Activities of Naphthoquinones against *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae), vector of dengue and *Biomphalaria glabrata* (Say, 1818), intermediate host of *Schistosoma mansoni*

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Abstract

Larvicidal (against *Aedes aegypti* Linnaeus, 1762) and molluscicidal (against *Biomphalaria glabrata* Say, 1818) activities of several natural and synthetic naphthoquinones were measured, with significant results. The best larvicidal compound is 3-bromojuglone, while the better molluscicides are 2-bromo- and 3-bromo-5-acetoxy-1,4-naphthoquinones together with the 3-bromo-5-methoxy derivative. The present results reinforce the potential use of substituted hydroxyquinones, their salts and halogenated quinones as very promising compounds against 4th instar larves of *Aedes aegypti*, the vector of dengue and against adult snail of *Biomphalaria glabrata*.

Key words: lapachol, isolapachol, juglone, bromonaphthoquinones, larvicidal activity, *Aedes aegypti*, *Biomphalaria glabrata*, dengue, schistosomiasis.

1. Introduction

Dengue

Mosquitoes are responsible for the spread of more diseases than any other group of arthropods. Of particular interest is *Aedes aegypti* because of its role as a vector for the arboviruses responsible for yellow fever and dengue haemorrhagic fever (DHF), both of which are endemic to Central and South America, Asia and Africa (Guzman and Kouri, 2003).

Dengue viruses occur as four antigenically related but distinct serotypes, which cause a broad range of diseases, including clinically asymptomatic forms, classic dengue fever (characterized by the sudden onset of fever, headache, retro-orbital pain and myalgia), and the more severe forms such as dengue haemorrhagic fever-dengue shock syndrome (Gibbons and Vaughn, 2002).

The incidence of dengue has grown dramatically around the world in recent decades (Kroeger and Nathan, 2006; WHO, 2008). Some 2.5 billion people – two fifths of the world's population – are now at risk from dengue. WHO estimated there may be 50 million dengue infections worldwide, every year. In 2007 alone, there were more than 890,000 reported cases of dengue in the Americas, of which 26,000 cases were DHF. The disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-east Asia and the Western Pacific, the last two being the most seriously affected (WHO, 2008). Today, dengue is the most important mosquito-borne viral disease affecting humans.

The increasing incidence, intensity and geographical expansion of dengue epidemics pose a growing threat to the health and economic well-being of populations living in endemic areas, where the introduction of new virus strains to

regions affected by existing serotypes is a risk factor for outbreaks and severe disease. Without vaccines, drugs, and even useful diagnostic tests, the only available responses to the challenge of dengue are clinical management, vector control for prevention and a surveillance system to identify outbreaks at an early and treatable stage (WHO, 2008).

In 2008, Brazil is experiencing its second most devastating dengue epidemic, with 734,000 reported dengue cases through October this year. However, this represents the most severe dengue epidemic in terms of deaths: 212 deaths were recorded, primarily in children. Deaths have been mainly reported for infections with dengue virus type 2 (DENV-2) in endemic areas that had previously experienced epidemics of DENV-3. Southeastern and Northeastern Brazilian cities have been most affected, and in some cities, the incidence has increased more than 2,000-fold. Additionally, and unfortunately, almost no results from scientific investigations on the dengue vector have been taken into account in local dengue control campaigns (de Oliveira, 2008*).*

The control of mosquito-borne diseases by destruction of the aquatic stages is a rapid and efficient mean of reducing and eliminating the transmission of the disease. The use of insecticides and many synthetic agents have been developed and employed in the field with considerable success. During outbreaks, emergency vector control measures can also include broad application of insecticides as space sprays using portable or truck-mounted machines or even aircraft. However, the mosquito-killing effect is transient, variable in its effectiveness because the aerosol droplets may not penetrate indoors to microhabitats where adult mosquitoes are sequestered, and the procedure is costly and operationally difficult. Regular monitoring of the vectors' susceptibility to widely used insecticides is necessary to

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ensure the appropriate choice of chemicals. Active monitoring and surveillance of the natural mosquito population should accompany control efforts to determine program effectiveness (WHO, 2008). However, one major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment.

The toxicity problem, together with the growing incidence of insect resistance, has called attention to the need for novel insecticides and for more detailed studies of naturally-occurring insecticides (Ansari et al., 2000; Casida and Quistad, 2000). Identification of novel effective mosquitocidal and larvicidal compounds is essential to combat increasing resistance rates, concern for the environment and food safety and to replace many unacceptable organophosphates and organochlorines and highly expensive synthetic pyrethroids. To be highly competitive and effective, the ideal chemical should have a combination of toxic effects and residual capacity. Acute toxicity is required at doses comparable to some commercial synthetic insecticides (Shaalan et al., 2005).

Schistosomiasis

Schistosomiasis is a major source of morbidity and mortality in developing countries including Africa, South America, the Caribbean, the Middle East, and Asia. However, with the increase in immigration from, and tourism to, these endemic areas, cases of schistosomiasis are now occurring throughout the developed world (Chitsulo, L., et al., 2000). While a number of species of *Schistosoma,* such as *S. haematobium, S. japonicum, S. mekongi* and *S. intercalatum*, can cause systemic human diseases, the main agent of human schistosomiasis is *S. mansoni*. This complex helminth infection requires alternate development between an intermediate snail host, belonging to the genus

Biomphalaria, and the final mammalian host, both of which are indispensable for maintaining the parasite. In humans, the nature of the disease is largely immunological and only indirectly due to the parasitic worm itself; the pathological lesions emanate from inflammatory reactions to the large number of parasite eggs which are retained in host tissues rather than excreted with the faeces or urine (to infect the snail). The majority of *Schistosome* species causes intestinal schistosomiasis, where the adult worm lives in the mesenteric venules of the host. However, the highest number of human infections is caused by *S. haematobium*, which has a predilection for the blood vessels around the bladder and causes urinary disease (WHO, 2005a). In Brazil, high infection rates persist among both the rural and urban poor (Kloos et al., 2008).

Currently, schistosomiasis control relies almost entirely on chemotherapy and yet only one drug, praziquantel, is available (Bergquist, 2008). This is a potentially dangerous situation which points to the need to identify effective new drugs. With regard to snail control, both environmental management and use of focal chemical molluscicides should be evaluated (WHO, 2005a). The high cost of synthetic molluscicides, together with the well-known environmental hazardous effects of the most used ones, for example, copper sulfate and niclosamide (Oliveira-Brett et al., 2002), and the possible development of snail resistance, promoted a considerable and systematic search for cheaper alternatives.

One strategy to discover new therapeutic leads is to investigate classes of compounds potentially bioactive or old active compounds for alternative uses.

As part of our continuing program of screening natural and synthetic quinones for molluscicidal and larvicidal activities (dos Santos and Sant'Ana, 1999, 2001; Lima et al., 2002a, Lima et al., 2002b, Lima et al., 2004, Ossowski et al., 2008), several

naphthoquinones (Figure 1) have been assayed for lethality against adults of *Biomphalaria glabrata* and 4th instar larves of *Ae. aegypti*, the vector of dengue. Lapachol (**1)** and its analogues are known to possess antitumor, antibiotic, antimalarial (de Andrade-Neto et al., 2004), anti-inflammatory, antiulcer (Hussain et al., 2007), leishmanicidal (Lima et al., 2004) and molluscicidal activities (dos Santos et al., 2000; 2001, Lima et al., 2002a, 2002b). Isolapachol (**2**) and its acetylderivative (**3**) also present significant activity against the mollusk *Biomphalaria glabrata* (adult snail and egg masses) (dos Santos et al., 2000 and 2001, Lima et al., 2002a) and against *Leishmania amazonensis* and *L. braziliensis* (Lima et al., 2004). Apart from that, other 2-hydroxynaphthoquinones were shown to exert insecticidal activities, for instance, quinones extracted from *Calceolaria andina* L., against tobacco cultures insects (Khambay et al, 1997; Khambay and Jewess, 2000). Very recently, naphthazarin (5,8-dihydroxy-1,4-naphthoquinone) derivatives (alkannin and shikonin) have shown activity against *Culex pipiens* (Michaelakis et al., 2008).

5-Hydroxy-1,4-naphthoquinone, juglone (**5**), which is found in walnut hulls and leaves, is toxic and behaves as a weak tumor promoter in mouse skin (Monks et al., 1990). Some toxic effects attributed to juglone include inhibitory effects on insect larval development and insect flight muscle mitochondria (Hejl and Koster, 2004). Other quinones (cordiaquinones) were also assayed as larvicides, using a different protocol in comparison with juglone (Ioset et al, 2000) as well as tectoquinones (Cheng et al., 2008) and anthraquinones, like emodin and derivatives (Yang et al, 2003).

2. Materials and Methods

2.1 Compounds

Lapachol (**1**) is from natural origin, extracted from the heartwood of several plants of the Bignoniaceae family, mainly from *Tecoma* and *Tabebuia* species. Isolapachol [2-hydroxy-3-(3-methyl-1-butenyl)-1,4-naphthoquinone] was easily synthesized, following established procedures, by reaction of 2-hydroxy-1,4-naphthoquinone (Aldrich) and isovaleraldehyde (Aldrich), in acidic medium (Hooker, 1936). Acetylisolapachol (**2**) was prepared by usual procedures (anhydrous sodium acetate and pyridine) (Lima et al., 2002a). Isolapachol lithium salt (**3**) was prepared by the use of an equivalent amount of LiOH in ethanol, distillation of the solvent and several washings with cold ether to eliminate residues of the initial compound and di-hydrolapachol (**4**) by catalytic hydrogenation, using PtO_2 on charcoal in ethanol) (Lima et al., 2002b). All the compounds show analytical and spectral (IR, NMR) data in full accord with the indicated structures.

All the juglone (**5**) derivatives **6-13** used in this study are known compounds and were prepared according to the methods described in the literature. Juglone (**5**) is a commercial material (Sigma-Aldrich, St. Louis, USA) and, when needed in large scale, was prepared according to the method by Tietze (Tietze et al., 2005). Acetylation of juglone under standard conditions afforded juglone acetate (**6**) (Fieser et al., 1937). Compound **7** was prepared by methylation of juglone (methyl iodide, silver (I) oxide) (Tietze et al., 2005; Garden and Thomson, 1957); compound **8**, prepared according to Grunwell (Grunwell et al., 1991) by hydrolysis of the corresponding acetate **9** (Grunwell et al., 1991). Methylation of **8** led to the corresponding methyl ether **10** (Jung and Hagenah, 1987; Echavarren et al., 1997). The 3-bromojuglone derivatives were prepared by selective bromination of juglone according to Brimble (Brimble and Brenstram, 2001), which yielded **11** as the major

isomer. From **11,** either by standard acetylation or methylation, compounds **12** (Tietze et al., 2007) or **13**, respectively, were obtained (Grunwell et al., 1991; Parker and Sworin, 1981). All the physico-chemical (IR, MS, ^{1}H-NMR, ^{13}C-NMR) data are compatible with the chemical structures of the compounds.

Culture media, parasites, assays.

Assay of larvicidal activity

The mosquito strains of Ae. aegypti, evaluated in the present study, are from Maceio, Alagoas State, Brazil, city very much affected by dengue, and the colonies are kept for more than three years in the laboratory of Entomology of Instituto de Química e Biotecnologia, Universidade Federal de Alagoas, Maceió, Alagoas. Eggs of this resistant population of Ae. aegypti were hatched by submersion in dechlorinated tap water at a temperature of 26 ± 1°C and fourth stage larvae were collected 72 h after hatching. Each bioassay was conducted on early fourth instar mosquito larvae collected from the same breeding site and at the same time, using standard methods (WHO, 2005b). Test compounds were dissolved in an appropriate mixture of dimethylsulphoxide (DMSO) and dechlorinated water, and the resulting solution diluted with dechlorinated water to give a final concentration of DMSO of 1 %. An appropriate volume of this test solution was added to a test tube containing twenty five larvae of Ae. aegypti in sufficient dechlorinated water (containing 1 % DMSO) in order to give a final volume of 100 mL. The test tubes were incubated in darkness at 25 - 27°C for 24 h and larval mortality was observed under laboratory lighting conditions. The positive control involved treatment with 3 µg mL^{-1} of Temephos [(O,O'-(thiodi-4,1-phenylene)bis(O,O-dimethylphosphorothiolate)],commercial sample obtained at FUNASA, Brazil, in dechlorinated water and the negative control was

water containing 1 % DMSO following the protocol reported by de Omena (de Omena et al., 2007). Temephos is an OP pesticide. It acts by inhibiting acetylcholinesterase, resulting in acetylcholine accumulation in neuromuscular synapses. The acute toxic effects of OP pesticides are caused by the hyperstimulation of muscarinic and nicotinic receptors, resulting in symptoms that range from increased secretions to death by respiratory depression (Porretta et al., 2008). The differences in toxicity values for Temephos in the present case and others (Porretta et al., 2008) are related to susceptible and resistant mosquito populations. In the present work, *Ae. aegypti* population is more resistant, so, the LC for Temephos is higher. LC_{10}, LC_{50} and LC_{90} were calculated using probit analysis with a reliability interval of 95 % (IC 95 %) (McLaughlin et al., 1991).

Assay of molluscicidal activity

A population of adult *B. glabrata* (Say, 1818) snails was maintained according to established procedures (dos Santos and Sant'Ana, 1999). Stock solutions of samples were prepared at different concentrations using dechlorinated water containing 0.1 % (v/v) dimethylsulphoxide (DMSO). Lethality against adult snails was determined (four replicates for each sample) as previously described (dos Santos and Sant'Ana, 1999). Both positive (niclosamide® at 3 μg mL^{-1}) and negative [dechlorinated water containing 0.1 % (v/v) DMSO] controls were included in the assay. Niclosamide has been in use as a molluscicide since the 1960s (Oliveira-Brett et al., 2002; Oliveira and Paumgartten, 2000) and is still the molluscicide of choice. Niclosamide is highly active at all stages of the snail life cycle, killing 100 % of *Biomphalaria glabrata* adults and egg masses at concentrations as low as 1.5 ppm after a two-hour exposure (WHO, 1965). Samples that produced mortality after 96 h of at least 40 % against

egg adult snails were submitted to accurate bioassay, as previously described (dos Santos and Sant'Ana, 1999).

Statistical methods

The LC_{10}, LC_{50} and LC_{90} values, as well as their 95 % intervals of confidence [IC_{95}], were determined through probit analysis (Finney, 1971) of the mortality data derived from the bioassays. When the data were insuficient to calculate an IC_{95}, the lethal concentration was obtained by logit transformation (Hafner et al., 1977). Since mortalities of the test organisms also occurred in the control treatments, the percentage mortality values were corrected using the Abbott (Abbott, 1925) formula.

3. Results and discussion

In this report, we evaluated the effect of several naphthoquinones against 4th instar larvae of *Ae. aegypti*, the vector of dengue and against adult snail of *Biomphalaria glabrata*. The assayed compounds belong to the general class of 1,4-naphthoquinones (NQ). They can be classified in two sub-classes. The first sub-class has oxygenated substituents at position 2 (compounds **1**-**4**), with additional saturated or unsaturated lipophilic side chains, located at C-3. T The second one is derived from 5-hydroxy-1,4-NQ (juglone) (**5**), with some compounds brominated at C-2 or C-3. The results (LC_{10}, LC_{50} and LC_{90} values) with corresponding 95 % IC for compounds **1** – **13** are listed (Table 1) that also shows the positive and negative controls (temephos for *Ae. aegypti* and niclosamide for *B. glabrata*). A single non-hydroxylated quinone (menadione, **14**) was also tested. To allow comparison with data from the literature, the results are reported in ppm as shown in Table 1(column 2).

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As pure compounds were employed, LC_{50} values < 5 ppm and LC_{90} values < 10 ppm for adult snails of *B. glabrata* and *Ae. aegypti* larvae were indicative of promising active compounds, while inactive compounds showed LC_{90} values >100 ppm.

Based on this and examining Table 1, compounds **3**, **4**, **5**, **6**, **8**, **9** and **11** were active against both targets, with compounds **12** and **7** being borderline. Apart from those, compounds **1**, **2**, **10**, **12**, **13** and **14** are significantly active molluscicides. Comparing both activities, with the exception of compound **11**, *Biomphalaria glabrata* seems to be more susceptible to quinones than *Ae. aegypti*.

Concerning the compounds as larvicides, within the quinones, based on µM concentrations (LC_{50} values in parenthesis, column 2), to allow better comparison, the order of activity (LC_{50}), from the more to the less active is:

11 (3.46) > **9** (3.98) > **8** (4.64) > **3** (15.24) > **4** (19.45) > **5** (20.61) > **6** (21.08) > **13** (21.62) > **12** (24.79) > **2** (33.94) > **10** (36.48) > **7** (42.12) > **14** (86.93) > **1** (108.7)

Lapachol was inactive, while isolapachol and derivatives stayed in the intermediate region. Compounds **11, 9** and **8** were the most active, with similar significant activity. Examining structural details, despite having only one representative of a non-oxygenated quinone (menadione, **14**), it seems that the presence of a further oxygen in the structure enhances the larvicidal effects. Juglone (**5**) and its O-acetylated (**6**) derivative have similar activity and are more active as larvicidal than its O-methylated (**7**) derivative, which suggests that a free OH (although strongly chelated), as such in **5**, or in the form of the acetylated precursor **6** (which might act as a prodrug-like species, being easily cleaved by esterases) is essential for the larvicidal activity. The hypothesis of partial chemical hydrolysis of the acetate in the aqueous media before

absorption should not be discarded. Introduction of a bromine group enhances the activity, for instance **5** vs. **8** and vs. **11**, or **6** vs. **9** or less marked vs.**12**, or **7** vs.**13**, except for the O-methoxy derivative **10**. These results indicate that the bromo substituent, in bromoquinones, is highly reactive because chemically it behaves as a vinylogous acid bromide and this high chemical reactivity might also occur in the biological target. Some bromonaphthoquinones are more active than the insecticides chlorpyrifos (LC_{50} = 1.1 ppm; LC_{90} = 2.5 ppm) (Cheng et al., 2008), pirimiphos - methyl (LC_{50} 0.16 ppm [0.12-0.19]) (Yang et al., 2003) and temephos (Table 1), assayed in the present case against the same resistant *Ae. aegypti* populations. Better activity is also verified when compared with anthraquinones (including hydroxyanthraquinones), where larvicidal activity values were higher than 20 ppm, including emodin (LC_{90} = 19.1 ppm) (Yang et al. 2003; Cheng et al., 2008), and other quinones, like tectoquinone (Cheng et al., 2008), which, in a similar test, showed LC_{90} of 8.8 ppm. Concerning cordiaquinones, non-hydroxylated quinones, the activities range between 12.5 and 25 ppm, in the majority of cases, less significant than the herein reported results (Ioset et al., 2000). Despite a different mosquito target (*Culex pipiens*), quinones [shikonin (3.9 ppm), shikalkin (8.73 ppm) and alkannin 12.35 ppm)] revealed generally high efficacy, in the range obtained in the present study, although they are structurally more complex (Michaelakis et al., 2008).

Molluscicidal Activities

Concerning molluscicidal activities, all the juglone derivatives showed significant molluscicidal activity (LC_{50} < 5 ppm; LC_{90} < 10 ppm). Some of the quinones presented LC_{50} values lower than 1 ppm [**4** (0.98), **9** (0.948), **10** (0.746), **12** (0.893), **13** (0.475)]. The sequence of molluscicidal activity (LC_{90} in µM) is:

13 (1.79**) > 10** (2.80**) ~ 12** (3.04**) > 9** (3.22**) > 4** (4.01**) > 8** (4,69**) > 6** (5.62**) ~ 2** (5.84**)

> 5 (8.48**) > 1** (9.31**) > 11** (9.90**) > 3** (12.29**) > 7** (13.08**) > 14** (17.43**)

Compounds **13**, **12**, **10**, **9**, **4** and **8** can be regarded as significantly active, especially considering that the herein presented values are related to LC_{50} and given in μM (LC_{50} < than 5 μM). Interestingly, and contrary to the above results as larvicidal agents, the methyl ethers **10** and **13** and the esters **12** as well as **9** are very active. The parent compounds bearing a hydroxy group (**8** and **11**) are less active, and in the parent juglone, with the OH and lacking the bromo atom, molluscicidal activity is lower. At this point no definitive conclusions in term of structure-activity relationships can be drawn and we are performing additional studies which will be reported in due course.

Literature data are reported as LC_{50} and in ppm. These values (Table 1) are similar to jatrophone (dos Santos and Santana, 1999) (LC_{50} = 1.16 ppm) and to extracts of *Annona crassifolia* (LC_{50} = 2.34 ppm) (dos Santos and Sant'Ana, 2001). The same trend concerning bromosubstituted naphthoquinones, herein presented, was envisaged with 2-bromo-3-methoxy-1,4-naphthoquinone (LC_{90} = 4.2 ppm) but not with 2,3-dibromo-1,4-naphthoquinone (LC_{90} = 28.4 ppm) (Camara et al., 2008). Despite being less active than niclosamide (LC_{50} = 0.06 ppm; LC_{90} = 0.10 ppm) (Oliveira and Paumgartten, 2000), they are much better than those presented by various recognized molluscicides such as muzigadial (LC_{50} = 5 to 10 ppm), warburganal (LC_{50} = 2 ppm), mukaadial (LC_{50} = 20 ppm) (Marston and Hostettman, 1985), chromene and pyrano[2,3-c]pyrazole derivatives (LC_{90} > 18 µM) (Abdelrazek et al, 2007), alkaloids like solanandaine, solasonine, solamargine (Silva et al., 2008),

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and some Baylis–Hillman adducts (LC_{90} = 9.23 ppm) (Vasconcellos et al., 2006), among others.

Conclusions

Results indicate that naphthoquinones, compared with other natural compounds with larvicidal activity, are very toxic against mosquito larvae. The present *in vitro* results reinforce the potential use of substituted hydroxyquinones and derivatives as very promising larvicidal drugs and suggest a continuing study within this class of compounds, with the aim of designing new products with better properties. Further studies on the insecticidal mode of action, their effects on non-target organisms and the environment, and formulations for improving the insecticidal potency and stability are needed for their practical use as a naturally or hemisynthetic occurring mosquito larval control agent, in search for an efficient biocontrol agent against larvae of the mosquito *Ae. aegypti* and snails of *B. glabrata*.

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6. Table(s)

Table 1: Larvicidal (against *Aedes aegypti*) and molluscicidal (against *B. glabrata*) activities of Quinones. Lethal Concentration, in ppm, (LC_{50} and LC_{90}, in μM).

COMPOUNDS	Larvicidal, in ppm [IC_{95}] (LC_{90} μM)	Molluscicidal, in ppm [IC_{95}] (corresponding LC, μM)
Lapachol (**1**)	LC_{50} **26.3*** (**108.7***) (Rodrigues et al., 2005)	LC_{10} **1.053** [0.364; 1.581] LC_{50} **2.252** [1.445; 2.806] (**9.31**) LC_{90} **4.818** [3.884; 7.351] (**19.91**) (Lima et al., 2002b)
Isolapachol acetate (**2**)	LC_{10} **5.753** [4.981 – 6.401] LC_{50} **9.640** [8.882 – 10.521] (**33.94**) LC_{90} **16.154** [14.286 – 19.169] (**56.88**)	LC_{50} **1.66** (**5.84**) LC_{90} **3.08** (**10.84**) (Lima et al., 2002a)
Isolapachol, lithium salt (**3**)	LC_{10} **2.02** [1.26 - 2.63] LC_{50} **3.78** [2.99 - 4.51] (**15.24**) LC_{90} **7.06** [5.83 - 9.46] (**28.47**)	LC_{10} **1.974** [1.424; 2.329] LC_{50} **3.049** [2.668; 3.439] (**12.29**) LC_{90} **4.710** [4.056; 6.258] (**18.99**)
Dihydrolapachol (**4**)	LC_{10} **2.485** [1.040 – 3.648] LC_{50} **4.745** [3.360 – 5.933] (**19.45**) LC_{90} **9.062** [7.086 – 15.744] (**37.14**)	LC_{50} **0.98** (**4.01**); LC_{90} **1.98** (**8.11**) (Lima et al., 2002) LC_{50} (**7.6**); LC_{90} (**12.5**) (Silva et al., 2005)
5-Hydroxy-1,4-NQ (juglone) (**5**)	LC_{10} **1.937** [1.389 – 2.397] LC_{50} **3.587** [3.035 – 4.065] (**20.61**) LC_{90} **6.641** [5.882 – 7.774] (**38.17**)	LC_{10} **0.237** [0.170 – 0.299] LC_{50} **1.475** [1.304 – 1.654] (**8.48**) LC_{90} **9.352** [7.664 – 11.942] (**53.75**)
5-Acetoxy-1,4-NQ (**6**)	LC_{10} **2.593** [1.415 – 3.758] LC_{50} **4.553** [2.405 - 5.844] (**21.08**) LC_{90} **7.993** [6.178 – 20.475] (**37.00**)	LC_{10} **0.390** [0.327 – 0.453] LC_{50} **1.213** [1.098 – 1.332] (**5.62**) LC_{90} **3.774** [3.392 – 4.252] (**17.47**)
5-Methoxy-1,4-NQ (**7**)	LC_{10} **5.789** [5.162 - 6.294] LC_{50} **7.919** [7.415 - 8.419] (**42.12**) LC_{90} **10.833** [10.081 - 11.900] (**57.62**)	LC_{10} **0.967** [0.865 – 1.064] LC_{50} **2.459** [2.324 – 2.599] (**13.08**) LC_{90} **6.252** [5.721 – 6.926] (**33.25**)
2-Bromo-5-hydroxy-1,4-NQ (**8**)	LC_{10} **0.773** [0.664 - 0.868] LC_{50} **1.391** [1.220 - 1.690] (**4.64**) LC_{90} **2.501** [1.982 - 3.718] (**6.71**)	LC_{10} **0.317** [0.277 – 0.358] LC_{50} **1.181** [1.092 – 1.276] (**4.69**) LC_{90} **4.404** [3.947 – 4.975] (**17.48**)
2-Bromo-5-acetoxy-1,4- NQ	LC_{10} **0.301** [0.171 - 0.442] LC_{50} **1.170** [0.891 - 1.451] (**3.98**)	LC_{10} **0.408** [0.355 – 0.457] LC_{50} **0.948** [0.883 – 1.014] (**3.22**)

|		
(9)	LC$_{90}$ **4.550** [3.636 - 6.078] (**15.48**)	LC$_{90}$ **2.205** [2.018 – 2.443] (**7.50**)
2-Bromo-5-methoxy-1,4- NQ (10)	LC$_{10}$ **5.538** [4.686 - 6.253] LC$_{50}$ **9.703** [8.827 - 10.749] (**36.48**) LC$_{90}$ **16.999** [14.739 - 20.844](**63.91**)	LC$_{10}$ **0.356** [0.315 – 0.394] LC$_{50}$ **0.746** [0.703 – 0.788] (**2.80**) LC$_{90}$ **1.563** [1.458 – 1.695] (**5.88**)
3-Bromo-5-hydroxy-1,4- NQ (11)	LC$_{10}$ **0.538** [0.459 - 0.599] LC$_{50}$ **0.873** [0.807 - 0.955] (**3.46**) LC$_{90}$ **1.417** [1.242 - 1.739] (**5.62**)	LC$_{10}$ **1.100** [0.993 – 1.201] LC$_{50}$ **2.494** [2.365 – 2.628] (**9.90**) LC$_{90}$ **5.654** [5.213 – 6.212] (**22.44**)
3-Bromo-5-acetoxy-1,4-NQ (12)	LC$_{10}$ **5.084** [4.551 - 5.529] LC$_{50}$ **7.287** [6.819 - 7.784] (**24.79**) LC$_{90}$ **10.445** [9.616 - 11.645] (**35.53**)	LC$_{10}$ **0.481** [0.441 – 0.518] LC$_{50}$ **0.893** [0.850 – 0.937] (**3.04**) LC$_{90}$ **1.659** [1.559 – 1.780] (**5.64**)
3-Bromo-5-methoxy-1,4- NQ (13)	LC$_{10}$ **2.656** [1.055 - 3.874] LC$_{50}$ **5.752** [3.977 - 7.664] (**21.62**) LC$_{90}$ **12.453** [9.068 - 25.069](**46.81**)	LC$_{10}$ **0.151** [0.128 – 0.174] LC$_{50}$ **0.475** [0,438 – 0.512] (**1.79**) LC$_{90}$ **1.492** [1.343 – 1.684] (**5.61**)
2-Methyl-1,4-NQ (14) (menadione)	LC$_{10}$ **9.692** [8.088 - 10.893] LC$_{50}$ **14.952** [13.661 - 16.488](**86.93**) LC$_{90}$ **23.065** [20.228 - 28.467] (**134.10**)	LC$_{10}$ **2.005** [1.872 - 2.112] LC$_{50}$ **2.998** [2.879 - 3.151] (**17.43**) LC$_{90}$ **4.481** [4.099 - 5.079] (**26.05**)
Temephos	LC$_{10}$ **0.468** [0.208 - 0.728] LC$_{50}$ **1.499** [1.058 - 1.936] (**3.45**) LC$_{90}$ **4.799** [3.567 - 7.773] (**11.06**) LC$_{100}$ **0.012** (WHO,1992)	Nd
Niclosamide	>**1000** ppm (Laurens et al., 1997)	LC$_{90}$ **0.10** ppm (Oliveira and Paumgartten, 2000)
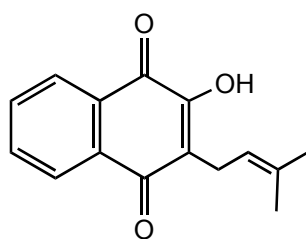
Lapachol (**1**)
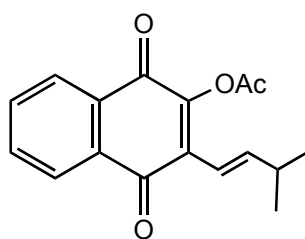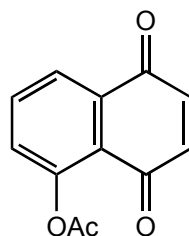
Isolapachol Acetate (**2**)
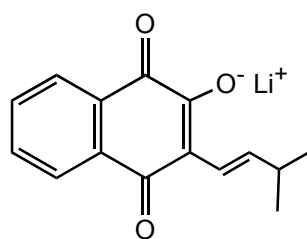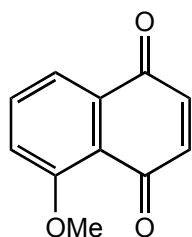
Isolapachol, lithium salt (**3**)
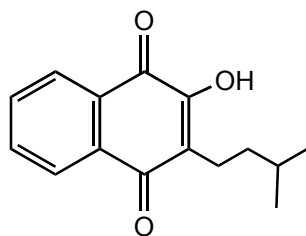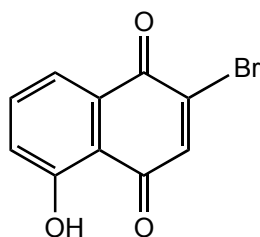
Dihydrolapachol (**4**)
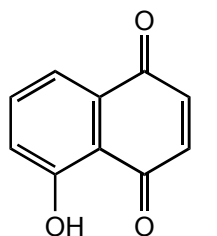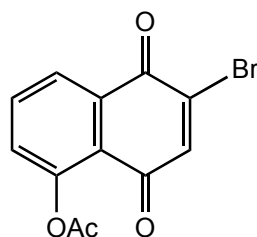
5-Hydroxy-1,4-naphthoquinone (juglone) (**5**)

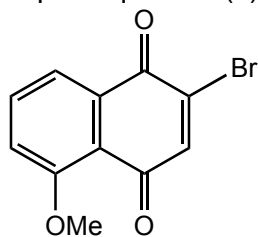5-Acetoxy-1,4- naphthoquinone (**6**)

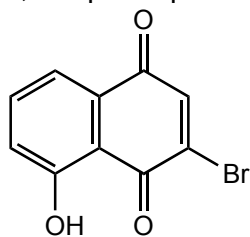5-Methoxy-1,4-naphthoquinone (**7**)
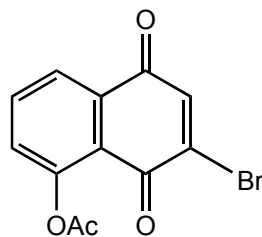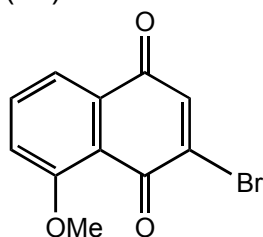
2-Bromo-5-hydroxy-1,4-naphthoquinone (**8**)

2-Bromo-5-acetoxy-1,4- naphthoquinone (**9**)
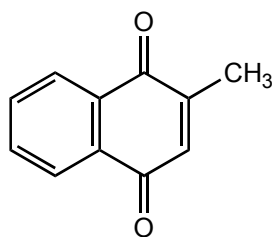
2-Bromo-5-methoxy-1,4- naphthoquinone (**10**)

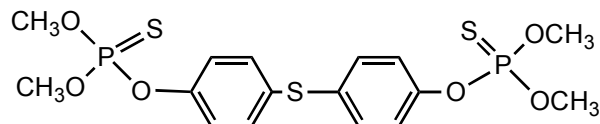3-Bromo-5-hydroxy-1,4- naphthoquinone (**11**)

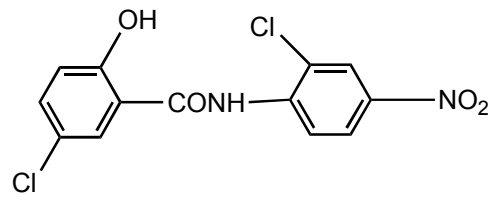3-Bromo-5-acetoxy-1,4- naphthoquinone (**12**)

3-Bromo-5-methoxy-1,4- naphthoquinone (**13**)

2-Methyl-1,4-naphthoquinone (**14**) (menadione)

Temephos

Niclosamide