

Synthesis, DFT Studies, Antioxidant and Anticancer Activities of Newly Synthesized Compounds Containing Lawsone Using Different Halo Reagents.

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CC License CC-BY-NC-SA 4.0	Abstract A novel binary compounds as 3-(1,3-diphenyl-1H-pyrazol-4-yl)-2-phenyl-2H benzo [f] indazole-4,9-dione (10) and fused compounds as 2-hydroxy-3-(4- hydroxy-7-methoxy-2-oxo-1,2 dihydroquinolin-3-yl) naphthalene-1,4-dione (7) and naphtha [2,3-b] furan-3,4,9 (2H)-trione (6) based on Lawsone ‘that plays as important precursor’ was synthesized by reaction with 1,3-diphenyl-1H-pyrazole-4-carbaldehyde and halo-compounds, such as 2-chloro-acetyl chloride or 3-bromo-4-hydroxy-7-methoxyquinolin-2(1H)-one. Spectra and elemental analysis of newly synthesized compounds were investigated. The target molecules were showed easy access to antioxidant (calorimetrical measurement) and antitumor (Ehrlich ascites carcinoma [EAC] cells) activities. Geometrical isomers (enol, Keto conformers, and syn, anti-conformers) were achieved by density functional theory (DFT) that conformed to the spectral analysis of the investigated compounds. Keywords: - <i>Lawsone, Halo-reagents, DFT studies, anti-oxidant, anti-tumor</i>
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Introduction

Lawsone is described as a medicinal plant.¹ Therefore; it was considered a common precursor for many synthetic compounds with a number of valuable biological applications like Juglone, Menadione (Vit. K3), Arizonin C1, Alkanin;² and its mannich bases (Figure 1).³ Lawsone has pharmaceutical activities as anticancer,^{4,5} anti-inflammatory,⁶ Leishmanicidal activity,⁷ antifungal, antiparasitic,⁸ antimalarial,⁹ and antimicrobial.^{10,11} In addition, a wide variety of applications of it, such as anticorrosion¹² and improving the solar cells.^{13,14}

Halo-reagents characterize an important class of alkylating and/or acylating reagents due to their widespread use as synthetic intermediate. Chloroacetyl chloride and chloro-acetic acid are playing important roles in the synthesis of pharmacological compounds like Lidocaine,¹⁵ 25- hydroxyl protopanaxadiol (25-OH-PPD, AD-2)¹⁶, and its esters.¹⁷ In addition to using these agents in synthesis herbicides as *N*-alkoxy Methyl-*N*-2,6-diethyl phenyl analogs¹⁸ and indicators in biologically active carbon (BAC) filter backwashing.¹⁹

Based on the aforementioned biological activity and in continuation of the research on literature.^{20, 21} we described herein to synthesis annulated naphtha[b]furan trione compounds containing Lawsone moiety by using chloro and aldehyde reagents, respectively, and investigate their antioxidant and antitumor activities.

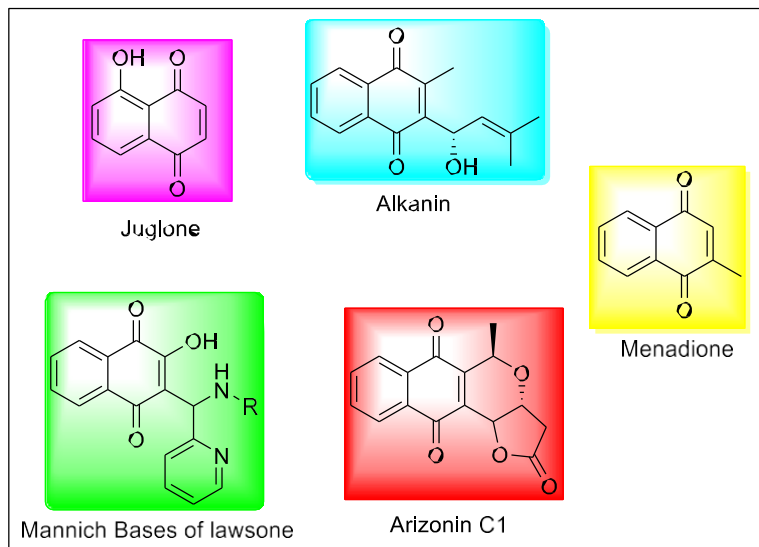


Figure 1 Some Bioactive Lawsone compounds

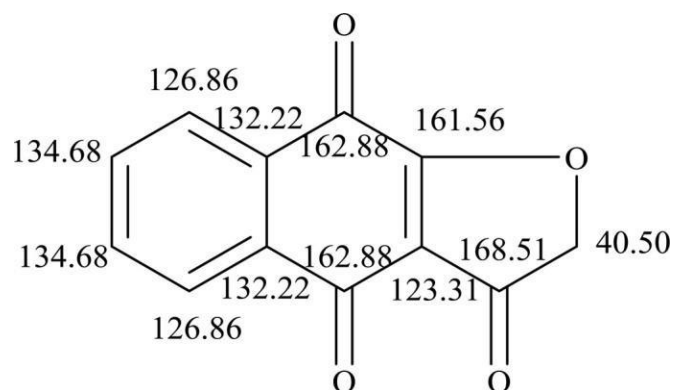
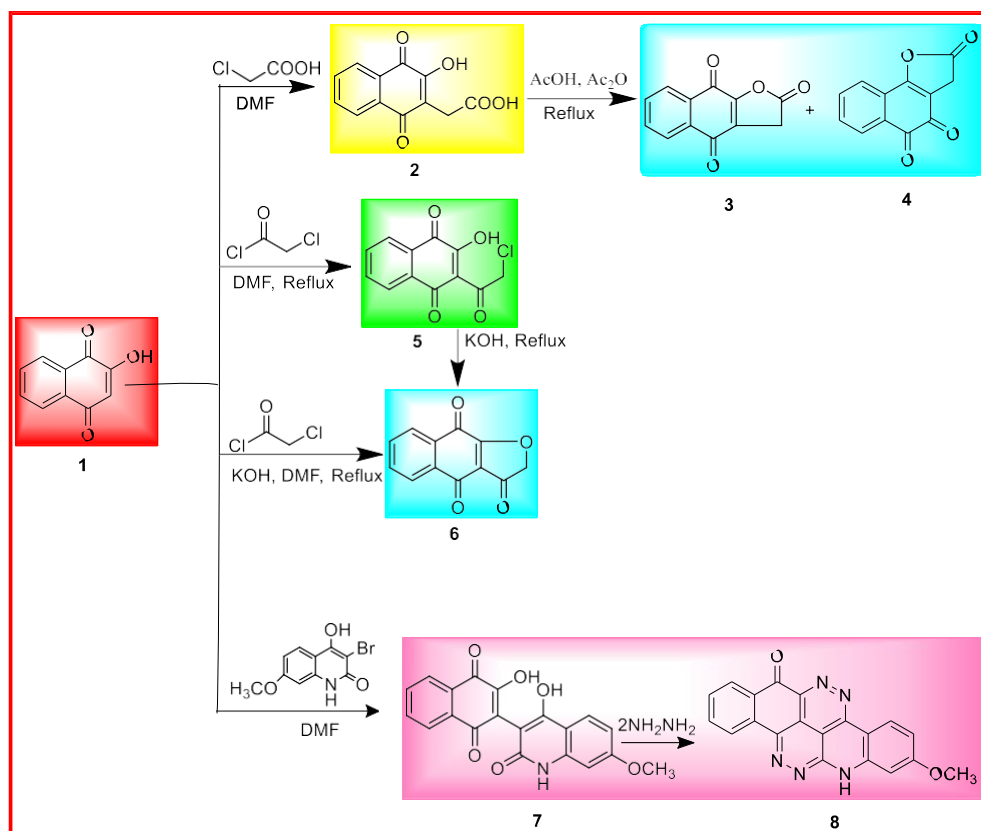
Results and discussion

Chemistry

Halo-molecules are highly reactive in the synthesis of various hetero-compounds with expected biological activity. Therefore, refluxing 1 with halo-compounds like 2-chloroacetic acid, 2-chloro acetyl chloride and 3-bromo-4-hydroxy-7-methoxy-quinolin-2(1H)-one²² in DMF formed 2-(3- hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl) acetic acid (**2**), 2-(2-chloroacetyl)-3-hydroxynaphthalene-1,4-dione (**5**), naphtha [2,3-b] furan-3,4,9(2H)-trione (**6**), and 2-hydroxy-3-(4-hydroxy-7-methoxy-2-oxo-1,2-dihydro-quinolin-3-yl) naphthalene-1,4-dione (**7**), respectively. The IR spectrum of compound(**2**) showed a new peak at 1780 cm^{-1} for the COOH group which appeared at 11.43ppm in its ^1H NMR spectrum in addition to the appearance of singlet signals at 2.89, 9.65ppm for the CH_2 and OH groups, indicating the formation of compound 2. Furthermore, the mass spectrum of compounds (**2**) and (**5**) showed a molecular ion peak at m/z 234 and 252, respectively. Also, the IR spectrum of compound (**6**) showed the disappearance of the band of the OH group, and its ^1H NMR, ^{13}C NMR spectrum showed the presence of signals at appropriate chemical shift values which proved its formation (**Figure 2**). Moreover, the ^1H NMR spectrum of compound (**7**) appeared singlet signals at 3.85, 11.3, and 12.6ppm for OCH_3 , NH, and OH (D_2O - exchangeable), respectively.

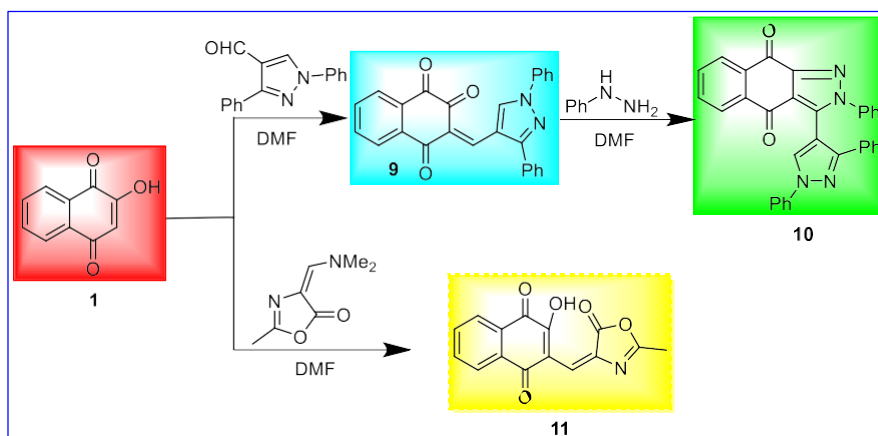
Furthermore, naphthalene derivative (**2**) cyclized via boiling in a mixture of $\text{AcOH}-\text{Ac}_2\text{O}$ (1:1) for 7hrs to afford naphtha [2,3-b]furan-2,4,9(3H)-trione (**3**), naphtha [1,2-b]furan-2,4,5(3H)-trione (**4**), respectively. The ^1H NMR of compounds 3 and 4 showed the disappearance of COOH signal at 11.43ppm of 2 indicating that the naphthalene 2 undergo cyclization to afford 3 and 4.

Moreover, compound (**7**) was chemically treatment with hydrazine hydrate to produce compound 8 after aromatization by some drops of hydrogen peroxide (H_2O_2) (**Scheme 1**). The ^1H NMR spectrum of 8 appeared singlet signals for OCH_3 and NH at 3.85 and 5.55, respectively. Owing to the very low solubility of most products, we were unable to obtain NMR spectra for some products.²³

Figure 2 ^{13}C NMR signal values of compound 6.

Scheme 1 Reaction lawsone-1 with halo-reagent to afford various hetero-compounds.

Lawsone has therapeutic importance so this study was also focused on synthesizing binary bio- active compounds based on Lawsone as a principle precursor. Therefore, Claisen–Schmidt condensation of 1 with 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde in DMF gave (z)-3-((1,3-diphenyl-1*H*- pyrazol-4-yl) methylene) naphthalene-1,2,4(3*H*)-trione (9). Moreover, 3-(1,3-diphenyl-1*H*-pyrazol- 4-yl)-2-phenyl-2*H*-benzo[*f*]indazole-4,9-dione (10) was formed by refluxing compound 9 with phenyl hydrazine in DMF. On the other hand, refluxing 1 with 4-((dimethyl-amino) methylene)-2- methyloxazol-5(4*H*)-one yielded (*E*)-2-hydroxy-3-((2-methyl-5-oxooxazol-4(5*H*)-ylidene) methyl)- naphthalene-1,4-dione (11) (Scheme 2). The ^1H NMR spectra of 9 and 11 gave evidence for the suggested structures by the appearance of singlet signal for CH at 9.45 and 7.96, respectively. While the structure of (10) was confirmed by the absence of this CH-signal. In addition to the MS fragmentation for 9–11 were supported the proposed structures. The stereoselective parameters for compounds 3–7, 9, and 11 were studied.



Scheme 2 Synthesis of some binary compounds 9–11.

Table 1 Binding energies and frontier orbital energies as obtained from DFT for compounds 3–7, 9, and 11.

Compound No.	Binding energy (ev)	Total energy (ev)	Dipole moment (Debye)	HOMO (ev)	LOMO (ev)	LOMO) — (HOMOE (ev)
3 (Keto)	—123.153	—20738.177	6.126	—6.291	—4.177	—2.114
3 (Enol)	—122.825	—20737.850	0.676	—5.673	—3.822	—1.851
4 (Keto)	—123.072	—20738.095	7.054	—5.852	—4.132	—1.720
4 (Enol)	—122.511	—20737.524	8.127	—5.204	—3.987	—1.217
5.1	—127.273	—33276.638	6.291	—6.040	—4.201	—1.839
5.2	—126.869	—33276.245	7.741	—6.082	—4.111	—1.971
5.3	—126.860	—33276.230	6.639	—5.354	—4.121	—1.233
5.4	—126.872	—33276.272	3.081	—5.376	—4.080	—1.296
5.5	—126.935	—33276.327	5.744	—5.870	—3.833	—2.037
5.6	—126.398	—33276.189	5.538	—6.211	—4.115	—2.056
6 (Keto)	—122.661	—20737.687	5.213	—5.952	—4.254	—1.698
6 (Enol)	—122.547	—20737.578	1.805	—5.794	—3.851	—1.943
7.1	—215.188	—34739.740	5.583	—5.287	—4.036	—1.251
7.2	—214.502	—34737.971	7.366	—5.389	—4.003	—1.386
7.3	—215.099	—34738.570	5.278	—5.566	—4.021	—1.545
7.4	—214.168	—34737.645	2.483	—5.562	—4.030	—1.532
7.5	—214.751	—34738.216	9.503	—5.318	—3.784	—1.534
7.6	—214.403	—34737.862	8.605	—5.656	—3.581	—2.075

7.7	—214.066	— 34737.536	7.016	—5.284	—3.904	—1.380
7.8	—213.676	— 34737.155	10.871	—5.579	—3.713	—1.866
7.9	—214.777	— 34738.243	12.887	—5.211	—3.921	—1.290
7.10	—214.554	— 34738.026	12.787	—5.353	—3.993	—1.360
7.11	—214.088	— 34737.563	12.965	—5.748	—4.038	—1.710
7.12	—214.779	— 34738.243	6.095	—5.623	—3.924	—1.699
9 (syn)	—255.188	— 36357.052	5.950	—5.482	—3.966	—1.516
9 (anti)	—254.110	— 36355.974	4.945	—5.473	—3.462	—2.011
11 (syn)	—161.939	— 27437.232	7.718	—5.948	—4.016	—1.923
11 (anti)	—162.457	— 27437.790	3.234	—5.812	—4.140	—1.672
3 (Keto)	—123.153	— 20738.177	6.126	—6.291	—4.177	—2.114
3 (Enol)	—122.825	— 20737.850	0.676	—5.673	—3.822	—1.851

Geometry studies

The geometry optimization of the molecular structure²⁴ for 3–7, 9, and 11 was performed by the density functional theory (DFT) method. Table 1 is obtained the main quantum data for some characters. Compounds 3, 4, and 6 have two isomers enol and keto conformers (Figures 3–5). From the calculations, the binding energy for compounds 3, 4, and 6 was showed the keto isomers have a more negative value of the binding energy with 0.328, 0.561, and 0.114 eV than the enol one, respectively. Due to the absence of OH peak in IR spectrum analysis for compounds 3, 4, and 6 and the presence of signal for CH₂ group at 2.89, 2.89, and 3.04 ppm in their ¹H NMR, these compounds exist in the keto conformer.

Compound 5 existed in six conformers 5.1–5.6 (Figure 6). Some conformers showed keto-enol forms like 5.1 and 5.2 among the structure ring and 5.3 and 5.5 in the branched-chain. Also, syn-anti conformers are displayed in 5.3 with 5.4 and 5.5 with 5.6. DFT calculations and spectral data achieved the same conformer

5.1. The DFT calculations showed it has the highest negative binding energy. IR spectrum appeared peaks at 1740 and 3443 for (CO–C₁) and (HO–C₂), respectively. Therefore, compound 5 exist in conformer 5.1.

Compound 7 existed in twelve conformers 7.1–7.12, shown in Figure 7. According to DFT calculations, conformer 7.1 is more stable than other conformers due to its highest negative value of binding energy. On the other hand, conformer 7.8 has the lowest binding energy, so its stability is the lowest. Spectral data of the conformer 7.1 are confirmed the DFT calculations. IR spectrum of compound 7 showed peaks for OH and NH groups at 2423 and 3100, respectively, and its ¹H NMR also confirmed the suggested structure by existing singlet signals at 12.6, 12, and 11.3 ppm (see the experimental part) approves its existence in conformer 7.1.

Figures 8 and 9 show the anti- and syn-conformers for compounds 9 and 11. The binding indicated the syn-isomer for compound 9 has a higher negative value than the anti-one by 1.078 eV. In the same way, the anti-isomer has a higher negative value than syn-one by 0.581 eV for compound 11. Therefore, the stability conforms for compound 9 is syn-conform, while compound 11 is anti-one (Table 1).

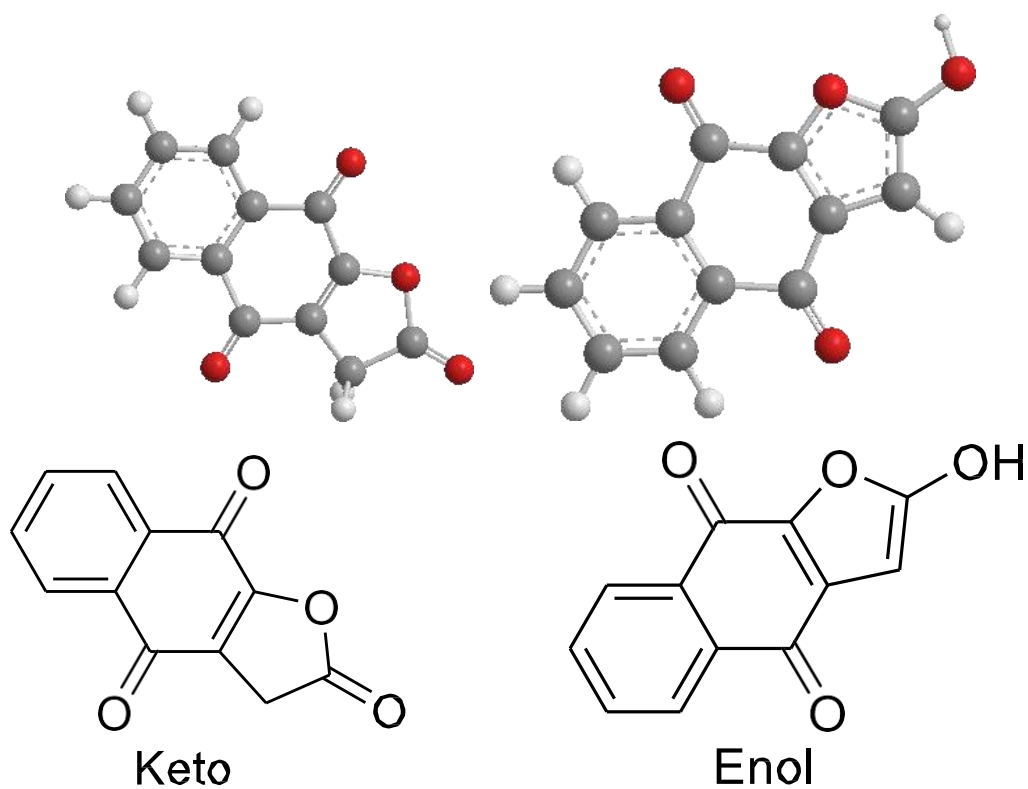


Figure 3 Optimum geometry calculation of compound 3.

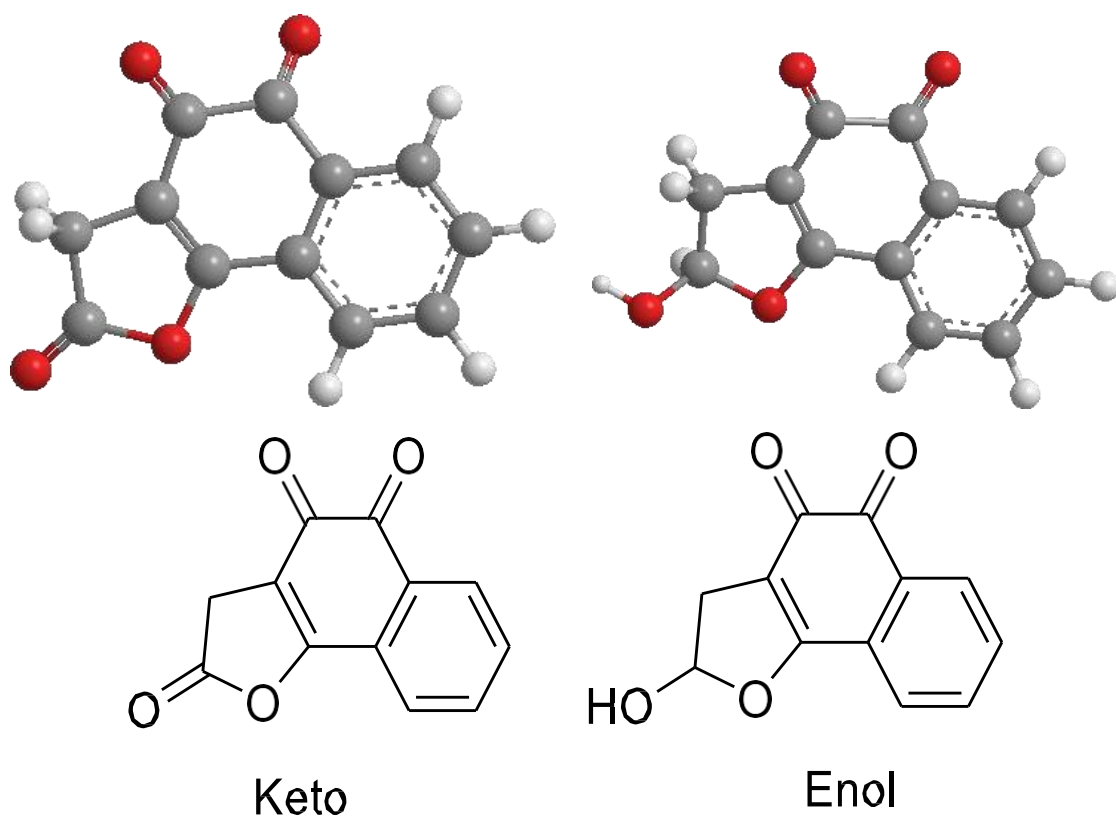


Figure 4 Optimum geometry calculation of compound 4.

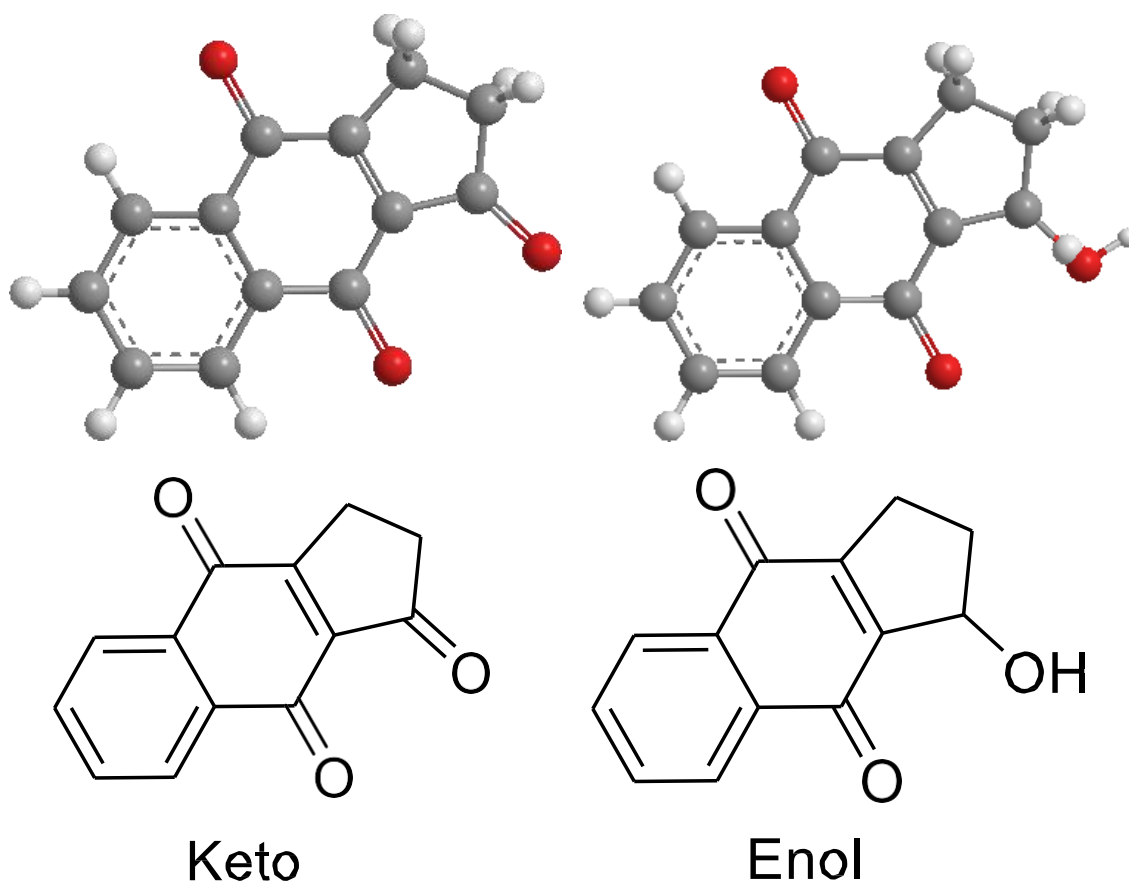
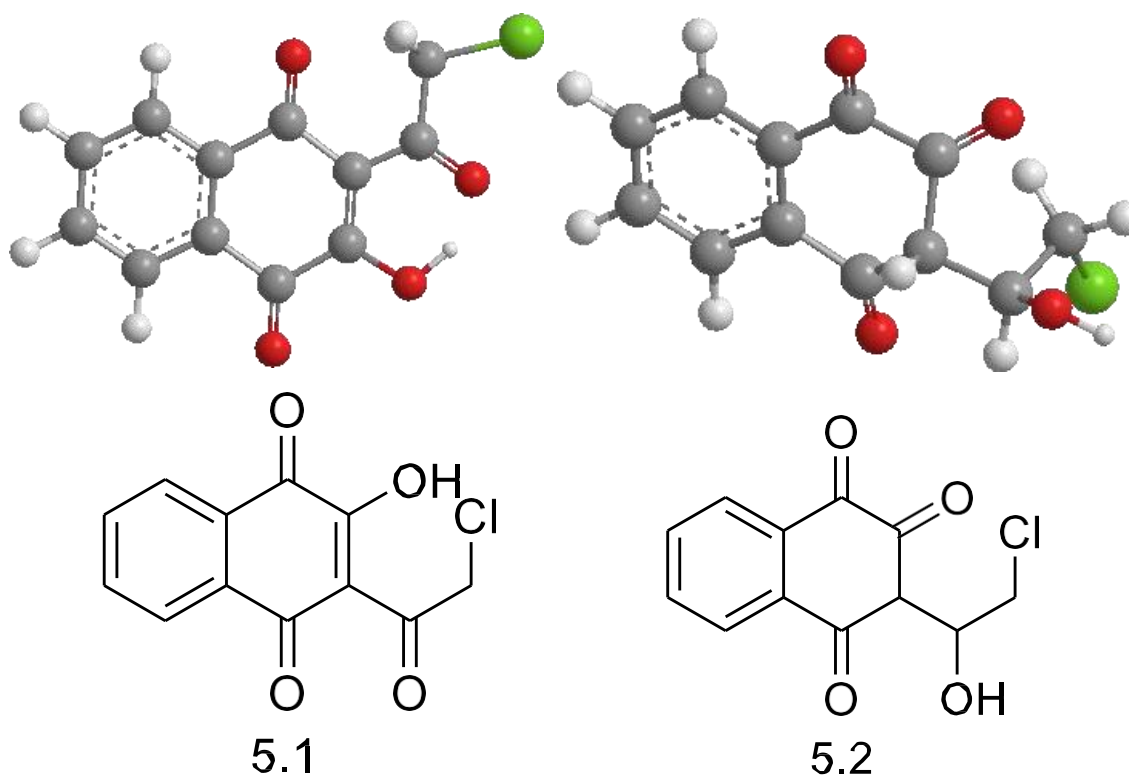


Figure 5 Optimum geometry calculation of compound 6.



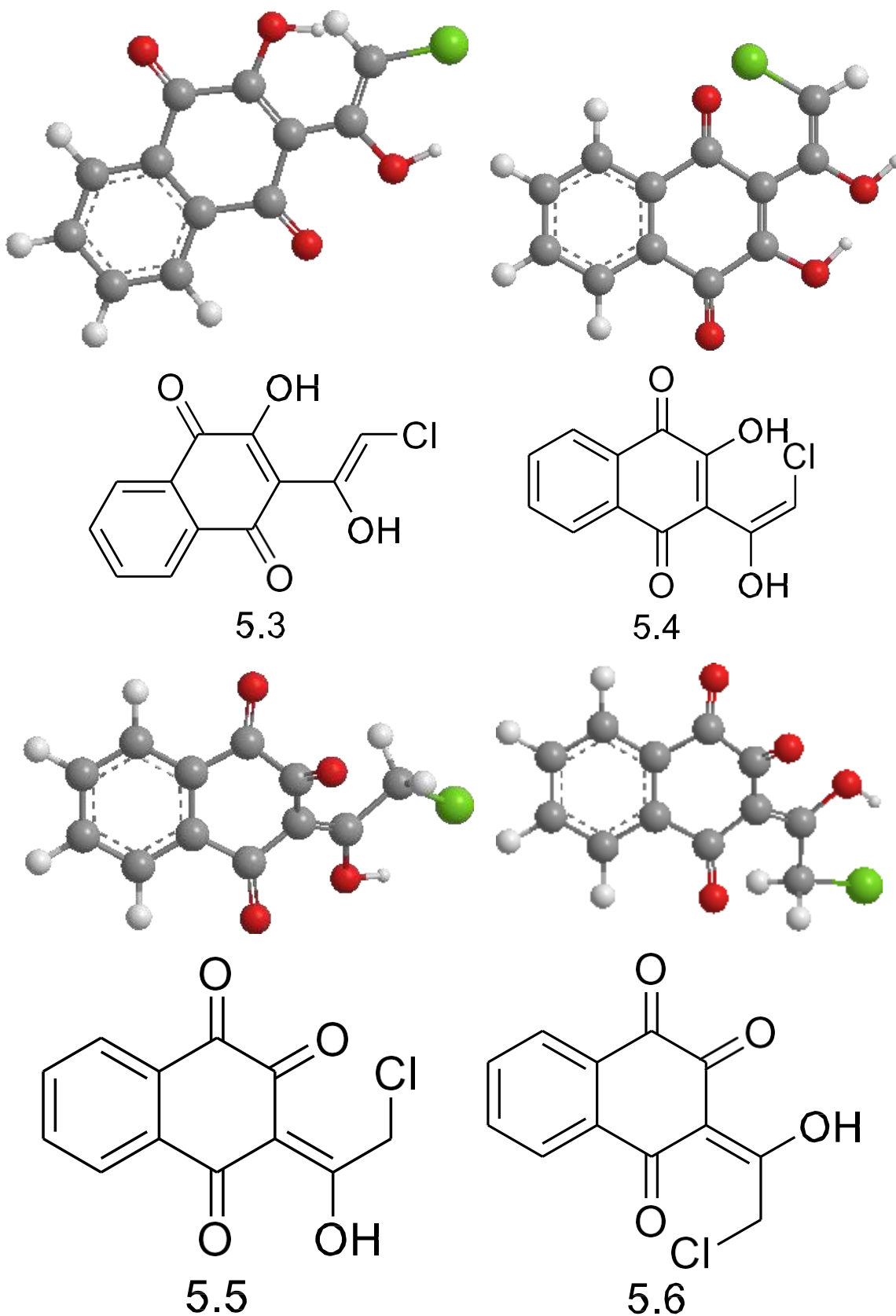
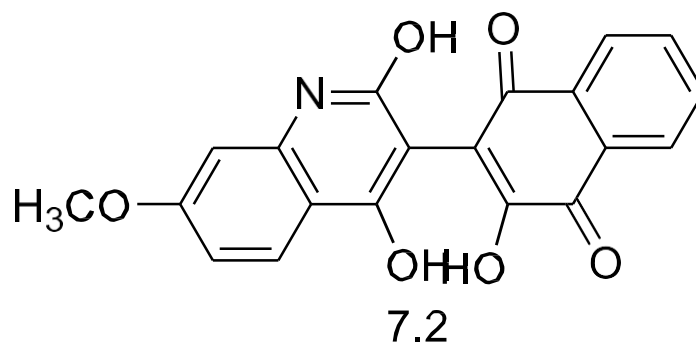
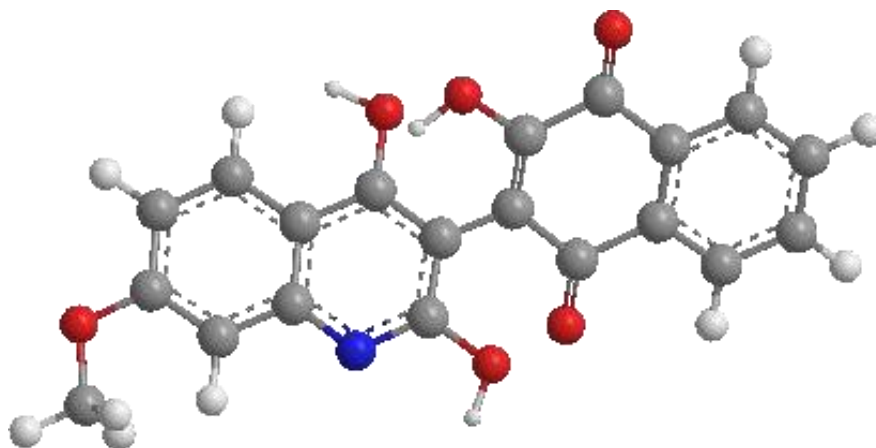
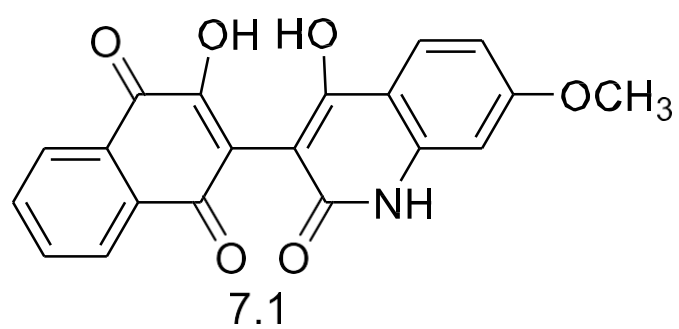
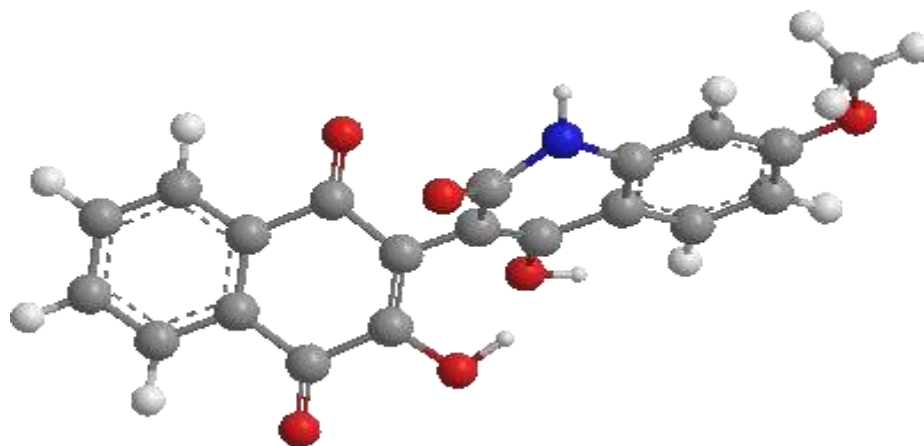
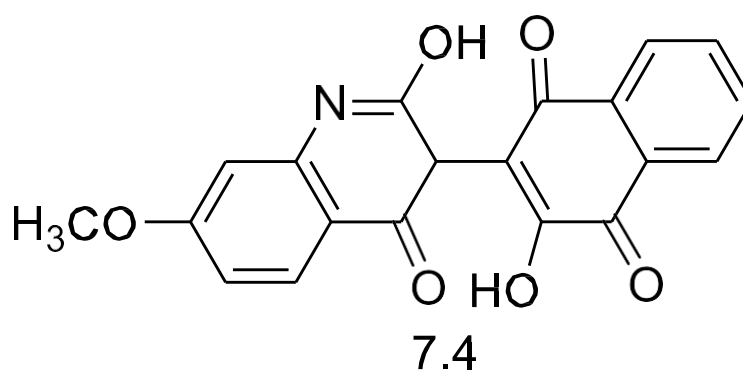
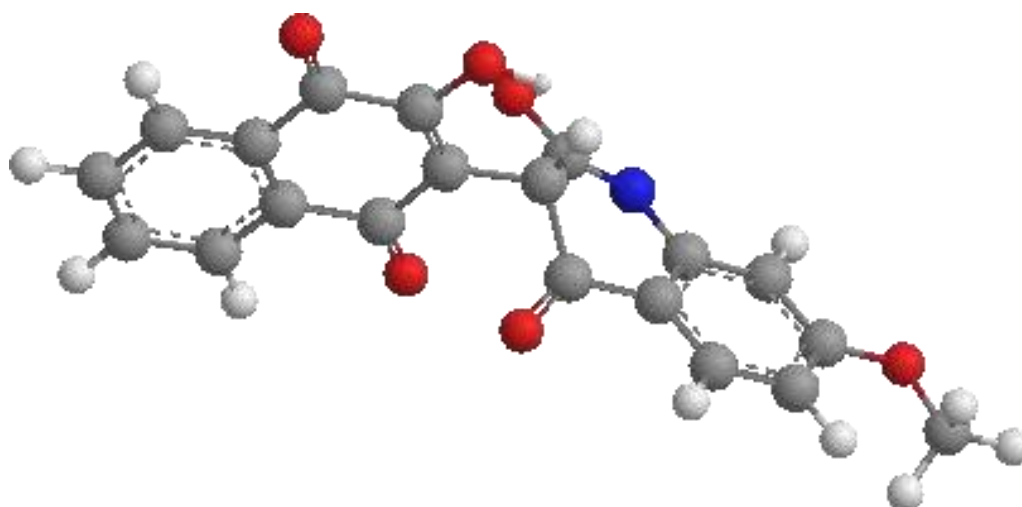
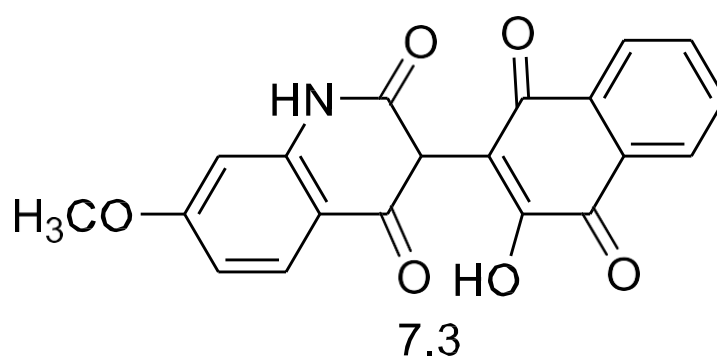
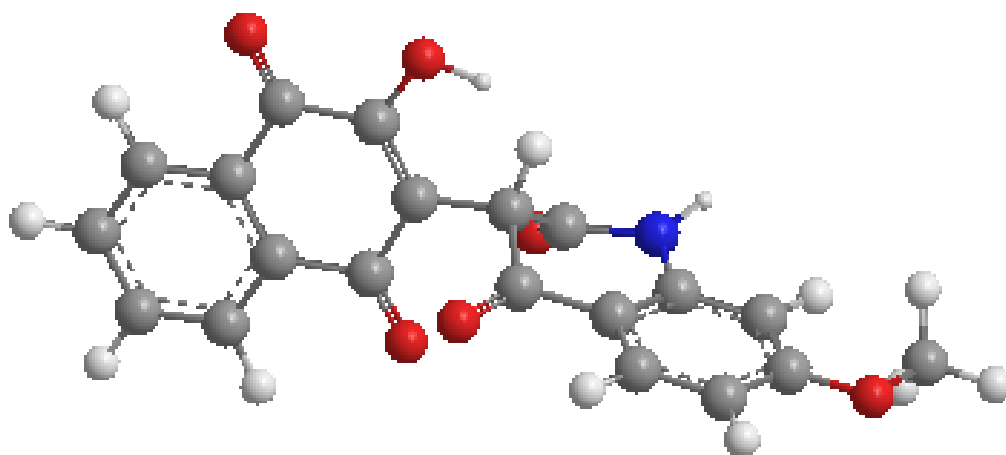


Figure 6 Optimum geometry calculation of compound 5.





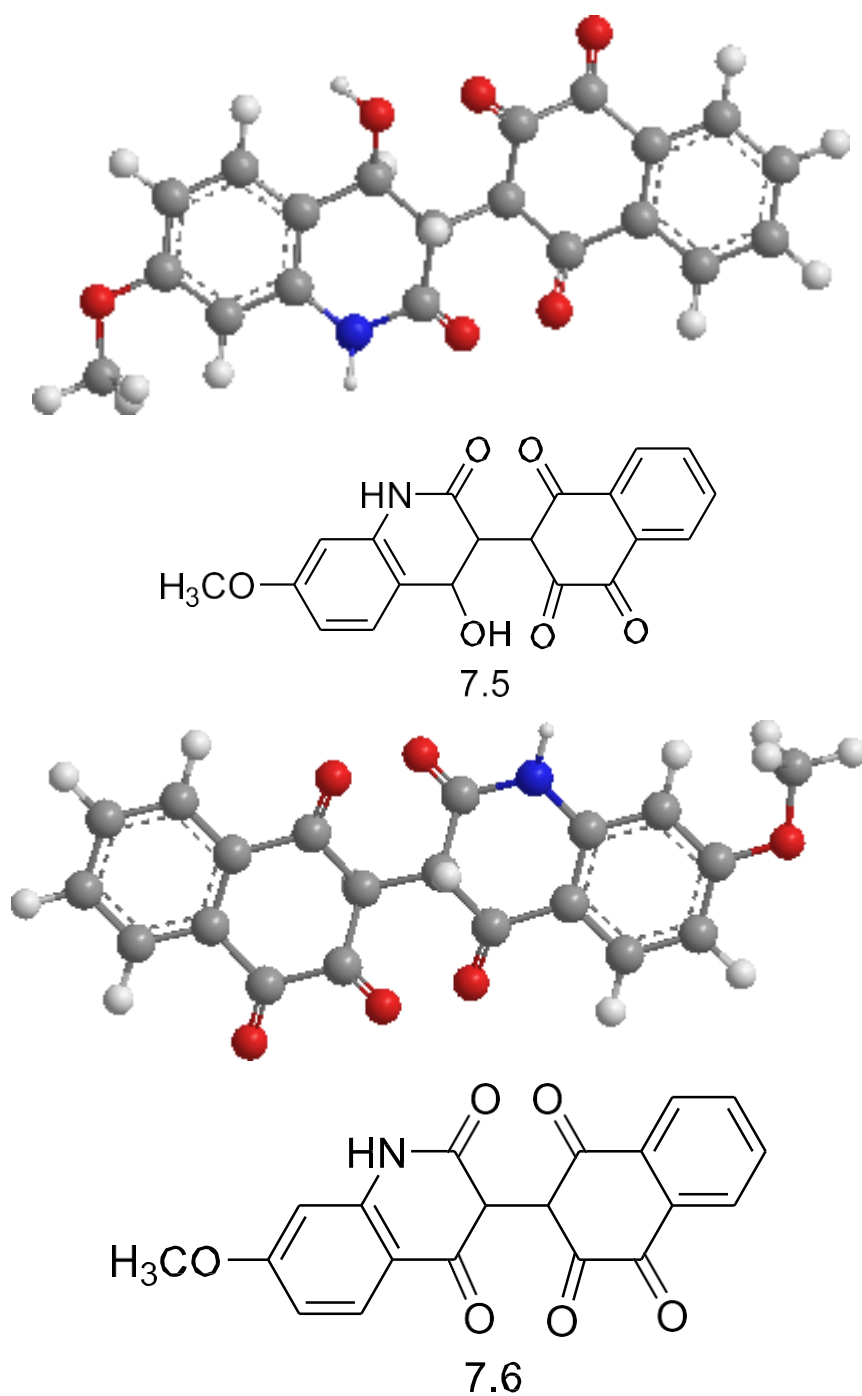
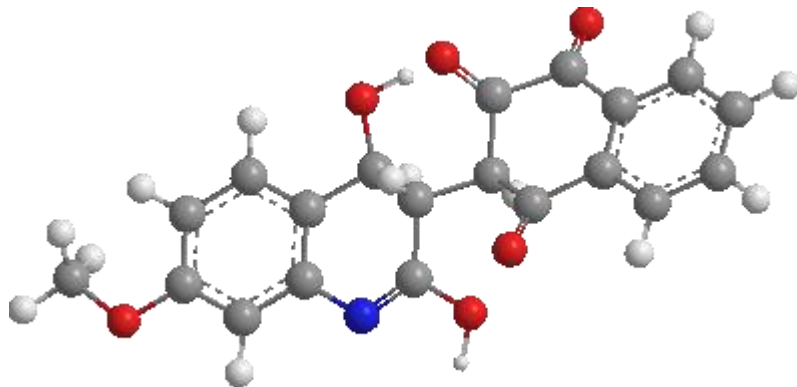
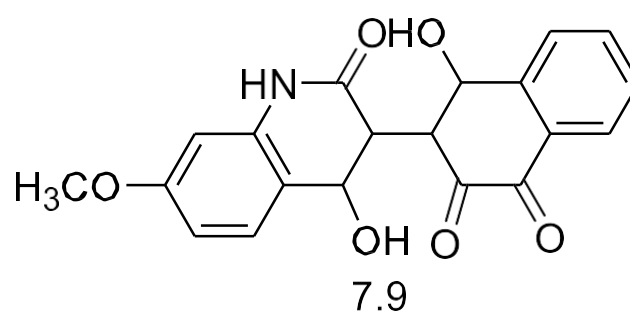
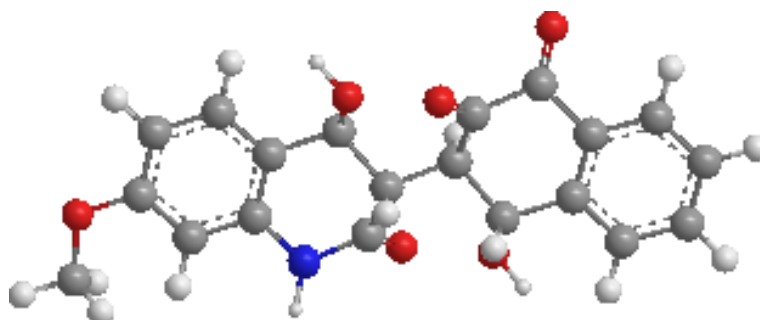
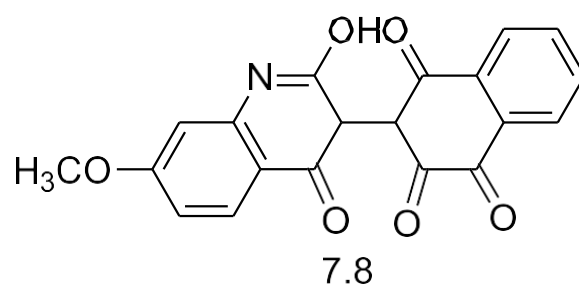
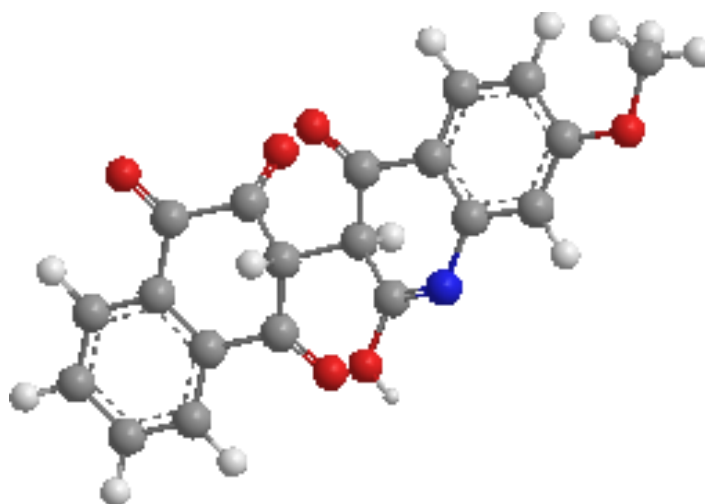
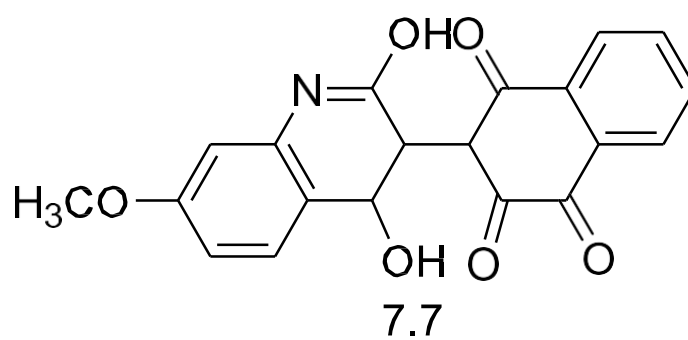
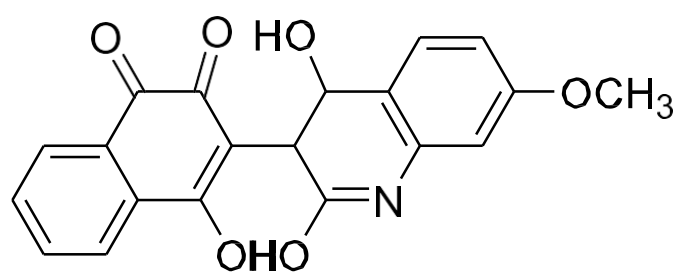
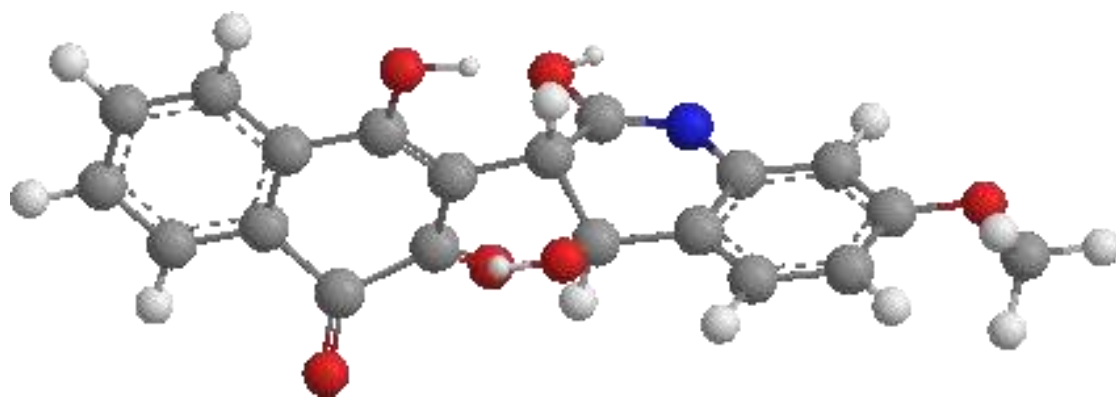


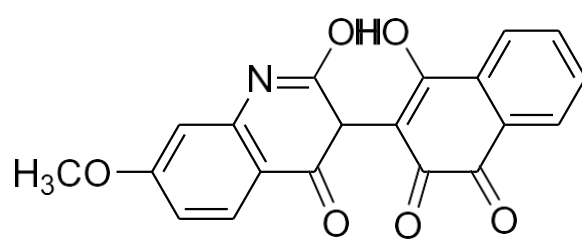
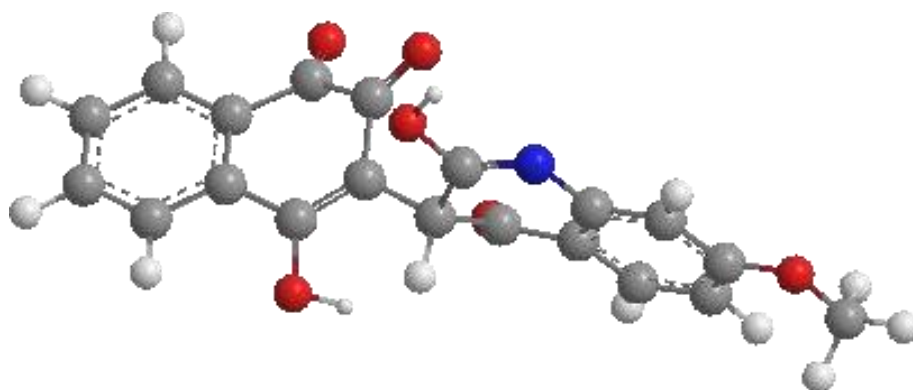
Figure 7 Optimum geometry calculation of compound 7.



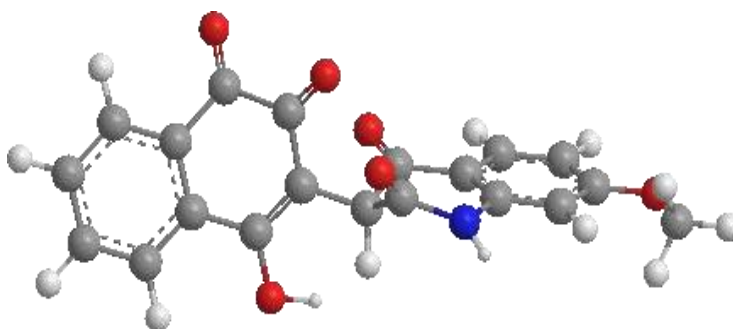


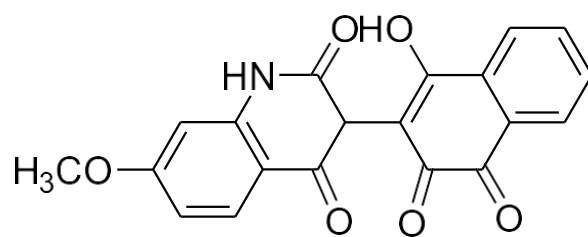


7.10



7.11





7.12

Figure 7 Continued

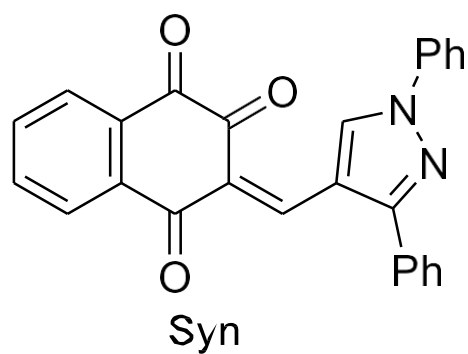
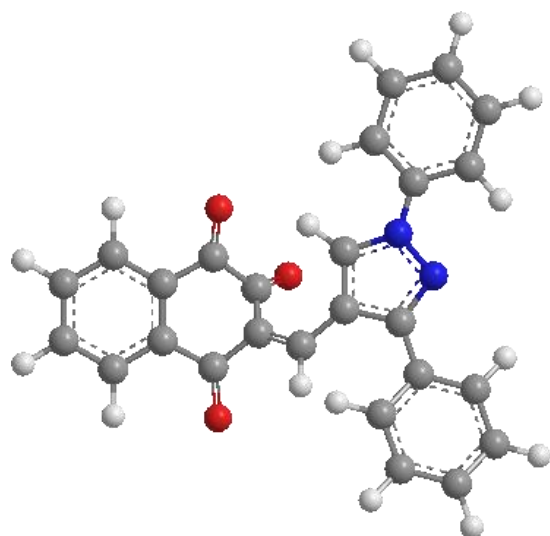
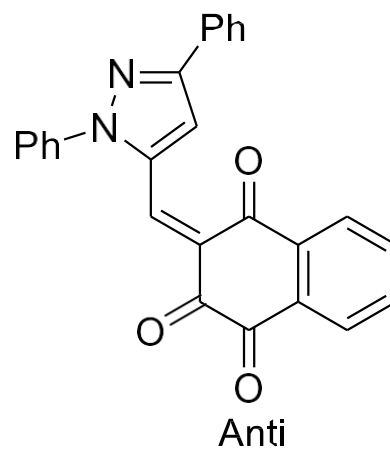
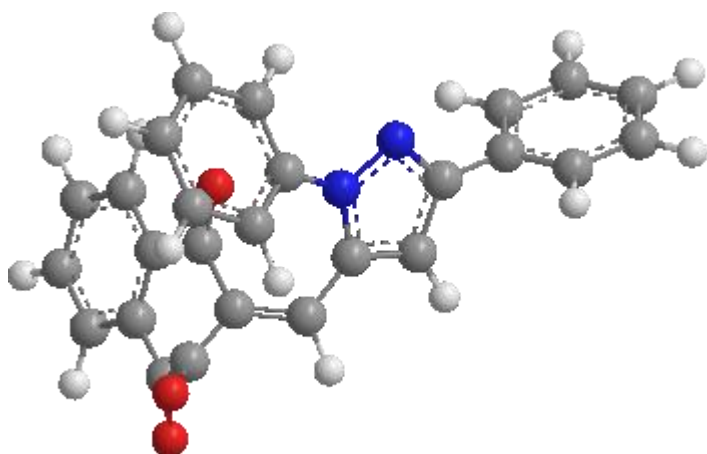


Figure 8 Optimum geometry calculation of compound 9.

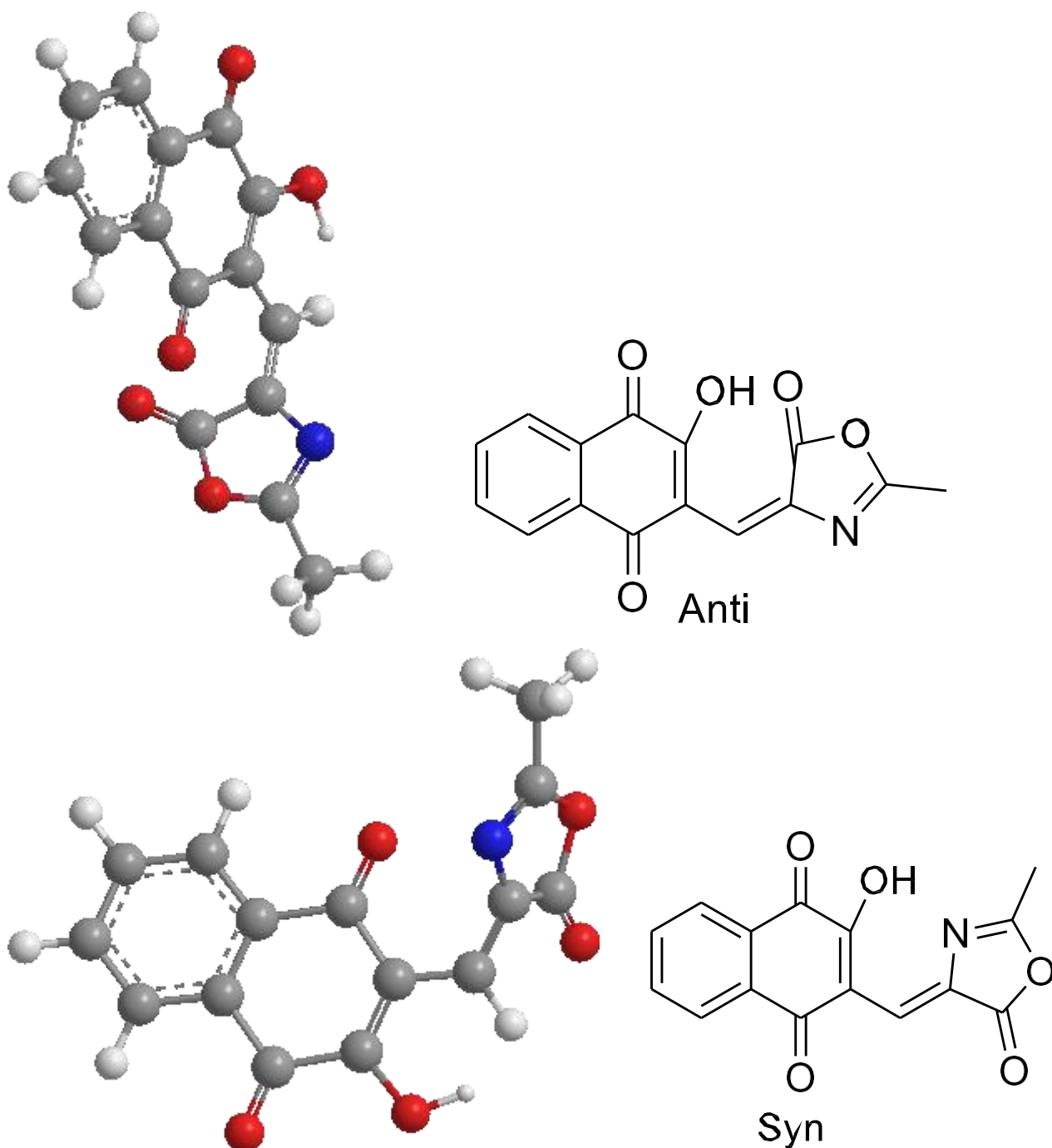
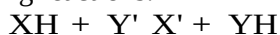


Figure 9 Optimum geometry calculation of compound 9.

Pharmacology

Antioxidant activity for ABTS

Different bioassay methods used for utilization the antioxidant activity like calculating free radicals (X') that disbursed as represented in the following reactions:²⁵



Calorimetric measurement exhibited a decrease in the intensity of the color of the free radical solution. The addition of compounds led to trapping free radical and hence X0 concentration was decreased and color changed. The antioxidant behavior for the tested compounds depended on the stable radical cation from '2, 20-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)' (ABTS). The standard antioxidant sample 'positive control' was ascorbic acid while the solvent without ABTS acted as a blank sample. The antioxidant data is represented in Table 2. The range between 85 and 83% for compounds 1 and 9 showed good antioxidant activity. However, the moderate activity played in the range between 55 and 45%, such as compounds 2, 3, 6, and 10. If we compared the tested compounds with ascorbic acid, compounds 4, 5, 7, 8, and 11 exhibited

weak antioxidant activity (Figure 10). The structure–activity relationships (SARs) for tested compounds exhibited the variability of antioxidant activity depending on the existence of naphthoquinone and pyrazole structure that increases the antioxidant activity. While the incorporation of chloride group into naphthoquinone rings construction decreases the antioxidant activity.

Antitumor activity

Effect of drugs on the viability of Ehrlich ascites carcinoma (EAC) cells in vitro

Synthesized molecules 1–11 were tested for cytotoxicity against proven model EAC²⁶ in vitro. Table 3 lists the results of values 100, 50, 25, and IC₅₀ of the tested compounds. According to the results, it is found that there is a positive relationship between the concentration of compounds and the percentage of dead cells. The most effective one that leads to the death of the cells is 100mM. The study shows that compounds 1, 2, 3, and 6 are more potent than the other compounds. Otherwise, compounds 4, 5, and 11 are moderate toxic compounds (Figures 11 and 12). SARs postulated that naphthoquinone and linear furanone naphthoquinone enhance the antitumor activity of the novel compounds. In contrast, introducing the fused pyrazole to the systems decrease their antitumor activity.

Conclusion

The synthetic compounds 2–11 are achieved by a smart, simple synthetic route, and their structures are confirmed by different spectral data analyses. Moreover, all possible isomers for compounds 2–11 were studied by DFT, which confirmed our spectral data analyses. The bioactive synthesized compounds showed a good activity compared with the standard samples.

Experimental

Chemistry

All melting points were uncorrected that obtained from the capillary melting point apparatus. Infrared (IR) spectra (cm⁻¹) were accomplished on Nicolet NEXUS 470 FTIR spectrophotometer in potassium bromide (KBr). Varian XL-300 spectrometer (300 MHz) was used to obtain ¹H NMR and ¹³C NMR spectra. Internal standard tetramethylsilane used as a reference point for chemical shifts was recorded in ppm by using DMSO-d₆ as solvent.

Microanalyses were performed on a Hosli-elemental analyzer and the data agreed with the calculated values. Mass spectra (MS) were measured on (Kratos, 70eV) MS equipment and/or a Varian MAT-311 spectrometer. The aforementioned spectral measurements and elemental microanalysis were carried out at the GLA University Mathura and Sophisticated analytical instrument facility CDRI-Lucknow, Uttar Pradesh, India.

Thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were used to follow up the reactions by using 0.2–0.4 mm silica gel 60 F254 (Merk, Kenilworth, NJ) plates using UV light (254 and 366 nm) for detection. 2-hydroxynaphthalene-1,4-dione (Lawsone) (1) was prepared according to published literature method.^{20,27,28} Biological activities were accomplished at the Hygia Institute of Pharmaceutical Education and Research Lucknow, Uttar Pradesh, India.

Preparation of 2-(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl) acetic acid (2)

Refluxing lawsone (1) (0.22g, 1.27mmol) with chloro acetic acid (0.12g, 1.27mmol) in DMF (15mL). After 6hrs crystalline product 2 was formed that's was filtered, dried, and crystallized from ethanol.

Brown crystals; yield (0.087g, 29%), M.P:246°C (dec.) (EtOH); R_f = 0.13 [ethyl acetate: pet. Ether (40:60)] (1:4); IR (KBr): ν /cm⁻¹ = 3442 (2 OH, HO–C₃ and HO–C₁), 1780 (CO–C₁), 1667 (CO–C₁), 1620 (CO–C₄); ¹H NMR (DMSO-d₆): δ : 2.89 (s, 2H, CH₂), 7.35–7.39 (m, 2H, HC-5, HC-8), 7.45–7.95 (m, 2H, HC-6, HC-7), 9.65 (s, 1H, OH), 11.43 (s, 1H, COOH); MS (EI, 70ev) m/z (%) = 234 (M⁺+2, 12), 233 (M⁺+1, 12), 184 (39), 149 (29), 99 (17), 58 (32), 57 (100, base peak). Anal. for C₁₂H₈O₅ (232.19): Calcd.: C 62.07, H 3.47%; Found: C 62.11, H 3.43%.

Preparation of naphtha [2,3-b] furan-2,4,9(3H)-trione (3) and naphtha [1,2-b] furan-2,4,5(3H)-tri-one (4)

The treatment of compound 2 (0.3 g, 1.29mmol) with acetic acid (Ac-OH) and acetic anhydride (Ac₂O) (1:1) gave a suspension solution. After refluxing this solution for 7 h. afford brown powder 4 on hot and then filtered off. The compound 3 was obtained via the addition of water to the filtrate then the solid formed as a brown powder was filtered off to give 3. Compounds 3 and 4 were recrystallized from the appropriate solvent.

Naphtho [2, 3-b] furan-2, 4, 9(3H)-trione (3)

Brown crystals; yield (0.09g, 32%), M.P: 300°C (DMF/H₂O); R_f = 0.13 [ethyl acetate: pet. Ether (40:60)] (1:4); IR (KBr): c/cm^{-1} = 1710 (CO-C₂), 1655 (CO-C₄), 1625 (CO-C₉); ¹H NMR (DMSO-d₆): δ : 2.89 (s, 2H, CH₂), 7.24–7.74 (m, 2H, CH-5, HC-8), 7.86–7.22 (m, 2H, CH-6, HC-7); MS (EI, 70ev) $m/z(\%)$ = 214 (M⁺, 29), 216 (M⁺+2, 24), 215 (M⁺+1, 24), 199 (26), 182 (23), 166 (22), 166 (22), 100 (29), 98 (41), 82 (33), 81 (36), 80 (100, base peak), 64 (64). Anal. For C₁₂H₆O₄ (214.17): Calcd: C 67.30, H 2.82%; Found: C 67.32, H 2.79%.

Naphtho[1,2-b] furan-2,4,5(3H)-trione (4)

Brown crystals; yield (0.05g, 18%), M.P: 195 °C (dec.) [CHCl₃: EtOH (5:0.5)]; R_f = 0.34 [ethylacetate: pet. Ether (40:60)] (1.5:4); IR (KBr): c/cm^{-1} = 1706 (CO-C₂), 1656 (CO-C₄), 1626 (CO-C₅); ¹H NMR (DMSO-d₆): δ : 2.89 (s, 2H, CH₂), 7.29–7.37 (m, 1H, CH-6), 7.52–7.68 (m, 1H, CH-7), 7.71–7.95 (m, 1H, CH-9), 7.99–8.42 (m, 1H, CH-8); MS (EI, 70ev) $m/z(\%)$ = 214 (M⁺, 21), 215 (M⁺+1, 19), 181 (22), 166 (M⁺-3O, 24), 76 (23), 65 (27), 56 (42), 55 (100, base peak), 54 (22). Anal. for C₁₂H₆O₄ (214.17): Calcd.: C 67.30, H 2.82%; Found: C 67.32, H 2.85%.

Preparation of 2-(2-chloroacetyl)-3-hydroxynaphthalene-1,4-dione (5)

Refluxing 1 (0.30g, 1.72mmol) with Chloroacetyl chloride (0.14mL, 1.72mmol) in DMF (15mL). The reaction was monitored by TLC and after 5hrs, it was completed then the reaction mixture was poured onto the Ice-water. The formed product was filtered off, dried, and recrystallized from EtOH/DMF (2:1) to yield compounds 5.

Pale yellow powder; yield (0.11g, 40%), M.P: 320°C; R_f = 0.59 [ethyl acetate: pet. Ether (40:60)] (3:4); IR (KBr): c/cm^{-1} = 3443 (OH-C₂), 1740 (CO-C₁), 1692 (CO-C₁), 1649 (CO-C₄); MS (EI, 70ev) $m/z(\%)$ = 252 (M⁺+ 2, 5), 251 (M⁺+1, 10), 250 (M⁺, 18), 247 (M⁺-3, 29), 233 (M⁺-OH, 10), 198 (M⁺-OH -Cl, 36), 118 (13), 100 (5), 98 (44), 90 (100, base peak). Anal. For C₁₂H₇ClO₄ (250.63): Calcd: C 57.51, H 2.82%; Found: C 57.64, H 2.95%.

Preparation of Naphtho [2,3-b] furan 3,4,9(2H)-trione (6)**Method A**

Refluxing a mixture of 1 (0.30g, 1.72mmol) and Chloroacetyl chloride (0.14mL, 1.72mmol) in DMF (15mL) for 13 h in the presence of the catalytic amount of potassium hydroxide. After completion of the reaction, Ice water was poured onto the reaction mixture to give precipitate 6 which was filtered off, dried then recrystallized.

Method B

Compound 5 (0.43g, 1.72mmol) refluxed in DMF (15mL) in the presence of a catalytic amount of potassium hydroxide for 10 h. The reaction mixture was monitored by TLC, and then poured onto Ice-water to give precipitate 6. It was filtered off, dried then recrystallized.

Brown powder; yield (0.22g, 60%), M.P: 320°C (EtOH/Acetone (1:1)); R_f = 0.15 [ethyl acetate: pet. Ether (40:60)] (1:1); IR (KBr): c/cm^{-1} = 1769 (CO-C₃), 1710 (CO-C₄), 1656 (CO-C₉); ¹H NMR (DMSO-d₆): δ : 3.04 (s, 2H, CH₂), 7.53–7.82 (m, 2H, HC-5, HC-8), 7.84–8.26 (m, 2H, HC- 6, HC-7); ¹³C NMR (100 MHz, DMSO): δ : 40.50, 123.31, 126.86, 126.86, 132.22, 132.22, 134.68, 134.68, 161.56, 162.88, 162.88, 168.51; MS (EI, 70ev) $m/z(\%)$ = 214 (M⁺, 30), 215 (M⁺+1, 5), 198 (M⁺-O, 10), 181 (36), 167 (26), 98 (86), 68 (21), 64 (45), 57 (100, base peak). Anal. For C₁₂H₆O₄ (214.17): Calcd: C 67.30, H 2.82%; Found: C 67.28, H 2.85%.

Preparation of 2-hydroxy-3-(4-hydroxy-7-methoxy-2-oxo-1, 2- dihydroquinolin-3-yl) naphthalene-1,4-dione (7)

Refluxing 1 (0.3g, 1.72mmol) with 3-bromo-4-hydroxy-7-methoxyquinolin-2(1H)-one (0.47mL, 1.72mmol) in DMF (15mL). After 21 h the reaction mixture was poured onto Ice-water to yield crystalline product 7. It was filtered off, dried then recrystallized from EtOH/DMF (5:0.5).

Brown powder; yield (0.45g, 71%), M.P:280°C (dec.); Rf = 0.36 [ethyl acetate: pet. ether(40:60)](1.5:4); IR (KBr): c/cm^{-1} = 3423 (2 OH, OH-C₂, OH-C₄), 3100 (NH), 2924 (OCH₃), 1685 (CO-C₄), 1673 (CO-C₁), 1623 (CO-C₂); ¹H NMR (DMSO-d₆): δ : 3.85 (s, 3H, OCH₃), 6.83–6.88 (m, 1H, HC-6⁰), 6.90–7.08 (m, 2H, HC-5⁰, HC-8⁰), 7.24–7.45 (m, 2H, HC-6, HC-7), 7.80–7.99 (m, 2H, HC-5, HC-8), 11.3 (s, 1H, NH, D₂O-exchangeable), 12 (s, 1H, OH- quinolone, D₂O exchangeable), 12.6 (s, 1H, OH- Lawsone, D₂O exchangeable); MS (EI, 70ev) m/z (%) = 363 (M⁺, 66), 364 (M⁺+1, 61), 362 (M⁺+1, 70), 347 (M⁺-O, 63), 345 (95), 317 (70), 191 (67), 122 (19), 106 (83), 74 (100, base peak).

Anal. for C₂₀H₁₃NO₆ (363.33): Calcd.: C 66.12, H 3.61, N 3.86%; Found: C 66.09, H 3.66, N 3.75%.

Preparation of polynuclear compound (8)

Compound 7 (0.3g, 0.82mmol) was refluxed with hydrazine hydrate (0.12mL, 2.40mmol) in DMF (15mL) in the presence of drops of H₂O₂. After 9 h the reaction was completed this was observed by TLC. After that, we added some ice-water to the mixture to precipitate 8. It was filtered off, dried then recrystallized from DMF/H₂O.

Brown powder; yield (0.22g, 76%), M.P: 300°C; Rf = 0.37 [ethyl acetate: pet. Ether (40:60)] (1.5:4); IR (KBr): c/cm^{-1} = 3421 (NH), 1660 (CO); ¹H NMR (DMSO-d₆): δ : 3.85 (s, 3H, OCH₃), 5.55 (s, 1H, NH, D₂O exchangeable), 6.55–7.72 (m, 7H, Ar-H). Anal. for C₂₀H₁₁N₅O₂ (353.33): Calcd.: C 67.99, H 3.14, N 19.82%; Found: C 67.87, H 3.17, N 19.85%.

Preparation of (Z)-3-((1,3-diphenyl-1H-pyrazol-4-yl) methylene) naphthalene-1,2,4(3H)-trione (9)

Lawsone (1) (0.4g, 2.30mmol) was refluxed with 1,3-diphenyl-1H-pyrazole-4-carbaldehyde (0.57 g, 2.30mmol) in DMF (15mL) for 10 h. The reaction mixture was diluted with ice water to produce 9. The product was filtered off, dried then recrystallized from CHCl₃.

Brown powder; yield (0.61g, 53%), M.P:188–190°C; Rf= 0.51 [ethyl acetate: pet. ether(40:60)](2.5:4); IR (KBr): c/cm^{-1} = 1710 (CO-C₁), 1660 (CO-C₂), 1624 (CO-C₄); ¹H NMR (DMSO-d₆): δ : 6.99–7.95 (m, 14H, Ar-H), 8.63 (s, 1H, CH pyrazole), 9.45 (s, 1H, CH=); MS (EI, 70ev) m/z (%) = 404 (M⁺, 13), 406 (M⁺+2, 7), 405 (M⁺+1, 8), 388 (M⁺-O, 9), 358 (10), 233 (13), 156 (9), 102 (12), 100 (11), 78 (12), 65 (18), 64 (100, base peak). Anal. For C₂₆H₁₆N₂O₃ (404.42): Calcd. C 77.22, H 3.99, N 6.93%; Found: C 77.14, H 3.87, N 6.86%.

Preparation of 3-(1,3-diphenyl-1H-pyrazol-4-yl)-2-phenyl-2H-benzoflindazole-4,9-dione (10)

Refluxing 9 (0.3g, 0.74mmol) with phenyl hydrazine (0.07mL, 0.74mmol) in DMF (10mL). After 15 h, the reaction was finished which was monitored by TLC. The reaction mixture was diluted by ice-water to afford 10. It was filtered off, dried then recrystallized from ethyl acetate/ chloroform (5:1).

Brown powder; yield (0.2g, 54%), M.P:133°C; Rf = 0.13 [ethyl acetate: pet. Ether (40:60)](1:4); IR (KBr): c/cm^{-1} = 1720 (CO-C₄), 1663 (CO-C₉); ¹H NMR (DMSO-d₆): δ : 7.26–8.43 (m, 19H, Ar-CH), 8.54 (s, 1H, CH=, pyrazole); MS (EI, 70ev) m/z (%) = 492 (M⁺, 51), 494 (M⁺+2, 63), 493 (M⁺+1, 54), 461 (67), 384 (13), 322 (M⁺-2Ph-O, 63), 320 (100, base peak), 219 (85), 143 (70), 100 (88), 66 (34). Anal. For C₃₂H₂₀N₄O₂ (492.53): Calcd. C 78.03, H 4.09, N 11.38%; Found: C 78.18, H 4.11, N 11.41%.

Preparation of (E)-2-hydroxy-3-((2-methyl-5-oxooxazol-4(5H)-ylidene) methyl) naphthalene -1,4-dione (11)

Lawsone (1) (0.23g, 1.32mmol) was refluxed with 4-((dimethyl amino) methylene)-2-methyloxazol-5(4H)-one (0.20g, 1.32mmol) in DMF (15mL) for 6 h. The reaction mixture was diluted with ice-water to precipitate 11. It was filtered off, dried then recrystallized from EtOH/DMF (4:1).

Brown powder; yield (0.12g, 32%), M.P:230°C (dec.); Rf = 0.24 [ethyl acetate: pet. Ether (40:60)] (1.5:4); IR (KBr): c/cm^{-1} = 3387 (OH), 2926 (CH₃), 1700 (CO-C₄), 1658 (CO-C₁), 1616 (CO-C₅); ¹H NMR (DMSO-d₆): δ : 1.25 (s, 3H, CH₃), 7.13–7.53 (m, 4H, Ar-CH), 7.96 (s, 1H, CH=), 8.40 (s, 1H, OH, D₂O exchangeable); MS (EI, 70ev) m/z (%) = 283 (M⁺, 31), 284 (M⁺+1, 15), 282 (M⁺-1, 15), 268 (M⁺ CH₃, 15), 266 (M⁺-OH, 18), 249 (23), 97 (12), 82 (6), 74 (32), 73 (100, base peak). Anal. For C₁₅H₉NO₅ (283.24): Calcd. C 63.61, H 3.20, N 4.95%; Found: C 63.53, H 3.17, N 4.91%.

Antioxidant screening assay (ABTS method)**Reagents:**

All chemicals used were of the highest quality available. While ABTS was bought from Wak and vitamin C was ordered from Sigma. However, the tested compounds; the ABTS solution (2mL, 60mM) and the 3 M MnO₂ solution (25 mg/mL) were prepared in phosphate buffer (pH 7M). The absorbance (A control) of the resulting green-blue solution (ABTS radical solution) is at ca. 0.5 at λ 734nm. Then, the solution of the test compound in spectroscopic grade methanol/ phosphate buffer (1:1) was added. The Inhibition percentage for each compound was calculated from

$$\% \text{ inhibition} = \frac{A(\text{control}) - A(\text{Test})}{A(\text{control})} \times 100$$

Vitamin C is used as a positive control, while methanol and phosphate (1:1) is a negative one. Also, methanol/phosphate (1:1) without ABTS acts as a blank sample.

Table 2 Antioxidant assay for the novel prepared compounds.

Method	ABTS Abs (control) - Abs (test/Abs (control) $\times$ 100	
	Absorbance of samples	%Inhibition (%)
Control of ABTS	0.520	0
Ascorbic acid	0.050	90.38
1 ⁽¹⁷⁾	0.081	84.54
2	0.261	50.19
3	0.232	55.72
4	0.348	33.58
5	0.291	44.46
6	0.264	49.61
7	0.385	26.52
8	0.318	39.31
9	0.088	83.20
10	0.283	45.99
11	0.293	44.08

Antitumor activity. The viability and percentage of viable cells were calculated by the trypan blue exclusion to know the cytotoxic effect on EAC for the tested compounds in concentration 1–25mg/mL DMSO. The required dilution that is suitable for the tumor cells was performed by saline solution (0.9%). The cells were discarded if the viability of the tumor cells was found less than 90%. This whole process was done three times.

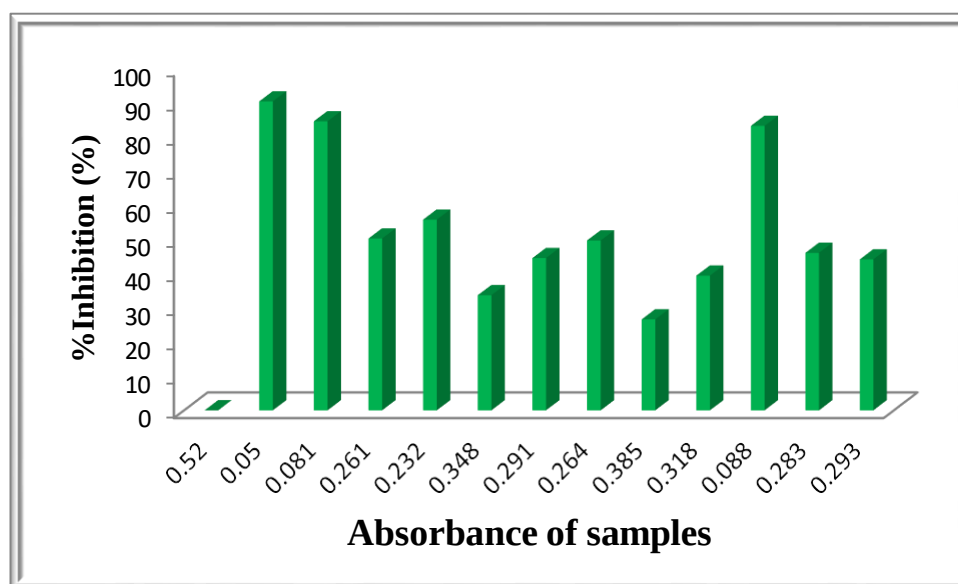
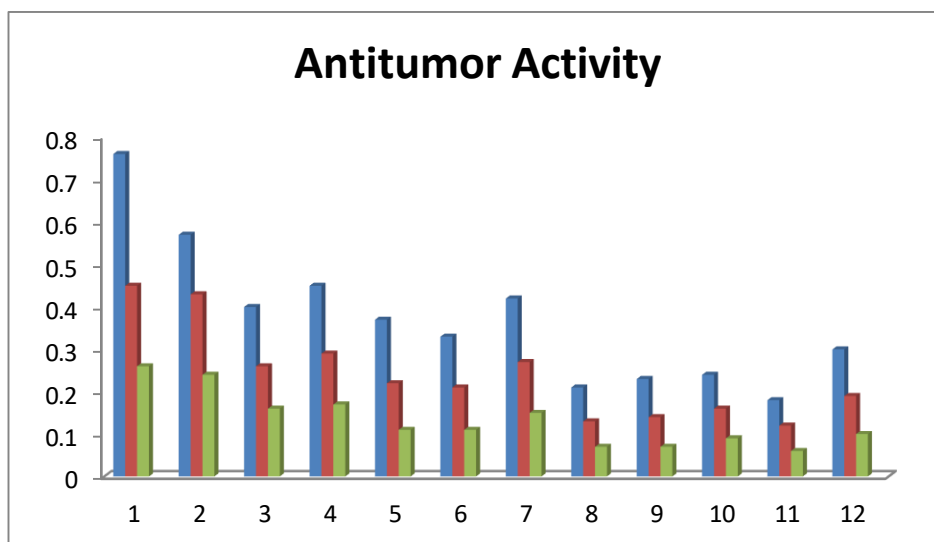
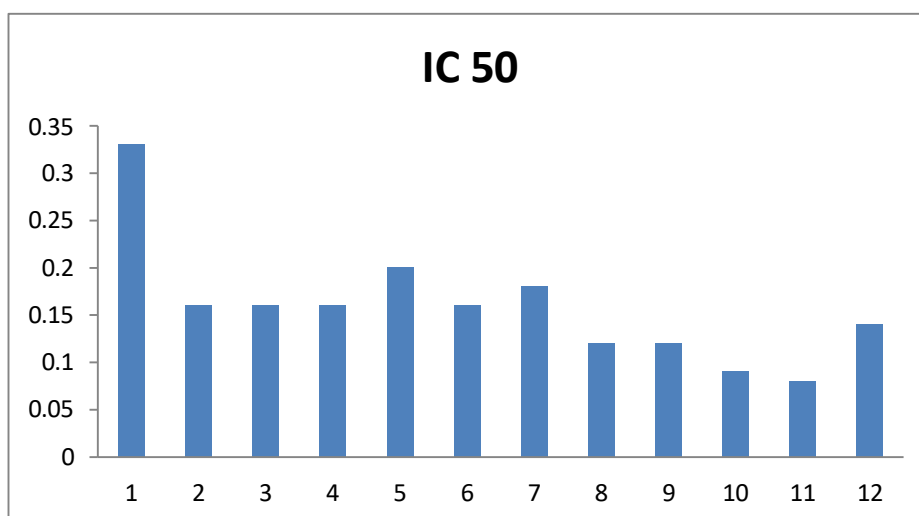
**Figure 10 Antioxidant activity screening assay.**

Table 3 Antitumor assay for the investigated compounds.

Compounds	%Dead			
	100mM	50mM	25mM	IC50 mM
5-Fu ^a	0.76	0.45	0.26	0.33
1 ⁽¹⁷⁾	0.57	0.43	0.24	0.16
2	0.4	0.26	0.16	0.16
3	0.45	0.29	0.17	0.16
4	0.37	0.22	0.11	0.20
5	0.33	0.21	0.11	0.16
6	0.42	0.27	0.15	0.18
7	0.21	0.13	0.07	0.12
8	0.23	0.14	0.07	0.12
9	0.24	0.16	0.09	0.09
10	0.18	0.12	0.06	0.08
11	0.3	0.19	0.1	0.14

Where the effective doses for the tested compounds are IC100, IC50, and IC25 at 25, 50, and 100mM, respectively. The dead % refers to the % of the dead tumor cells and 5-FU is 5-fluorouracil as a well-known cytotoxic agent.

**Figure 11 Antitumor activity for the novel prepared compounds.****Figure 12 IC50 for the novel prepared compounds.**

Disclosure statement

No potential conflict of interest was reported by the author(s).

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