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Substituted 1,4-naphthoquinones as a new class of antimycobacterial agents

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Abstract

Naphthalene-1,4-dione compounds 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. The synthesized compounds were screened against Mycobacterium tuberculosis (M. tb) $H_{37}Rv$, where most of the compounds exhibited significant in vitro antitubercular activities and may serve as a lead for further optimization. Compounds ***1, 18, 20*** *&* ***25*** *have IC90 values ranging from 3.14-3.43 µg/mL. The objective of our study is to generate new leads that operate through a different mode of action and to optimize their structure to display potent efficacy.*

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

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INTRODUCTION

Tuberculosis (TB) caused by *M. tb*, is a chronic infectious disease, infecting approximately one-third of the world's population as estimated by the World Health Organization (WHO) [1]. The WHO estimates that 500,000 new cases of multidrug resistant TB (MDRTB) occur globally every year [2]. The synergy of this disease with HIV infection and the emergence and spread of MDRTB and extensively drug resistant TB (XDRTB) pose an additional threat and continues to claim millions of lives [3,4].

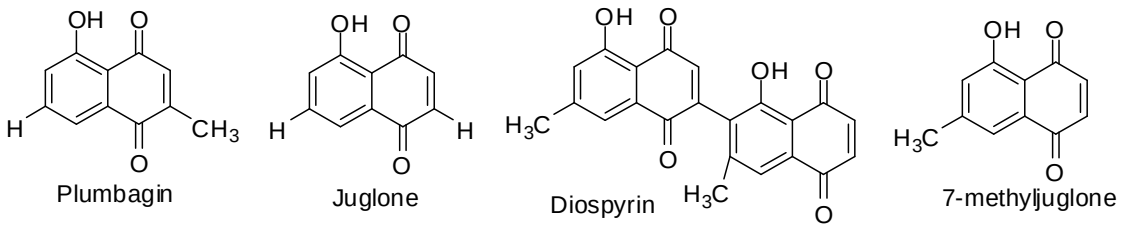
Traditionally, the chemotherapy of TB has relied heavily on a limited number of drugs. The frontline chemotherapeutic agents are isonicotinic acid hydrazide, rifampicin, ethambutol, streptomycin, ethionamide, pyrazinamide, fluoroquinolones etc [5]. The directly observed short

course chemotherapy (DOTS) is available to treat the disease, but this treatment is old, slow and inefficient by the current standards. MDRTB is resistant to at least rifampicin (RIF) and isoniazid (INH), and requires 18–24 months of treatment with expensive second line drugs some of which are injectable agents. Therefore, it is crucial that MDRTB should be detected as soon as possible, and measures implemented to effectively control its further spread. Therefore, there is an urgent need to develop new drugs, to shorten the duration of the treatment and acting through a novel mechanism of action for the chemotherapy of tuberculosis [6].

Although a number of lead molecules exist today to develop new drugs, no new chemical entity has emerged for clinical use for over the last 45 years in the treatment of this disease [7,8]. Only within the last few years several promising drug candidates have been identified and are undergoing clinical evaluation.

Naturally occurring compounds containing a quinone group are useful as synthetic intermediates and biologically active compounds. Naphthalene-1,4-diones derivatives have been known to possess a wide spectrum of biological activities such as antibacterial, antifungal, antiinflammatory, anticancer, antidiabetic and antimalarial activities [9,10]. Plumbagin and juglone (Fig. 1) have strong sterilizing activity against mycobacterium, potentially with a unique mechanism of action. Naphthoquinones and other compounds with antimycobacterial activity against *M. tb* have previously been isolated from *Euclea natalensis* species. This has led to the identification of diospyrin (Fig. 1) as active constituent against drug-resistant strains of *M. tb* [11]. Diospyrin is a dimer of 7-methyljuglone (Fig. 1). 7-methyljuglone has superior intracellular and extracellular inhibition of *M. tb* relative to the anti-TB drugs streptomycin and ethambutol [12] and synergistically enhance the antitubercular activity of isoniazid and rifampicin both extracellularly and intracellularly [13]. Diospyrin and 7-methyljuglone exhibited MICs of 8.0 and 0.5 µg/mL respectively against drug-sensitive *M. tb* [12].

Figure 1. Naturally occurring naphthoquinones



Towards the development of new class of compounds which are structurally different from known antituberculosis drugs, we have earlier reported several substituted naphthoquinones and the aminoquinoline derivatives as novel antitubercular agents with significant antitubercular activities [14,15].

Many amino and heterocyclic naphthalene-1,4-diones have also been used for the synthesis of numerous biologically important compounds [16,17]. The interesting biological profile resulting from the presence of heteroatom, nitrogen, oxygen or sulfur in the naphthalene-1,4-diones prompted us to synthesize derivatives **1**-**30** according to Scheme 1, possessing nitrogen atom at 2 position of naphthalene-1,4-dione and evaluating them for their *in vitro* antimycobacterial

activities. These derivatives were synthesized following standard procedures starting from 2 and 3-substituted naphthalene-1,4-diones [9,10]. The structures of all the compounds were established on the basis of spectroscopic analysis and analytical data. It is expected that these naphthalene-1,4-diones may be effective against drug-resistant strains of *M. tb* and make them excellent leads for synthesizing derivatives for antimycobacterial activity.

RESULTS AND DISCUSSION

Antimycobacterial activity

The *in vitro* primary screen was conducted against *M. tb* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA) [18]. The minimum inhibitory concentrations (MIC) of compounds 1-30 against *M. tb* $H_{37}Rv$ were determined in liquid media and are reported in Table 1. Compounds were tested in ten 2-fold dilutions, typically from 100 µg/mL to 0.19 µg/mL. The IC_{90} and IC_{50} are defined as the concentration effecting a reduction in fluorescence of 90% and 50% relative to controls. The IC_{90} value of ≤10 µg/mL is considered "active" for antitubercular activity. The secondary screening for determination of VERO cell cytotoxicity was done in parallel with the TB Dose Response assay. After 72 hours exposure, viability was assessed using Promega's Cell Titer Glo Luminescent Cell Viability Assay, a homogeneous method of determining the number of viable cells in culture based on quantitation of the ATP present. Ultimately, the CC_{50} is divided by the IC_{90} to calculate an SI (Selectivity Index) value. SI values of ≥ 10 were considered for further testing.

The compounds **1, 18, 20** & **25** have IC_{90} values ranging from 3.14-3.43 µg/mL and IC_{50} values ranging from 2.78-3.13 µg/mL, exhibiting the greatest activity amongst all the screened derivatives. Of these compounds, **20** was the most potent one with SI of 11.31 followed by **18**, **25** and **1** with SI values of 2.02, 3.73 and 1.22 respectively. Derivatives **4, 15, 22** and **27** also have IC_{90} values ranging from 6.44-9.34 µg/mL. Earlier studies into the antibacterial activity of 1,4-naphthoquinone derivatives have demonstrated that a nitrogen substituted aromatic heterocycle substituent at C-2 position enhanced the antibacterial activity [14,15]. The naphthoquinone derivatives synthesized during this study has established the discovery of a new series of analogues with significant and promising activity against drug-sensitive *M. tb* cultures. These effective derivatives are ideally suited for further modifications to obtain more efficacious antimycobacterial compounds. For the therapeutic development of more potent and non toxic antitubercular agents, further investigations on the structural modifications are currently underway and results will be reported in due course.

Experimental Section

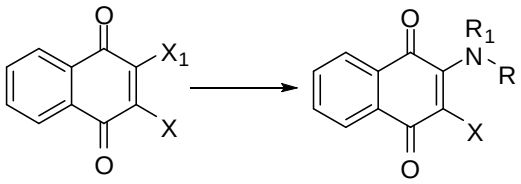
General Procedures. Melting points were recorded on a Büchi capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Nicolet Impact-410 FTIR spectrometer. ^1H spectra were recorded on a 300 MHz Bruker FT-NMR spectrometer in $CDCl_3$ & MeOD solution. The chemical 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.

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

Compound	MABA- $H_{37}Rv$ (IC_{90} µg/mL)	MABA- $H_{37}Rv$ (IC_{50} µg/mL)	CTG Vero cell (CC_{50} µg/mL)	SI	ClogP
1	3.437	3.132	4.189	1.218	3.98
2	23.593	21.68			4.84
3	35.752	34.93			5.19
4	6.861	5.979	7.076	1.031	4.42
5	49.76	44.132			3.80
6	14.153	12.29			2.79
7	47.53	38.703			3.60
8	30.714	22.025			5.75
9	64.965	41.989			6.16
10	25.736	22.766			6.16
11	39.177	23.673			5.39
12	>100	96.21			4.97
13	13.785	10.138			4.08
14	51.773	45.652			5.39
15	8.200	6.041	23.640	2.882	4.52
16	39.135	27.984			3.46
17	82.036	65.457			5.02
18	3.140	2.779	6.341	2.019	4.13
19	83.862	62.068			3.97
20	3.371	2.967	38.159	11.31	5.19
21	25.059	23.106			3.97
22	6.440	4.506	7.250	1.125	1.39
23	19.487	14.89			3.12
24	16.031	15.626			3.89
25	3.167	2.802	11.799	3.7256	4.94
26	11.74	10.515			4.49
27	9.342	6.771	27.547	2.9487	4.99
28	74.282	71.282			5.76
29	17.864	13.521			1.27
30	12.82	11.661			5.51

Scheme 1. Synthesis of naphthoquinone derivatives



X = H, CH_3, Cl
X_1 = H, Cl

1. X = H, R = Ph, R_1 = H
2. X = Cl, R = Ph-4-COOH, R_1 = H
3. X = Cl, R = Ph-4-F, R_1 = H
4. X = H, R = Ph-4-F, R_1 = H
5. X = H, R = CH_2Ph, R_1 = H
6. X = Cl, R = CH_3, R_1 = H
7. X = H, R = CH_2Ph, R_1 = CH_3
8. X = Cl, R = Ph-2,6-CH_3, R_1 = H
9. X = Cl, R = Ph-3-CF_3, R_1 = H
10. X = Cl, R = Ph-2-CF_3, R_1 = H
11. X = H, R = Ph-3-CF_3, R_1 = H
12. X = H, R = Ph-2,6-CH_3, R_1 = H
13. X = H, R = Ph-4-OCH_3, R_1 = H
14. X = H, R = Ph-2-CF_3, R_1 = H
15. X = H, R = Ph-2-NO_2, R_1 = H
16. X = H, R = R_1 = -$(CH_2)_5$-
17. X = H, R = Ph-2-Cl-4-OCH_3, R_1 = H
18. X = H, R = -$(CH_2)_4$-CH_3, R_1 = H
19. X = H, R = Ph-4-$COCH_3$, R_1 = H
20. X = H, R = -$(CH_2)_6$-CH_3, R_1 = H
21. X = H, R = Ph-4-COOH, R_1 = H
22. X = H, R = -CH_2COOH, R_1 = H
23. X = H, R = -CH(COOH)-CH_2Ph, R_1 = H
24. X = Cl, R = -CH(COOH)-CH_2Ph, R_1 = H
25. X = CH_3, R = Ph-4-F, R_1 = H
26. X = CH_3, R = Ph, R_1 = H
27. X = H, R = Ph- 2-Cl, R_1 = H
28. X = Cl, R = Ph-2-Cl, R_1 = H
29. X = H, R = $CH_2CH_2NH_2$, R_1 = H
30. X = CH_3, R = Ph-2-Cl, R_1 = H

General method for the synthesis of derivatives 1-30 (Scheme 1)
A solution containing 0.500 g of 2-bromo-1,4-naphthoquinone and equivalent quantity of the corresponding amine, 0.100 g of anhydrous potassium carbonate and 20 ml of absolute ethanol were heated under reflux for 6-8 hours. The resulting solution was filtered and evaporated to give a residue, which was then chromatographed on a silica gel column and eluted with hexane: ethyl acetate to give the corresponding 2-substituted naphthoquinone derivatives **1-30**.

2-phenylamino-1,4-naphthoquinone (**1**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and aniline (0.197 g, 2 mmol) as a dark brown solid, yield 0.205 g, 39 %, mp. 196-8°C [Lit.198-9°C]. ^{1}H NMR ($CDCl_3$): δ 8.22-8.20 (m, 2H), 8.14-8.10 (m, 2H), 7.82-7.80 (m, 2H), 7.78 (m, 1H), 7.73-7.68 (m, 2H), 7.36 (s, 1H); Maldi TOF TOF: m/z 250.265 [$(M+H)^+$, 100%]; Anal. Calc for C16H11NO2 (249.26): C, 77.10; H, 4.45; N, 5.62; found: C, 77.37; H, 4.67; N, 5.70.

4-(3-chloro-1,4-dioxo1,4-dihydronaphthalen-2-ylamino)benzoic acid (**2**): Obtained from 2,3-dichloro-1,4-naphthoquinone (0.500 g, 2 mmol) and p-amino benzoic acid (0.302 g, 2 mmol) as a brown solid, yield 0.303 g, 42 %, mp. 300° (decomp) [Lit. 310°C decomp]. ^{1}H NMR ($CDCl_3$): δ 8.22 (m, 4H), 7.83 (m, 4H); Maldi TOF TOF: m/z 329.638 [$(M+2H)^+$, 100%]; Anal. Calc for C17H10ClNO4 (327.72): C, 62.30; H, 3.08; N, 4.27; found: C, 62.37; H, 3.20; N, 4.34.

2-chloro-3-(4-fluoro-phenylamino)-1,4-naphthoquinone (**3**): Obtained from 2,3-dichloro-1,4-naphthoquinone (0.500 g, 2 mmol) and p-fluoro aniline (0.245 g, 2 mmol) as an oil, yield 0.300

g, 45 % [Lit. bp 400°C]. ^{1}H NMR ($CDCl_3$): δ 8.22 (m, 2H), 7.83 (m, 2H), 7.72 (m, 2H), 7.08 (m, 2H); Maldi TOF TOF: m/z 302.576 [$(M+H)^+$, 100%]; Anal. Calc for C16H9ClFNO2 (301.70): C, 63.70; H, 3.01; N, 6.30; found: C, 64.17; H, 3.00; N, 6.42.

2-(4-fluoro-phenylamino)-1,4-naphthoquinone (**4**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and p-fluoro aniline (0.235 g, 2 mmol) as a viscous oil, yield 0.209 g, 37 % [Lit. bp 419°C]. ^{1}H NMR ($CDCl_3$): δ 8.22-8.14 (m, 2H), 8.13-8.11 (m, 2H), 7.27 (s, 1H), 7.16-7.07 (m, 4H); Maldi TOF TOF: m/z 269.589 [$(M+2H)^+$, 100%]; Anal. Calc for C16H10FNO2 (267.25): C, 71.91; H, 3.77; N, 5.24; found: C, 72.27; H, 3.90; N, 5.42.

2-benzylamino-1,4-naphthoquinone (**5**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and benzylamine (0.226 g, 2 mmol) as a yellow solid, yield 0.250 g, 45 %, mp.156-8°C [Lit. 158-9°C]. ^{1}H NMR ($CDCl_3$): δ 8.06 (m, 2H), 7.73 (dt, 1H, J = 6.30, 1.36 Hz), 7.63 (dt, 1H, J = 6.30, 1.32 Hz), 7.38 (m, 2H), 7.33 (m, 3H), 5.79 (s, 1H), 4.38 (s, 2H); Maldi TOF TOF: m/z 264.324 [$(M+H)^+$, 100%]; Anal. Calc for C17H13NO2 (263.29): C, 77.55; H, 4.98; N, 5.32; found: C, 78.37; H, 5.00; N, 5.50.

2-chloro-3-methylamino-1,4-naphthoquinone (**6**): Obtained from 2,3-dichloro-1,4-naphthoquinone (0.500 g, 2 mmol)and methylamine (0.070 g, 2 mmol) as a yellow solid, yield 0.186 g, 38 %, mp.165-6°C [Lit. 164-7°C]. ^{1}H NMR ($CDCl_3$): δ 8.14 (dd, 1H, J = 7.04, 0.60 Hz), 8.02 (dd, 1H, J = 6.88, 0.74 Hz), 7.72 (dt, 1H, J = 6.42, 1.13 Hz), 7.61 (dt, 1H, J = 6.48, 1.08 Hz), 3.44 (s, 3H); Maldi TOF TOF: m/z 222.764 [$(M+H)^+$, 100%]; Anal. Calc for C11H8ClNO2 (221.64): C, 59.61; H, 3.64; N, 6.32; found: C, 59.77; H, 3.80; N, 6.50.

2-(benzyl-methyl-amino)-1,4-naphthoquinone (**7**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and N-benzylmethylamine (0.256 g, 2 mmol) as a viscous oil, yield 0.200 g, 34 %. ^{1}H NMR ($CDCl_3$): δ 8.12 (dd, 1H, J = 6.64, 0.98 Hz), 8.06 (dd, 2H, J = 6.28, 1.02 Hz), 7.90 (dd, 1H, J = 4.96, 1.60 Hz), 7.76 (m, 2H), 7.65 (m, 2H), 7.58 (m, 1H), 5.73 (s, 1H), 4.39 (s, 2H), 2.95 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 182.7, 178.8, 166.3, 137.8, 136.2 (2C), 132.4, 131.8, 129.1 (2C), 128.2 (2C), 127.2 (2C), 126.4, 110.8, 58.5, 42.0; Maldi TOF TOF: m/z 279.629 [$(M+2H)^+$, 100%]; Anal. Calc for C18H15NO2 (277.32): C, 77.96; H, 5.45; N, 5.05; found: C, 78.07; H, 5.76; N, 5.20.

2-chloro-3-(2,6-dimethyl-phenylamino)-1,4-naphthoquinone (**8**): Obtained from 2,3-dichloro-1,4-naphthoquinone (0.500 g, 2 mmol) and 2,6-dimethylaniline (0.267 g, 2 mmol) as an oil, yield 0.288 g, 42 % [Lit. bp 425°C]. ^{1}H NMR ($CDCl_3$): δ 8.21-8.19 (m, 2H), 7.82-7.79 (m, 2H), 6.96 (d, 2H, J = 7.44 Hz), 6.70 (t, 1H, J = 7.40 Hz), 2.23 (s, 6H); Maldi TOF TOF: m/z 313.693 [$(M+2H)^+$, 100%]; Anal. Calc for C18H14ClNO2 (311.76): C, 69.35; H, 4.53; N, 4.49; found: C, 69.47; H, 4.58; N, 4.60.

2-chloro-3-(3-trifluoromethyl-phenylamino)-1,4-naphthoquinone (**9**): Obtained from 2,3-dichloro-1,4-naphthoquinone (0.500 g, 2 mmol) and 3-trifluoromethyl aniline (0.355 g, 2 mmol) as a viscous oil, yield 0.372 g, 48 % [Lit. bp 406°C]. ^{1}H NMR ($CDCl_3$): δ 8.21 (m, 2H), 8.15 (dd, 1H, J = 6.60, 1.08 Hz), 7.82 (m, 2H), 7.74 (dt, 1H, J = 4.04, 1.34 Hz), 7.48 (m, 1H), 7.25 (m,

1H); Maldi TOF TOF: m/z 353.689 $(M+2H)^+$, 100%]; Anal. Calc for C17H9ClF3NO2 (351.71): C, 58.05; H, 2.58; N, 3.98; found: C, 58.27; H, 2.90; N, 4.00.

2-chloro-3-(2-trifluoromethyl-phenylamino)-1,4-naphthoquinone (**10**): Obtained from 2,3-dichloro-1,4-naphthoquinone (0.500 g, 2 mmol) and 2-trifluoromethyl aniline (0.355 g, 2 mmol) as a dark viscous oil, yield 0.371 g, 48 % [Lit. bp 450°C]. ^{1}H NMR ($CDCl_3$): δ 8.22-8.17 (m, 4H), 7.84-7.79 (m, 4H); Maldi TOF TOF: m/z 353.722 $[(M+2H)^+$, 100%]; Anal. Calc for C17H9ClF3NO2 (351.71): C, 58.05; H, 2.58; N, 3.98; found: C, 58.37; H, 3.00; N, 4.20.

2-(3-trifluoromethyl-phenylamino)-1,4-naphthoquinone (**11**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and 3-trifluoromethyl aniline (0.340 g, 2 mmol) as a viscous oil, yield 0.308 g, 46 % [Lit. bp 416°C]. ^{1}H NMR ($CDCl_3$): δ 8.11 (dt, 2H, J = 6.68, 0.92 Hz), 7.78 (dt, 1H, J = 6.18, 1.36 Hz), 7.69 (dt, 1H, J = 6.22, 1.32 Hz), 7.58-7.46 (m, 2H), 7.24 (s, 1H), 6.98 (dd, 1H, J = 7.64, 1.02 Hz), 6.82 (dd, 1H, J = 6.04, 2.02 Hz), 3.84 (bs, 1H); Maldi TOF TOF: m/z 319.561 $[(M+2H)^+$, 100%]; Anal. Calc for C17H10F3NO2 (317.26): C, 64.36; H, 3.18; N, 4.41; found: C, 64.47; H, 4.01; N, 4.60.

2-(2,6-dimethyl-phenylamino)-1,4-naphthoquinone (**12**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and 2,6-dimethylaniline (0.256 g, 2 mmol) as an oil, yield 0.263 g, 45 % [Lit. bp 432°C]. ^{1}H NMR ($CDCl_3$): δ 8.21-8.19 (m, 2H), 7.82-7.79 (m, 2H), 6.94 (d, 2H, J = 7.44 Hz), 6.82 (s, 1H), 6.64 (t, 1H, J = 7.40 Hz), 2.31 (s, 6H); Maldi TOF TOF: m/z 279.516 $[(M+2H)^+$, 100%]; Anal. Calc for C18H15NO2 (277.32): C, 77.96; H, 5.45; N, 5.05; found: C, 78.47; H, 5.91; N, 5.30.

2-(4-methoxy-phenylamino)-1,4-naphthoquinone (**13**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and 4-methoxy aniline (0.260 g, 2 mmol) as a dark brown solid, yield 0.253 g, 43 %, mp. 156-8°C [Lit. 158°C]. ^{1}H NMR ($CDCl_3$): δ 8.10 (m, 2H), 7.75 (dt, 1H, J = 6.24, 1.33 Hz), 7.66 (dt, 1H, J = 6.26, 1.31 Hz), 7.20 (q, 1H, J = 2.95 Hz), 6.96 (q, 1H, J = 3.37 Hz), 6.74 (m, 1H), 6.65 (m, 1H), 6.23 (s, 1H), 3.84 (s, 3H); Maldi TOF TOF: m/z 281.666 $[(M+2H)^+$, 100%]; Anal. Calc for C17H13NO3 (279.29): C, 73.11; H, 4.69; N, 5.02; found: C, 73.47; H, 4.91; N, 5.30.

2-(2-trifluoromethyl-phenylamino)-1,4-naphthoquinone (**14**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and 2-trifluoromethyl aniline (0.340 g, 2 mmol) as a viscous oil, yield 0.315 g, 47 % [Lit. bp 455°C]. ^{1}H NMR (MeOD): δ 8.17-8.06 (m,1H), 7.92-7.88 (m, 1H), 7.86-7.80 (m, 1H), 7.79-7.73 (m, 1H), 7.68-7.65 (m, 1H), 7.50-7.46 (m, 2H), 7.42 (s, 1H); Maldi TOF TOF: m/z 318.619 $[(M+H)^+$, 100%]; Anal. Calc for C17H10F3NO2 (317.26): C, 64.36; H, 3.18; N, 4.41; found: C, 65.17; H, 4.00; N, 4.30.

2-(2-nitro-phenylamino)-1,4-naphthoquinone (**15**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and 2-nitro aniline (0.290 g, 2 mmol) as an oil, yield 0.235 g, 38 %. ^{1}H NMR (MeOD): δ 8.53-8.48 (m, 1H), 8.11-8.06 (m, 2H), 7.36 (t, 2H, J = 7.14 Hz), 6.90 (d, 2H, J = 8.32 Hz), 6.65 (t, 2H, J = 7.74 Hz); ^{13}C NMR ($CDCl_3$): δ 182.5, 178.2, 158.8, 140.2, 137.8, 136.2 (2C), 132.4, 131.8, 129.1 (2C), 125.2 (2C), 120.4, 114.1, 112.8; Maldi TOF TOF: m/z 295.532 $[(M+H)^+$, 100%]; Anal. Calc for C16H10N2O4 (294.26): C, 65.31; H, 3.43; N, 9.52; found: C, 65.87; H, 4.11; N, 9.60.

2-piperidin-1-yl-1,4-naphthoquinone (**16**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and piperidine (0.180 g, 2 mmol) as a yellowish solid, yield 0.214 g, 42 %, mp. 94-6°C [Lit. 94-6°C]. ^{1}H NMR (MeOD): δ 8.00-7.97 (m, 2H), 7.74-7.60 (m, 2H), 5.99 (s, 1H), 3.58 (m, 4H), 1.77 (m, 6H); Maldi TOF TOF: m/z 241.604 [$(M)^{+}$, 100%]; Anal. Calc for C15H15NO2 (241.29): C, 74.67; H, 6.27; N, 5.81; found: C, 75.17; H, 6.86; N, 6.00.

2-(2-chloro-4-methoxy-phenylamino)-1,4-naphthoquinone (**17**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and 2-chloro-4-methoxy aniline (0.332 g, 2 mmol) as a viscous oil, yield 0.285 g, 43 %. ^{1}H NMR (MeOD): δ 8.17-8.06 (m, 2H), 7.80-7.68 (m, 2H), 7.21 (m, 1H), 7.04 (m, 1H), 6.80 (m, 1H), 6.62 (m, 1H), 3.94 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 182.6, 180.0, 158.6, 153.0, 136.6 (2C), 135.8, 132.8 (2C), 127.6 (2C), 124.8, 116.4, 113.4 (2C), 112.6, 56.0; Maldi TOF TOF: m/z 315.646 [$(M+2H)^{+}$, 100%]; Anal. Calc for C17H12ClNO3 (313.73): C, 65.08; H, 3.86; N, 4.46; found: C, 65.47; H, 4.11; N, 4.30.

2-pentylamino-1,4-naphthoquinone (**18**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and amylamine (0.184 g, 2 mmol) as a viscous oil, yield 0.205 g, 40 % [Lit. bp 390°C]. ^{1}H NMR (MeOD): δ 8.05 (m, 2H), 7.83-7.73 (m, 2H), 5.70 (s, 1H), 2.92 (m, 2H), 1.73-1.67 (m, 4H), 1.40 (m, 2H), 9.95 (m, 3H); Maldi TOF TOF: m/z 244.605 [$(M+H)^{+}$, 100%]; Anal. Calc for C15H17NO2 (243.30): C, 74.05; H, 7.04; N, 5.76; found: C, 74.47; H, 7.15; N, 6.10.

2-(4-acetyl-phenylamino)-1,4-naphthoquinone (**19**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and 4-amino acetophenone (0.285 g, 2 mmol) as an oil, yield 0.234 g, 38 %. ^{1}H NMR (MeOD): δ 8.05-8.03 (m, 2H), 7.94 (m, 2H), 7.80-7.76 (m, 2H), 7.60-7.59 (m, 1H), 7.45-7.43 (m, 1H), 6.67 (s, 1H), 2.48 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 198.0, 182.5, 180.0, 158.6, 145.2, 135.8 (2C), 132.8, 131.2 (2C), 130.0 (2C), 127.6 (2C), 116.4 (2C), 112.0, 26.8; Maldi TOF TOF: m/z 293.570 [$(M+2H)^{+}$, 100%]; Anal. Calc for C18H13NO3 (291.30): C, 74.22; H, 4.50; N, 4.81; found: C, 74.47; H, 4.65; N, 4.90.

2-heptylamino-1,4-naphthoquinone (**20**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and heptylamine (0.243 g, 2 mmol) as a dark viscous oil, yield 0.245 g, 43 %. ^{1}H NMR ($CDCl_3$): δ 8.06 (m, 2H), 7.76 (m, 1H), 7.66 (m, 1H), 7.37 (s, 1H), 3.20 (m 2H), 1.70 (m 2H), 1.36 (m, 8H), 0.90 (m, 3H); ^{13}C NMR ($CDCl_3$): δ 184.0, 182.6, 160.2, 136.6 (2C), 132.2 (2C), 127.2 (2C), 104.6, 45.8, 31.8 (2C), 28.8, 27.6, 22.6, 14.8; Maldi TOF TOF: m/z 273.683 [$(M+2H)^{+}$, 100%]; Anal. Calc for C17H21NO2 (271.35): C, 75.25; H, 7.80; N, 5.16; found: C, 65.47; H, 8.15; N, 5.45.

4-(1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-benzoic acid (**21**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and p-amino benzoic acid (0.290 g, 2 mmol) as a dark brown oil, yield 0.290 g, 47 % [Lit. bp 523°C].. ^{1}H NMR (MeOD): δ 8.14 (m, 1H), 8.08 (m, 1H), 7.82-7.74 (m, 5H), 6.67 (m 2H); Maldi TOF TOF: m/z 295.598 [$(M+2H)^{+}$, 100%]; Anal. Calc for C17H11NO4 (293.27): C, 69.62; H, 3.78; N, 4.78; found: C, 69.97; H, 4.15; N, 4.87.

(1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-acetic acid (**22**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and glycine (0.158 g, 2 mmol) as a brown oil, yield 0.220 g, 45 % [Lit. bp 476°C].. ^{1}H NMR (MeOD): δ 8.10 (m, 2H), 7.77 (m, 2H), 7.70 (m, 1H), 4.48 (s,

2H); Maldi TOF TOF: m/z 233.485 [$(M+2H)^+$, 100%]; Anal. Calc for C12H9NO4 (231.20): C, 62.34; H, 3.92; N, 6.06; found: C, 62.47; H, 4.10; N, 6.10.

2-(1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-3-phenyl-propionic acid (**23**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and phenylalanine (0. 348 g, 2 mmol) as dark viscous oil, yield 0.270 g, 40 %. ^{1}H NMR (MeOD): δ 8.04 (m, 1H), 7.88 (m, 1H), 7.68 (m, 1H), 7.66 (m, 1H), 7.53 (m, 1H), 7.50-7.46 (m, 4H), 7.18 (m, 1H), 3.46 (m, 1H), 3.31 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 184.0, 182.2, 175.4, 160.2, 138.0, 137.2, 136.2 (2C), 132.0 (2C), 128.8 (2C), 127.8 (2C), 126.0 (2C), 104.0, 60.2, 38.0; Maldi TOF TOF: m/z 322.639 [$(M+H)^+$, 100%]; Anal. Calc for C19H15NO4 (321.33): C, 71.02; H, 4.71; N, 4.36; found: C, 71.20; H, 4.85; N, 4.38.

2-(3-chloro-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)- 3-phenyl-propionic acid (**24**): Obtained from 2,3-dichloro-1,4-naphthoquinone (0.500 g, 2 mmol) and phenylalanine (0.364 g, 2 mmol) as a viscous oil, yield 0.337 g, 43 % [Lit. bp 536°C]. ^{1}H NMR (MeOD): δ 8.18 (m, 2H), 7.87 (m, 2H), 7.31 (m, 5H), 3.78 (m, 1H), 3.32 (m, 1H), 3.00 (m, 1H); Maldi TOF TOF: m/z 357.740 [$(M+2H)^+$, 100%]; Anal. Calc for C19H14ClNO4 (355.77): C, 64.14; H, 3.97; N, 3.94; found: C, 64.47; H, 4.15; N, 4.10.

2-(4-fluoro-phenylamino)-3-methyl-1,4-naphthoquinone (**25**): Obtained from 2-methyl-1,4-naphthoquinone (0.500 g, 3 mmol) and 4-fluoro aniline (0.323 g, 3 mmol) as a dark coloured oil, yield 0.368 g, 45 % [Lit. bp 425°C]. ^{1}H NMR ($CDCl_3$): δ 8.22 (m, 2H), 7.88 (m, 2H), 7.74 (m, 2H), 7.06 (m, 2H), 2.75 (s, 3H); Maldi TOF TOF: m/z 282.551 [$(M+H)^+$, 100%]; Anal. Calc for C17H12FNO2 (281.28): C, 72.59; H, 4.30; N, 4.98; found: C, 72.87; H, 4.45; N, 5.10.

2-methyl-3-phenylamino-1,4-naphthoquinone (**26**): 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)-1,4-naphthoquinone (**27**): Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and 2-chloroaniline (0.270 g, 2 mmol) as a dark brown solid, yield 0.268 g, 45 %, mp. 150-52°C [Lit. 149-51°C]. ^{1}H NMR (MeOD): δ 8.04 (d, 1H, J = 7.64 Hz), 7.84 (d, 1H, J = 7.80 Hz), 7.64 (t, 1H, J = 6.38 Hz), 7.44 (m, 1H), 7.24 (t, 1H, J = Hz), 7.07 (d, 1H, J = 7.96 Hz), 6.93 (t, 1H, J = 7.66 Hz), 6.72 (d, 1H, J = 8.0 Hz), 6.52 (t, 1H, J = 7.60 Hz); Maldi TOF TOF: m/z 284.539 [$(M+H)^+$, 100%]; Anal. Calc for C16H10ClNO2 (283.71): C, 67.74; H, 3.55; N, 4.94; found: C, 67.87; H, 4.00; N, 4.98.

2-chloro-3-(2-chloro-phenylamino)-1,4-naphthoquinone (**28**): Obtained from 2,3-dichloro-1,4-naphthoquinone (0.500 g, 2 mmol) and 2-chloroaniline (0.280 g, 2 mmol) as a dark coloured oil, yield 0.280 g, 40 % [Lit. bp 429°C]. ^{1}H NMR (MeOD): δ 7.94 (d, 1H, J = 7.60 Hz), 7.87 (d, 1H, J = 7.56 Hz), 7.64 (t, 1H, J = 7.52 Hz), 7.52 (t, 1H, J = 7.50 Hz), 7.17 (d, 1H, J = 7.84 Hz), 7.03 (t, 1H, J = 7.66 Hz), 6.82 (d, 1H, J = 8.00 Hz), 6.62 (t, 1H, J = 7.62 Hz); Maldi TOF TOF: m/z 320.674 [$(M+2H)^+$, 100%]; Anal. Calc for C16H9Cl2NO2 (318.15): C, 60.40; H, 2.85; N, 4.40; found: C, 60.87; H, 3.15; N, 4.20.

2-(2-amino-ethylamino)-1,4-naphthoquinone (**29**). Obtained from 2-bromo-1,4-naphthoquinone (0.500 g, 2 mmol) and ethylenediamine (0.128 g, 2 mmol) as a viscous oil, yield 0.192 g, 42 % [Lit. bp 404°C]. ^{1}H NMR (MeOD): δ 8.46 (m, 1H), 8.38 (m, 1H), 7.72-7.70 (m, 2H), 7.36 (s, 1H), 3.37 (m, 2H), 2.73 (m, 2H); Maldi TOF TOF: m/z 217.370 [$(M+H)^+$, 100%]; Anal. Calc for C12H12N2O2 (216.24): C, 66.65; H, 5.59; N, 12.96; found: C, 67.17; H, 6.05; N, 13.10.

2-(2-chloro-phenylamino)-3-methyl-1,4-naphthoquinone (**30**): 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.

CONCLUSIONS

The present study on the synthesis and evaluation of naphthalene-1,4-dione derivatives has established the discovery of a new series of analogues with significant and promising activity against drug-sensitive *M. tb* cultures. These effective derivatives are ideally suited for further modifications to obtain more efficacious antimycobacterial compounds. For the therapeutic development of more potent and non toxic antitubercular agents, further investigations on the structural modifications are currently underway and results will be reported in due course.

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