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Research Article

Synthesis and Macrofilaricidal Activity of Substituted 2-Hydroxy/5-Hydroxy/2-Methyl-1,4-Naphthoquinones

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT Lymphatic filariasis is a disfiguring disease caused by parasitic worms that destroy the human lymphatic system leading to substantial morbidity. The current drug of choice for the treatment of filariasis is diethylcarbamazine and ivermectin with albendazole which are only effective against the microfilaria, leaving the adult worm unaffected, requiring the development of "adulticidal drugs." Thirty amino substituted 2-hydroxy/5-hydroxy/2-methyl-1,4-naphthoquinones were synthesized via the reaction of 2-hydroxy/5-hydroxy/2-methyl-1,4-naphthoquinones with different primary and secondary amines. Compounds **1–30** were evaluated for in vitro antifilarial activity against the adult bovine filarial worm *Setaria digitata* as assessed by worm motility and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assays. The mutagenecity, tumerogenecity, irritantancy, reproductive toxicity, drug score, druglike, and cLog*P* properties were calculated using OSIRIS property predictor. Ten compounds showed macrofilaricidal activity with ED_{50} values ranging between 0.086 and 7.6 μM. Taking into account the biological effects and the promising drug-like profiles of these compounds, these represent valid leads for the development of antifilarial agents against adult filarial worm. Drug Dev Res 74 : 216–226, 2013.

Key words: macrofilaricide; filariasis; naphthoquinone; *Setaria digitata*; ADME

INTRODUCTION

Lymphatic filariasis (LF) is a disfiguring disease caused by parasitic worms that damage the human lymphatic system leading to morbidity (http://www.filariasis.org—Global Alliance to Eliminate Lymphatic Filariasis). The parasitic worms include *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* that are transmitted by mosquito vectors. The drugs currently used for the treatment of LF are diethyl carbamazine and ivermectin with albendazole. These treatments are not effective in fully killing the longer-lived adult worm and therefore are intended at reducing transmission and pathology. This requires the necessity for the development of an "adulticidal" drug.

Derivatives of 1,4-naphthoquinones are extensively distributed in nature with many plants containing these substances being used in folk medicine for the therapy of different diseases [Watt and Breyer-Brandwijik, 1962; Duke, 1985; Gafner et al., 1996]. Systematic studies of naphthoquinone derivatives have led to the discovery of compounds with anticancer [O'Brien, 1991; Lamson and Plaza, 2003; Taper et al., 2004], antitumor [Lin et al., 1989], antifungal [Gershon and Shanks, 1975; Gafner et al., 1996; Tandon et al., 2004; Chung and Mi, 2005], antibacterial [Machado

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et al., 2003; Medina et al., 2004], antiviral [Rastogi and Dhawan, 1990], molluscicidal [Celso et al., 2008], and antithrombotic [Jin et al., 2004] activities. The inhibitory activity of certain naphthoquinones on HIV-1 protease has also been decribed [Brinworth and Fairlie, 1995; Mazumder et al., 1996]. The antiparasitic effects of naphthoquinones against *Trypanosoma cruzi* [Salmon-Chemin et al., 2001], *Toxoplasma gondii*, *Leishmania* sp. [Touraire et al., 1996], and *Plasmodium* sp. [Bullock et al., 1970; Lin et al., 1991] have been studied, while the hydroxyl-1,4-napthoquinone, atovaquone [Williams and Clark, 1998] has been approved by the Food and Drug Administration for the treatment of pneumonia due to *Pneumocystis carinii*.

The development of new antifilarial drugs based on new molecular targets present in adult worms is a challenging task [Nisha et al., 2007]; however, it is important as there is no adulticidal drug available for killing the adult filarial worms. Polyamines have macrofilaricidal activity and represent a nucleus which may be used for synthesis of effective compounds [Kinnamon et al., 1999]. N-alkyl amines have been studied as the pharmacophore for antifilarial drug development [Srivastava et al., 2000]. Aminoquinones are used as medicines [Elslager et al., 1970; Kallmayer and Tappe, 1987) and herbicides [William and Anja, 2001].

The product formed from the reaction of amine with various quinones has significant scope for investigation. Earlier we have reported the antifilarial potential of plumbagin and substituted 1,4-naphthoquinones [Nisha et al., 2002, 2010; Lakshmy et al., 2009] that suggested that this chemical class is worthy of additional investigation. The present work provides a preliminary account of results obtained from the reaction of 2-hydroxy/5-hydroxy/2-methyl-1,4-naphthoquinone with primary and secondary amines and their screening against bovine filarial worm *Setaria digitata* (Nematoda: Filariodea) for antifilarial activity. Absorption, distribution, metabolism, excretion, and toxicity (ADME/tox) are key properties that need to be considered early on any drug development project and this has been done using the FAFDrugs online program developed by Maria et al. [2006]. The prediction of properties such as mutagenecity, tumerogenecity, irritant, reproductive toxicity, drug score, druglike, and cLog*P* was also done using the online program OSIRIS Property Explorer developed by Sander [2001].

MATERIALS AND METHODS

General Procedures

Chemicals and solvents purchased from Sigma-Aldrich (St. Louis, MO) or Merck (Mumbai, India) were used without additional purification. Melting points were taken in glass capillary tubes on Melting point apparatus (Techno Instruments Pvt. Ltd, Bangalore, India) and were corrected using KSPII (KRUSS, GmbH, Hamburg, Germany). ^{1}HNMR spectra were recorded on a Bruker 400 MHz NMR spectrometer (Billerica, MA), chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), or m (multiplet). The FT-IR spectrum was taken in accordance with the KBr disc technique on Shimadzu FT-IR model 8300 (Kyoto, Japan). Chromatographic separation was performed on glass columns with silica gel 60 (230–400 mesh, Merck). Compound purity was determined using HPLC (Thermo Finnigan, San Jose, CA) composed of Spectra System P4000, solvent delivery system Spectra System AS3000, autosampler, and photodiode array detector SN4000. Output signals were supervised *via* a Chromquest 4.0 chromatography workstation (Thermo Finnigan, San Jose, CA). A 3 μm, Supelcosil ABZplus analytical column (Sigma-Aldrich, St. Louis, MO) (150 × 4.6 mm) and a mobile phase combination of acetonitrile-water (70 : 30) at a flow rate of 1 mL/min at 40°C and detection at 280 nm were utilized for the analysis. Retention times were recorded for all the compounds. The purity of target compounds was ≥95%. Thin layer chromatography (TLC) was executed with F_{254} (Merck, GmbH, Darmstadt, Germany) coated aluminium sheets. Synthesis was done in a combinatorial library synthesizer, Miniblock XT (Mettler-Toledo Bohdan, New Jersey). Absorbance measurements were prepared using Spectra-Max Plus (Molecular Devices, Sunnyvale, CA, USA) with SoftmaxPro software. All incubations were completed in a New Brunswick Scientific CO_2 incubator (Edison, NJ, USA).

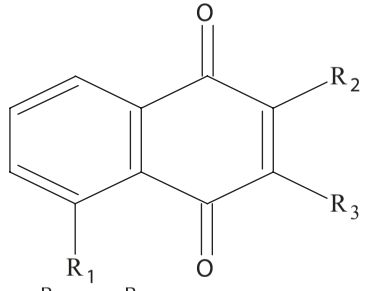
Synthesis of Substituted Naphthoquinones

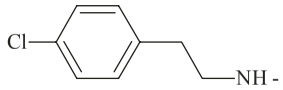
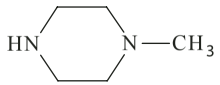
To a stirred solution of substituted amine (6.96 mM) in ethanol (4 mL), substituted napthoquinone (2.32 mM) was added slowly in 2 mL of dichloromethane (DCM). Stirring was continued for 5–6 h at room temperature [Nisha et al., 2010]. The color of the reaction mixture was changed from yellow to deep black. The reaction was monitored by TLC. Chromatographic purification of the crude product was carried out by using column chromatography. The analytical and spectroscopic data for the synthesized compounds described here are shown in Table 1.

In Vitro Screening for Antifilarial Activity Against *S. digitata*

Adults of the cattle filarial parasite of *S. digitata* were used to screen macrofilaricidal activity. Adult

TABLE 1. Analytical and Spectroscopic Data for the Synthesized Compounds 1–30



Compound code	R_1	R_2	R_3	Yield (%)	Mp (°C)	Rf†	R_t (min)	FT-IR υ (per cm)	^{1}H NMRδ
1	H	OH	$CH_3CH_2CH_2NH$ -	26.47	147.2	0.81	2.66	3362, 3066, 1676, 1591	8.13(d, 1H), 8.11(d, 1H), 7.84(t, 1H), 7.52(t, 1H), 6.99(s, 1H), 6.32(s, 1H), 4.38(m, 2H), 1.27 (m, 2H), 0.98 (t, 3H)
2	H	OH	$(CH_3)_2CHNH$ -	57.59	162.6	0.35	2.60	3169, 1678, 1641	8.12(d, 1H), 8.10(d, 1H), 7.79(t, 1H), 7.71(t, 1H), 7.59(s, 1H), 6.36(s, 1H), 4.15(m, 1H), 1.25(d, 6H)
3	H	OH	$CH_3CH_2(CH_3)CHNH$ -	55.18	157.1	0.78	2.54	3169, 1678, 1642, 1577	8.12(d, 1H), 8.09(d, 1H),7.79(t, 1H), 7.73(t, 1H), 7.21(s,1H) 6.55(s, 1H), 4.11(m, 1H), 2.04 (m, 2H), 1.25(d, 3H), 0.87(t, 3H)
4	H	OH	$(CH_3)_2CHCH_2(CH_3)CHNH$ -	55.83	137.8	0.84	2.69	3049, 2956, 1681, 1641	8.09(d, 1H), 8.07(d, 1H), 7.77(t, 1H), 7.68(t, 1H), 7.01(s, 1H), 5.75 (s, 1H), 3.49(m, 1H), 1.42(m, 1H), 1.37 (m, 2H), 1.25 (d, 3H), 0.91(dd, 6H)
5	H	OH		14.07	180	0.32	2.85	3385, 3059, 1676, 1612	8.97(d, 1H), 7.93(d, 1H), 7.81(t, 1H), 7.65(t, 1H), 7.54(t, 1H), 7.37(d, 2H), 7.26(d, 2H), 5.47(s, 1H), 2.92 (t, 2H), 0.89(t, 2H)
6	H	OH		23.9	206	0.87	2.58	3387, 3072, 1678, 1641	8.13(d, 1H), 8.11(d, 1H), 7.79 (t, 1H), 7.71(t, 1H), 6.99(s, 1H), 3.49(t, 4H), 2.41(t, 4H), 1.13(s, 3H)
7	H	CH_3	CH_3CH_2NH -	37.44	94	0.87	2.79	3286, 2928, 1670, 1606	8.04(d, 1H), 8.03(d, 1H), 7.74(t, 1H), 7.70(t, 1H), 5.83(s, 1H), 3.23(m, 2H), 1.67(s, 3H), 1.33(t,3H)
8	H	CH_3	$CH_3CH_2CH_2NH$ -	46.81	81	0.91	3.28	3329, 2958, 1668, 1602	8.16(d, 1H), 8.04(d, 1H), 7.71 (t, 1H), 7.64(t, 1H), 6.01(s, 1H), 4.72(m, 2H), 1.68(m, 2H), 1.29(s, 3H), 0.998(t, 3H)
9	H	CH_3	$(CH_3)_2CHNH$ -	15	83	0.94	2.65	3293, 2970, 1676, 1597	8.09(d, 1H), 8.07(d, 1H), 7.68 (t, 1H), 7.57(t, 1H), 5.60(s, 1H), 4.16(m, 1H), 2.20(s, 3H), 1.26 (d, 6H)
10	H	CH_3	$CH_3CH_2CH_2CH_2NH$ -	42.87	69	0.80	3.45	3327, 2955, 1670, 1602	8.06(d, 1H), 7.98(d, 1H), 7.68 (t, 1H), 7.64(t, 1H), 5.71(s, 1H), 3.55(m, 2H), 2.23(s, 3H), 1.63 (m, 2H), 1.43(m, 2H), 0.96(t, 3H)

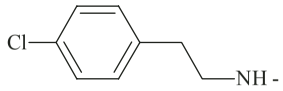
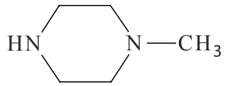
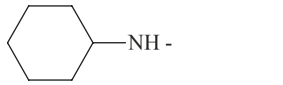
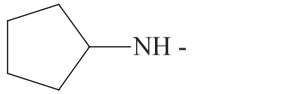
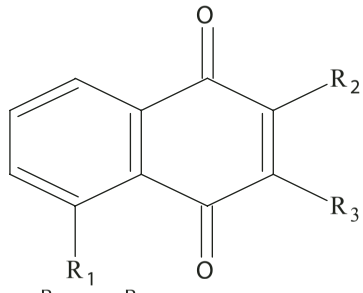
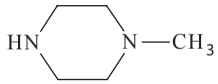
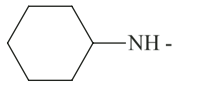
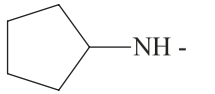
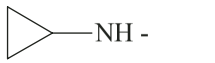
11	H	CH_3	$CH_3CH_2(CH_3)CHNH$ -	14.53	*	0.87	2.99	3333, 2968, 1668, 1604	8.10(d, 1H), 8.08(d, 1H), 7.67 (t, 1H), 7.55(t, 1H), 5.67(s, 1H), 3.95(m, 1H), 2.02(s, 3H), 1.60 (m, 2H), 1.24(d, 3H), 0.98(t, 3H)
12	H	CH_3	$(CH_3)_2NCH_2CH_2CH_2NH$ -	17.57	*	0.18	1.18	3333, 2943, 1668, 1600	8.05(d, 1H), 7.96(d, 1H), 7.66 (t, 1H), 7.62(t, 1H), 5.65(s, 1H), 3.69(t, 2H), 3.49(t, 2H), 2.46(s, 3H), 2.42(m, 2H), 1.21(s, 6H)
13	H	CH_3	$(CH_3CH_2)_2NCH_2CH_2CH_2NH$ -	23.67	*	0.58	1.15	3348, 2968, 1670, 1604	8.06(d, 1H), 8.05(d, 1H), 7.65(t, 1H), 7.54(t, 1H), 6.74(s, 1H), 3.79(t, 2H), 2.54(m, 4H), 2.17(s, 3H), 1.77(t, 2H), 1.28(m, 2H), 1.06(t, 6H)
14	H	CH_3	$(CH_3CH_2CH_2CH_2)_2$ $NCH_2CH_2CH_2NH$ -	29.74	*	0.84	1.32	3350, 2956, 1668, 1606	8.07(d, 1H), 8.04(d, 1H), 7.66(t, 1H), 7.53(t, 1H), 6.51(s, 1H), 3.65(t, 2H), 2.53(t, 4H), 2.42(t, 2H), 1.15(s, 3H), 1.44(m, 2H), 1.39(m, 4H), 1.28(m, 4H), 0.88(t, 6H)
15	H	CH_3	$(CH_3)_2CHCH_2(CH_3)CHNH$ -	13.5	*	0.94	3.93	3331, 2958, 1668, 1604	8.09(d, 1H), 8.00(d, 1H), 7.69(t, 1H), 7.59(t, 1H), 5.57(s, 1H), 4.09(m, 1H), 2.21(s, 3H), 1.71(m, 1H), 1.49(m, 2H), 1.21(d, 3H), 0.92(dd, 6H)
16	H	CH_3		37.84	112	0.89	3.45	3342, 2943, 1666, 1604	8.08(d, 1H), 7.98(d, 1H), 7.69(t, 1H), 7.59(t, 1H), 7.29(d, 2H), 7.16(d, 2H), 5.68(s, 1H), 3.78(t, 2H), 2.89(t, 2H
17	H	CH_3		13.87	205	0.58	1.84	3342, 2929, 1664, 1620	8.11(d, 1H), 8.03(d, 1H), 7.72 (t, 1H), 7.62(t, 1H), 3.29(t, 4H), 2.49(t, 4H), 2.32(s, 3H), 2.17(s, 3H)
18	H	CH_3		16.01	73	0.91	4.25	3321, 2924, 1668, 1599	8.11(d, 1H), 8.03(d, 1H), 7.72(t, 1H), 7.62(t, 1H), 3.29(t, 4H), 2.49(t, 4H), 2.32(s, 3H), 2.17(s, 3H)
19	H	CH_3		29.21	53	0.86	3.59	3336, 2949, 1600, 1568	8.01(d, 1H), 7.89(d, 1H), 7.59(t, 1H), 7.49(t, 1H), 5.69(s, 1H), 4.26(s, 3H), 2.30(m, 1H), 2.16(m, 4H), 1.95(m, 4H)
20	H	CH_3		36.4	85	0.77	2.63	3309, 1668, 1600	8.03(d, 1H), 7.89(d, 1H), 7.61 (t, 1H), 7.49(t, 1H), 5.88(s, 1H), 2.36(s, 3H), 2.10(m, 2H), 0.83(m, 2H)
21	H	CH_3	$CH_3OCH_2CH_2NH$ -	29.34	65	0.76	2.23	3338, 2901, 1606, 1572	8.03(d, 1H), 7.92(d, 1H), 7.58(t, 1H), 7.51(t, 1H), 5.89(s, 1H), 3.68(t, 3H), 3.54(t, 2H), 3.35(s, 3H), 2.16(s, 3H)
22	OH	H	$CH_3CH_2CH_2CH_2NH$ -	19.16	137	0.86	2.95	3331, 2958, 1614, 1595	13.04(s, 1H), 7.52(d, 1H), 7.40(t, 1H), 7.17(d, 1H), 6.00(s, 1H), 5.56(s, 1H), 3.14(m, 2H), 1.62(m, 2H), 1.38(m, 2H), 0.92(t, 3H
23	OH	H	$(CH_3)_2NCH_2CH_2CH_2NH$ -	21.37	98	0.28	2.08	3340, 2945, 1616, 1593	13.21(s, 1H), 7.81(s, 1H), 7.69 (d, 1H), 7.62(d, 1H), 7.42(t, 1H), 5.59(s, 1H), 3.28(t, 2H), 2.46(t, 2H), 2.29(s, 6H), 1.83(m, 2H)

TABLE 1. Continued



Compound code	R_1	R_2	R_3	Yield (%)	Mp(°C)	Rf†	R_t (min)	FT-IR υ (per cm)	^{1}H NMRδ
24	OH	H	$(CH_3CH_2)_2NCH_2CH_2CH_2NH$ -	51.46	70	0.34	2.18	3132, 2970, 1626, 1599	13.21(s, 1H), 7.67(d, 1H), 7.56(d, 1H), 7.43(t, 1H), 5.61(s, 1H), 4.59(s, 1H), 3.32(m, 2H), 3.67(m, 4H), 2.45(m, 2H), 1.87(m, 2H), 1.26(t, 6H)
25	OH	H	$(CH_3CH_2CH_2CH_2)_2$ $NCH_2CH_2CH_2NH$ -	18.16	79	0.77	2.20	3340, 2956, 1612, 1591	13.01(s, 1H), 7.67(d, 1H), 7.57(d, 1H), 7.56(t, 1H), 5.57(s, 1H), 4.59(s, 1H), 3.97(t, 2H), 3.37(t, 4H), 2.67(t, 2H), 2.44(m, 2H), 1.87(m, 4H), 1.26(m, 4H), 0.920(t, 6H)
26	OH	H		24.0	209	0.19	2.26	3387, 2939, 1627, 1566	12.02(s, 1H), 7.61(d, 1H), 7.59(d, 1H), 7.22(t, 1H), 6.05(s, 1H), 3.59(t, 4H), 2.62(t, 4H), 1.66(s, 3H)
27	H	H		46.77	105	0.98	2.89	3343, 1671, 1628	8.09(d,1H), 8.02(d, 1H), 7.71(t, 1H), 7.59(t, 1H), 5.85(s, 1H), 5.75(s, 1H), 3.29(m, 1H), 2.03(m, 4H), 1.77(m, 4H), 1.37(m, 2H)
28	H	H		39.30	75	0.95	2.57	3325, 1676, 1616	8.09(d, 1H), 8.04(d, 1H), 7.73 (t, 1H), 7.69(t, 1H), 5.76(s, 1H), 5.73(bs, 1H), 3.78(m, 1H), 2.04 (m, 4H), 1.65(m, 4H)
29	H	H		48.11	134	0.91	2.13	3350, 1671, 1623	8.09(d, 1H), 8.03(d, 1H), 7.72(t, 1H), 7.63(t, 1H), 6.01(s, 1H), 5.98(s, 1H), 2.51(m, 1H), 0.89 (m, 2H), 0.66(m, 2H)
30	H	H	$CH_3OCH_2CH_2NH$ -	39.70	110	0.42	1.95	3236, 1681, 1634	8.08(d, 1H), 8.05(d, 1H), 7.72(t, 1H), 7.63(t, 1H), 6.16(s, 1H), 5.74(s, 1H), 3.64(t, 2H), 3.40(s, 3H), 3.34(t, 2H)

*Liquid.

†TLC eluent – Petroleum ether: Chloroform: Ethyl acetate: Methanol-1 : 3 : 2 : 1 for compounds **1–6**, **12–14,** and **23–25**;
Petroleum ether: Chloroform: Ethyl acetate-4 : 10 : 1 for compounds **7–11**, **15–22,** and **26–30**.

MP, melting point; Rf, retention factor; R_t, retention time; TLC, thin layer chromatography.

female *S. digitata* worms collected from the peritoneal cavity of freshly slaughtered cattle were washed with normal saline (0.85%) to free them from extraneous material and transferred to Dulbecco's modified Eagle medium (DMEM) containing 0.01% streptopenicillin and supplemented with 10% heat-inactivated fetal calf serum and were used within an hour as reported earlier [Nisha et al., 2002, 2010; Lakshmy et al., 2009].

Worm Motility Assay

Stock solutions of compounds **1–30** were prepared in dimethylsulfoxide (DMSO)/ethyl alcohol depending upon the solubility of the compound at 30 mg/mL. For the assays, the compounds were further diluted to the appropriate concentration using complete assay medium. The DMSO/alcohol concentration in the medium was kept below 1%. Preliminary screening was done at a concentration of 0.1 mg/mL. A simultaneous control with an equal volume of the vehicle in the DMEM was included. Two adult female *S. digitata* worms were introduced into each Petri dish with three replicates for both test and control. Worms were incubated at 37°C for 24 and 48 h in an incubator. After the incubation period, the number of immobilized worms was counted. Immediately after counting, the worms were washed twice with fresh medium and transferred to another set of Petri dishes containing fresh medium, without the test solution, to assess whether any of the immobile worms regained motility. If the worms did not revive, the condition was considered irreversible and the concentration lethal. Each experiment was repeated twice.

MTT-Formazan Colorimetric Assay for Viability of Worms

Compounds **1–30** were further screened for viability of adult *S. digitata* through an (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) reduction assay [Comley et al., 1989]. Yellow MTT is reduced to purple formazan by mitochondrial enzymes present in living cells. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion is used as a measure of viable (living) cells. During the assay, the formazan formed is extracted with DMSO and is quantified. As the values of absorption correlate with formazan formation, worm viability was estimated as percentage inhibition in formazan formation relative to control worms. Adult female worms were used for this assay. After the exposure of the worms to the compounds (0.1 mg/mL) in DMEM at 24 and 48 h incubation period, the worms were further incubated for 30 min individually in phosphate-buffered saline (pH 7.4, 0.5 mL) containing MTT (0.25 mg/mL). A control was set up with untreated adult females but exposed to DMSO as described previously. At the end of the MTT incubation, worms were transferred to a microliter plate containing 400 µL of spectroscopic-grade DMSO and equilibrated at room temperature for 1 h, with occasional gentle shaking to extract the color developed. The absorbance of the resulting formazan solution was then determined at 492 nm in a microplate spectrophotometer relative to DMSO blank. Compounds showing greater than 50% inhibition in formazan formation with respect to control at 0.1 mg/mL were considered effective and were further screened at lower concentrations to generate ED_{50} values (the dose that gives a 50% response, determined by a sigmoid plot obtained by plotting the logarithm of the dose on the *x*-axis and the percentage response on the *y*-axis).

Calculated ADME Properties of Substituted 1,4-Naphthoquinones

FAFDrugs is an online service that allows users to process compounds *via* simple ADME/tox filtering rules, for example, molecular weight, polar surface area, logP, or number of rotatable bonds. Compounds **1–30** were transformed to SMILES coordinates to generate these calculated ADME properties. The parameters were either in compliance with Lipinski's rule of five or set as default. The prediction of properties such as mutagenecity, tumerogenecity, irritant, reproductive toxicity, drug score, druglike, and cLog*P* was done using the online program OSIRIS Property Explorer available in the Organic Chemistry Portal [Sander, 2001]. cLog*P* data were acquired using the OSIRIS property explorer that uses the Chou and Jurs (1979] algorithm, based on computed atom contributions.

RESULTS

Thirty amino substituted 1,4-naphthoquinones were synthesized by the reaction between alcoholic solutions of substituted amines and substituted 1,4-naphthoquinones in DCM followed by chromatographic purification. The yield was found to vary from 13.5 to 57.6%.

The results of preliminary screening of compounds **1–30** at 0.1 mg/mL for antifilarial activity *in vitro* against adult bovine filarial worm *S. digitata* by worm motility assay by visual observation are shown in

TABLE 2. ***In Vitro*** **Macrofilaricidal Activity of Compounds 1–30 Against** ***S. digitata*** **Adult by Worm Motility and MTT Reduction Assays**

List of compounds	% MTT reduction at 0.1 mg/mL ± SE ($n = 12$)		Worm motility at 0.1 mg/mL		ED_{50} value μM	
	24 h	48 h	24 h	48 h	24 h	48 h
1	75.52 ± 0.94	87.01 ± 0.23	+++	+++	30.61	5.78
2	86.41 ± 0.24	90.28 ± 0.27	+++	+++	3.85	0.086
3	82.15 ± 0.15	91.85 ± 0.11	+++	+++	18.21	2.43
4	83.72 ± 1.01	96.04 ± 0.27	+++	+++	28.4	0.37
5	67.24 ± 0.28	91.50 ± 0.48	+++	+++	136.3	2.42
6	77.99 ± 0.77	97.03 ± 0.82	+++	+++	116.13	1.84
7	20.49 ± 2.25	40.45 ± 0.48	+	+	–	–
8	18.26 ± 2.50	39.28 ± 0.93	+	+	–	–
9	27.36 ± 2.64	48.64 ± 0.18	+	+	–	–
10	46.26 ± 0.13	64.90 ± 0.93	+	++	–	–
11	23.99 ± 1.90	44.50 ± 0.86	+	+	–	–
12	31.42 ± 3.05	56.03 ± 1.68	+	++	–	–
13	24.36 ± 1.16	48.28 ± 0.99	+	+	–	–
14	69.31 ± 1.85	88.19 ± 0.38	+++	+++	49.88	12.53
15	36.00 ± 2.04	48.57 ± 0.49	+	+	–	–
16	31.41 ± 1.96	34.12 ± 1.68	+	+	–	–
17	30.58 ± 2.53	37.87 ± 2.80	+	+	–	–
18	61.93 ± 1.10	74.36 ± 0.24	++	+++	93.26	52.44
19	62.33 ± 0.54	72.53 ± 0.34	++	+++	277.29	29.37
20	25.40 ± 0.60	38.61 ± 0.35	+	+	–	–
21	72.47 ± 0.57	85.0 ± 0.45	+++	+++	131.95	13.5
22	73.73 ± 0.73	85.27 ± 0.24	+++	+++	214.0	26.9
23	85.65 ± 0.55	90.29 ± 0.21	+++	+++	36.5	7.3
24	86.49 ± 0.61	93.87 ± 0.44	+++	+++	61.6	7.6
25	80.42 ± 0.97	86.01 ± 1.84	+++	+++	101.0	44.2
26	90.78 ± 0.33	92.2 ± 0.37	+++	+++	8.04	4.85
27	24.45 ± 3.01	44.72 ± 0.29	+	+	–	–
28	66.38 ± 1.11	71.30 ± 0.29	+++	+++	16.50	14.70
29	88.68 ± 0.65	96.93 ± 0.91	+++	+++	12.34	2.64
30	34.29 ± 1.19	46.54 ± 0.57	+	+	–	–

+ Active worms; ++ Sluggish worms; +++ Immotile or dead worms.

Table 2. Worm motility assay results showed that the worms treated with compounds **1**, **2**, **3**, **4**, **5**, **6**, **14**, **18**, **19**, **21–26**, **28**, and **29** were completely paralyzed or dead after 48 h incubation, whereas with compounds **7–13**, **15–17**, **27**, and **30**, the worms showed active or sluggish movement even after 48 h incubation. The results of *in vitro* macrofilaricidal screening at 0.1 mg/mL by MTT reduction assay are also shown in Table 2. Seventeen compounds showed greater than 50% inhibition in formazan formation. No macrofilaricidal activity was observed for compounds **7–9**, **11**, **13**, **15–17**, **20**, **27**, and **30** at 0.1 mg/mL (<50% reduction in MTT assay) while **10** and **12** showed moderate activity. The results of the motility assay were confirmed by the MTT assay results. The effective compounds were further screened at lower concentrations to get ED_{50} values and the results are given in Table 2. Ten compounds *viz*., **2**, **4**, **6**, **5**, **3**, **29**, **26**, **1**, **23**, and **24,** showed promising macrofilaricidal activity with ED_{50} values of 0.086, 0.37, 1.84, 2.42, 2.43, 2.64, 4.85, 5.78, 7.3, and 7.6 μM, respectively, at 48 h incubation. Calculated ADME properties of substituted 1,4-Naphthoquinones by FAF-Drugs online program and OSIRIS property explorer are given in Table 3.

DISCUSSION

There is an obvious requirement for the development of efficient, complementary chemotherapeutic move that results in a long-term decline of the pathology-inducing worm stages, for example, adult worms in lymphatic filariasis or to a macrofilaricidal effect. An exhaustive review of literature [Nisha and Kalyanasundaram, 2007] illustrated that considerable efforts have been focused on developing an effective and safe drug that could kill or permanently sterilize adult filarial worms. Although many of the studied compounds showed promising activity, none reached the final stage as an adulticidal drug either due to toxicity or poor absorption and other practical reasons. Many plant-derived compounds are also being studied

TABLE 3. OSIRIS Calculations for Compounds 1–30

Comp	MW	M	T	I	R_T	cLog*P*	Drs	Ars	FB	RB	DL	DS
1	231	G	G	G	G	1.71	2	4	3	13	–0.41	0.62
2	231	G	G	G	G	1.65	2	4	2	13	–0.05	0.66
3	245	G	G	G	G	2.11	2	4	3	13	0.29	0.67
4	273	G	G	R	G	2.91	2	4	4	13	–0.64	0.32
5	327	G	G	G	G	3.13	2	4	4	19	0.95	0.58
6	272	G	G	G	G	1.07	1	5	1	19	5.1	0.94
7	215	G	G	G	G	2.23	1	3	2	13	–0.95	0.57
8	229	G	G	G	G	2.69	1	3	3	13	–0.54	0.58
9	229	G	G	G	G	2.63	1	3	2	13	–0.19	0.62
10	243	G	G	G	G	3.15	1	3	4	13	–2.36	0.44
11	243	G	G	G	G	3.09	1	3	3	13	0.07	0.61
12	272	G	G	G	G	1.94	1	4	5	13	2.54	0.88
13	300	G	G	G	G	2.81	1	4	7	13	3.86	0.84
14	356	G	G	G	G	4.67	1	4	11	13	–0.09	0.45
15	271	G	G	R	G	3.9	1	3	4	13	–0.85	0.28
16	325	G	G	G	G	4.12	1	3	4	19	0.80	0.51
17	270	G	G	G	G	2.05	0	4	1	19	4.89	0.92
18	269	G	G	G	G	3.38	1	3	2	19	–6.36	0.36
19	255	G	G	G	G	3.06	1	3	2	18	–3.65	0.40
20	227	G	G	G	G	2.42	1	3	2	16	–0.96	0.54
21	246	G	G	G	G	1.73	1	4	4	13	–0.68	0.61
22	245	G	G	G	G	2.46	2	4	4	13	1.01	0.75
23	274	G	G	G	G	1.25	2	5	5	13	5.91	0.93
24	302	G	G	G	G	2.12	2	5	7	13	7.15	0.89
25	358	G	G	G	G	3.98	2	5	11	13	3.18	0.69
26	272	G	G	G	G	1.36	1	5	1	19	8.31	0.94
27	255	G	G	G	G	2.99	1	3	2	19	–5.84	–0.38
28	241	G	G	G	G	2.67	1	3	2	18	–3.10	0.42
29	213	G	G	G	G	2.03	1	3	2	16	–0.39	0.61
30	231	G	G	G	G	1.34	1	4	4	13	–0.17	0.67

MW, Molecular weight; M, mutagenic; T, tumorigenic; I, irritant; RT, reproductive toxicity; R, red; G, green; Drs, hydrogen donors; Ars, hydrogen acceptors; FB, flexible bonds; RB, rigid bonds; ClogP, fragment based prediction of logP (octanol / water); DL, drug likeness; DS, drug score donors.

for macrofilaricidal activity [Chatterjee et al., 1992; Singh et al., 1994; Nisha et al., 2008]. Our recent work on alkylamino-1,4-naphthoquinones resulted in few macrofilaricidal lead molecules [Nisha et al., 2010].

The 1,4-naphthoquinone scaffold has received attention as a pharmacophore for the design of antitumor and antimalarial agents [Baggish and Hill, 2002; Tandon et al., 2004]. The mechanism of action of naphthoquinones has not been completely elucidated. Exceptional biological activity is imparted on the 1,4-naphthoquinone pharmacophore due to the presence of two carbonyl groups that have the ability to accept electrons to produce the corresponding radical anion or di-anion species, as well as their acid-base properties [Tandon et al., 2004]. Atovaquone, a hydroxyl-1,4-napthoquinone is an antiparasitic drug that selectively targets the mitochondrial respiratory chain of the malaria parasite [Fry and Pudney, 1992; Baggish and Hill, 2002]. The 1,4-naphthoquinone structure is common in many natural products linked with antifungal, antibacterial, antiviral, and antitumour activities [O'Brien, 1991]. 1,4-naphthoquinone pharmacophore is known to impart cytotoxity in a number of drugs, for example, streptonigrin [McBride et al., 1966], actinomycins [Reich et al., 1962], mitomycins [Keyes et al., 1991], and 2-hydroxynaphthoquinone derivatives [Hatzigrigoriou et al., 1993]. In addition to imparting antifungal and cytotoxic activity, 1,4-naphthoquinones exhibit significant antiparasitic activity [Williams and Clark, 1998; Lanfranchi et al., 2012]. Introduction of nitrogen in two different positions of the naphthoquinone core, at C-5 and at C-8 of menadione through a two-step, straightforward synthesis based on the regioselective hetero-Diels–Alder reaction, improved the solubility of polysubstituted 1,4-naphthoquinone derivatives. The antimalarial and the antischistosomal activities of these polysubstituted *aza*-1,4-naphthoquinone derivatives were evaluated and led to the selection of distinct compounds for antimalarial *versus* antischistosomal action [Lanfranchi et al., 2012]. The structure–activity relationships (SARs) revealed that the presence of nitrogen-containing substituents at the 2-position was associated with an increase in activity—specifically, the

presence of an arizidinyl substituent at the 2-position of the naphthoquinone ring was associated with a substantial enhancement in antimalarial activity, while compounds with non-arizidinyl nitrogen-containing substituents at the 2-position demonstrated even greater activity [Lin et al., 1991].

Our current research on substituted 1,4-napthoquinones has resulted in 10 antifilarial molecules of which two (**2** and **4**) showed excellent macrofilaricidal activity *in vitro* against adult bovine filarial worm *S. digitata*. The ED_{50} values for the most effective macrofilaricidal compounds **2** and **4** were 0.086 and 0.37 μM, respectively. SAR studies were carried out to find out the influence of chemical structure modification on macrofilaricidal activity. Comparison of the macrofilaricidal activity of compounds **1–30** showed the following trend in killing the adult *S.digitata in vitro*: **2 > 4 > 6 > 5 > 3 > 29 > 26 > 1 > 23 > 24**. SAR studies showed that when position 2 of the quinone ring was occupied by a hydroxyl group, the macrofilaricidal activity was more compared with a methyl group in the same position along with isopropylamino and 4-(methylpentan-2-yl)amino substitution at position 3. Our earlier studies [Nisha et al., 2010] showed that 1,4-napthoquinone with propylamino, isopropylamino, isobutylamino, 1,3-dimethylbutylamino, and piperazinylamino substitutions in the quinone ring exhibited macrofilaricidal activity with ED_{50} values of 36, 3.6, 3.1, 0.91, and 1.2 μM, respectively. In the present study, an enhancement in macrofilaricidal activity was observed with a hydroxyl substitution at position 2 of the quinone ring, along with similar substituted amino groups at position 3 except for piperazinylamino substitution (ED_{50} values were 5.78, 0.086, 2.43, 0.37, and 1.84 μM, respectively, for 3-propylamino, 3-isopropylamino, 3-isobutylamino, 3-(1,3-dimethylbutylamino), and 3-piperazinylamino substituted 2-hydroxy-1,4-napthoquinone). However, a methyl substitution at position 2 instead of hydroxyl group has reduced the macrofilaricidal activity as seen in compounds **7–21**. Only compounds with 3-(dibutylamino)propylamino (**14**), cyclohexylamino (**18**), cyclopentylamino (**19**), and 2-methoxyethylamino (**21**) groups at C3 position showed macrofilaricidal activity with ED_{50} ranging between 12.53 and 52.44 μM. When the hydroxyl group was in the 5th carbon of the 1,4-napthoquinone, compounds with substitutions of butylamino (**22**), 3-(dimethylamino) propylamino (**23**), 3-(diethylamino)propylamino (**24**), 3-(dibutylamino)propylamino (**25**), and piperazinylamino (**26**) groups at C3 exhibited macrofilaricidal activity ED_{50} ranging between 4.85 and 26.9 μM. As observed in the case of compounds **28** and **29**, cyclopentylamino and cyclopropylamino substitutions at C3 position of the quinone ring showed macrofilaricidal activity with ED_{50} values 14.7 and 2.64 μM, respectively.

The results generated for ADME/tox properties of the compounds using FAFDrugs ADME/tox filtering and OSIRIS Property Explorer showed that all the compounds are in agreement with Lipinski's rule of five and passed through the filter. In general, an orally active drug has: not more than five hydrogen bond donors (OH and NH groups), not more than 10 hydrogen bond, acceptors (notably N and O), a molecular weight under 500, a LogP under 5. These features are referred to as Lipinski's rule of five and can be used as a rule of thumb to indicate whether a molecule is likely to be orally bioavailable (bioactive). Molecular property evaluation of the analogs was done using the Osiris Property Explorer (Table 3). The chemical structure of the lead compound was entered and various drug-relevant properties like druglike, drug score, etc., were calculated. Prediction results were assessed and color coded. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red (R). Whereas green (G) indicates druglike conform behavior. The toxicity predicted by OSIRIS property explorer clearly shows that the amino substituted compounds have less toxicity in terms of mutagenicity, tumorigenicity, reproductive toxicity. Red indicates unfavorable toxicity. Compounds **4** and **15** with 4-methylpentan-2-ylamino substitution may be irritants as indicated by the red color. A positive value for druglikeness indicates that the molecule contains predominantly fragments that are present in commercial drugs. The "druglikeness" was improved in most of the substituted compounds except in the case of cyclohexylamino substitution. Toxicity assessment using Osiris revealed that except for **4** and **15**, all other compounds had a good calculated ADME profile which minimizes the toxicity risk of napthoquinone analogs in humans.

In conclusion, our studies have shown that amino substituted 1,4-napthoquinones exhibit antifilarial activity against adult filarial worms and the activity is enhanced by the presence of hydroxyl group at position 2 of the quinone ring and the activity is diminished by the presence of a methyl group. The compounds **2**, **3**, **5**, and **6** may be exploited as leads for the development of effective macrofilaricidal agents.

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