6-trichlorocyclohexa-2,5-diene-1,4-dione (5):[28] *p*-chloranil (8.14 mmol, **1**) was treated with 1.0 equiv. of sodium-2-methyl-2-propanethiolate ($(CH_3)_3CSNa$, 8.14 mmol, **2**) in $CHCl_3/CH_2Cl_2$ (50 ml) at 55 °C. The reaction progress was monitored by thin layer chromatography (TLC). The reaction mixture was extracted with $CHCl_3$ and H_2O (3 × 25 ml). The $CHCl_3$ phase was dried with anhydrous Na_2SO_4, and the solvent was removed under vacuum. Chromatography on silica gel, eluted with a petroleum ether/chloroform mixture, yielded compound **5**. R*f* (1PET/1$CHCl_3$): 0.7; Yield: 195 mg (8 %). UV ($CHCl_3$), λ_{max} (log ε): 296 (4.23), 506 (2.91). ^1H NMR ($CdCl_3$), δ (ppm): 1.38 (s, 9H, $3CH_3$). ^{13}C NMR ($CdCl_3$), δ (ppm): 173.33, 170.28 (C=O); 148.67, 144.27, 142.02, 140.48 ($C_{quinone}$); 53.92 (C_{tert}); 32.38 (CH_3); MS, *m/z*: 298.0 ($[M]^-$, 100 %), (299.9, 96 %). Anal. calc. for $C_{10}H_9Cl_3O_2S$ (299.6): C, 40.09; H, 3.03; S, 10.70. Found: C, 40.10; H, 3.01; S, 10.65.

Synthesis of 2,5-bis(*tert*-butylthio)-3,6-dichlorocyclohexa-2,5-diene-1,4-dione (6) and 2,3,5-tris(*tert*-butylthio)-6-chlorocyclohexa-2,5-diene-1,4-dione (7): *p*-chloranil (8.0 mmol, **1**) was treated with 2.0 equiv. of sodium-2-methyl-2-propanethiolate ($(CH_3)_3CSNa$, 16 mmol, **2**) in CH_2Cl_2 (60 ml). The reaction progress was monitored by TLC. The reaction mixture was extracted with $CHCl_3$ and H_2O (3 × 25 ml), and the $CHCl_3$ phase was dried with anhydrous Na_2SO_4. The solvent was removed under vacuum. Chromatography on silica gel eluted with petroleum ether/chloroform mixtures yielded compounds **6** and **7**.

2,5-bis(*tert*-butylthio)-3,6-dichlorocyclohexa-2,5-diene-1,4-dione (6): R*f* (1PET/1$CHCl_3$): 0.5; Yield: 225 mg (16 %). UV ($CHCl_3$), λ_{max} (log ε): 264 (3.90), 296 (3.90), 486 (3.16). ^1H NMR

($CdCl_3$), δ (ppm): 1.38 (s, $6CH_3$). ^{13}C NMR ($CdCl_3$), δ (ppm): 173.78 (C=O); 151.28 (=CS); 141.36 (C–Cl); 53.36 (C_{tert}); 32.48 (CH_3). MS, *m/z*: 351.9 ($[M]^-$, 100 %). Anal. calc. for $C_{14}H_{18}Cl_2O_2S_2$ (353.33): C, 47.59; H, 5.13; S, 18.15. Found: C, 47.55; H, 5.18; S, 18.20.

2,3,5-tris(*tert*-butylthio)-6-chlorocyclohexa-2,5-diene-1,4-dione (7): Yield: 23 mg (2%). UV ($CHCl_3$), λ_{max} (log ε): 268 (4.02), 424 (3.35). ^1H NMR ($CdCl_3$), δ (ppm): 1.393 (s, 9H, $3CH_3$), 1.388 (s, 9H, $3CH_3$), 1.375 (s, 9H, $3CH_3$). ^{13}C NMR ($CdCl_3$), δ (ppm): 178.00, 174.64 (C=O); 153.17, 150.58, 150.02, 144.40 (=CS, C–Cl); 53.00, 52.90, 52.74 (C_{tert}); 32.52, 32.46, 32.42 (CH_3). MS, *m/z*: 406.8 ($[M+H]^+$, 100%) and MS: *m/z*: 428.9 ($[M+Na]^+$, 37%). Anal. calc. for $C_{18}H_{27}ClO_2S_3$ (407.05): C, 53.11; H, 6.69; S, 23.63. Found: C, 53.09; H, 6.64; S, 23.67.

Synthesis of 2,3,5-tris(4-*tert*-butylphenylthio)-6-chlorocyclohexa-2,5-diene-1,4-dione (8):[29] *p*-chloranil (**1**) (1.48 g, 6.0 mmol) was stirred into a mixture of MeOH and $CHCl_3$. To this solution, equiv. amount of 4-*tert*-butylbenzenethiol (**3**) (1.0 g, 6.0 mmol) was added, causing an immediate color change. The reaction mixture was stirred at 65 °C for 5 minutes, then allowed to cool to room temperature. The reaction progress was monitored by TLC. The reaction mixture was extracted with $CHCl_3$ and H_2O, and the $CHCl_3$ phase was dried over anhydrous Na_2SO_4. The solvent was removed under vacuum. Purification by silica gel chromatography with petroleum ether/$CHCl_3$ (3:1) yielded compound **8**. Yield: 15%. UV ($CHCl_3$), λ_{max} (log ε): 252 (4.95), 416 (4.09). ^1H NMR ($CdCl_3$), δ (ppm): 7.10 – 7.25 (m, 12H), 1.24 (s, 9H, $3CH_3$), 1.22 (s, 18H, $6CH_3$). ^{13}C NMR ($CdCl_3$), δ (ppm): 175.13, 172.02 (C=O); 152.05, 151.57, 151.35, 146.44, 146.20, 145.55, 139.90, 132.46, 131.70, 131.25, 129.34, 129.06, 127.03, 126.28, 126.25 ($C_{quinone}$, C_{arom}); 34.70, 34.66 (C_{tert}); 31.25, 31.21 (CH_3). MS, *m/z*: 635.4 ($[M+H]^-$, 100%). Anal. calc. for $C_{36}H_{39}ClO_2S_3$ (635.34): C, 68.06; H, 6.19; S, 15.14. Found: C, 68.10; H, 6.14; S, 15.19.

Synthesis of 2,3,5,6-tetrakis(isopropylthio) cyclohexa-2,5-diene-1,4-dione (**9**):[30,31] *p*-Chloranil (**1**) (2.0 g, 8.13 mmol) was reacted with an equiv. of sodium 2-propanethiolate (**4**) (0.8 g, 8.13 mmol) in $CHCl_3$ at room temperature. The reaction progress was monitored by TLC. The reaction mixture was extracted with $CHCl_3$ and H_2O, and the $CHCl_3$ phase was dried over anhydrous Na_2SO_4. The solvent was removed under vacuum. Purification by silica gel chromatography with *n*-hexane/$CHCl_3$ (1:1) yielded compound **9**. R_f (1PET:1$CHCl_3$): 0.3. Yield: 30%. UV ($CHCl_3$), λ_{max} (log ε): 248 (4.20), 366 (3.83), 398 (3.80). ^1H NMR ($CdCl_3$), δ (ppm): 4.03 (septet, J = 6.7 Hz, 4H, S–C<u>H</u><), 1.32 (d, J = 6.7 Hz, 24H, $8CH_3$). ^{13}C NMR ($CdCl_3$), δ (ppm): 175.06 (C=O), 147.22 (C-S), 38.72 (S–C<), 23.83 (CH_3). MS, *m/z*: 405.08 ($[M+H]^+$, 100 %). Anal. calc. for $C_{18}H_{28}O_2S_4$ (404.67): C, 53.42; H, 6.97; S, 31.69. Found: C, 53.45; H, 6.94; S, 31.65.

2.3. *Electrochemistry*

The electrochemical characteristics of benzoquinone derivatives were examined using the staircase cyclic voltammetry (CV) technique at different scan rates (50 – 1000 mV/s) within a potential range of +0.3 to –2 V. The CV studies were conducted with an Ivium Vertex potentiostat/galvanostat system (Netherlands), employing a traditional three-electrode cell configuration consisting of a platinum wire counter electrode, an Ag/AgCl reference electrode with double-junction system, and a glassy carbon working electrode (GCE).

The measurements were conducted in 1×10^{-3} M benzoquinone solutions, with 0.1 M tetrabutylammonium perchlorate (TBAP) as the supporting electrolyte, dissolved in acetonitrile (ACN) under a nitrogen atmosphere.

2.4. *Cell lines and culture conditions and compound **5** treatment*

The antiproliferative activity of compound **5** was evaluated against triple negative breast cancer (TNBC) cell line MDA-MB 231, obtained from the American Type Culture Collection. The cells were cultured in a 1:1 mixture of Ham's F12 medium (Sigma Aldrich, USA). Compound **5** was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution, which was then diluted in fetal bovine serum (FBS-free DMEM) and applied to MDA-MB-231 cells at certain concentrations. Untreated cells and those treated solely with DMSO were used as controls.

2.5. *Cell viability and proliferation assays (MTS assay)*

Cell viability and proliferation were assessed using the MTS assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; Promega, Madison, WI). Cell counting was performed using a hemocytometer, and viable cells were identified through trypan blue exclusion. Among all synthesized compounds, mono- (thio) substituted benzoquinone (**5**) was selected to evaluate its antiproliferative effect. The identified cells were seeded into 96-well plates (greiner BIO-ONE 655180, Germany) at a density

of $1.20 \cdot 10^3$ cells/well and treated with different concentrations of compound **5** (5, 10, 20, 50, 100, 200, and 500 μM).

After 24 h of treatment, a solution containing MTS and phenazine methosulfate (20:1 v/v) was added to the cells. Following 2 – 3 h of incubation at 37 °C, cell viability was determined by measuring absorption at 490 nm using ELISA Reader (Promega Glomax Multi Detection System, USA), which measures the generation of formazan by metabolically active cells.

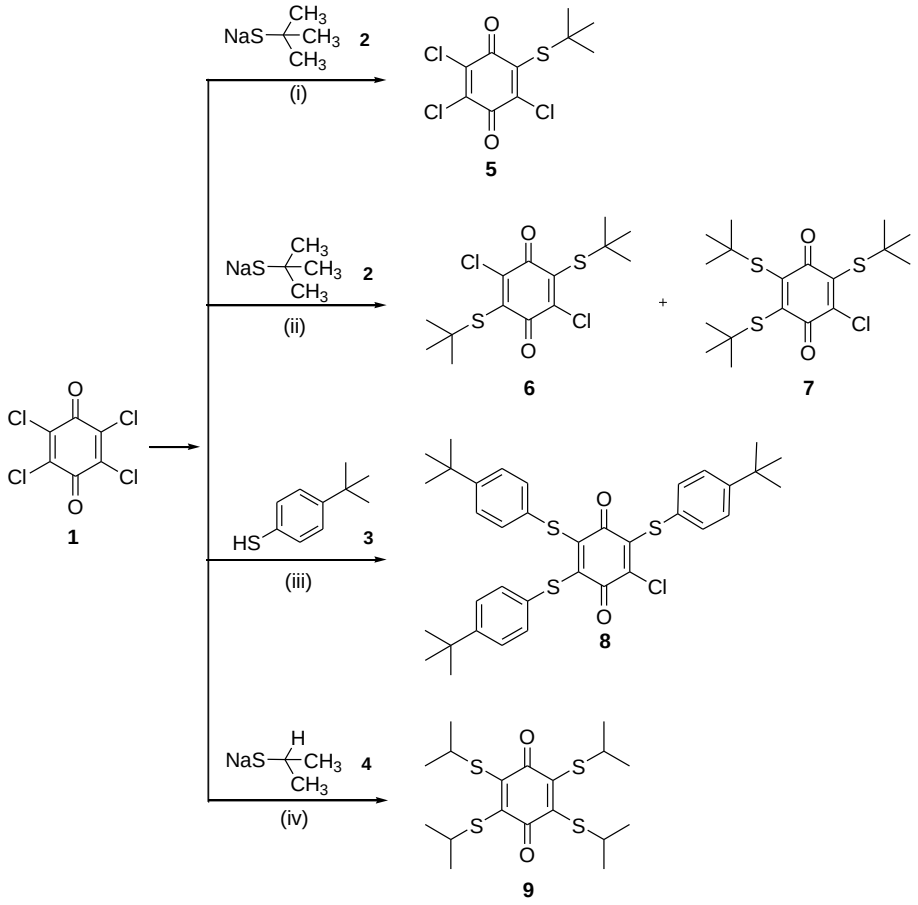
2.6. *Colony formation assays*

MDA-MB-231 cells were seeded in 6-well plates at a density of $1.5 \cdot 10^3$ cells/well, treated with compound **5,** and incubated at 37 °C for 2 weeks to allow colony formation. Following incubation, the colonies were washed with PBS, stained with 0.5% (w/v) crystal violet, and the visible colonies were counted. All experiments were independently repeated at least twice.

3. RESULTS AND DISCUSSION

3.1. *Chemistry*

The mono(thio)-substituted benzoquinone (**5**) was obtained via a substitution reaction of 2,3,5,6-tetrachloro-1,4-benzoquinone (*p*-chloranil, **1**) with an equivalent amount of sodium-2-methyl-2-propanethiolate (**2**), according to patent application method.[28] Bis- and tris(thio)-substituted 1,4-benzoquinones (**6** and **7**) were prepared using **1** (1 equiv.) and **2** (2 equiv.), respectively. Similarly, *p*-chloranil **1** reacted nucleophilically with 4-*tert*-butylbenzenethiol (**3**) and sodium 2-propanethiolate (**4**) to yield tris- and tetrakis(thio)-substituted products (**8** and **9**), respectively, as shown Scheme 1. Structures of synthesized compounds were elucidated by various techniques, including ^{1}H NMR, ^{13}C NMR, MS (ESI), UV-Vis, and cyclic voltammetry (CV).



Scheme 1. General scheme for mono-, bis- tris- and tetrakis(thio)-substituted 1,4-benzoquinone derivatives (**5**–**9**).
Reagents and conditions: (i) 1 equiv. **2**, 55 °C, $CHCl_3/CH_2Cl_2$; (ii) 2 equiv. **2**, CH_2Cl_2; (iii) 1 equiv. **3**, $MeOH/CHCl_3$ (5 min. 65 °C) and then room temperature; and (iv) 1 equiv. **4**, $CHCl_3$, room temperature

In the ^{13}C NMR spectrum, symmetrical carbonyl carbons of 1,4-benzoquinone derivatives (bis- and tetrakis(thio)-substituted structures) appeared as a single signal at 173.78 ppm (for **6**) and 175.06 ppm (for **9**). In contrast, two carbonyl carbon signals were observed for mono- and tris(thio)-substituted structures: 173.33, 170.28 ppm (for **5**); 178.00 and 174.64 ppm (for **7**); and 175.13 and 172.02 ppm (for **8**). Notably, for bis(thio)-substituted quinone (**6**), the single carbonyl signal at 173.78 ppm was assigned to the 2,5-position of the thio groups due to the symmetrical structure.

In the ^{1}H NMR spectra, compounds **5**, **6**, and **7** exhibited proton signals corresponding to CH_3 groups at approximately 1.38 ppm. Compound **8** showed a multiplet at 7.10 – 7.25 ppm for aryl group protons (12H) and a singlet at approximately 1.23 ppm for methyl protons (27H). For compound **9**, a septet signal at 4.03 ppm was observed for the –S–C<u>H</u>< group (J = 6.7 Hz), while a doublet at 1.32 ppm corresponds to CH_3 groups (J = 6.7 Hz), consistent with expectations.

For compound **5**, the molecular ion was detected at *m/z* $[M]^-$ = 298.0 (100%), as expected. Additionally, the isotopic peak at *m/z* 299.9 (96%) confirmed the presence of three chlorine atoms. Compound **6** exhibited a base peak ($[M]^-$) at *m/z* = 351.9, as expected. The mass spectra of compounds **7** and **9** were characterized by the presence of protonated ion peaks ($[M+H]^+$) at *m/z* 406.8 and 405.08, respectively, aligning with the expected values.

3.2. *Electrochemistry*

One of the most commonly used redox couples in chemical processes is quinone-hydroquinone.[32–34] To investigate the impact of thio-groups on electronic perturbation, several novel benzoquinone derivatives (**5**, **6**, **7**, **8**, and **9**) were examined using CV in the potential range of +0.3 to –2.0 V (vs. Ag/AgCl with double-junction system) by varying the scan rates from 50 to 1000 mV/s. The cyclic voltammograms of five benzoquinone derivatives, recorded at a scan rate of 100 mV/s, are shown in Figure 1. Two distinct redox couples (I and II) were identified for these benzoquinone derivatives.

For each compound, parameters such as the peak current ratio (I_{pa}/I_{pc}), the dependence of peak current on the square root of the scan rate ($I_p/v^{1/2}$), the peak-to-peak separation (ΔE_p), and the effect of scan rate variation on ($I_p/v^{1/2}$) were evaluated, as shown Table 1. In the I_{pc} vs. $v^{1/2}$ plot, the straight lines passing through the origin indicate reversible processes.[35] This behavior was observed in two distinct processes within quinone structures. The first process involved the reduction of quinone to the semiquinone radical (Q^-, I_a/I_c), while the second process corresponded to the reduction of semiquinone to the quinone dianion (Q^{2-}, II_a/II_c).[36–38] The redox processes of benzoquinone derivatives **5**, **6**, and **8** were found to be reversible, as illustrated in Figure 1. The separations of peak potentials ($\Delta E_p = E_{pa} - E_{pc}$) for the two redox couples were calculated to be 60 – 100 mV for these compounds, with peak current ratios (i_{pa}/i_{pc}) close to unity at 100 mV/s. Additionally, ΔE_p and $i_p/v^{1/2}$ ratios did not show appreciable change with varying scan rates.

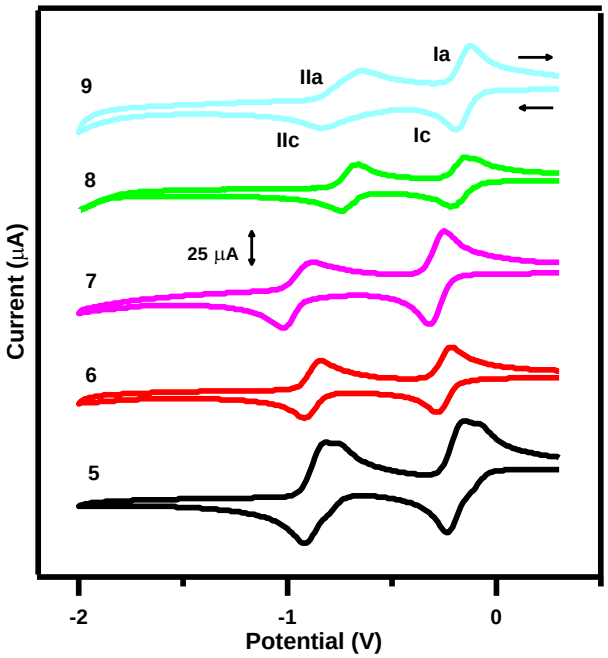


Fig. 1. Cyclic voltammograms of 1×10^{-3} M benzoquinone derivatives (**5**, **6**, **7**, **8**, and **9**) containing 0.1 M tetrabutylammonium perchlorate (TBAP) in acetonitrile (ACN) at potential range of +0.3 V to –2.0 V (Scan rate: 100 mV/s, E_{step}: 10 mV)

In contrast, benzoquinone derivatives **7** and **9** exhibited a reversible first redox process and a quasi-reversible second redox process. Notably, the peak potentials for derivatives **5**, **6**, and **7** changed significantly with the reduction of chlorine (Cl) group in their structure. The replacement of the electron-withdrawing chlorine group in compound **5** with thio-groups in compounds **6** and **7** caused a significant cathodic shift in the two one-electron reduction processes. The electron-donating nature of the thio-groups made the reduc-

tion of compounds **6** and **7** more challenging compared to compound **5**.[39]

The cathodic shift for the first and second waves of compound **7**, relative to compound **5**, was approximately 90 mV and 60 mV, respectively, at 100 mV/s. The presence of a benzene ring on the thiolate group in compound **7** further increased electron density on the quinoid structure. Additionally, the $E_{1/2}$ potentials for the thio-substituted benzoquinone compound **8** were less negative compared to those of compound **7** in both one-electron reduction steps, indicating that compound **8** is more easily reduced (Fig. 1).

Additionally, increasing the number of thiolate (–SR) groups, along with the number of methyl groups attached to the thiolate groups, significantly affected the reduction potentials. The initial reversible redox couples for benzoquinone derivatives **7** and **9** were observed at –285 mV and –155 mV, respectively. The second waves for these compounds were recorded at –950 mV and –740 mV. Compared to compound **7**, both redox couples of compound **9**, which contains four thiolate groups on the benzoquinone ring and two methyl groups on each thiolate group, shifted towards less negative potentials.

T a b l e 1

*Electrochemical parameters obtained from cyclic voltammograms of 1 × 10^{-3} M benzoquinone derivatives (**5**, **6**, **7**, **8**, and **9**) containing 0.1 M tetrabutylammonium perchlorate (TBAP) in acetonitrile (ACN) in the potential range from +0.3 V to –2.0 V (Scan rate: 100 mV/s, E_{step}: 10 mV)*

Compound	$i_{pa}v^{-1/2}c^{-1}$ ×10^{-2} ($AV^{-1/2}$ $s^{1/2}$ mol^{-1} cm^3) Wave I	$i_{pa}v^{-1/2}$ c^{-1} ×10^{-2} ($AV^{-1/2}$ $s^{1/2}$ mol^{-1} cm^3) Wave II	E_{pa} (mV) Wave I	E_{pa} (mV) Wave II	$i_{pc}v^{-1/2}$ c^{-1} ×10^{-2} ($AV^{-1/2}$ $s^{1/2}$ mol^{-1} cm^3) Wave I	$i_{pc}v^{-1/2}$ c^{-1} ×10^{-2} ($AV^{-1/2}$ $s^{1/2}$ mol^{-1} cm^3) Wave II	E_{pc} (mV) Wave I	E_{pc} (mV) Wave II	$E_{1/2}$ (mV) Wave I	$E_{1/2}$ (mV) Wave II	i_{pa}/i_{pc} Wave I	i_{pa}/i_{pc} Wave II	ΔE_p (mV) Wave I	ΔE_p (mV) Wave II
5	0.97	0.88	–160	–820	1.15	0.81	–230	–920	–195	–870	0.95	1.07	70	100
6	0.54	0.48	–220	–840	0.54	0.52	–280	–920	–250	–880	1.03	1.08	60	80
7	0.91	0.57	–250	–880	1.08	0.76	–320	–1020	–285	–950	0.94	0.78	70	140
8	0.48	0.27	–150	–660	0.52	0.35	–220	–730	–185	–695	0.99	1.00	70	70
9	0.96	1.19	–120	–640	0.99	1.27	–190	–840	–155	–740	1.11	1.14	70	200

i_{pa} and i_{pc} are the anodic and cathodic peak currents, respectively, while E_{pa} and E_{pc} are the anodic and cathodic peak potentials, respectively. ΔE_p is the peak-to-peak separation ($\Delta E_p = E_{pa} - E_{pc}$).

3.3. *Quantum chemical studies*

The complete geometry optimization and energy calculations of all tested benzoquinone derivatives were performed using the TD-DFT method[40] with GAUSSIAN 09.[41] The Becke three-parameter hybrid exchange functional and the Lee-Yang-Parr correlation functional (B3LYP)[42,43] along with the 6-311G(d,p) basis set, were employed for all atoms of quinoid compounds during the TD-DFT calculations.

The following equations were utilized to estimate quantum chemical descriptors based on the one-electron energies of the frontier molecular orbitals (FMO), specifically the Lowest Unoccupied Molecular Orbital (LUMO) and Highest Occupied Molecular Orbital (HOMO). Equations (1) – (6):

$$I = -E_{\mathrm{HOMO}} \quad (1)$$

$$A = -E_{\mathrm{LUMO}} \quad (2)$$

$$\Delta E_{\mathrm{gap}} = E_{\mathrm{LUMO}} - E_{\mathrm{HOMO}} \quad (3)$$

$$\mu = -\left(\frac{\mathrm{I+A}}{2}\right) \quad (4)$$

$$\eta = \frac{\mathrm{I-A}}{2} \quad (5)$$

$$\sigma = \frac{1}{\eta} \quad (6)$$

where I represents ionization potential, A denotes the electron affinity, μ is the electronic chemical potential, η represents the chemical hardness, and σ corresponds to the global softness.

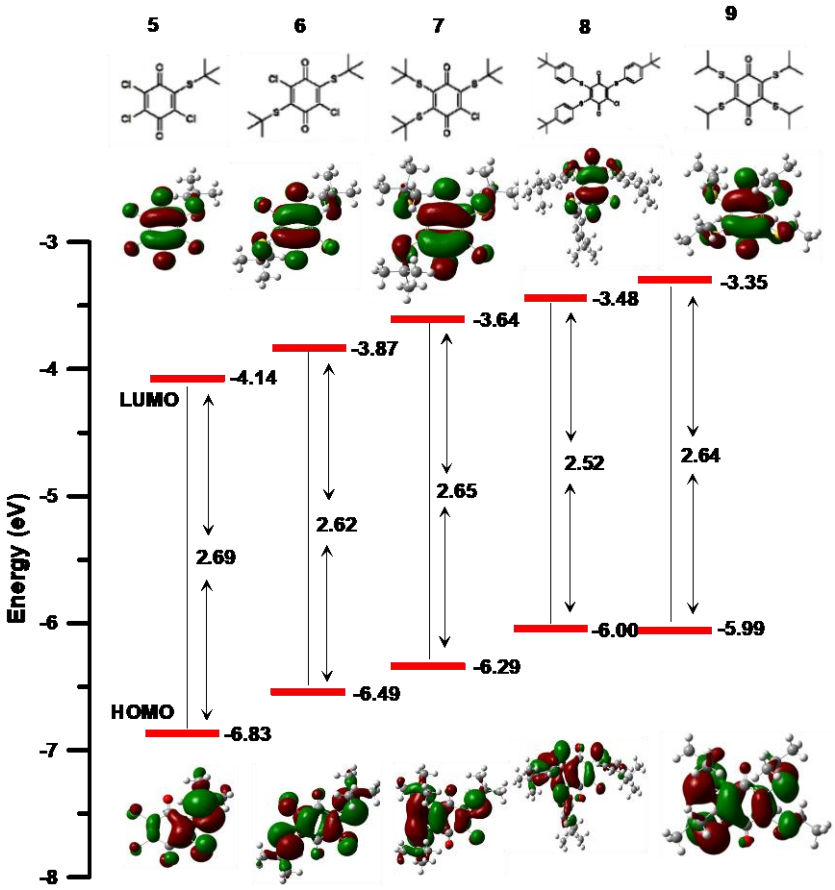


Fig. 2. FMO and energy diagrams for benzoquinone derivatives (**5**, **6**, **7**, **8**, and **9**) computed using the B3LYP/6–311G(d,p) basis set

T a b l e 2

*Quantum chemical descriptors of the benzoquinone derivatives (**5**, **6**, **7**, **8**, and **9**) calculated at B3LYP/6–311G(d,p)*

Compound	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE_{gap}	I	A	μ (eV)	η (eV)	σ (eV)
5	–6.83	–4.14	2.69	6.83	4.14	–5.60	1.34	0.74
6	–6.49	–3.87	2.62	6.49	3.87	–5.18	1.31	0.76
7	–6.29	–3.64	2.65	6.29	3.64	–4.97	1.33	0.75
8	–6.00	–3.48	2.52	6.00	3.48	–4.74	1.26	0.79
9	–5.99	–3.35	2.64	5.99	3.35	–4.67	1.32	0.76

In order to elucidate the experimental findings detailed above, we performed full geometry optimizations for all compounds using density functional theory.[40] The HOMO and LUMO energies, which reflect the molecules' chemical activity, were used to compute the energy difference between the frontier orbitals of the donor and acceptor orbitals corresponding to the charge transfer transition (ΔE_{gap} = $LUMO_{acceptor}$ – $HOMO_{donor}$).[44] In addition, contour plots and 3D visualizations of the frontier orbitals (HOMOs and LUMOs) calculated using the B3LYP/6-311G(d,p) basis set, along with their corresponding energy levels and gaps, are shown in Figure 2. The results for benzoquinone derivatives (**5**, **6**, **7**, **8**, and **9**) are summarized in Table 2.

The substitution of –SR groups in place of Cl groups in benzoquinone derivatives (**5**, **6**, and **7**) did not significantly affect ΔE_{gap}, as shown in Figure 2. Among these compounds, the highest ΔE_{gap} value was calculated for compound **5**, while the smallest ΔE_{gap} value was observed for compound **6**. In particular, the HOMO and LUMO energy values of compound **5** were determined to be –6.83

eV and –4.14 eV, respectively, using TD-DFT, resulting in a ΔE_{gap} of 2.69 eV. The ΔE_{gap} values for compounds **6** and **7** were 2.62 eV and 2.65 eV, respectively.

For compound **8**, the addition of a benzene ring (an electron-donating group) significantly decreased both the HOMO and LUMO energies, as well as the ΔE_{gap} value. The HOMO, LUMO, and ΔE_{gap} for compound **8** were calculated as –6.00 eV, –3.48 eV, and 2.52 eV, respectively. When compared to compounds **7** and **9**, it is evident that the reduction of methyl groups on the substituted group and the replacement of the Cl atom with an –SR group caused noticeable changes in HOMO and LUMO energies, while ΔE_{gap} remained relatively stable. The band gaps for compounds **7** and **9** were calculated as 2.65 eV and 2.64 eV, respectively.

As depicted in Figure 2, the HOMOs of benzoquinone derivatives **5**, **6**, **7**, and **9** are primarily localized over the –S and –Cl atoms, with smaller distributions over the quinonoid core and O atoms. Notably, the CH_3 groups in the –SR substituents did not contribute to the HOMO orbitals of these derivatives. For compound **8**, the benzene ring attached to the –SR substituent had minimal influence on the HOMO. Conversely, the LUMOs of all compounds showed higher amplitude localized on the quinone skeleton,[45] with additional localization on the –Cl and –S groups in the benzoquinone derivatives.

Various descriptors are employed to express molecular reactivity in chemical processes. Among them, the HOMO-LUMO energy gap (ΔE_{gap}) is a critical factor for predicting the reactivity of both organic and inorganic compounds.[46] The electron-donating ability of a molecule is described by E_{HOMO}, while its electron-accepting ability corresponds to E_{LUMO}. Molecules with a narrow HOMO-LUMO gap (ΔE_{gap}) are considered "soft," indicating lower stability and higher reactivity.[47]

Table 2 summarizes the values for E_{HOMO}, E_{LUMO}, ΔE_{gap}, I, A, μ, η, and σ, which collectively assess the activity of benzoquinone derivatives. Based on Table 2, the ranking of ΔE_{gap} (eV) for all molecules is as follows: **8** (2.52) < **6** (2.62) < **9** (2.64) < **7** (2.65) < **5** (2.69). This order is corroborated by the hardness values (η, eV): **8** (1.26) < **6** (1.31) < **9** (1.32) < **7** (1.33) < **5** (1.34).

The selection of an optimal system for binding to cancer cells can be guided by quantum molecular descriptors.[48] The calculated energy gap, chemical hardness, and chemical potential values suggest the predicted binding affinities of benzoquinone derivatives to cancerous tissues in the following order: **8** > **6** > **9** > **7** > **5**.

3.4. *Cell viability and clonogenic formation*

The cytotoxicity effects of compound **5** on MDA-MB-231 cells were evaluated by increasing concentration of the compound (5, 10, 20, 50, 100, 200, and 500 μM) following a 24 h treatment period. The MTS assay was conducted to assess cell viability. The analysis showed that treatment with compound **5** significantly inhibited the proliferation of MDA-MB-231 cells compared to untreated (NT) and DMSO-treated control cells, 50 % cytotoxic concentration (IC_{50}) found as 50 μM (Fig. 3).

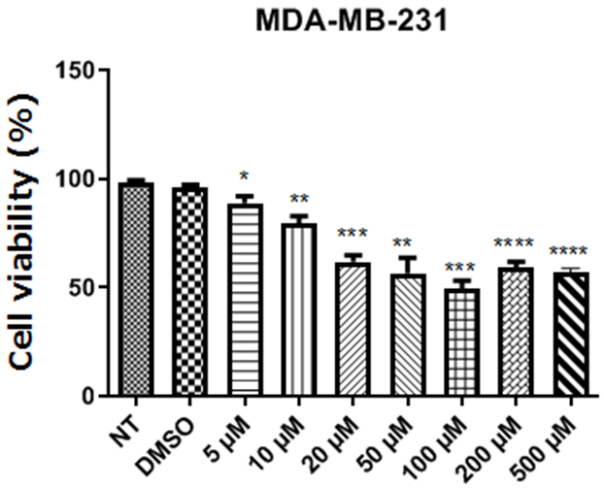


Fig. 3. Cells treated with increasing concentrations of compound **5**; cell viability was assessed using the MTS assay after 24 h. The data are presented as means ± standard deviations (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, Students t-test)

The effects of compound **5** on colony formation in MDA-MB-231 cells were investigated. Treatment with compound **5** (5 – 25 μM) resulted in a significant reduction in the number of colonies at concentrations of 10 μM and higher, compared to NT and DMSO-treated cells (Fig. 4).

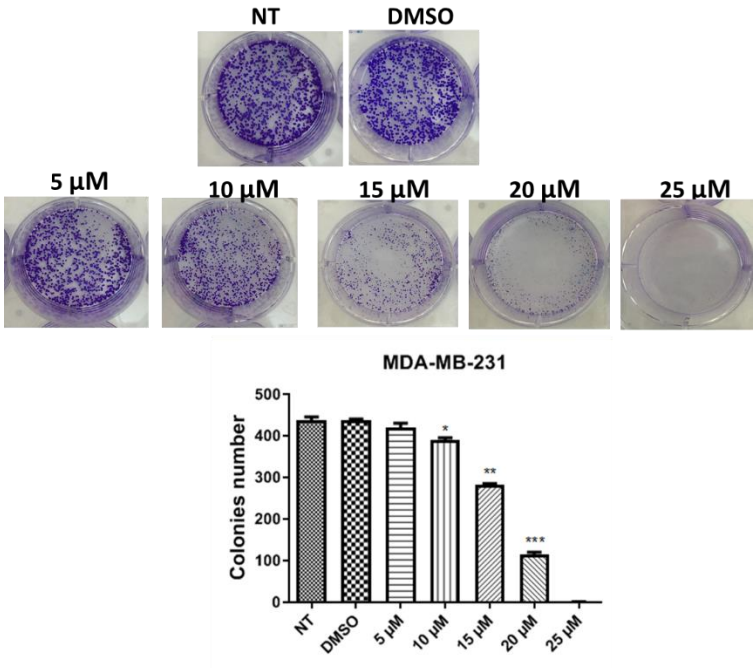


Fig. 4. The effects of compound **5** on colony formation of MDA-MB-231 cells

4. CONCLUSIONS

In conclusion, several 1,4-benzoquinone derivatives (**5** – **9**) containing sulfur atoms were successfully synthesized and characterized using elemental analysis, UV-Vis, ^{1}H-NMR, ^{13}C-NMR, and MS (ESI) spectroscopy. The electrochemical properties and theoretical calculations of these compounds were investigated. The reduction potentials of the benzoquinone derivatives varied significantly with a decreasing number of chlorine (Cl) groups in their structure. Substituting a Cl group on the benzoquinone ring with a thio group resulted in a marked cathodic shift in the two one-electron reduction processes. Benzoquinones with a benzene ring linked to the thiolate group exhibited less negative reduction potential compared to those with only a thio substitution. Additionally, an increase in thiolate (–SR) groups and their attached methyl groups significantly influenced the reduction potential.

Based on ΔE_{gap}, chemical hardness, and chemical potential parameters, the predicted binding affinity of benzoquinone derivatives to cancerous tissues is ranked as follows: **8** > **6** > **9** > **7** > **5**. Among the synthesized compounds, compound **5** demonstrated significant cytotoxicity in MDA-MB-231 cells during in vitro studies. Future studies will focus on the direct synthesis of target compounds for cancerous tissues, as predicted by theoretical calculations.

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