



Synthesis and Evaluation of Anti-tuberculosis and Anti-cancer Activities of Hydroxynaphthoquinone Derivatives

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ABSTRACT

Hydroxy-1,4-naphthoquinone derivatives associated with a variety of side chains have been synthesized from 2-hydroxy-1,4-naphthoquinone **1**, butylamine, allylamine and selected aldehydes. Their biologically significant activities, anti-*Mycobacterium tuberculosis* (H37Ra strain), anti-cancer (MCF7-breast cancer and NCI-H187-small cell lung cancer) were studied and reported. It was found that (2*E*,4*E*)-1,4-dioxo-1,4-dihydronaphthalen-2-yl-5(benzo[*d*][1,3]dioxol-5-yl)penta-2,4-dienoate **6** showed significant anti-MCF7 and anti-NCI-H187 activities with IC₅₀ value at 3.84 µg/mL and 2.24 µg/mL and was found non-cytotoxic. In addition, 2-((allylamino)(phenyl)methyl)-3-hydroxynaphthalene-1,4-dione **2f** also showed similar bioactivities to those of compound **6** and it showed anti-TB activity with MIC value 25 µg/mL however it was found to display cytotoxic activity against Vero cells.

Keywords: naphthoquinone; hydroxy-1,4-naphthoquinone; anti-tuberculosis; anti-cancer

1. INTRODUCTION

Currently cancer and tuberculosis (TB) are the leading causes of death. They are the worldwide health problem and likely to become the most common diseases in the future [1-3]. Many of the available anticancer and anti-TB drugs are unable to differentiate between normal and neoplastic cells, thus there is a pressing need for new anticancer and anti-TB agents with high potency, less toxicity in normal tissue and devoid of unwanted side effects [4-5]. In recent years, a large number of research studies for development of

anticancer and anti-TB agents. Accordingly, the synthesis of a newer class of anticancer and anti-TB compounds is still in need. 1,4-Naphthoquinone pharmacophore is known to impart pronounced biological effects, such as antitumor [6-10], antimycobacterial [11], anti-inflammatory, antiallergic [12], antimalarial [13,14] and antileishmanial [15] activities. In the present study, two new 2-hydroxy-1,4-naphthoquinone derivatives were synthesized and their biological activities against cancer and tuberculosis were evaluated.

2. MATERIALS AND METHODS

2.1 General Procedure for Synthesis of 2-hydroxy-1,4-naphthoquinone Derivatives 2a-2g

2-Hydroxy-1,4-naphthoquinone **1** (0.5 mmol), amine **2** (0.5 mmol) were dissolved in ethanol (10 mL). The mixture was heated at 45°C for 5 min. The aldehyde (0.6 mmol) was then added with vigorous stirring. The product occurred as a red precipitate in 1 h. After 3 h, the mixture was filtered, washed with ethanol (15-20 ml) and then with diethyl ether (10 ml). After evaporation of solvent, purified by preparative gel filtration chromatography (sephadex LH-20 methanol as eluent) compounds **2a-2g** were obtained as an orange solid are shown in Figure 1.

2.2 General Procedure for Synthesis of 2-hydroxy-1,4-naphthoquinone Derivatives 6

Piperine **3** (2.8530 g, 0.01 mol) was refluxed with ethanolic KOH (2N, 10 ml) for 25 h. Ethanol was removed under vacuum to obtain the solid potassium salt of piperic acid, which was dissolved in hot water 50 ml, acidified with 35% HCl to give the yellow precipitate. Recrystallization from ethanol afford piperic acid **4**. To a solution of piperic acid (1 equiv) in dried THF at room temperature under nitrogen atmosphere was added oxalyl chloride (5 equiv) and stirred at room temperature for 6 h. The reaction mixture was evaporated to give the crude piperic acid chloride **5** as an orange residue. To the crude piperic acid

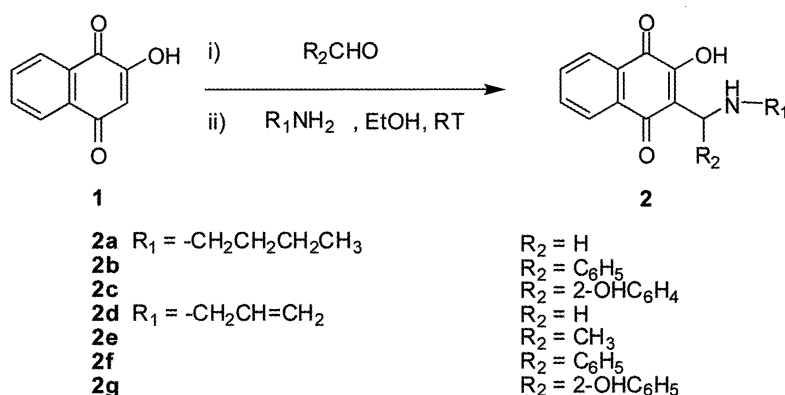


Figure 1. The synthesis of 2-hydroxy-1,4-naphthoquinone derivatives **2a-2g**.

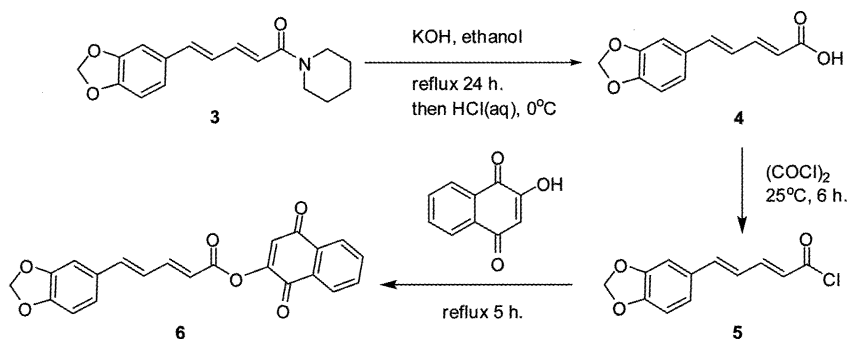


Figure 2. The synthesis of 2-hydroxy-1,4-naphthoquinone derivatives **6**.

chloride in dried THF was added the solution of 2-hydroxy-1,4-naphthoquinone (1 equiv) followed by triethylamine (1.5 equiv) and refluxed for 5 h. to obtain the compounds of derivatives **6** as shown in Figure 2 [16].

The NMR data 400 MHz ^1H -NMR and 100 MHz ^{13}C -NMR spectra were performed on a Bruker DRX 400 NMR spectrometer using tetramethylsilane (TMS) as the internal standard using CDCl_3 and $\text{DMSO}-d_6$ as the solvents. The high resolution mass spectra were performed on ESI-Q-TOF-MS (Micromass, Manchester, UK). Thin layer chromatography (TLC) was carried out on aluminium-sheet Merck silica gel 60 F254. Melting points were determined with a GALLENKAMP apparatus and are uncorrected [17].

2.3 *In vitro* Bioassay Studies

All the compounds were evaluated for *in vitro* anti-tuberculosis activity against *M. tuberculosis*, MCF-7-breast cancer and

NCI-H187-small cell lung cancer at National Center for Genetic Engineering and Biotechnology (BIOTEC). The synthesized compounds were tested by serial dilution against *M. tuberculosis* H37Ra strain, using the Green fluorescent protein microplate assay (GFPMA) [18] to determine the actual minimum inhibitory concentration (MIC) and tested by serial dilution anti MCF-7 and NCI-H187-small cell lung cancer, using the resazurin microplate assay (REMA) [19].

3. RESULTS AND DISCUSSION

The synthesis of 2-hydroxy-1,4-naphthoquinone derivatives **2a-2g** and **6** were performed by a modified process previously reported [13]. The syntheses of 2-hydroxy-1,4-naphthoquinone derivatives **2a-2g** were achieved by a Mannich reaction between 2-hydroxy-1,4-naphthoquinone, butylamine or allyl amine and selected aldehydes. The 2-hydroxy-

Table 1. Cytotoxicity against Vero cells (African green monkey kidney), anti-TB and anti cancer of 2-hydroxy-1,4-naphtho quinone derivatives.

Compounds	Cytotoxicity activity	$\text{IC}_{50}(\mu\text{g}/\text{mL})^a$	Anti-TB MIC ($\mu\text{g}/\text{mL})^b$	MCF7 $\text{IC}_{50}(\mu\text{g}/\text{mL})^c$	NCI-H187 $\text{IC}_{50}(\mu\text{g}/\text{mL})^d$
2a	Non-cytotoxic	-	-	-	7.94
2b	Non-cytotoxic	-	-	-	-
2c	Cytotoxic	26.30	-	-	30.87
2d	Cytotoxic	12.11	-	18.24	8.03
2e	Cytotoxic	8.13	-	9.70	3.96
2f	Cytotoxic	8.78	25.00	6.34	11.14
2g	Cytotoxic	7.03	-	8.40	4.94
6	Non-cytotoxic	-	-	3.84	2.24

^a Cytotoxicity against Vero cells : % cell growth > 50% non-cytotoxic, % cell growth \geq 50% non-cytotoxic (IC_{50} included)

^b MIC of positive control; Rifampicin = 0.003-0.012 $\mu\text{g}/\text{ml}$, Streptomycin = 0.156-0.313 $\mu\text{g}/\text{ml}$, Isoniazid = 0.023-0.046 $\mu\text{g}/\text{ml}$, Ofloxacin = 0.391-0.781 $\mu\text{g}/\text{ml}$

^c IC_{50} of positive control; Doxorubicine = 1.29 $\mu\text{g}/\text{ml}$

^d IC_{50} of positive control; Doxorubicine = 0.441 ± 0.195 $\mu\text{g}/\text{ml}$, Doxorubicine = 0.094 ± 0.032 $\mu\text{g}/\text{ml}$

1,4-naphthoquinone derivatives **2a-2g** were then prepared by condensation of 2-hydroxy-1, 4-naphthoquinone **1** with the corresponding aldehyde in the presence of butylamine or allyl amine in absolute ethanol (Figure 1). The synthesis of 2-hydroxy-1, 4-naphthoquinone derivative **6** was achieved by esterification reaction between 2-hydroxy-1,4-naphthoquinone and piperic acid chloride (Figure 2). Compounds **2a-2g** and **6** were obtained in a range of 5-80% yields.

Compounds **2a-2g** and **6** were subjected to test for their biological activities, as shown in Tables 1. It was found that compounds **2a**, **2b** and **6** were non-cytotoxic against Vero cells (African green monkey kidney) and compounds **2c**, **2d**, **2e**, **2f** and **2g** were cytotoxic. The determinations of the anti-*Mycobacterium tuberculosis* (anti-TB) H37Ra strain with green fluorescent protein microplate assay (GFPMA) method found that only compound **2f** showed significant anti-TB with a MIC value of 25 µg/mL.

Their anti-cancer (MCF7-breast cancer and NCI-H187-small cell lung cancer) activities with resazurin microplate assay (REMA) indicated that compounds **2d-2g** and **6** showed positive results for MCF7-breast cancer while compounds **2a-2c**

showed inactive results. For the activity to NCI-H187-small cell lung cancer, it was found that most of the compounds showed positive results except for compound **2b**.

4. CONCLUSIONS

Eight novel 2-hydroxy-1,4-naphthoquinone derivatives were synthesized. Among those, compound **6** is non-cytotoxic and showed potent activity against MCF7-breast cancer and NCI-H187-small cell lung cancer cell lines with the IC₅₀ values of 3.84 and 2.24 µg/mL, respectively. It is believed that pharmacological activities of compound **6** are derived from the presence of piperidine

side chain of the naphthoquinone core. [20-22] Compound **2a** also exhibited good result in NCI-H187-small cell lung cancer with the IC₅₀ value of 7.94 µg/mL and it was non-cytotoxic. In addition compounds **2c-2g** showed similar bioactivities to those of compounds **6** and **2a** but they displayed cytotoxic activity against vero cells. In the future, synthesis of other 2-hydroxy-1, 4-naphthoquinone derivatives as well as structural modification of the previously synthesized compounds will be investigated for evaluation of their bioactivities for their potentials to antituberculosis and anti-cancer drug development.

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[17] 17.1 2-((butylamino)methyl)-3-hydroxynaphthalene-1,4-dione (2a)

Orange solid. Yield = 22%. Mp 168.5-170.0°C. ^1H NMR (400 MHz, DMSO d_6): δ 7.93 (1H, d, J = 7.1 Hz, ArH), 7.81 (1H, d, J = 7.1 Hz, ArH), 7.70 (1H, t, J = 7.5 Hz, ArH), 7.56 (1H, t, J = 7.4 Hz, ArH), 3.93 (2H, s, Napht- CH_2N), 2.83 (2H, t, J = 7.6 Hz, NH- CH_2), 1.63-1.54 (2H, m, CH_2), 1.35-1.25 (2H, m, CH_2CH_3), 0.87 (3H, t, J = 7.4 Hz, CH_3). ^{13}C NMR (101 MHz, DMSO- d_6) δ 184.64, 178.48, 171.74, 135.13, 133.60, 131.63, 130.56, 125.32, 125.07, 107.53, 45.74, 41.35, 27.32, 19.30, 13.52. ESIMS, calcd for $\text{C}_{15}\text{H}_{18}\text{NO}_3$: 260.1287 $[\text{M} + \text{H}^+]$. Found: 260.1285.

17.2 2((butylamino) (phenyl)methyl)-3-hydroxynaphthalene-1,4-dione (2b)

Orange solid. Yield = 73%. Mp 185.0-186.0°C. ^1H NMR (400 MHz, DMSO d_6): δ 7.90 (1H, d, J = 6.8 Hz, ArH), 7.81 (1H, d, J = 7.3 Hz, ArH), 7.70 (1H, t, J = 7.2 Hz, ArH), 7.61-7.56 (3H, m, ArH and PhH), 7.37-7.29 (3H, m, PhH), 5.50 (1H, s, Napht- CHN), 2.87 (2H, t, J = 7.7 Hz, NH- CH_2), 1.65-1.55 (2H, m, CH_2), 1.32-1.24 (2H, m, CH_2CH_3), 0.83 (3H, t, J = 7.4 Hz, CH_3). ^{13}C NMR (101 MHz, DMSO- d_6) δ 184.31, 178.49, 170.63, 138.66, 134.62, 133.75, 131.51, 130.88, 128.34, 127.91, 127.75, 125.35, 125.08, 111.10, 58.85, 45.42, 27.63, 19.27, 13.46. ESIMS, calcd for $\text{C}_{21}\text{H}_{22}\text{NO}_3$: 336.1600 $[\text{M} + \text{H}^+]$. Found: 336.1602.

17.3 2-((butylamino)(2-hydroxyphenyl)methyl)-3 hydroxynaphthalene-1, 4-dione (2c)

Orange solid. Yield = 30%. Mp 176.5-177.0°C. ^1H NMR (400 MHz, DMSO d_6): δ 7.91 (1H, d, J = 6.4 Hz, ArH), 7.86 (1H, d, J = 6.7 Hz, ArH), 7.72 (1H, t, J = 7.4 Hz, ArH), 7.62 (1H, t, J = 7.5 Hz, ArH), 7.31 (1H, d, J = 7.7 Hz, PhH), 7.15 (1H, t, J = 8.1 Hz, PhH), 6.87 (1H, d, J = 8.1 Hz, PhH), 6.74 (1H, t, J = 7.1 Hz, PhH), 5.73

(1H, s, Napht- CHN), 2.90 (2H, t, J = 7.4 Hz, NH- CH_2), 1.65-1.54 (2H, m, CH_2), 1.36-1.25 (2H, m, CH_2CH_3), 0.84 (3H, t, J = 7.4 Hz, CH_3). ^{13}C NMR (101 MHz, DMSO- d_6) δ 183.96, 179.43, 171.46, 155.33, 134.43, 133.78, 131.52, 131.05, 129.44, 128.56, 125.44, 125.09, 123.47, 119.02, 115.82, 109.81, 54.01, 45.61, 27.73, 19.24, 13.44. ESIMS, calcd for $\text{C}_{21}\text{H}_{22}\text{NO}_4$: 352.1549 $[\text{M} + \text{H}^+]$. Found: 352.1545.

17.42-((allylamino)methyl)-3-hydroxynaphthalene-1,4-dione (2d)

Orange solid. Yield = 42%. Mp 159.0-160.8°C. ^1H NMR (400 MHz, DMSO d_6): δ 7.93 (1H, d, J = 6.9 Hz, ArH), 7.80 (1H, d, J = 7.6 Hz, ArH), 7.69 (1H, t, J = 7.4 Hz, ArH), 7.56 (1H, t, J = 7.5 Hz, ArH), 5.98-5.88 (1H, m, CHvinyl), 5.51-5.37 (2H, m, CH_2vinyl), 3.92 (2H, s, Napht- CH_2N), 3.53 (2H, d, J = 6.7 Hz, CH_2NH). ^{13}C NMR (101 MHz, DMSO- d_6) δ 184.73, 178.71, 171.70, 135.08, 133.70, 131.78, 130.69, 129.58, 125.38, 125.13, 122.26, 107.69, 48.29, 40.91. ESIMS, calcd for $\text{C}_{14}\text{H}_{14}\text{NO}_3$: 244.0974 $[\text{M} + \text{H}^+]$. Found: 244.0971.

17.5 2-(1-(allylamino)ethyl)-3-hydroxynaphthalene-1,4-dione (2e)

Orange solid. Yield = 5%. Mp 162.0-163.0°C. ^1H NMR (400 MHz, DMSO d_6): δ 7.91 (1H, d, J = 7.6 Hz, ArH), 7.82 (1H, d, J = 7.6 Hz, ArH), 7.71 (1H, t, J = 7.2 Hz, ArH), 7.58 (1H, t, J = 7.4 Hz, ArH), 5.90-5.80 (1H, m, CHvinyl), 5.39-5.32 (2H, m, CH_2vinyl), 4.50 (1H, q, J = 6.8 Hz, Napht- CHN), 3.50-3.42 (2H, m, CH_2NH), 1.42 (3H, d, J = 6.8 Hz, CH_3). ^{13}C NMR (101 MHz, DMSO- d_6) δ 184.34, 178.28, 170.68, 134.87, 133.61, 131.56, 130.65, 129.55, 125.26, 125.00, 121.80, 111.16, 50.54, 46.82, 17.37. ESIMS, calcd for $\text{C}_{15}\text{H}_{16}\text{NO}_3$: 258.1130 $[\text{M} + \text{H}^+]$. Found: 258.1130.

17.6 2-((allylamino)(phenyl)methyl)-3-hydroxynaphthalene-1,4-dione (2f)

Orange solid. Yield = 80%. Mp 191.0-

192.4°C. ^1H NMR (400 MHz, DMSO d_6): δ 7.90 (1H, d, $J = 7.7$ Hz, ArH), 7.81 (1H, d, $J = 7.6$ Hz, ArH), 7.68 (1H, t, $J = 7.6$ Hz, ArH), 7.58-7.56 (3H, m, ArH and PhH), 7.37-7.26 (3H, m, PhH), 5.95-5.84 (1H, m, CHvinyl), 5.50 (1H, s, Napht-CHN), 5.38-5.30 (2H, m, CH_2 vinyl), 3.52 (2H, d, $J = 5.6$ Hz, CH_2NH). ^{13}C NMR (101 MHz, DMSO- d_6) δ 184.26, 178.36, 170.69, 138.52, 134.69, 133.74, 131.56, 130.86, 129.28, 128.40, 127.95, 127.79, 125.35, 125.09, 122.51, 110.92, 57.71, 47.53. ESIMS, calcd for $\text{C}_{20}\text{H}_{18}\text{NO}_3$: 320.1287 $[\text{M}+\text{H}^+]$. Found: 320.1295.

17.7 2-((allylamino)(2-hydroxyphenyl)methyl)-3-hydroxynaphthalene-1,4-dione (2g)

Orange solid. Yield = 14%. Mp 183.0-184.5°C. ^1H NMR (400 MHz, DMSO d_6): δ 7.90 (1H, d, $J = 7.6$ Hz, ArH), 7.86 (1H, d, $J = 7.6$ Hz, ArH), 7.71 (1H, t, $J = 7.2$ Hz, ArH), 7.61 (1H, t, $J = 7.2$ Hz, ArH), 7.31 (1H, d, $J = 7.7$ Hz, *o*-OHPhH), 7.13 (1H, t, $J = 7.1$ Hz, *o*-OHPhH), 6.85 (1H, d, $J = 8.0$ Hz, *o*-OHPhH), 6.74 (1H, t, $J = 7.5$ Hz, *o*-OHPhH), 5.96-5.86 (1H, m, CHvinyl), 5.74 (1H, s, Napht-CHN), 5.38-5.31 (2H, m, CH_2 vinyl), 3.53 (2H, d, $J = 7.0$ Hz, CH_2NH). ^{13}C NMR (101 MHz, DMSO- d_6) δ 183.93, 179.48, 171.46, 155.23, 133.82, 131.70, 131.54, 131.12, 129.51, 129.27, 128.61, 127.55, 125.60, 125.48, 122.33, 119.09, 115.97, 109.66, 53.04, 47.78. ESIMS, calcd for $\text{C}_{20}\text{H}_{18}\text{NO}_4$: 336.1236 $[\text{M}+\text{H}^+]$. Found: 336.1236.

17.8 (2E,4E)-1,4-dioxo-1,4-dihydronaphthalen-2-yl-5(benzo[*d*][1,3]dioxol-5-yl)penta-2,4-dienoate (6)

Yellow solid. Yield = 57%. Mp 208-209°C. ^1H NMR (400 MHz, DMSO d_6): δ 8.07-8.02 (2H, m, Napht-ArH), 7.95-7.90 (2H, m, Napht-ArH), 7.63 (1H, dd, $J = 15.2, 9.8$ Hz, aH), 7.29

(1H, s, ArH), 7.17 (2H, dd, $J = 16.8, 12.6$ Hz, g and dH), 7.11 (1H, s, Napht-ArH), 7.07 (1H, d, $J = 8.0$ Hz, ArH), 6.97 (1H, d, $J = 8.0$ Hz, ArH), 6.25 (1H, d, $J = 15.1$ Hz, bH), 6.08 (2H, s, CH_2). ^{13}C NMR (100 MHz, DMSO- d_6) δ 184.46, 178.52, 163.45, 154.15, 149.18, 148.73, 148.10, 142.98, 134.88, 134.47, 131.57, 130.62, 130.18, 126.43, 126.20, 126.07, 124.40, 123.98, 116.71, 108.65, 105.92, 101.55. ESIMS, calcd for $\text{C}_{22}\text{H}_{14}\text{O}_6\text{Na}$: 397.0688 $[\text{M}+\text{Na}^+]$. Found: 397.0705.

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