

Chemoprevention of Schistosomiasis: *In vitro* Antiparasitic Activity of Nineteen Plant-derived and Synthetic Simple Naphthoquinones and Naphthols against *Schistosoma Mansoni* Adult Worms

Lizandra G. Magalhães^{1*}, G. Subba Rao², Ingrid A. O. Soares¹, Fernanda R. Badocco¹, Wilson R. Cunha¹, Vanderlei Rodrigues³, and Govind J. Kapadia⁴

¹Research Group on Natural Products, Center for Research in Sciences and Technology, University of Franca, Franca, Brazil

²Global Biotechnology Resource Center, 145 Rosewood Drive, Streamwood, IL 60107, USA

³Department of Biochemistry and Immunology, University of São Paulo, Medicine School of Ribeirão Preto, Ribeirão Preto, Brazil

⁴Department of Pharmaceutical Sciences, College of Pharmacy, Howard University, Washington, DC 20059, USA

Abstract

Schistosomiasis, a debilitating disease dating back to ancient times, is currently endemic in 78 tropical and subtropical countries with 243 million people requiring treatment. Current treatment of schistosomiasis depends primarily on a single drug, praziquantel which is less effective against larval stage of the parasite and has potential for development of resistance. Thus, there is urgent need for development of new, effective and inexpensive antischistosomal drugs. Simple naphthoquinone (NAPQ) secondary metabolites in plants are known to act as phytotoxins in preventing bacterial, fungal and parasitic attacks. The present study reports antischistosomal activity of nineteen plant-derived and synthetic simple NAPQs and naphthols against *Schistosoma mansoni* adult worms under *in vitro* conditions. Four of the tested compounds met the WHO's Special Program for Research and Training in Tropical Diseases (TDR) *in vitro* criterion for "hit" and lead compound (100% mortality of adult worms at a concentration of $\leq 5 \mu\text{g/ml}$ when incubated for 48 h): plant-derived naphthazarin, two synthetic NAPQs, 1, 4-NAPQ and 2-methy-1, 4-NAPQ (menadione) and synthetic 1-amino-2-naphthol hydrochloride. Structure-antischistosomal activity studies with 1, 4-NAPQs indicated the importance of the number and position of hydroxyl and methyl groups, particularly at C-2, C-5 and C-8 positions of the parent NAPQ molecule, which play important role in stabilizing quinone moiety and formation of reactive oxygen species essential for antiparasitic effect. The results call for further *in vivo* studies on the chemopreventive potential of plant-derived naphthazarin and synthetic 1,4-NAPQ, menadione and 1-amino-2-naphthol, all of which have met the WHO/TDR *in vitro* criterion for their consideration as lead schistosomicidal candidates.

Keywords: *Schistosoma mansoni*; 1, 2- and 1, 4-naphthoquinones; Naphthols; Chemoprevention

Introduction

Schistosomiasis is a tropical disease caused by trematodes of the genus *Schistosoma* [1] and World Health Organization (WHO) considers this water-borne, communicable disease a major public health problem of the 21st century [2]. While an estimated 779 million people are at risk worldwide [3], in 2011 at least 243 million people required treatment for this parasitic disease in 78 tropical and subtropical countries, including China. Schistosomiasis has been the scourge of humanity since ancient times. There is evidence that it was prevalent in ancient Egypt as early as 1250-1000 BC by the discovery of calcified schistosomes eggs in the kidneys of mummies [4]. Similar discoveries have been made with Chinese mummies dating back to 400 BC [5]. It was first described as a tropical parasitic disease by the German physician Theodore Maximillian Bilharz in 1851 while working in a Cairo hospital [6]. Oliver Tracy Logan, an American physician, reported the first clinical case of schistosomiasis in Hunan province of China in 1905 [7]. Bilharz's pioneering studies traced the origin of this chronic infection to the larval form of *Schistosoma* parasite released by fresh water snails which act as the intermediate host and cause infection in humans by penetrating skin when it comes in contact with infested water used for swimming and bathing. In the human body, the larvae develop into adult schistosomes and the females release eggs in the blood vessels. Symptoms of the disease are caused by the body's reaction to the eggs and not by the worms themselves [2]. Adult *S. mansoni* parasites can survive in the mesenteric veins and enter the hepatic portal circulation system for up to 30 years without being

eliminated by the immune attack in their human host [8].

People are at risk of schistosomiasis infection (also known as bilharzia, named after its discoverer) mostly in developing countries due to domestic, recreational and agricultural activities which expose them to infested water. The adverse economic and health consequences of this disease are considerable. In tropical countries, it is the second most socioeconomically devastating parasitic disease after malaria. In children it can cause anemia, stunting and learning disability, although they can be reversed by prompt treatment. Chronic schistosomiasis in adults can affect their ability to work and lead to infertility which is irreversible. Urogenital schistosomiasis results in kidney damage and fibrosis of the bladder and ureter, and if untreated, could advance to cancers. More than 200,000 deaths per year in sub-Saharan Africa are attributable to chronic schistosomiasis [2].

***Corresponding author:** Lizandra Guidi Magalhães, Research Group on Natural Products, Center for Research in Sciences and Technology, University of Franca, Franca, 14404 600, São Paulo, Brazil; Tel: +55-16-3711-8878; E-mail: lizandra.magalhaes@unifran.edu.br

Received July 20, 2014; Accepted July 23, 2014; Published August 12, 2014

Citation: Magalhães LG, Rao GS, Soares IAO, Badocco FR, Cunha WR, et al. (2014) Chemoprevention of Schistosomiasis: *In vitro* Antiparasitic Activity of Nineteen Plant-derived and Synthetic Simple Naphthoquinones and Naphthols against *Schistosoma Mansoni* Adult Worms. J Trop Dis 2: 146. doi: 10.4172/2329-891X.1000146

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Despite recent advances in treatment and control of schistosomiasis, over 700 million people live in areas considered endemic in 78 countries including Africa, South America and China, and it continues to spread to new geographic regions [2,9]. Bilharz identified the genus *Schistosoma* among which *S. mansoni* is the most prevalent species infecting millions in Africa, the Middle East, the Caribbean, Brazil, Suriname and Venezuela. Currently, praziquantel (PZQ), first introduced in 1975, is the drug of choice for treatment of *S. mansoni* infection with oxamniquine as an infrequently used alternative for adults [10]. However, both drugs have limited effect on juvenile schistosomes and on already developed liver and spleen lesions [11]. Additionally, development of resistance to these drugs is a major concern [12,13]. Schistosomiasis treatment will reach crisis state if parasites acquire resistance to PZQ. Thus, current reliance on essentially a single drug in controlling such a widespread disease as schistosomiasis has raised alarm among medical practitioners and scientific investigators [9]. There is consensus on urgent need for research in developing new and affordable drugs against this debilitating parasitic disease which is considered a neglected infection primarily affecting developing countries.

Plants produce variety of secondary metabolites among which simple naphthoquinones (NAPQs) are known to act as phytotoxins to ward off bacterial, fungal and parasitic attacks [14]. Recent studies have found several NAPQs of plant origin to be potent antibacterial, antifungal and cytotoxic chemopreventive agents [15-19]. Also, antiparasitic activity of the following plant-derived NAPQs have been reported: lapachol commonly occurring in *Bignoniaceae* plants [20] and plumbagin [21] as leishmanicidal; plumbagin and 2-methylnaphthazarin from *Nepenthes thorelii* as antimalarials [22,23]; plumbagin against *Fasciola gigantica* [24]; juglone against *Hymenolepes nana* [25] and lapachol against *Trypanosoma cruzi* [26]. Earlier studies have shown antiparasitic effect of synthetic NAPQs, such as menadione on *S. mansoni* [27] and blockage of cercarial skin penetration by NAPQ derivatives [28]. Recently, *in vitro* anthelmintic effect of plant-derived NAPQs, plumbagin against *S. mansoni* adult worms [29,30] and lapachol potassium salt against *S. mansoni* cercariae [31] have been reported.

In continuation of our investigations of plant-derived and related synthetic antiparasitic compounds [32-34], we have studied nineteen plant-derived and synthetic simple NAPQs and naphthols derivatives (Figure 1) against *S. mansoni* adult worms under *in vitro* conditions. The objectives of the present study included comparison of their chemical structure and available antioxidation potential data with antischistosomal activity.

Material and Methods

Chemicals

The following chemicals were purchased from Aldrich Chemical Company, Milwaukee, WI, USA: 5-hydroxy-1,4-naphthoquinone (lawsone) (NAP-1); 5,8-dihydroxy-1,4-naphthoquinone (naphthazarin) (NAP-2); 5-hydroxy-1,4-naphthoquinone (juglone) (NAP-3); 2-hydroxy-3-(3-methylbut-2-enyl)-1,4-naphthoquinone (lapachol) (NAP-4); 1,4-naphthoquinone (NAP-5); 2-methyl-1,4-naphthoquinone (menadione, vitamin K3) (NAP-6); 2-amino-3-chloro-1,4-naphthoquinone (herbicide ACNQ) (NAP-7); 2,3-dichloro-1,4-naphthoquinone (NAP-8); 2,3-dibromo-1,4-naphthoquinone (NAP-9); 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone (NAP-10); bis-lawsone (NAP-12); 1,2-naphthoquinone (NAP-13); 1,2-naphthoquinone-4-sulphonoc acid sodium salt (NAP-15); 1-

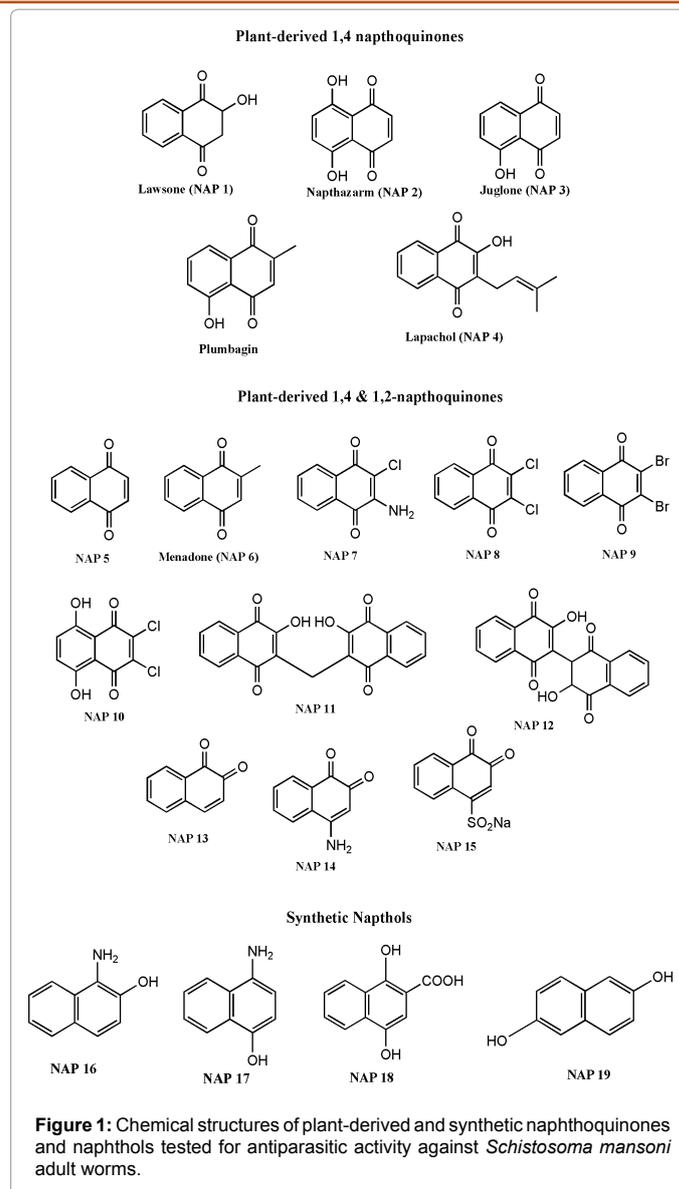


Figure 1: Chemical structures of plant-derived and synthetic naphthoquinones and naphthols tested for antiparasitic activity against *Schistosoma mansoni* adult worms.

amino-2-naphthol hydrochloride (NAP-16); 1-amino-4-naphthol hydrochloride (NAP-17) 1,4-dihydroxy-2-naphthoic acid (NAP-18); praziquantel (PZQ) and dimethyl sulfoxide (DMSO). 2,6-Dihydroxynaphthalene (NAP-19) was procured from TCI America, Portland, OR, USA. Methylene-bis-lawsone (NAP-11) and 4-amino-1,2-naphthoquinone (NAP-14) were synthesized as reported earlier [35,36].

Animals and parasites

The life cycle of the *S. mansoni* strain Luis Evangelista (LE) is maintained by passage through *Biophalaria glabrata* snails and Balb/c mice at the Parasitology Research Laboratory, the University of Franca. This maintenance is authorized by the Ethical Committee for Animal Care of the University of Franca (Protocol: 028/12) in accordance with the internationally accepted principles for laboratory animal handling and care. Female mice (Balb/c weight 20-22 g) were infected with 200 cercariae and after 56±2 days of infection, *S. mansoni* adult worms were recovered under aseptic conditions by perfusion of the livers and mesenteric veins [37]. The worms were washed in RPMI 1640 medium (Gibco Life Technologies, Gaithersburg, MD, USA), kept at pH 7.5 with HEPES mM buffer, and supplemented with penicillin

(100 UI/ml), streptomycin (100 µg/ml) and 10% bovine fetal serum (Gibco).

In vitro studies with *S. mansoni* adult worms

The procedure used has been described in detail earlier [33]. Briefly, one adult *S. mansoni* worm pair (one male and one female) in RPMI 1640 medium were placed in each of 24-well plates. A preliminary screening of test compounds was performed at a concentration of 100 µM. The compounds were prepared in DMSO and added to the RPMI 1640 medium containing the worms after a period of 24 h of adaptation to the culture medium. The parasites were kept for 72 h in a constant temperature incubator at 37°C in an atmosphere of 5% CO₂ and monitored every 24 h for motor activity and mortality. Alteration in motor activity was classified as either slight, defined as a reduction in movement compared with the negative control or significant, defined as minimal movement observed for 1 min. The worms were considered dead when no movement was observed for at least 2 min [38]. The test compounds that killed all parasites (100%) during 24, 48 or 72 h incubation periods were further evaluated at concentrations of 6.125 µM to 100 µM. The minimum lethal concentration (MLC) which is the minimum concentration needed to kill all worms and the 50% lethal concentration (LC50) which is the concentration that kills 50% of worms for these test compounds were determined after incubating for 24 h, 48h and 72 h [39]. The LC50 values were calculated from nonlinear regression dose-response mortality graphs. All experiments were carried out in quadruplicate (8 worms in each experiment) and repeated two times (total of 16 worms). Adult worms incubated with the highest concentration of solvent (0.1% DMSO) served as negative control while those incubated with PZQ (12.5 µM) served as positive control.

Statistical analysis

Results are expressed as the mean ± S.D. The statistical tests were performed with the Graphpad Prism (version 5.0) software. Data were statistically analyzed by one-way analysis of variance, followed by Dunnet's comparison.

Results

In the preliminary screening, the nineteen plant-derived and synthetic NAPQ and naphthol compounds (Figure 1) were tested at 100 µM concentration and the following ten compounds (52.6% of compounds tested) were found to cause death of 100% of adult *S. mansoni* worms when incubated for 24 h (Table 1): two plant-derived 1,4-NAPQs, naphthazarin (NAP-2) and juglone (NAP-3); synthetic analogs, five 1,4-NAPQs (NAP-5, NAP-6, NAP-8, NAP-9 and NAP-10); 1,2-NAPQ-4-sulfonic acid (NAP-15) and two 1-amino-naphthols (NAP-16 and NAP-17). Among the synthetic NAPQs, 1,2-NAPQ (NAP-13) caused 100% mortality of worms when incubated for 48 and 72 h, while herbicide ACNQ (NAP-7) and methylene-bis-lawsone (NAP-11) caused 100% mortality of parasites only when incubated for 72 h. A 25% reduction in mortality of adult schistosomes was observed with NAP-7 at 24 h which increased to 50% at 48 h of incubation. With synthetic 4-amino-1, 2-NAPQ, (NAP-14) and naphthol carboxylic acid (NAP- 18), worm mortality remained at 25% when incubated for 48 or 72 h with no lethal effect observed at 24-h incubation period. Also, there was no difference in mortality rate between male and female *S. mansoni* adult worms.

Among the plant-derived 1, 4-NAPQs studied, lawsone (NAP-1) and lapachol (NAP-4), and the synthetic bis-lawsone (NAP-12) and 2, 6-naphthol (NAP-19) were inactive (0% mortality) against *S. mansoni*

Compound	Mortality ± SD ^a (%)		
	24h	48h	72h
Negative control (DMSO) ^b	0 ± 0.00	0 ± 0.00	0 ± 0.00
Positive control (PZQ) ^c	100 ± 0.00 ^d	100 ± 0.00 ^d	100 ± 0.00 ^d
Plant-derived			
1,4-naphthoquinones			
NAP-1 (Lawsone)	0 ± 0.00	0 ± 0.00	0 ± 0.00
NAP-2 (Naphthazarin)	100 ± 0.00 ^d	100 ± 0.00 ^d	100 ± 0.00 ^d
NAP-3 (Juglone)	100 ± 0.00 ^d	100 ± 0.00 ^d	100 ± 0.00 ^d
NAP-4 (Lapachol)	0 ± 0.00	0 ± 0.00	0 ± 0.00
Synthetic 1,4- and 1,2-naphthoquinones			
NAP-5	100 ± 0.00 ^d	100 ± 0.00 ^d	100 ± 0.00 ^d
NAP-6 (Menadione)	100 ± 0.00 ^d	100 ± 0.00 ^d	100 ± 0.00 ^d
NAP-7 (ACNQ)	25 ± 17.68	50 ± 17.68 ^d	100 ± 0.00 ^d
NAP-8	100 ± 0.00 ^d	100 ± 0.00 ^d	100 ± 0.00 ^d
NAP-9	100 ± 0.00 ^d	100 ± 0.00 ^d	100 ± 0.00 ^d
NAP-10	100 ± 0.00 ^d	100 ± 0.00 ^d	100 ± 0.00 ^d
NAP-11	50 ± 17.68 ^d	81 ± 17.68 ^d	100 ± 0.00 ^d
NAP-12	0 ± 0.00	0 ± 0.00	0 ± 0.00
NAP-13	0 ± 0.00	100 ± 0.00 ^d	100 ± 0.00 ^d
NAP-14	0 ± 0.00	25 ± 17.68	25 ± 17.68
NAP-15	100 ± 0.00 ^d	100 ± 0.00 ^d	100 ± 0.00 ^d
Synthetic naphthols			
NAP-16	100 ± 0.00 ^d	100 ± 0.00 ^d	100 ± 0.00 ^d
NAP-17	100 ± 0.00 ^d	100 ± 0.00 ^d	100 ± 0.00 ^d
NAP-18	0 ± 0.00	25 ± 17.68	25 ± 17.68
NAP-19	0 ± 0.00	0 ± 0.00	0 ± 0.00

^aMean percentage ±SD of two independent experiments using 8 adult *S. mansoni* worms in each experiment incubated for 24 – 72 h. Worms were considered dead when no movement was observed for at least 2 min

^bDMSO solvent (0.1%) + RPMI medium

^cPZQ (praziquantel) tested at a concentration of 12.5 µM.

^dIndicate statistically significant differences compared with negative control group (P<0.001)

Table 1: Effect of plant-derived and synthetic naphthoquinone and naphthol compounds at 100 µM concentration on mortality of *S. mansoni* adult worms *in vitro*.

when tested at 100 µM concentration for 24, 48 and 72 h (Table 1). The synthetic naphthol carboxylic acid (NAP-18) and 4-amino-1, 2-NAPQ (NAP-14) were inactive when incubated with worms for 24 h but exhibited 25% mortality rate upon increasing incubation period to 48 and 72 h. Decreased parasite motor activity was observed with synthetic 1, 2-NAPQ (NAP-13) when incubated for 48 and 72 h, and with methylene-bis-lawsone (NAP-11) after 72-h incubation only. All compounds that did not cause death of *S. mansoni* adult worms at 100 µM concentration (NAP-1, NAP-4, NAP-12, NAP-14, NAP-18 and NAP-19) when incubated for 24, 48 and/or 72 h showed decrease (25 to 100%) in motor activity (Table 2). The positive control (PZQ at 12.5 µM concentration) exhibited 100% mortality of worms after 24-h incubation period while the negative control (DMSO solvent + RPMI medium) caused no parasite mortality at all incubation periods tested (24 to 72 h) (Tables 1 and 2).

Among the plant-derived 1, 4-NAPQs, naphthazarin (NAP-2) was the most active antischistosomal compound with MLC of 25 µM and LC50 of 20.33 µM when tested for 24-h incubation period. For similar incubation period of 24-h, the most active synthetic compounds were, in order of potency, 1-amino-2-naphthol hydrochloride (NAP-16), 1,4-NAPQs NAP-5 and NAP-10 with MLC of 25 µM and LC50 of 11.77, 14.40 and 20.33 µM, respectively. Between 2- and 4-naphthols

Compound	Motor Activity Reduction ± SD ^a (%)					
	24h		48h		72h	
	Slight	Significant	Slight	Significant	Slight	Significant
Negative control (DMSO) ^b	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Positive control (PZQ) ^c	-	-	-	-	-	-
Plant-derived 1,4-naphthoquinones						
NAP-1 (Lawsonia)	100 ± 0.00 ^d	0 ± 0.00	75 ± 17.68 ^d	25 ± 17.68	75 ± 17.68 ^d	25 ± 17.68
NAP-2 (Naphthazarin)	-	-	-	-	-	-
NAP-3 (Juglone)	-	-	-	-	-	-
NAP-4 (Lapachol)	75 ± 17.68 ^d	0 ± 0.00	75 ± 17.68 ^d	0 ± 0.00	100 ± 0.00 ^d	0 ± 0.00
Synthetic 1,4- and 1,2-naphthoquinones						
NAP-5	-	-	-	-	-	-
NAP-6 (Menadione)	-	-	-	-	-	-
NAP-7(ACNQ)	25 ± 17.68	50 ± 17.68 ^d	0 ± 0.00	50 ± 17.68 ^d	-	-
NAP-8	-	-	-	-	-	-
NAP-9	-	-	-	-	-	-
NAP-10	-	-	-	-	-	-
NAP-11	0 ± 0.00	44 ± 17.68 ^d	0 ± 0.00	25 ± 17.68 ^d	-	-
NAP-12	100 ± 0.00	0 ± 0.00	100 ± 0.00 ^d	0 ± 0.00	100 ± 0.00 ^d	0 ± 0.00
NAP-13	0 ± 0.00	100 ± 0.00 ^d	-	-	-	-
NAP-14	50 ± 26.52 ^d	50 ± 26.52 ^d	25 ± 17.68	25 ± 17.68	0 ± 0.00	50 ± 17.68 ^d
NAP-15	-	-	-	-	-	-
Synthetic naphthols						
NAP-16	-	-	-	-	-	-
NAP-17	-	-	-	-	-	-
NAP-18	25 ± 17.68	25 ± 17.68	25 ± 17.68	44 ± 17.68 ^d	25 ± 17.68	44 ± 17.68 ^d
NAP-19	25 ± 17.68 ^d	0 ± 0.00	25 ± 17.68 ^d	0 ± 0.00	25 ± 17.68	0 ± 0.00

^aMean percentage ±SD of two independent experiments using 8 adult *S. mansoni* worms in each experiment incubated for 24-72 h. 'Slight' indicates a reduction in movement compared with the negative control and

'Significant' indicates minimal movement observed during 1 minute. 0 = no reduction; 25 to 100 are percentage reductions and '-' indicates worms dead.

^bDMSO solvent (0.1%) + RPMI medium.

^cPZQ (praziquantel) tested at a concentration of 12.5 µM.

^dStatistically significant differences compared with negative control group (P<0.001).

Table 2: Effect of plant-derived and synthetic naphthoquinone and naphthol compounds at 100 µM concentrations on motor activity reduction of *S. mansoni* adult worms *in vitro*.

with amino group at C-1 position, NAP-16 and NAP-17, the former derivative exhibited higher antiparasitic activity except at extended incubation periods of 48 and 72 h (LC50 = 10.04 vs 11.77 µM). For 72-h incubation period, the synthetic 1,4-NAPQ menadione (NAP-6) was the most active among the nineteen compounds tested against *S. mansoni* in the present screening with MLC of 12.5 µM and LC50 of 5.80 µM (Table 3).

Discussion

On-going research on plant-derived simple NAPQs have demonstrated their remarkable antiparasitic, antimicrobial and cytotoxic properties, and provided lead for development of new synthetic analogs with higher potency and lower toxicity [16, 21]. Recently, we reported potent antimalarial activity of a novel class of amino 1, 4-NAPQs against *Plasmodium falciparum* [32] and leishmanicidal activity of halogenated 1, 4-NAPQs against *Leishmania donovani* [34]. The present investigation is a continuation of our research on antiparasitic activity of NAPQs which compares antischistosomal activity of nineteen plant-derived and synthetic simple NAPQs and naphthols (Figure 1) against *S. mansoni* adult worms under *in vitro* experimental conditions.

WHO's Special Program for Research and Training in Tropical Diseases (TDR) has outlined the following definition and activity criteria for hit and lead compound for the control of schistosomiasis: *in vitro* studies, 100% mortality of adult worms at a concentration of ≤ 5 µg/ml when incubated for 48 h and *in vivo* studies, 80% worm reduction after five injections at a dose of 100 mg/ kg body-weight

Compound	MLC (µM)			LC ₅₀ (µM)		
	24h	48h	72h	24h	48h	72h
PZQ ^c	3.12	3.12	1.56	0.86	0.86	0.78
Plant-derived 1,4-naphthoquinones						
NAP-2 (Naphthazarin)	25.00	25.00	25.00	20.33	11.77	11.77
NAP-3 (Juglone)	100.00	100.00	50.00	34.16	32.14	25.00
Synthetic 1,4- and 1,2-naphthoquinones						
NAP-5	25.00	25.00	25.00	14.40	12.98	12.98
NAP-6(Menadione)	50.00	25.00	12.50	11.77	10.64	5.80
NAP-7 (ACNQ)	> 100	> 100	100.00	70.92	53.85	52.15
NAP-8	50.00	50.00	50.00	22.24	20.22	10.22
NAP-9	50.00	50.00	50.00	37.94	37.94	25.97
NAP-10	25.00	25.00	25.00	20.33	12.50	12.50
NAP-13	100.00	50.00	50.00	54.18	26.93	25.02
NAP-14	> 100	> 100	100.00	84.30	84.30	58.71
NAP-15	100.00	50.00	50.00	48.47	37.94	37.94
Synthetic naphthols						
NAP-16	25.00	25.00	25.00	11.77	11.77	11.77
NAP-17	100.00	50.00	50.00	20.15	10.04	10.04

^aMLC is the minimal lethal concentration needed to kill all 16 adult worms after 24-, 48- or 72-h incubation period.

^bLC50 is the lethal concentration needed to kill 50% of the adult worms after 24-, 48- or 72-h incubation period.

^cPZQ (praziquantel) positive control.

Table 3: Minimal lethal concentration (MLC)^a and 50% Lethal Concentration (LC50)^b of plant-derived and synthetic naphthoquinone and naphthol compounds active against *S. mansoni* adult worms *in vitro*.

of test animal/day [30,40]. Among the nineteen plant-derived and synthetic simple NAPQs and naphthols tested in the present *in vitro* study with *S. mansoni* adult worms, four compounds met the WHO/TDR *in vitro* criterion for “hit” and lead compound: plant-derived naphthazarin (NAP-2), two synthetic 1,4-NAPQs (NAP-5 and NAP-6) and synthetic 1-amino-2-naphthol hydrochloride (NAP-16) with 100% mortality of worms at 25 μ M concentration (equivalent to 4.75, 3.95, 4.30 and 4.9 μ g/ml, respectively) when incubated for 48 h (Table 3). These are recommended for further *in vivo* studies in animal models of schistosomiasis. Among the above four active compounds, menadione (NAP-6) is vitamin K3 which is a metabolite of orally ingested vitamin K [41] and found to be the third most active compound among the lead candidates that met WHO/TDR *in vitro* antiparasitic criteria against *S. mansoni* adult worms. It is noteworthy that in a recent study, the plant-derived plumbagin (Figure 1) has also met the above WHO/TDR criterion at 4.70 μ g/mL concentration when incubated for 48 h [30] and it is reported to be more active than the currently used antischistosomal drug PZQ under the *in vitro* experimental conditions employed [29].

Lawsonone (NAP-1) and lapachol (NAP-4) among the plant-derived 1,4-NAPQs, and bis-lawsonone (NAP-12) and 2,6-naphthol (NAP-19) among the synthetic compounds evaluated in this investigation were completely inactive (0% mortality) against *S. mansoni* when tested at 100 μ M concentration when incubated for 24, 48 and 72 h (Table 1). In an earlier study [31], the potassium salt of lapachol (NAP-4) was found to be active against *S. mansoni* eggs with $LC_{50} < 3$ μ g/ml which may be attributable to higher solubility of the salt tested.

Although plant-derived 1,4-NAPQ juglone (NAP-3), synthetic halogenated 1,4-NAPQs (NAP-8, NAP-9 and NAP-10), 1-amino-4-naphthol hydrochloride (NAP-17) and 1,2-NAPQ sulfonic acid sodium salt (NAP-15) caused 100% mortality of *S. mansoni* adult worms when incubated for 24, 48 and 72 h (Table 1), they failed to meet the WHO/TDR criteria for consideration as “hit” and lead compound for further antischistosomal study (Table 3). Similarly, the remaining synthetic 1,4-NAPQs, herbicide ACNQ (NAP-7) and methylene-bis-lawsonone (NAP-11), and 1,2-NAPQs (NAP-13 and NAP-14) and naphthol carboxylic acid (NAP-18) did not meet the WHO/TDR criteria for further study against schistosomiasis.

Several structure-activity relationship (SAR) studies with NAPQs for identifying best candidates for treating parasitic diseases have been conducted earlier [28,42]. However, to-date no clinically viable antischistosomal compound has emerged from such endeavors. In the present investigation, comparison of chemical structures of active compounds against *S. mansoni* adult worms that met the WHO/TDR *in vitro* criterion for further antischistosomal study with those of lesser or no such activity (Figure 1 and Table 1)[30] indicated the dependence of antiparasitic activity on the number and nature of substituents in the parent NAPQ molecule. Thus, retention of antischistosomal activity with the addition of methyl group at C-2 position in the parent 1, 4-NAPQ molecule, as in menadione (NAP-6) and plumbagin, and hydroxyl group(s) at C-5 or C-5 and C-8 positions, as in plumbagin and naphthazarin (NAP-2), was observed. This follows their reported NADPH-dependent oxidoreductase enzyme inhibitory activity (naphthazarin, 100%, plumbagin 85% and menadione 82%) [43]. However, there was substantial loss in antiparasitic activity when methyl group was absent at C-2 position with hydroxyl group at C-5 position, as in juglone (NAP-3) and complete loss of activity when hydroxyl group was present at C-2 position instead of methyl group, as in lawsonone (NAP-1) and lapachol (NAP-4). This indicates the importance of location of hydroxyl (a strong electron donor) and methyl (a weak electron donor) groups in stabilizing the quinone

moiety in the parent 1, 4-NAPQ structure. Such differential bioactivity of 1, 4-NPQs with the two functional groups, hydroxyl and methyl, at C-2/C-5 positions has also been observed with plumbagin (as both antimutagenic and antioxidant), juglone (antimutagenic only) and menadione (antioxidant only) when tested in the Ames mutagenic and DNA oxidative damage assays [44]. Other comparative studies with lawsonone (NAP-1) and lapachol (NAP-4) have confirmed such inhibitory effect of hydroxyl group at C-2 position on antileishmanial activity [45] and pro-oxidant, cytochrome P450-linked monooxygenase systems [46].

The mechanism by which NAPQs exerts their *in vitro* schistosomicidal effect is not clear at the present time. However, it is generally recognized that mechanisms of antiparasitic activity involve modulation of parasite redox cycling (reduction and oxidation cycle of quinones in flavin enzymes such as NADPH-cytochrome P450 and mitochondrial NADH-ubiquinone oxidoreductase) and production of reactive oxygen species (ROS) leading to severe oxidative stress by oxidizing essential macromolecules, such as proteins, lipids and DNA [47,48]. Studies have shown that NAPQs are potent electrophiles and they are also capable of directly reacting with thiol groups of *S. mansoni* parasite proteins and glutathione resulting in inhibition of enzymes essential for parasite survival [43,49]. While NAPQs are capable of acting both as modulator of redox cycling and as electrophiles, one of these two mode of actions may play a dominant role in eliciting antiparasitic activity of individual compounds based on their chemical structure [40,50,51].

In conclusion, the present *in vitro* antischistosomal studies with nineteen plant-derived and synthetic simple NAPQs and naphthols against *S. mansoni* adult worms identified plant-derived naphthazarin (NAP-2), synthetic NAPQs (NAP-5 and NAP-6) and 1-amino-2-naphthol hydrochloride (NAP-16) as hit and lead compounds that met the WHO/TDR *in vitro* criterion for further *in vivo* studies in animal models of schistosomiasis. Although several plant-derived and synthetic NAPQs tested in this study are known to exhibit diverse antiparasitic effects, the structural requirement for this group of antischistosomal compounds appears to be different from those required for their activity against other parasites afflicting humans. While this is the first report on the antiparasitic activity of synthetic amino derivatives of simple naphthols, their mode of action against schistosomes remains to be delineated. Based on the results of this *in vitro* investigation, further chemopreventive *in vivo* studies are called for on the plant-derived naphthazarin (NAP-2) and synthetic menadione (NAP-6), 1,4-NAPQ (NAP-5) and 1-amino-2-naphthol (NAP-16), all of which have met the WHO/TDR *in vitro* criterion for their consideration as lead schistosomicidal candidates.

Acknowledgements

GJK thanks Dean Anthony Wutoh for continued support and Dr. Xionghao Lin for assistance in drawing of chemical structures. This research was supported in part by São Paulo Research Foundation, Brazil - FAPESP (Process: 2013/11164-4; 2014/02018-7), National Counsel of Technological and Scientific Development, Brazil – CNPQ and the Howard University Fund for Academic Excellence.

Conflict of Interest

The authors declare that they have no conflict of interest.

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