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ORIGINAL PAPER

Oxime derivatives with larvicidal activity against *Aedes aegypti* L.

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Abstract Oximes containing secondary metabolites constitute an important group of bioactive compounds and have been described and frequently updated in the literature due to their pharmacological properties. Thus, the aim of this study was to evaluate the larvicidal activity of a series of fourteen structurally related [1,4]-Benzoquinone monooximes on third-instar *Aedes aegypti* larvae and to investigate structure-activity relationships (SAR) of these compounds. Results of larvicidal assay revealed that all oximes were found to have larvicidal activity. Compound 2,6-dimethyl-[1,4]-benzoquinone oxime tosylate (**11**) was the most bioactive (LC_{50}= 9.858 ppm), followed by 2-methyl-[1,4]-benzoquinone oxime tosylate (**9**) (LC_{50}=14.450 ppm). [1,4]-benzoquinone oxime (**1**) exhibited the lowest potency, with an LC_{50}=121.181 ppm. The molecular characteristics which may help to understand the assayed compounds larvicidal activity were identified. SAR indicates that the addition of alkyl groups attached to the ring, number, position in the unsaturated cyclic structure, and size of these groups influence the larvicidal activity. Moreover, the lipophilicity seems to play an important role in increasing the larvicidal effect, because, in general, tosyl-containing products were more potent than products containing free OH.

Keywords Oxime · Dengue · *Aedes aegypti* · Larvicidal activity · Structure-activity

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Introduction

Dengue is a serious mosquito-borne viral infection transmitted to humans by the bite of the female mosquitoes of the genus *Aedes* (Diptera: Culidae), especially *Aedes aegypti* L. and caused by any of four closely related virus serotypes, also called genotypes: DENV1 to DENV4. Infection with a serotype elicits lifelong immunity to that serotype but does not protect against the others, and sequential infections can show severe outcome due to antibody-dependent enhancement, which may be a greater risk factor for severe dengue. This illness is often fatal and contributes significantly to disease burden, poverty, and social debility worldwide, principally in tropical and developing countries (Zareen et al. 2011; Torres et al. 2014).

According to the World Health Organization (WHO), in 2013, dengue behaved as a classic epidemic for zones of the Americas, with the largest historical cases ever registered. American countries reported more than 2.3 million dengue infections, including 37,692 cases of severe dengue and 1280 deaths, with a mortality rate of about 0.05 % mostly among children. The average incidence rate of dengue for the year 2014 was 430.8 per 100,000 individuals (Pan American Health Organization 2014).

Three strategies for the prevention and control of dengue are possible: vaccines, antivirus, and mosquito control programs. Unfortunately, dengue virus vaccines are still under development, antivirus candidates are yet ongoing basic

research studies, and vector control programs are costly and difficult to sustain (Cafferata et al. 2013). Although the larvicidal products are effective, the indiscriminate use has triggered the emergence of negative environmental impacts, development of resistance in mosquito populations, and human health concerns (Zahran and Abdelgaleil 2011). These complications have stimulated numerous researches to develop new strategies using active principles from plants as well as their synthetic and semisynthetic analogues to selective larval control of *Ae. aegypti*.

Several studies have reported the larvicidal activity of natural quinones against different vector mosquitoes such as *Ae. aegypti* (Kiprono et al. 2004; Rodrigues et al. 2005), *Aedes albopictus* (Cheng et al. 2008), and *Culex pipiens* (Michaelakis et al. 2009). De Sousa et al. (2010) revealed the larvicidal activity of six structurally related *para*-benzoquinones against *Ae. aegypti* larvae, elucidating their structure-activity relationship.

Oxime derivatives constitute an important group of bioactive compounds and have been reported in the literature due to their varied pharmacological properties, such as antitumor (Soga et al. 2001), antidepressant (De Sousa et al. 2006), analgesic, antiarrhythmic (Schenone et al. 2000), anticonvulsivant, and antimicrobial (Karakurt et al. 2001). This class of compounds can be potentially derivable by the oximation of *para*-benzoquinones and some studies have described the larvicidal effect of these derivatives (Sun et al. 2008, 2010; Li et al. 2014).

These facts led us to ascertain the larvicidal potential of various *para*-benzoquinone mono-oxime derivatives. Thus, the present work describes the synthesis and characterization of a series of 14 structurally related *para*-benzoquinone oximes, as well as the evaluation of their larvicidal activity on third-instar larvae *Ae. aegypti*. Additionally, we investigated the structure-activity relationship of these compounds.

Material and methods

Preparation of the oxime derivatives

General

^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX-400 spectrometer at 400 and 100 MHz, respectively, with $CDCl_3$ as solvent. Chemical shifts are reported in ppm downfield from a tetramethylsilane internal standard. Infrared spectra were recorded on a Bomen Michelson model 102 FTIR or a Hartman & Braun MB, and the most intense or representative bands are reported (in cm^{-1}). Melting points were determined on a Micro Química model APF 301 apparatus and are uncorrected. Solvents and reagents were used directly from the supplier or purified when required by standard procedures. The phenols, sodium nitrite, and *para*-toluenesulfonyl chloride are all commercial products (Aldrich) and were used as obtained.

General procedure for the synthesis of the 1,4-benzoquinone mono-oximes (1-7)

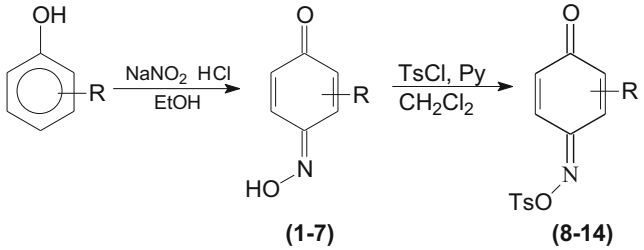
The phenol (73.5 mmol) was dissolved in 10 M hydrochloric acid (50 ml) and 95 % ethanol (50 ml). Sodium nitrite (7.5 g, 108.7 mmol) was added at 0 °C over 5 min with stirring, and the reaction mixture was then stirred for an hour at 0 °C. Ethanol (10 ml) was then added and stirring continued for a further hour at room temperature. The reaction mixture was diluted with water (500 ml) and extracted with diethyl ether. The ethereal extract was then washed with 10 % aqueous sodium carbonate solution. The aqueous solution, on acidification with 3 M hydrochloric acid, yielded a dirty yellow precipitate which was filtered. The solid residue was washed with hexane to eliminate soluble impurities to give the product (**1–7**) (Scheme 1).

Compound 1 44 % yield
^{1}H *NMR* ($CDCl_3$, *400 MHz*) δ: 6.47 (CH, m); 7.29 (CH, m); 7.79 (CH, m). ^{13}C *NMR* ($CDCl_3$, *100 MHz*) δ: 124.4; 129.2; 131.2; 138.2; 149.4; 187.8. *IR* (ν_{max}., *KBr*, cm^{-1}): 3412, 1628, 1558, 1457. Mp 126.4–127.0 °C.

Compounds 2a and 2b 82 % yield
^{1}H *NMR* ($CDCl_3$, *400 MHz*) δ: 2.02(CH_3, d, J=1.3 Hz); 2.06 (CH_3, d, J=1.5 Hz); 6.48 (CH, d, J=9.8 Hz); 6.49 (CH, d, J=10.1 Hz); 7.10 (CH, m); 7.21 (CH, dd, J=9.8 Hz, 2.8 Hz); 7.65 (CH, m); 7.75 (CH, dd, J= 10.1 Hz, 2.8 Hz). ^{13}C *NMR* ($CDCl_3$, *100 MHz*) δ: 15.7; 16.3; 121.4; 124.0; 129.2; 131.2, 134.3; 137.3; 137.8, 139.8; 149.7; 149.8; 188.1; 188.2. *IR* (ν_{max}., *KBr*, cm^{-1}): 3241, 2951, 1637, 1607, 1428, 1029. Mp 102.2–103.0 °C.

Compound 3 75 % yield
^{1}H *NMR* ($CDCl_3$, *400 MHz*) δ: 2.2 (CH_3, d, J= 1.3 Hz); 6.30 (CH, m); 6.37 (CH, dd, J= 10.1 Hz, 2.1 Hz); 7.7 (CH, d, J=10.1 Hz). ^{13}C *NMR* ($CDCl_3$, *100 MHz*) δ: 17.3; 124.4; 127.6; 129.6; 147.1; 149.9; 186.9. *IR* (ν_{max}., *KBr*, cm^{-1}): 3508, 2955, 1634, 1599, 1422, 1027. Mp 136.3–136.9 °C.

Compound 4 83 % yield
^{1}H *NMR* ($CDCl_3$, *400 MHz*) δ: 1.97 (CH_3, d, J=1.1 Hz); 1.99 (CH_3, d, J=1.2 Hz); 6.98 (CH, q, J=1.1 Hz), 7.54 (CH, q, J=1.2 Hz). ^{13}C *NMR* ($CDCl_3$, *100 MHz*) δ: 16.0; 16.6; 120.8; 134.0; 136.9; 139.5; 149.6; 188.1. *IR*



Scheme 1 Synthesis of the mono-oximes and their tosyl derivatives

(ν_{max}., *KBr*, cm^{-1}): 3190, 2961, 1632, 1605, 1423, 1183, 1049. Mp 138.4–139.0 °C.

Compound 5 84 % yield
^{1}H *NMR* (*$CDCl_3$*, *400 MHz*) δ: 2.01 (CH_3, d, *J*=1.5 Hz); 2.20 (CH_3, d, *J*=1.2 Hz); 6.27 (CH, q, *J*=1.5 Hz); 7.60 (CH, q, *J*=1.2 Hz). ^{13}C *NMR* (*$CDCl_3$*, *100 MHz*) δ: 15.8; 16.8; 121.5; 128.1; 138.5; 146.9; 150.1; 187.9. *IR* (ν_{max}., *KBr*, cm^{-1}): 3219, 2941, 1642, 1610, 1409, 1233, 1025. Mp 126.2–127.1 °C.

Compound 6 65 % yield
^{1}H *NMR* (*$CDCl_3$*, *400 MHz*) δ: 1.13 (CH_3, d, *J*=6.8 Hz); 2.20 (CH_3, d, *J*=1.3 Hz); 3.06 (CH, d hept, *J*=1.2 Hz, 6.8 Hz); 6.26 (CH, q, *J*=1.3 Hz); 7.56 (CH, d, *J*=1.2 Hz). ^{13}C *NMR* (*$CDCl_3$*, *100 MHz*) δ: 16.9; 21.5; 26.4; 118.4; 128.5; 146.2; 147.9; 150.2; 186.9. *IR* (ν_{max}., *KBr*, cm^{-1}): 3480, 2961, 1638, 1604, 1439, 1241, 1055. Mp 142.6–143.2 °C.

Compound 7 60 % yield
^{1}H *NMR* (*$CDCl_3$*, *400 MHz*) δ: 1.20 (CH_3, d, *J*=6.8 Hz); 2.04 (CH_3, d, *J*=1.5 Hz); 3.35 (CH, d hept, *J*=0.7 Hz, 6.8 Hz); 6.34 (CH, d, *J*=0.7 Hz); 7.68 (CH, q, *J*=1.5 Hz). ^{13}C *NMR* (*$CDCl_3$*, *100 MHz*) δ: 15.8; 22.4; 26.6; 122.0; 124.4; 138.4; 148.8; 156.8; 188.6. *IR* (ν_{max}., *KBr*, cm^{-1}): 3239, 2964, 1637, 1613, 1428, 1235, 1048. Mp 153.5–154.2 °C.

Tosylation of the 1,4-Benzoquinone mono-oximes (***1–7***)

The 1,4-benzoquinone mono-oxime (**1–7**) (4.6 mmol) was dissolved in dry CH_2Cl_2 (4.5 ml) and pyridine (1.3 ml) and cooled to 0 °C. *Para*-toluenesulfonyl chloride (6.0 mmol) was added portion-wise, the flask was purged with N_2, and the mixture was warmed to room temperature and stirred mechanically for 24 h or until completion by TLC. The reaction was diluted with Et_2O and washed successively with water, 2 M HCl (aq.) and water. The organic layer was dried with $MgSO_4$, filtered, and concentrated. The tosylated product (**8–14**) was obtained after purification by flash column chromatography, using as eluent a mixture of 9:1 hexane:ethyl acetate (Norris and Sternhell 1969; De Sousa et al. 2006).

Compound 8 78 % yield
^{1}H *NMR* (*$CDCl_3$*, *400 MHz*) δ: 2.49 (CH_3, s); 6.54 (CH, m); 6.57 (CH, m); 7.17 (CH, dd, *J*=10.1 Hz, 2.3 Hz); 7.41 (CH, d, *J*=8.5 Hz); 7.64 (CH, dd, *J*=10.1 Hz, 2.3 Hz); 7.93 (CH, d, *J*=8.5 Hz). ^{13}C *NMR* (*$CDCl_3$*, *100 MHz*) δ: 21.7; 124.5; 129.1; 129.9; 132.5; 134.3; 135.3; 146.1; 153.1; 170.5; 186.6. *IR* (ν_{max}., *KBr*, cm^{-1}): 1646, 1597, 1391, 1194, 1177, 1098.

Compounds 9a and 9b 85 % yield
^{1}H *NMR* (*$CDCl_3$*, *400 MHz*) δ: 2.02 (CH_3, d, *J*=1.5 Hz); 2.07 (CH_3, d *J*=1.5 Hz); 2.48 (CH_3, s); 6.53 (CH, d, *J*=10.1 Hz); 6.54 (CH, d, *J*=10.1 Hz); 6.96 (CH, m); 7.09 (CH, dd, *J*=10.1 Hz, 2.5 Hz); 7.38 (CH, d, *J*=8.5 Hz); 7.40 (CH, d, *J*=8.5 Hz); 7.46 (CH, m); 7.57 (CH, dd, *J*=10.1 Hz, 2.5 Hz); 7.91 (CH, d, *J*=8.5 Hz); 7.93 (CH, d, *J*=8.5 Hz). ^{13}C *NMR* (*$CDCl_3$*, *100 MHz*) δ: 15.7; 16.4; 21.7; 120.9; 124.1; 129.0; 129.1; 129.8; 131.1; 131.5; 132.4; 132.5; 134.3; 135.1; 145.9; 153.4; 153.6; 186.3; 186.4. *IR* (ν_{max}., *KBr*, cm^{-1}): 2926, 1641, 1634, 1387, 1193, 1176, 1091.

Compound 10 80 % yield
^{1}H *NMR* (*$CDCl_3$*, *400 MHz*) δ: 2.13 (CH_3, d, *J*=1.3 Hz); 2.47 (CH_3, s); 6.33 (CH, m); 6.45 (CH, dd, *J*=10.3 Hz, 2.0 Hz); 7.38 (CH, d, *J*=8.3 Hz); 7.57 (CH, d, *J*=10.3 Hz); 7.91 (CH, d, *J*=8.3 Hz). ^{13}C *NMR* (*$CDCl_3$*, *100 MHz*) δ: 17.2; 21.7; 124.8; 129.2; 129.8; 131.0; 131.4; 133.6; 145.0; 146.1; 153.6; 186.1. *IR* (ν_{max}., *KBr*, cm^{-1}): 2974, 1649, 1593, 1386, 1196, 1175, 1092.

Compound 11 90 % yield
^{1}H *NMR* (*$CDCl_3$*, *400 MHz*) δ: 1.99 (CH_3, d, *J*=1.5 Hz); 2.05 (CH_3, d, *J*=1.5 Hz); 2.45 (CH_3, s); 6.87 (CH, q, *J*=1.5 Hz); 7.36 (CH, *J*=8.3 Hz); 7.40 (CH, q, *J*=1.5 Hz); 7.90 (CH, *J*=8.3 Hz). ^{13}C *NMR* (*$CDCl_3$*, *100 MHz*) δ: 15.9; 16.6; 21.7; 120.7; 129.1; 129.8; 130.9; 131.8; 140.9; 143.4; 145.7; 153.6; 186.7. *IR* (ν_{max}., *KBr*, cm^{-1}): 2922, 1638, 1612, 1377, 1192, 1177, 1092.

Compound 12 88 % yield
^{1}H *NMR* (*$CDCl_3$*, *400 MHz*) δ: 2.00 (CH_3, d, *J*=1.5 Hz); 2.09 (CH_3, d, *J*=1.2 Hz); 2.45 (CH, s); 6.30 (CH, q, *J*=1.2 Hz); 7.36 (CH,

d, J=8.3 Hz); 7.37 (CH, q, J=1.5 Hz); 7.90 (CH, d, J=8.3 Hz). ^{13}C *NMR* ($CDCl_3$, *100 MHz*) δ: 15.9; 19.8; 21.7; 121.3; 129.1; 129.8; 130.9; 131.7; 142.8; 144.8; 145.9; 153.9; 186.4. *IR* (ν_{max}., *KBr*, cm^{-1}): 2926, 1654, 1602, 1377, 1191, 1176, 1091.

Compound 13 79 % yield

^{1}H *NMR* ($CDCl_3$, *400 MHz*) δ: 1.10 (CH_3, d, J=6.8 Hz); 2.08 (CH_3, d, J=1.5 Hz); 2.46 (CH_3, s); 3.04 (CH, d hept, J=1.3 Hz, 6.8 Hz); 6.30 (CH, q, J=1.5 Hz); 7.27 (CH, d, J=1.3 Hz); 7.37 (CH, d, J=8.3 Hz); 7.92 (CH, d, J=8.5 Hz). ^{13}C *NMR* ($CDCl_3$, *100 MHz*) δ: 16.7; 21.5; 21.7; 26.9; 118.2; 129.2; 129.7; 131.4;.131.7; 144.2;.145.8; 152.2;.154.0;.185.6. *IR* (ν_{max}., *KBr*, cm^{-1}): 2964, 1647, 1599, 1382, 1195, 1181, 1095.

Compound 14 80 % yield

^{1}H *NMR* ($CDCl_3$, *400 MHz*) δ: 1.07 (CH_3, d, J=7.1 Hz); 2.01 (CH_3, d, J=1.5 Hz); 2.45 (CH_3, s); 3.08 (CH, d hept, J=0.7 Hz, 7.1 Hz); 6.31 (CH, d, J=0.7 Hz); 7.36 (CH, J=8.3 Hz); 7.40 (CH, q, J=1.5 Hz); 7.89 (CH, J=8.3 Hz). ^{13}C *NMR* ($CDCl_3$, *100 MHz*) δ: 15.8; 21.7; 21.9; 27.4; 121.6; 127.3; 129.1; 129.7; 131.7; 142.3; 145.8; 152.4; 154.5; 186.8. *IR* (ν_{max}., *KBr*, cm^{-1}): 2958, 1654, 1386, 1195, 1176, 1091.

Chemical structures of the compounds prepared are shown in Fig. 1.

Larvicidal assay

Larval mortality assays were conducted according to the methodology described by Lima et al. (2014) with some modifications. The larvicidal activity of the oxime derivatives was evaluated on field-collected (Aracaju City, Sergipe State, Brazil) third-instar larvae of mosquito *Ae. aegypti*. The eggs of *Ae. aegypti* were produced in the insectary of the Federal University of Sergipe attached to paper strips. Paper strips having approximately 1000 eggs were placed in a polyethylene container with dechlorinated water (1 L) and cat food (Purina®) to allow larvae development. The container was kept in the insectary for hatching and monitoring of larvae development for 3 to 4 days. The concentration ranges were determined by a preliminary curve concentration-response with 20 larvae. A stock solution (20,000 ppm) of the compounds was prepared employing 20 mg of each compound, 10 % of Tween-80 (*v*/*v*), 30 % of DMSO (*v*/*v*), and 60 % of dechlorinated water (*v*/*v*). From the stock solution, a series of concentrations was prepared ranging from 10 to 500 ppm. Then, twenty larvae were collected with a Pasteur pipette and placed on a 25-ml graduated cylinder. The volume was completed to 20 ml with dechlorinated water and transferred to disposable cups containing variable volumes of the stock solution. For each test, negative control was prepared employing the same number of larvae in Tween-80 (0.1 ml), DMSO (0.3 ml), and dechlorinated water (19.6 ml). Three replicates were used for each concentration and the control treatments. The organophosphorate temephos was used as positive control on final concentrations ranging from 0.0117 to 0.0663 ppm. Mortality count was performed after 24 h exposure to variable concentration of title solutions. The larvae were considered dead when they did not respond to stimulus with a Pasteur pipette.

Statistical analysis

The larvicidal assays mortality data were submitted to *Probit* analysis (Finney 1971) to measure the lethal concentration for 50 % mortality (LC_{50}) and 95 % confidence intervals (CI) values of the compounds (Table 1). In all cases where deaths had occurred in the control treatments between 5 and 20 %, percentage mortality values were corrected applying the Abbott's formula (1925):

$$\%\ \text{Deaths} = \frac{\%\ \text{test mortality} - \%\ \text{control mortality} \times 100}{100 - \%\ \text{mortality}}$$

When mortality in control was higher than 20 %, the test was discarded and repeated. Compounds activity was considered significantly different if the 95 % confidence limits did not overlap.

Results and discussion

In the present investigation, thirteen analogues of the [1,4]-benzoquinone oxime (**1**) were synthesized and subjected to larvicidal toxicity bioassays on third-instar larvae of *Ae. aegypti*, the main vector of dengue. In addition, the structure-activity relationship (SAR) was investigated. The larvae mortality count was conducted after 24 h of treatment to variable concentrations of the testing solutions.

The results of the larvicidal activity values were measured in terms of LC_{50}, expressed in ppm. LC_{50} values and 95 % confidence limits (CI) of the [1,4]-benzoquinone oxime and its analogues are presented in Table 1. The results of larvicidal bioassay revealed that all the oximes were found to have larvicidal activity, with LC_{50} values lower than 122 ppm, and the rates of mortality were directly proportional to concentration. Compound **11** (2,6-dimethyl-[1,4]-benzoquinone oxime tosylate) displayed the largest larvicidal activity with an LC_{50}= 9.858 ppm, followed by 3-methyl-[1,4]-benzoquinone oxime tosylate (**9**) (LC_{50}=14.450 ppm). [1,4]-benzoquinone oxime

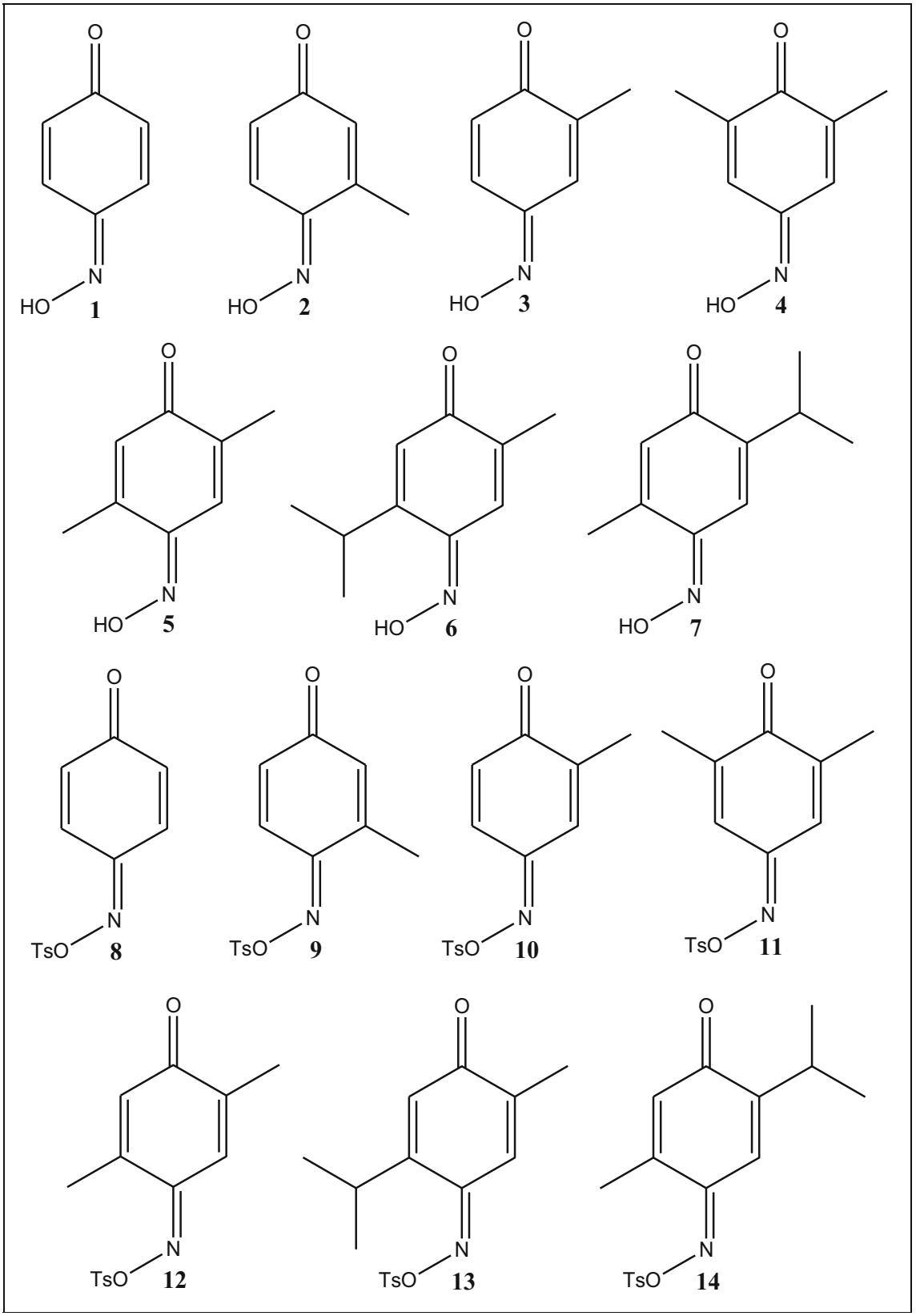


Fig. 1 Chemical structures of the evaluated compounds

Table 1 Larvicidal activity (LC_{50}) and 95 % confidence interval (CI) of oxime derivatives on third-instar larvae of *Ae. aegypti*

Compound	LC_{50} ppm (CI 95 %)	Compound	LC_{50} ppm (CI 95 %)
1	121.181 (107.916–134.484)	**9**	14.450 (12.905–15.959)
2	118.350 (99.724–136.461)	**10**	32.487 (24.118–42.392)
3	79.764 (70.932–87.730)	**11**	9.858 (7.832–12.117)
4	120.215 (104.582–139.058)	**12**	16.536 (14.789–18.227)
5	84.741 (76.668–94.763)	**13**	71.411 (51.137–61.072)
6	78.434 (69.442–85.677)	**14**	51.335 (36.855–65.202)
7	50.370 (46.776–53.163)	*Temephos*	0.042 (0.035–0.05)
8	29.653 (22.502–37.195)	–	–

Probit analysis was conducted on mortality data collected after a 24-h exposure to different concentrations of testing solutions to establish the lethal concentration for 50 % mortality (LC_{50}) and 95 % confidence interval (CI) values for the respective compounds and temephos. The compounds' activity was considered significantly different if the 95 % confidence limits did not overlap

(**1**), which is not substituted by alkyl groups, exhibited the lowest potency, with an LC_{50}=121.181 ppm. The organophosphate temephos (positive control) showed an excellent larvicidal effect, with an LC_{50} value of 0.042 ppm, while the negative control (Tween-80, DMSO, and water) did not cause significant mortality of the larvae during the experiments (mortality ≤5 %). In general, compounds that contain conjugated C–C double bonds in their chemical structure show good larvicidal and repellent activities (Dias and Moraes 2014). A study performed by García et al. (2005) reported that the monoterpenoid pulegone (presence of conjugated C–C double bond) displayed an excellent acute repellent activity against *Tribolium castaneum* adults (LD_{50}=0.44 μM.cm^{-2}), while dihydropulegone (absence of conjugated C–C double bond) was inactive. Similar results also were found by Santos et al. (2011). These authors showed that the number of conjugated C–C double bonds appears to collaborate to an increase in larvicidal potency.

According to the functional and structural characteristics and the observed larvicidal activity (SAR), the assayed compounds can be divided into two classes. The first class is made up of *para*-benzoquinone oxime derivatives with presence or absence of alkyl groups, while the second group involves tosylated oxime derivatives with additional alkyl groups.

To investigate if the introduction of a methyl group in the chemical structure influences the larvicidal activity, [1,4]-benzoquinone oxime (**1**) was compared to 3-methyl-[1,4]-benzoquinone oxime (**2**) and 2-methyl-[1,4]-benzoquinone oxime (**3**). Compound **3** (LC_{50}=79.764 ppm) was more bioactive than **1** (LC_{50}=121.181 ppm), indicating that the addition of a lipophilic group improves the activity. However, compounds **1** and **2** presented equivalent LC_{50} values (LC_{50}=121.181 and 118.350 ppm, respectively). Similar results were found by Yang et al. (2003) and Cheng et al. (2008). These authors demonstrated that the presence of methyl groups in unsubstituted quinones promoted a significant increase of larvicidal activity against different mosquito species.

Furthermore, compounds **2** and **3** have a methyl differing from each other in the position of this group in the ring. In compound **2**, the methyl group is attached to the carbon C-3, while in **3** this substituent is attached to the carbon C-2. This modification in the position of alkyl group resulted in different larvicidal activity between these compounds. Compound **3** was more potent (LC_{50}=79.764 ppm) than **2** (LC_{50}=118.350 ppm), suggesting that the disposition of the methyl substituent on the benzoquinone ring has significant influence in the biological activity, and there is an improvement in the activity when the substitution occurs at the C-2 position (Me group close to the carbonyl). Analogous results were observed in the study performed by Cheng et al. (2008). These authors observed that anthraquinolinic compounds that possess a methyl substituent at the carbon C-2, such as tectoquinone, exhibited a strong larvicidal activity against both *Ae. aegypti* and *Ae. albopictus* larvae, with LC_{50} values of 3.3 and 5.4 μg/ml, respectively. Additionally, Morimoto et al. (2002) demonstrated that the presence of a methyl substituent located at the carbon C-2 of 2-methyl-anthraquinone was important for antifeedant activity against the common cutworm *Spodoptera litura*.

The effect of introducing a second methyl group in the larvicidal activity and at different positions on the ring was further evaluated. The addition of a methyl group in compound **3** (LC_{50}=79.764 ppm) at C-6 and C-5 positions resulted in 2,6-dimethyl-[1,4]-benzoquinone oxime (**4**) and 2,5-dimethyl-[1,4]-benzoquinone oxime (**5**), respectively. The compounds **4** and **5** exhibited an LC_{50}>84 ppm, showing that oximes with two Me groups attached to the ring are less bioactive than the monosubstituted oximes. In addition, it also was observed that the change in the position of the second methyl group in cyclohexane skeleton alters the larvicidal potency (**4**: LC_{50}=120.215 ppm; **5**: LC_{50}=84.741 ppm). These results are consistent with those described earlier.

Replacement of a methyl at carbon 2 by an isopropyl group contributed to increase the larvicidal activity; 2-isopropyl-5-

methyl-[1,4]-benzoquinone oxime (**7**) (LC_{50}=50.370 ppm) was more bioactive than **5** (LC_{50}=84.741 ppm). This effect emphasizes the importance of lipophilicity for the larvicidal activity, suggesting the relevance of the addition of alkyl groups for the larvicidal effect. This fact was similar to the result of De Sousa et al. (2010), who described that the presence of bulky alkyl substituents, such as an isopropyl group, attached to the cyclohexane ring of *para*-benzoquinones, enhanced the larvicidal activity against the mosquito larvae *Ae. aegypti*.

In order to ascertain whether the position of the isopropyl group influences the larvicidal activity, 2-isopropyl-5-methyl-[1,4]-benzoquinone oxime (**7**) and 5-isopropyl-2-methyl-[1,4]-benzoquinone oxime (**6**) were compared. Interestingly, compound **7** (LC_{50}=50.370 ppm) was more bioactive than **6** (LC_{50}=78.434 ppm), indicating that the addition of isopropyl groups in different positions in the unsaturated cyclic structure results in different biological effects. Furthermore, introduction of lipophilic substituents located at C-2 appears to be more important for the larvicidal activity when compared with the substitution in the other positions of the cyclohexane ring because **3** was more bioactive than **2**, and **7** was more potent than **6**.

With the exception of compounds 5-isopropyl-2-methyl-[1,4]-benzoquinone oxime tosylate (**13**) and 2-isopropyl-5-methyl-[1,4]-benzoquinone oxime tosylate (**14**), all the tosylated products were found to be more bioactive than their respective 1,4-benzoquinone oximes. For example, the larvicidal activity of the compound **11** (2,6-dimethyl-[1,4]-benzoquinone oxime tosylate; LC_{50}=9.858 ppm) was 12-fold better than compound **4** (2,6-dimethyl-[1,4]-benzoquinone oxime; LC_{50}=120.215 ppm), compound **9** (3-methyl-[1,4]-benzoquinone oxime tosylate; LC_{50}=14.450 ppm) was 8-fold more active than **2** (3-methyl-[1,4]-benzoquinone 4-oxime; LC_{50}= 118.350 ppm), and compound **12** (2,5-dimethyl-[1,4]-benzoquinone oxime tosylate; LC_{50}=16.536 ppm) was 5-fold more toxic against *Ae. aegypti* larvae than **5** (2,5-dimethyl-[1,4]-benzoquinone oxime; LC_{50}=84.741 ppm). These results indicate that the introduction of a tosyl moiety considerably increases potency, resulting in better larvicidal activity. A plausible explanation for this result is the presence of a tosyl-protecting group in the chemical structure, thus enhancing overall lipophilicity of the molecule. Research has demonstrated that tosyl-containing products present promising insecticidal activity. Aliyu (2007, 2008), for example, reported that tosylated compounds of *p*-nitrophenol, *p*-aminophenol, 1,2-propanediol, and ethanolamine showed interesting insecticidal and herbicidal activities, with LC_{50} values less than 50.0 μg/ml. Additionally, several studies have shown that the lipophilicity seems to play an important role in increasing the larvicidal potency (Mathew et al. 1996; Santos et al. 2010; 2011; Lucia et al. 2013; Silva et al. 2013; Dias and Moraes 2014; Lima et al. 2014). Generally, the presence of lipophilic moieties in the chemical structure can facilitate the ability of penetration of the compounds in the larval cuticle and as a consequence, such molecules can achieve their aims and interact with an active site (Lopez et al. 2005).

Unlike what happened earlier, the introduction of a methyl at carbon C-2 has not improved the larvicidal effect, since the compound 2-methyl-[1,4]-benzoquinone oxime (**10**) and [1,4]-benzoquinone oxime tosylate (**8**) presented equivalent LC_{50} values (LC_{50}=32.487 and 29.653 ppm, respectively). However, the addition of a methyl at carbon C-3 {3-methyl-[1,4]-benzoquinone oxime tosylate (**9**), LC_{50}= 14.450 ppm} enhances the larvicidal potency, evidencing that the addition and position of methyl groups in the in the quinone core affects the biological activity.

The addition of a second methyl group in the compound **10** at C-6 and C-5 positions resulted in the compounds 2,6-dimethyl-[1,4]-benzoquinone oxime tosylate (**11**) and 2,5-dimethyl-[1,4]-benzoquinone oxime (**12**), respectively. Compounds **11** and **12** showed different larvicidal effects, with LC_{50} values of 9.858 and 15.536 ppm, respectively, showing that oximes tosylated with two methyl groups attached to the cyclohexane ring are more potent than the monosubstituted tosylated oximes (**10**, LC_{50}=32.487 ppm). In addition, it was observed that changing the position of the second methyl group in the cyclohexane skeleton also affects the larvicidal potency. Replacement of a methyl at carbon C-2 or C-5 by an isopropyl group caused a decrease in larvicidal effect, since that compounds 2-isopropyl-5-methyl-[1,4]-benzoquinone oxime tosylate (**14**) (LC_{50}=51.355 ppm) and 5-isopropyl-2-methyl-[1,4]-benzoquinone oxime tosylate (**13**) (LC_{50}= 71.411 ppm) were less toxic against *Ae. aegypti* larvae than **12** (LC_{50}=15.536 ppm). This fact may be explained by the presence of two closely neighboring bulky groups, which may cause steric hindrance to a possible nucleophilic attack. Furthermore, change in the position of the isopropyl group in the cyclohexane skeleton did not affect larvicidal potency, since the compounds **13** and **14** had a similar potency. A study performed by Praba and Velmurugan (2007) demonstrated that steric effects are not favorable for activity mainly due to the larger surface area (volume). A comparison between structurally related compounds showed that the isopropyl esters of carboxylic acids (bulkier derivatives) were less bioactive than the methyl derivatives and the unsubstituted carboxylic acid.

Conclusion

This paper presents a study on the larvicidal activity and SAR of a series of 14 structurally related *para*-benzoquinone oximes. All the assayed compounds exhibited a good larvicidal activity against *Ae. aegypti* larvae. Structure-activity relationships indicate that the addition of different alkyl groups (methyl or isopropyl) in different positions, the introduction of a

tosyl protecting group, and the lipophilicity of the oximes influence the larvicidal potency. Moreover, the results obtained suggest that the tested compounds are a promising class of larvicide substances, which adequate structural modifications in the tosylated products can enable the development of new larvicide agents potentially suitable to control the dengue mosquito. However, the compounds tested herein require more studies to verify mammalian and environmental toxicity.

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Conflict of interest We declare that we have no conflict of interest.

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