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Der Pharma Chemica, 2010, 2(4): 309-315
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X

Synthesis and biological evaluation of alkyl/arylamino derivatives of naphthalene-1,4-dione as antimycobacterial agents

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ABSTRACT

*1,4-naphthoquinone structure is common in various natural products and possess a wide spectrum of biological activities. In the present study, a series of 1,4-naphthoquinone compounds were synthesized by reacting substituted naphthoquinones with alkyl or aryl amines in presence of a base, and screened against Mycobacterium tuberculosis (M. tb) $H_{37}Rv$. Most of the compounds exhibited significant in vitro antitubercular activities and may serve as a lead for further development as a novel class of antituberculosis agents. Compound **9** has IC_{90} value of 2.40µg/mL. The objective of our study is to generate new leads, as there is an urgent demand for new and more effective anti-TB drugs possessing new modes of action.*

Keywords: Tuberculosis, *Mycobacterium tuberculosis*, Antimycobacterial activity, Naphthalene-1,4-diones

INTRODUCTION

The re-emergence of tuberculosis (TB) as a global health problem over the past few decades, accompanied by the rise of multidrug-resistant strains of Mycobacterium tuberculosis, emphasizes the need for the discovery of new therapeutic drugs against this disease[1,2]. The spread of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) strains make the treatment of this disease extremely difficult and threatening to public health worldwide[3,4]. Currently, there are limited effective and safe antituberculous medications available, many of which are complicated by numerous side effects, drug-interactions and the need for long duration of therapy. The goals of tuberculosis control are to cure active disease,

prevent relapse, reduce transmission and avert the emergence of drug-resistance. Therefore, the ability of new chemotherapeutic agents should be to meet these aims more efficiently.

Plant containing 1,4-naphthoquinone derivatives showed promising antitubercular activity with novel mode of action and their synthetic/semisynthetic derivatives devote toxicity. Naphthoquinones and other compounds isolated from *Euclea natalensis* and *E. undulata* have been reported with antimycobacterial activity against *Mycobacterium* tuberculosis (*M. tb*)[5]. The studies on the intracellular activity of naphthoquinones and triterpenes isolated from *E. natalensis* roots, has established that 7-methyljuglone (Figure 1), a naphthoquinone has superior intracellular and extracellular inhibition of *M. tb* relative to the anti-TB drugs streptomycin and ethambutol[6]. 7-methyljuglone and diospyrin (dimer of 7-methyljuglone) (Fig. 1), exhibited MICs of 0.5 and 8.0 µg/ml respectively against drug-sensitive *M. tb*[2]. 7-methyljuglone was the most active compound, with an MIC as low as 1.55 µg/ml against pathogenic *M. bovis*[7]. Shinanolone (Fig. 1), isolated from an ethanol extract of *E. natalensis*, exhibited antimycobacterial activity against *M. tb* (MIC=100 µg/ml), and antibacterial activity against a variety of test organisms[8].

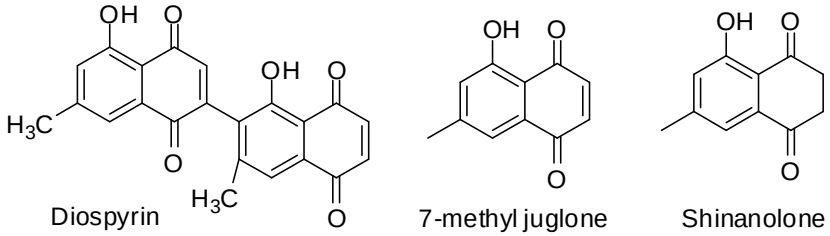
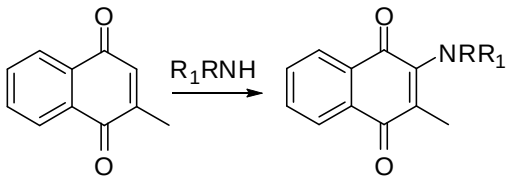


Figure 1. Naturally occurring naphthoquinones

The aminonaphthoquinone moiety is present in several natural products such as rifamycins[9], kinamycins[10], rifampicins[11] etc., and has been used as a synthetic intermediate for the synthesis of several biologically important compounds[12-14]. The alkylamino derivatives of naphthoquinones and related compounds exhibit potent antitumor and antimalarial activities[15,16]. We have recently reported the synthesis of a series of 1,4-naphthoquinone derivatives[17] and their significant *in vitro* antitubercular activities against *M. tb* $H_{37}Rv$. This paper reports the synthesis and antimycobacterial activities of alkyl/arylamino derivatives of 2-methyl-1,4-naphthoquinone. The general method for the preparation of alkylamino naphthoquinones involved a direct addition of alkyl or aryl amine to the quinone ring of 2-methyl naphthoquinone [18]. The ^{1}H NMR and infrared spectra of compounds **1-13** are consistent with their composition and structure. The ^{1}H NMR spectra exhibit signals in the δ 7.5-8.20 ppm region as multiplets are attributed to the four naphthoquinone aromatic hydrogens H5-H8. The results of *in vitro* antimycobacterial activities are given in Table 1.

Scheme 1. Synthesis of naphthoquinone derivatives



RESULTS AND DISCUSSION

Antimycobacterial activity

In vitro evaluation of the antitubercular activity against *M. tuberculosis* strain H37Rv was carried out within the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF) screening program for the discovery of novel drugs for the treatment of tuberculosis according to procedures previously published by the TAACF organization[19]. According to TAACF the IC_{90} of the compounds is determined as a primary screen. Any compound having an $IC_{90} \leq 10$ mg/mL is considered active for antitubercular activity.

The compounds **9, 5, 2** & **3** have IC_{90} values ranging from 2.40-10.88 µg/mL and IC_{50} values ranging from 1.69-8.80 µg/mL, exhibiting the greatest activity amongst all the screened derivatives (Table 1). Compound **9** was the most potent with SI of 3.94. Earlier studies into the antibacterial activity of 1,4-naphthoquinone derivatives have demonstrated that a nitrogen substituted aromatic heterocyclic substituent at C-2 position enhanced the antibacterial activity[17]. Compounds with methyl substitution at position 2 of the naphthoquinone ring were less active than compounds without methyl substitution as reported in earlier studies[17]. This new series of naphthoquinone analogues have significant and promising activity against drug-sensitive *M. tb* cultures and further modifications can lead to better antimycobacterial compounds. The compound **9** can be a lead for antimycobacterial activity and further work on these compounds is in progress which will be reported in due course.

Table 1. *In vitro* antimycobacterial activities and in silico parameters of naphthoquinone derivatives against drug-sensitive strain of *M. tb* H37Rv

S. No.	R =, R_1 =	MABA- H37Rv (IC_{90}, IC_{50} µg/ml)	CTG Vero cell (CC_{50} µg/ml)	SI	ClogP
1	H, -$C(CH_3)_3$	44.18, 37.97			3.77
2	-$C(CH_2)_5$	10.83, 8.49			3.97
3	-$(CH=CH)_2$	10.88, 8.80			3.13
4	CH_3, CH_2Ph	36.98, 21.06			4.11
5	H, Ph-2-CF_3	10.68, 8.46			4.57
6	H, CH_2COOH	12.32, 11.32			1.91
7	H, CH_2Ph	90.56, 83.81			4.32
8	H, CH_3	13.19, 11.53			2.54
9	H, Ph-4-CH_3	2.40, 1.69	9.466	3.944	4.99
10	H, $CH_2CH_2NH_2$	96.09, 66.14			1.79
11	H, Ph-2,6-CH_3	11.63, 10.91			5.49
12	H, Ph	11.74, 10.52			4.49
13	H, Ph-2-Cl	12.82, 11.66			5.51

MATERIALS AND METHODS

General Procedures. Melting points were recorded on a Büchi capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Nicolet Impact-410 FTIR spectrometer. ^{1}H spectra were recorded on a 300 MHz Bruker FT-NMR spectrometer in $CDCl_3$ & MeOD solution. The chemical shifts are reported in δ (ppm) relative to internal standard tetramethylsilane (TMS) and coupling constants *J* are given in Hz. Mass spectrometry was conducted using MALDI TOF-TOF mass spectrometer (Bruker, Ultra flex). Elemental analyses were recorded on an Elementar Vario EL analyzer. All chromatographic purifications were performed with silica gel (60-120 mesh), whereas all TLC development was done on silica gel coated (Merck Kiesel 60 F254, 0.2 mm thickness) plates. All chemicals were purchased from Aldrich Chemical Company (USA) and were used as received unless otherwise noted. Solvents used for the chemical synthesis were of laboratory and analytical grade, and were used without further purification unless otherwise stated.

General method for the synthesis of derivatives 1-13 (Scheme 1)
A solution containing 0.500 g of 2-methyl-1,4-naphthoquinone and equivalent quantity of the corresponding amine, 0.100 g of anhydrous potassium carbonate and 25 ml of absolute ethanol were heated under reflux for 4-6 hours. The resulting solution was filtered and evaporated to give a residue, which was chromatographed on a silica gel column and eluted with hexane: ethyl acetate to give the corresponding 2, 3-substituted naphthoquinone derivatives **1-13**.

2-(tert-butylamino)-3-methylnaphthalene-1,4-dione (**1**): Obtained from 2-methyl-1,4-naphthoquinone (0.500 g, 3 mmol) and t-butylamine (0.212 g, 3 mmol) as an oil, yield 0.298 g, 42 %. ^{1}H NMR ($CDCl_3$): δ 7.86-7.88 (m, 2H), 7.54 (m, 2H), 2.53 (s, 3H), 1.24-1.30 (m, 9H); Maldi TOF TOF: m/z 244.320 [$(M+H)^+$, 100%]; Anal. Calc for C15H17NO2 (243.30): C, 74.05; H, 7.04; N, 5.76; found: C, 74.37; H, 7.20; N, 5.96.

2-methyl-3-(piperidin-1-yl) naphthalene-1,4-dione (**2**): Obtained from 2-methyl-1,4-naphthoquinone (0.500 g, 3 mmol) and piperidine (0.247 g, 3 mmol) as viscous oil, yield 0.334 g, 45 %, [Lit. Bp. 402±45°C]. ^{1}H NMR ($CDCl_3$): δ 7.82-7.88 (m, 2H), 7.60 (d, 2H, J = 7.11 Hz), 3.01 (d, 4H, J = 6.20 Hz), 2.60 (s, 3H), 1.50-1.57 (m, 6H); Maldi TOF TOF: m/z 256.322 [$(M+H)^+$, 100%]; Anal. Calc for C16H17NO2 (255.31): C, 75.27; H, 6.71; N, 5.49; found: C, 75.87; H, 7.20; N, 5.84.

2-methyl-3-(1H-pyrrol-1-yl) naphthalene-1,4-dione (**3**): Obtained from 2-methyl-1,4-naphthoquinone (0.500 g, 3 mmol) and pyrrole (0.195 g, 3 mmol) as a dark oil, yield 0.283 g, 41 %. ^{1}H NMR ($CDCl_3$): δ 7.86-7.89 (m, 2H), 7.57 (d, 2H, J = 7.23 Hz), 7.32 (d, 2H, J = 6.3 Hz), 6.15-6.22 (m, 2H), 2.55 (s, 3H); Maldi TOF TOF: m/z 238.350 [$(M+H)^+$, 100%]; Anal. Calc for C15H11NO2 (237.35): C, 75.94; H, 4.67; N, 5.90; found: C, 76.23; H, 5.20; N, 6.00.

2-benzyl-(methyl) amino-3-methylnaphthalene-1,4-dione (**4**): Obtained from 2-methyl-1,4-naphthoquinone (0.500 g, 3 mmol) and N-benzyl methylamine (0.353 g, 3 mmol) as a dark coloured oil, yield 0.330 g, 39 %. ^{1}H NMR ($CDCl_3$): δ 7.90 (m, 2H), 7.62 (m, 2H), 7.27.38 (m, 5H), 4.36 (s, 2H), 3.14 (s, 3H), 2.46 (s, 3H); Maldi TOF TOF: m/z 292.349 [$(M+H)^+$, 100%];

Anal. Calc for C19H17NO2 (291.34): C, 78.33; H, 5.88; N, 4.81; found: C, 78.78; H, 6.20; N, 4.94.

2-methyl-3-(2-trifluoromethyl)-phenylamino- naphthalene-1,4-dione (**5**): Obtained from 2-methyl-1,4-naphthoquinone (0.500 g, 3 mmol) and 2-trifluoromethyl aniline (0.468 g, 3 mmol) as a viscous oil, yield 0.480 g, 50 %. ^{1}H NMR ($CDCl_3$): δ 7.88 (m, 2H), 7.62 (m, 2H), 7.54 (s, 1H), 7.10 (2H), 6.56 (s, 1H), 4.5 (s, 1H), 2.46 (s, 3H); Maldi TOF TOF: m/z 332.304 [$(M+H)^+$, 100%]; Anal. Calc for C18H12F3NO2 (331.29): C, 65.26; H, 3.65; N, 4.23; found: C, 65.37; H, 3.80; N, 4.64.

2-(3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)acetic acid (**6**): Obtained from 2-methyl-1,4-naphthoquinone (0.500 g, 3 mmol) and glycine (0.218 g, 3 mmol) as a brown coloured oil, yield 0.370 g, 52 %, [Lit. Bp.473.8±45°C]. ^{1}H NMR ($CDCl_3$): δ 7.90 (m, 2H), 7.62 (m, 2H), 4.20 (s, 2H), 2.48 (s, 3H); Maldi TOF TOF: m/z 246.244 [$(M+H)^+$, 100%]; Anal. Calc for C13H11NO4 (245.23): C, 63.67; H, 4.52; N, 5.71; found: C, 64.17; H, 4.65; N, 5.74.

2-(benzylamino)-3-methylnaphthalene-1,4-dione (**7**): Obtained from 2-methyl-1,4-naphthoquinone (0.500 g, 3 mmol) and benzyl amine (0.310 g, 3 mmol) as a brown liquid, yield 0.362 g, 45 %. ^{1}H NMR ($CDCl_3$): δ 7.88 (m, 2H), 7.76 (m, 2H), 7.40 (m, 2H), 7.36 (m, 3H), 3.96 (s, 2H), 2.34 (s, 3H); Maldi TOF TOF: m/z 278.503 [$(M+H)^+$, 100%]; Anal. Calc for C18H15NO2 (277.32): C, 77.96; H, 5.45; N, 5.05; found: C, 78.37; H, 5.68; N, 5.34.

2-methyl-3-(methylamino)naphthalene-1,4-dione (**8**): Obtained from 2-methyl-1,4-naphthoquinone (0.500 g, 3 mmol) and methyl amine (0.270 g, 3 mmol) as a yellow solid, yield 0.263 g, 45 %, mp. 127°C [Lit. Mp. 127-129°C]. ^{1}H NMR ($CDCl_3$): δ 7.92 (m 2H), 7.58 (m, 2H), 3.00 (s, 3H), 2.46 (s, 3H); Maldi TOF TOF: m/z 202.229 [$(M+H)^+$, 100%]; Anal. Calc for C12H11NO2 (201.22): C, 71.63; H, 5.51; N, 6.96; found: C, 72.37; H, 5.86; N, 7.14.

2-methyl-3-(p-tolylamino)naphthalene-1,4-dione (**9**): Obtained from 2-methyl-1,4-naphthoquinone (0.500 g, 3 mmol) and 4-methyl aniline (0.312 g, 3 mmol) as a brown viscous oil, yield 0.338 g, 42 %. ^{1}H NMR ($CDCl_3$): δ 7.92 (m, 2H), 7.58 (m, 2H), 7.12 (m, 2H), 6.34 (m, 2H), 2.46 (s, 3H), 2.40 (s, 3H); Maldi TOF TOF: m/z 278.322 [$(M+H)^+$, 100%]; Anal. Calc for C18H15NO2 (277.32): C, 77.96; H, 5.45; N, 5.05; found: C, 78.37; H, 6.20; N, 5.34.

2-(2-aminoethylamino)-3-methylnaphthalene-1,4-dione (**10**): Obtained from 2-methyl-1,4-naphthoquinone (0.500 g, 3 mmol) and ethylene diamine (0.176 g, 3 mmol) as a oil, yield 0.288 g, 43 %. ^{1}H NMR ($CDCl_3$): δ 7.88 (m, 2H), 7.62 (m, 2H), 3.22 (t, 2H, J = 4.56 Hz), 2.80 (t, 2H), 2.48 (s, 3H), 2.30 (s, 2H); Maldi TOF TOF: m/z 231.270 [$(M+H)^+$, 100%]; Anal. Calc for C13H14N2O2 (230.26): C, 67.81; H, 6.13; N, 12.17; found: C, 68.37; H, 6.20; N, 12.34.

2-(2,6-dimethylphenylamino)-3-methylnaphthalene-1,4-dione (**11**): Obtained from 2-methyl-1,4-naphthoquinone (0.500 g, 3 mmol) and 2,6-dimethyl aniline (0.352 g, 3 mmol) as a viscous liquid, yield 0.355 g, 42 %. ^{1}H NMR ($CDCl_3$): δ 7.94 (m, 2H), 7.64 (m, 2H), 6.82 (m, 1H), 7.14 (m, 2H), 2.46 (s, 3H), 2.26 (m, 6H); Maldi TOF TOF: m/z 292.355 $(M+H)^+$, 100%]; Anal. Calc for C19H17NO2 (291.34): C, 78.33; H, 5.88; N, 4.81; found: C, 78.89; H, 6.20; N, 4.34.

2-methyl-3-phenylamino-1,4-naphthoquinone (**12**): Obtained from 2-methyl-1,4-naphthoquinone (0.500 g, 3 mmol) and aniline (0.270 g, 3 mmol) as a brown solid, yield 0.336 g, 44 %, mp. 164-5°C [Lit. 165°C]. ^{1}H NMR ($CDCl_3$): δ 8.20 (m, 2H), 8.14-8.10 (m, 2H), 7.82 (m, 2H), 7.78 (m, 1H), 7.68 (m, 2H), 2.76 (s, 3H); Maldi TOF TOF: m/z 265.515 [$(M+2H)^+$, 100%]; Anal. Calc for C17H13NO2 (263.29): C, 77.55; H, 4.98; N, 5.32; found: C, 78.07; H, 4.95; N, 5.10.

2-(2-chloro-phenylamino)-3-methyl-1,4-naphthoquinone (**13**): Obtained from 2-methyl-1,4-naphthoquinone (0.500 g, 3 mmol) and 2-chloroaniline (0.370 g, 3 mmol) as a viscous oil, yield 0.345 g, 40 %. ^{1}H NMR (MeOD): δ 7.59 (m, 1H), 7.50 (m, 1H), 7.36 (m, 1H), 7.26 (m, 1H), 7.12 (d, 1H, J = 7.80 Hz), 6.98 (t, 1H, J = 7.62 Hz), 6.78 (d, 1H, J = 8.00 Hz), 6.58 (t, 1H, J = 7.62 Hz), 2.76 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 181.3, 180.2, 150.8, 145.2, 135.8 (2C), 132.4 (2C), 131.2 (2C), 130.0 (2C), 126.2, 125.4, 121.6, 104.8, 12.2; Maldi TOF TOF: m/z 298.750 [$(M+H)^+$, 100%]; Anal. Calc for C17H12ClNO2 (297.74): C, 68.58; H, 4.06; N, 4.70; found: C, 68.87; H, 4.15; N, 4.90.

Acknowledgements
Authors are thankful to the Tuberculosis Antimicrobial Acquisition and Coordination Facility (TAACF), which provided antimycobacterial data through a research and development contract with the U.S. National Institute of Allergy and Infectious Diseases.

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