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Synthesis and evaluation of new oxamniquine derivatives

Silvio Barberato Filho [a], Cybele Gargioni [b], Pedro Luiz Silva Pinto [b], Silvia Gabriel Chiodelli [b], Sylvia Amaral Gurgel Vellosa [b], Rita Maria da Silva [b], Maria Amélia Barata da Silveira [a,*]

[a] *Department of Pharmacy, Faculty of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 580, CEP 05508-900 São Paulo, Brazil*

[b] *Schistosomiasis Sector, Adolfo Lutz Institute, Av. Dr. Arnaldo, 355, CEP 01246-902 São Paulo, Brazil*

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Abstract

Oxamniquine derivatives were synthesized and evaluated as novel schistosomicide agents. Oxamniquine (1,2,3,4-tetrahydro-2-[[(1-methylethyl)amino]methyl]-7-nitro-6-quinolinemethanol) was submitted to the Mannich reaction, using formaldehyde, paraformaldehyde and acetaldehyde as reagents, and gave three unexpected products: two of them were cyclized on the alkylamine side chain and another etherified on the aminequinolinemethanol group. The three compounds were biologically evaluated using mice infected with *Schistosoma mansoni* and showed promising activities, but had higher toxicities. For studies on structure–activity relationships, results demonstrate that the side alkylamine group can be modified with preserved activity, but that this modification is associated with increased toxicity. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Drug design; Prodrug; Oxamniquine; *Schistosoma mansoni*; Schistosomicide

1. Introduction

Schistosomiasis is an infectious disease caused by a trematode of the *Schistosoma* genus and coexists with poverty, ignorance, precarious habitation and improper hygienic and structural conditions (Davis, 1986; World Health Organization, 1988; El-Garem, 1998).

Schistosomiasis is, after malaria, the most endemic parasitic disease over the world and is responsible for 500,000 deaths, annually (Newton and White, 1999). It is estimated that approximately 200 million people are infected globally and 600 million are exposed to risk of infection (Dupré et al., 1999). In Brazil, there are 8–10 million peoples infected and about 30 million at risk of infection.

Thus, the chemotherapy has become the most effective means of endemic disease control (Silveira, 1989) and therapeutic drugs available are: metrifonate, oxamniquine and praziquantel. In some countries only oxamniquine and praziquantel are used, although the latter is not used against schistosomiasis but in cerebral cisticercosis (Ko-

* Corresponding author. Tel.: +55-11-381-83793; fax: +55-11-381-54418.

E-mail address: ameusp@usp.br (M.A.B. da Silveira).

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rolkovas, 1999) and a risk of resistance always exists (Bruce et al., 1987). Conversely, design of a new drug is too expensive and schistosomicides are not economically attractive to industry (Shekhar, 1991; World Health Organization, 1993; Purewal and Stainsby, 1994; Dimasi, 1995; Harman, 1999).

With the purpose of obtaining new derivatives of oxamniquine with some therapeutic advantages, the Mannich reaction was used. This reaction is widely applied by the pharmaceutical industry for obtaining prodrugs (Tramontini, 1973) but it was not still used to obtain new schistosomicides. New antimalarials (Sweeney and Pick, 1984; Barlin and Tan, 1985; Scott et al., 1987; Newton and White, 1999), uricosuric agents (Bundgaard and Johansen, 1981), antihelmintics (Jain and Chaurasia, 1990), antibacterial agents (Magarian and Nobles, 1967; Shah et al., 1991), cardiotonics (Thiele et al., 1966) and antineoplasic agents (Schönenberger and Adam, 1965; Werner et al., 1970) were achieved with success.

2. Materials and methods

2.1. Materials

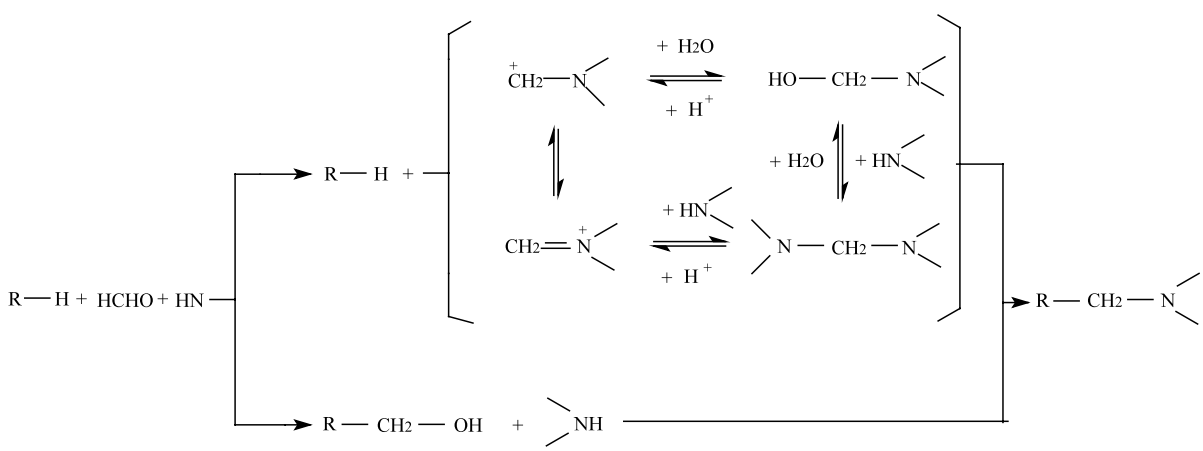
Oxamniquine from Pfizer; praziquantel from Merck; acetaldehyde and 37% formaldehyde solution from Merck; paraformaldehyde from Quimibrás. Other solvents and reagents, from Merck, are of analytical grade.

2.2. Apparatus

The infrared data were recorded on a FTIR-BOMEM MB-120 in KBr pellets and characteristic peaks are given as cm^{-1}. The ^{1}H and ^{13}C NMR spectra were recorded on a BRUCKER AC-200 and Advance DPX-300 spectrometer with tetramethylsilane as the internal reference and DMSO-d_6 as the solvent. Chemical shifts are given in ppm, coupling constants in Hertz, and splitting patterns are designated as follows: s, singlet; brs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

2.3. General synthetic method

Mannich's reaction consists in the condensation of ammonium, primary or secondary amines or their salts with an aldehyde and a compound with an active hydrogen. The product is called the Mannich base. Concentrated HCl is often used as catalyst besides aluminium, benzenesulfonic acid, alkylbenzenesulfonic acids, chloromethylsilane, copper(II) acetate, copper(II) chloride, copper(II) iodide, silver nitrate and triethylamine (Miocque and Vierfond, 1973; Heaney et al., 1988). The Scheme 1 shows the reaction mechanism (Tramontini, 1973).



Scheme 1. General synthetic method.

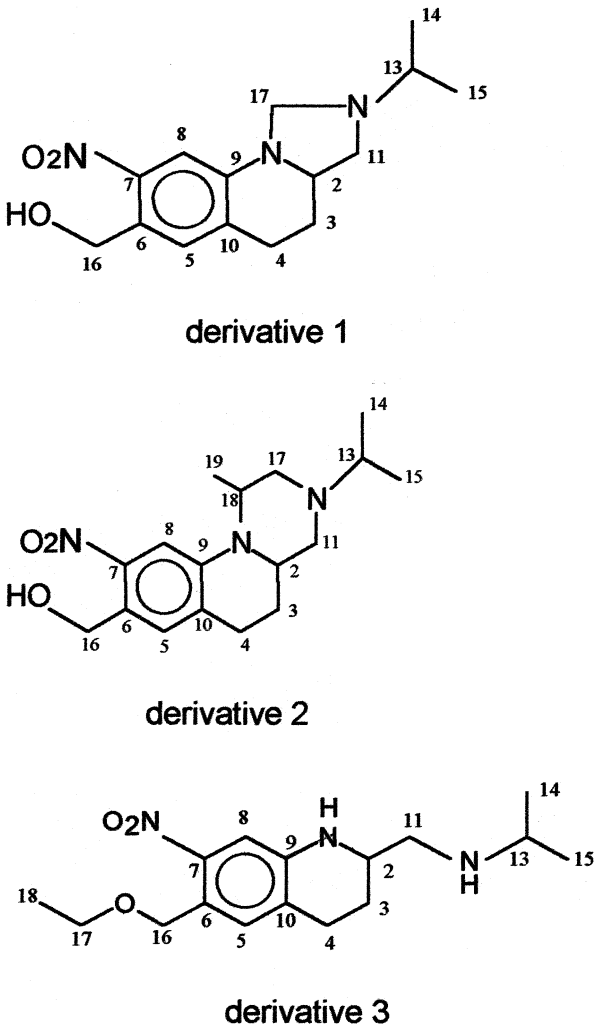


Fig. 1. Structure of new oxamniquine derivatives.

Oxamniquine and praziquantel were used as amines. Despite data in the literature, praziquantel does not react in this way and oxamniquine reaction produces unexpected results. The products obtained are presented in Fig. 1.

*2.3.1. Synthesis of derivative **1***

A mixture of oxamniquine (0.5 g) and 37% formaldehyde solution (20 ml) was stirred at room temperature for 18 h. The pale yellow precipitate was separated under pressure filtration and recrystallized from isopropanol, yielding 80% of a pale yellow product with a melting point of 158 °C. IR: 3150 (str. OH); 2866 (str. CH); 1621 (ass. str. C=C); 1516 (ass. str. NO_2); 1343 (sim. str. NO_2); 1220 (ang. def. OH); 1076 (prim. str. CO alcohol); 913 (str. NO arom.); 841 (ang. def. CH arom.). ^{1}H NMR (DMSO-d_6): 1.12 (d, d; 3H, 3H); 2.11 (m, 2H); 2.54 (m, 1H); 2.78 (t, 2H); 3.40 (d, 2H); 3.54 (m, 1H); 3.64 (s, 1H); 4.46 (s, 1H); 4.65 (d, 2H); 5.26 (t, OH); 6.93 (s, 1H); 7.35 (s, 1H). ^{13}C NMR (DMSO-d_6 ppm): 21.5 (C_{14}, C_{15}); 23.8 (C_3); 27.2 (C_4); 53.1 (C_{13}); 56.5 (C_2); 57.0 (C_{11}); 59.9 (C_{16}); 69.2 (C_{17}); 104.7 (C_8); 124.3 (C_{10}); 127.7 (C_6); 128.5 (C_5); 142.4 (C_9); 146.4 (C_7).

*2.3.2. Synthesis of derivative **2***

A mixture of oxamniquine (0.5 g) and 0.06 g of solid paraformaldehyde in 20 ml absolute ethanol and 1 ml concentrated HCl was stirred for 7 days at room temperature. The product was separated by filtration under pressure, yielding 60% of a product with a melting point of 186 °C. IR: 3433 (str. OH); 2888 (str. CH); 2341 (amine hydrochl.); 1620 (str. C=C); 1521 (assim. NO_2); 1338 (sim. NO_2); 901 (str. C–N in nitroaromatic); 841 (def. CH arom. out pl.). ^{1}H NMR (DMSO-d_6): 1.12 (d, d; 3H, 3H); 1.15 (d, 3H); 2.11 (m, 2H); 2.32 (t, 2H); 2.65 (d, m, 2H, 1H); 2.72 (d, 2H); 2.78 (m, 1H); 3.40 (m, 1H); 4.51 (d, 2H); 4.56 (t, OH); 6.95 (s, 1H); 7.22 (s, 1H). ^{13}C NMR (DMSO-d_6 ppm): 15.1 (C_{19}); 21.1 (C_{14}, C_{15}); 23.5 (C_3); 26.8 (C_4); 53.5 (C_{13}); 56.2 or 56.6 (C_2); 56.2 or 56.6 (C_{18}); 65.4, 68.4 or 68.7 (C_{11}); 65.4, 68.4 or 68.7 (C_{16}); 65.4, 68.4 or 68.7 (C_{17}); 105.2 (C_8); 120.1 (C_5); 127.3 (C_{10}); 129.3 (C_6); 142.2 (C_9); 147.2 (C_7).

*2.3.3. Synthesis of derivative **3***

A mixture of oxamniquine (0.5 g) and acetaldehyde (0.079 g) were added to 20 ml absolute ethanol and 1 ml concentrated HCl. The mixture was stirred for 48 h at room temperature. After evaporation of the solvent, the product was recrystallized from isopropanol, yielding 40% of a product with a melting point 217 °C. IR: 3306 (str. NH); 2859 (str. CH); 1626 (str. C=C); 1521 (assim. str. NO_2); 1338 (sim. str. NO_2); 1297 (assim. str. C–O–C); 1116 (sim. str. C–O–C); 905 (arom. nitrocompound str. C–N); 841 (def. C–H arom. out pl.). ^{1}H NMR (DMSO-d_6): 1.10 (t, 3H); 1.26 (d, 3H); 1.29 (d, 3H); 1.69 (m, 2H); 2.49 (t,

2H); 2.75 (t, 2H); 2.99 (m, 1H); 3.35 (m, 2H); 3.69 (m, 1H); 4.55 (s, 2H); 7.12 (s, 1H); 7.16 (s, 1H); 8.94 (brs, NH); 9.2 (brs, NH). ^{13}C NMR (DMSO-d_6 ppm): 15.2 (C_{18}); 18.6 (C_{15}); 18.8 (C_{14}); 23.9 (C_3); 24.6 (C_4); 47.3 (C_{13}); 48.8 (C_{11}); 50.4 (C_2); 65.5 or 68.6 (C_{16}); 65.5 or 68.6 (C_{17}); 108.5 (C_8); 120.5 (C_5); 125.9 (C_6); 130.1 (C_{10}); 144.0 (C_9); 146.4 (C_7).

2.4. Elemental analysis

Elemental analysis was performed on a Perkin–Elmer-24013 and the results were consistent with the elemental formula (within 0.4% of theoretical values Table 1).

2.5. Biological in vivo tests

Biological evaluation was made by the Schistosomiasis Sector at the Adolfo Lutz Institute, São Paulo, Brazil and was performed on male 'Swiss' mice, weighing 18–22 g, infected subcutaneously with 50 *Schistosoma mansoni BH cepa* cercareae. For confirmation of infection, fecal analysis by the direct egg method was performed 45 days after inoculation. The mice were distributed in seven groups of 15 animals and one group of 14 animals:

1. Untreated group—14 infected mice.
2. Vehicle group—15 infected mice which received the vehicle alone (10% Cremophor EL solution in 0.1 M phosphate buffer, pH 7.4).
3. Mansil group—15 infected mice which received commercial *Mansil* 50 mg/ml syrup.
4. Oxamniquine group—15 infected mice which received oxamniquine in 10% Cremophor EL, phosphate buffer pH 7.4.
5. Derivative **1** group—15 infected mice treated with derivative **1** in 10% Cremophor EL, phosphate buffer pH 7.4.
6. Derivative **2** group—as above, but using derivative **2**.
7. Derivative **3** group—as above, but using derivative **3**.

Each derivative was suspended in 10% Cremophor solution in phosphate buffer, pH 7.4 administered per os, in a single dose, by gastric sond, 100 mg/kg weight, representing the ED_{99} as is often used in biological tests. Each animal was sacrificed 10 days after treatment. The adult worms were quantified by a method of perfusion of the mesenteric and portal veins. From each mouse two thin gut fragments (about 1 cm) were removed for quantification of the number of eggs and verification of stages of development–quantitative oogram method (Pellegrino and Faria, 1965; Pellegrino and Katz, 1968).

2.6. Toxicological assays

These evaluations were performed by the Adolfo Lutz Institute, São Paulo, Brazil using the method of Reed and Muench (1938) on 50 mice divided into five groups. Each derivative was given in a single dose, by mouth, aided with gastric sond. The volume of all doses given to the mice was 0.025 ml/g weight, not exceeding 0.5 ml by animal (Turner, 1965). For this, five solutions with 92, 72, 52, 32 and 12 mg/ml of each derivative were prepared. Group I received dose equivalent to 300 mg/kg of derivative **1**; group II, 800 mg/kg; group III, 1300 mg/kg; group IV, 1800 mg/kg; and group V, 2300 mg/kg. DL_{50} was calculated by observation of occurring deaths till the

Table 1
Elemental analysis of new oxamniquine derivatives

Derivatives	Elemental formula	% Calculated (found)				
		Carbon	Hydrogen	Nitrogen	Oxygen	Chloro
1	$C_{15}H_{21}N_3O_3$	61.84 (61.42)	7.27 (7.21)	14.42 (14.08)	16.47 (17.29)	
2	$C_{17}H_{24}N_3O_3{\cdot}HCl$	57.54 (57.21)	7.10 (7.11)	11.84 (11.87)	13.53 (14.04)	9.99 (9.77)
3	$C_{16}H_{25}N_3O_3{\cdot}HCl$	55.89 (55.90)	7.62 (7.27)	12.22 (12.28)	13.96 (14.44)	10.31 (10.11)

4th day of treatment. This experiment was repeated for derivatives **2** and **3**.

2.7. Statistical analysis

Results were expressed as averages and standard deviation of the averages. The statistical evaluation of the results was accomplished by analysis of variance (one-way ANOVA), with the same or smaller critical level to 0.05 for null rejection. In the presence of significance in ANOVA, contrast analysis among the averages was conducted being applied a posteriori Dunnett's test for comparison with control groups. For the measures in percentage of the oogram method, non-parametric test of Wilcoxon and Kruskal–Wallis was used. For the accomplishment of the statistical calculations, JUMP (JMP) program, version 3.1.4, Copyright 1989–1995, SAS Institute Inc., License 40-2301 for Windows was applied.

3. Results and discussion

Three new oxamniquine derivatives were obtained, in spite of the unexpected behavior of the oxamniquina and praziquantel when submitted to the Mannich reaction. Oxamniquine reacts quickly with formaldehyde resulting in the identified derivative **1**. This derivative presents low chemical reactivity making unfeasible the subsequent stages of Mannich reaction. Variation of solvents, temperature and time of reaction were not suitable to alter the product of reaction.

The formaldehyde replacement by paraformaldehyde has resulted in the identified derivative **2**, while the substitution by acetaldehyde has resulted in the derivative **3**.

Side alkylamine group ciclization of the derivatives **1** and **2** is the main structural difference with the oxamniquine. Derivative **3** is an eterified aminequinolinemethanol group derivative from oxamniquine. The mechanism of this reaction is being studied in our recent work and seems not acetaldehyde dependant, but of the solvents and catalysts involved.

Praziquantel did not reacts under these conditions.

The structures of the three derivatives were confirmed by data analysis. It should be pointed out that the ^{13}C NMR oxamniquine data obtained differs from those of the literature (Ahmad et al., 1991): attribution was slightly altered for C_6, C_7, C_9 and C_{10}, considering that the electronic effect of the $-NO_2$ group may displace C_7 and C_9 with more ease than C_6 and C_{10}. This observation is applied for all three new derivatives.

Two methods used in the evaluation of new schistosomicides were mainly selected for preliminary screening: quantitative oogram and adult worms recovery by portal and mesenteric perfusion technique.

The three new derivatives present as much high insolubility as oxamniquine. Thus, the method used for biological evaluation showed that Cremophor EL is the most employed suspending agent for these cases. It simulates the vehicle used in Mansil, a commercial pharmaceutical formulation of oxamniquine (Foster et al., 1971; Jewsbury, 1972; Foster and Cheetham, 1973; Fripp, 1973; Shaw and Brammer, 1983; Tanaka et al., 1989; Penido et al., 1994).

Pharmacological evaluation through the quantitative oogram showed oviposition suppression with all the three new derivatives (Table 2). While in the control groups (not treated and vehicle) were found eggs of all the stages, the new derivatives presented equivalent results to the groups that received standard treatment (Mansil and oxamniquine).

Using the perfusion technique, the search of adult worms also confirmed the pharmacological activity of the three new derivatives (Table 3). The average number of parasites recovered from control groups was quite superior to that of the other groups. Derivative **3** presented superior results to derivatives **1** and **2**; besides better results than those observed in the standard treatment groups.

Toxicological evaluation is very important, being one of the decisive steps of drug development. DL_{50} of the new derivatives were determined and compared to DL_{50} of standard oxamniquine, measured by the same way (Reed and Muench, 1938). The obtained results were consistent with literature.

The new three derivatives showed more toxicity than oxamniquine (Table 4). Derivative **1** presents DL_{50} close to oxamniquine. Derivatives **2** and **3** present bigger toxicity than oxamniquine.

Table 2
Quantitative oogram

Group average (%)							
Egg stage	Controls		Standard treatment		New derivatives		
	Not treated	Vehicle	Mansil	Oxamniquine	Derivative **1**	Derivative **2**	Derivative **3**
1st stage	6.32	6.49	0.00*	0.00*	0.00*	0.00*	0.00*
2nd stage	14.51	11.48	0.00*	0.00*	0.00*	0.00*	0.00*
3rd stage	24.20	22.27	0.00*	0.00*	0.00*	0.00*	0.00*
4th stage	8.47	11.11	3.67*	2.75*	3.07*	2.33*	3.95*
5th stage	33.67*	42.11*	73.85	47.32*	68.86	34.06*	66.00
Unviable	12.82*	6.54*	22.48*	49.93	28.07	63.61	30.05*

Wilcoxon/Kruskal–Wallis: 1st stage, $\chi^2 = 90.66$; 2nd stage, $\chi^2 = 99.68$; 3rd stage, $\chi^2 = 99.52$; 4th stage, $\chi^2 = 28.60$; 5th stage, $\chi^2 = 36.41$; Unviable, $\chi^2 = 48.92$.
* $P < 0.0001$.

Table 3
Worm recovery by mesenteric and portal perfusion

Groups							
	Controls		Standard treatment		New derivatives		
	Not treated	Vehicle	Mansil	Oxamniquine	Derivative **1**	Derivative **2**	Derivative **3**
Average	9.86	13.40*	0.40**	0.20**	1.73**	2.40**	0.13**
Standard deviation	5.48	5.32	1.06	0.56	1.58	2.85	0.35
Minimum	0	2	0	0	0	0	0
Maximum	22	22	4	2	5	10	1
Number of mice	14	15	15	15	15	15	15

ANOVA $F_{(6, 97)} = 43.24$; $P < 0.0001$.
* $P < 0.05$;
** $P < 0.01$ (Dunnett's test).

Although the three new derivatives have showed promising activity, all of them presented toxicity larger than oxamniquine. For studies on structure–activity relationships, the observed results are indications that the side alkylamine group can be modified with preserved pharmacological activity, but those modifications are associated with increased toxicity.

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Table 4
DL_{50} values of oxamniquine and derivatives obtained by experimental determination

Oxaminiquine and derivatives	DL_{50} (mg/kg)
Oxamniquine	1300
Derivative **1**	1135
Derivative **2**	697
Derivative **3**	632

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