New imidazolidinic bioisosters: potential candidates for antischistosomal drugs

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The emergence of strains of Schistosoma *resistant to praziquantel has drawn attention to the search for new schistosomacide drugs. Imidazolidinic derivatives have performed outstandingly against adult* S. mansoni *worms when evaluated in vitro. The molecular modification of imidazolidine by way of bioisosteric replacement gives rise to variations in its biological response. This study verifies the potential of substituent groups in the derivatives (Z)3-benzyl-5-(2-fluoro-benzylidene)-imidazolidine-2,4-dione* ***NE4****, 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-ona* ***PT5****, 3-benzyl-5-(3-fluoro-benzylidene)-1-methyl-2-thioxo-imidazolidin-4-one* ***JT53****; 3-benzyl-1-methyl-5-(4-methyl-benzylidene)-2-thioxo-imidazolidin-4-one* ***JT63****; 3-benzyl-1-methyl-5-(4-methoxi-benzylidene)-2-thioxo-imidazolidin-4-one* ***JT68****; 3-(4-chloro-benzyl)-1-methyl-5-(4-methoxi-benzylidene)-2-thioxo-imidazolidin-4-one* ***JT69****; 3-(4-phenyl-benzyl)-1-methyl-5-(4-methoxi-benzylidene)-2-thioxo-imidazolidin-4-one* ***JT72*** *by determining the viability in vitro of adult* S. mansoni *worms in the presence of these derivatives. The susceptibility of the worms obtained from mice and kept in culture in the presence of different concentrations was determined by way of schistosomacide kinetic, observed every 24 h over a period of eight days. The results show that the worms were more sensitive to the* ***PT5*** *derivative at a concentration of 58 µM which killed 100% of the worms after 24 h of contact, also giving rise to alterations in the tegument surface of the worms with the formation of bubbles and peeling. These observations suggest a strong electronic contribution of the arylazo grouping in the biological response.*

Key words: *Schistosoma mansoni* - imidazolidines - in vitro susceptibility

Schistosomiasis is still a major public health problem in Africa, Eastern Europe, Asia, and South America (WHO 1993). For some species of *Schistosoma* that infect humans, praziquantel, an isoquinolin-4-one, is the only effective drug (Davis & Wegner 1979). However, its continuous use for more than 25 years, against infections caused by both cestodes and trematodes, has made the parasites resistant (Silva et al. 2005). At present, various research groups are dedicating themselves to identifying new schistosomacide agents obtained from natural (Hamed & Hetta 2005, Tallima et al. 2005, Massoud et al. 2005) and synthetic sources (Bahgat et al. 2005, Abass & Mostafa 2005).

The imidazolidines are a broad class of bioactive compounds that also have schistosomacide properties. Niridazol, 1-(5-nitro-thiazol-2-yl)-imidazolidin-2-one, the drug used in the last century, has been widely used for clinical purposes (Cioli et al. 1995).

The schistosomacide properties of imidazoline derivatives has been shown in the case of adult *S. mansoni* worms in in vitro studies (Oliveira et al. 2004**,** Albuquerque et al. 2005). It is known that the structural variations lead to alterations in the physical properties and reactivity of the chemical compounds, thereby giving rise to changes in the distribution in the cells and tissues and in access to the active enzyme and receptor centers. The molecular modification of imidazolidine by bioisosteric replacement produces a biological response. This study evaluated the schistosomacide effect of imidazolidine derivatives with different substituent groups by determining their in vitro viability against adult *S. mansoni* worms.

New 3-benzyl-5-benzylidene-1-methyl-2-thioxo-imidazolidine **JT** derivatives were obtained in four stages. At first, *N*-methyl-glicine or sarcosine reacts with ammonium thiocianate to form 1-methyl-2-thioxo-imidazolidin-4-one. At the same time, Cope esters are obtained by the reaction of substituted benzadehydes with ethyl cyanoacetate in the presence of piperidene (Cope et al. 1941). To obtain the intermediary substitutes in position 5 of the 2-thioxo-imidazolidine nucleus, an addition reaction like that of Michael's was used reacting 1-methyl-2-thioxo-imidazolidin-4-one with the different Cope esters, to obtain the 5-benzylidene-1-methyl-2-thioxo-imidazolidin-4-one derivatives, which react with benzyl bromide to produce the 3-benzyl-5-benzylidene-1-methyl-2-thioxo-imidazolidin-4-one derivatives **JT**.

The synthesis and the physico-chemical properties of the derivatives (*Z*)3-benzyl-5-(2-fluoro-benzylidene)-imidazolidine-2,4-dione **NE4** (Lima et al. 1992) and 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-one **PT5**

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(Brandão et al. 1997) are described in the literature. Crystallographic study of **NE4** proved it to have a *Z* configuration (Simone et al. 1996).

MATERIALS AND METHODS

The BH strain of *S. mansoni* was kept in the laboratory after it had passed through *Biomphalaria glabrata* molluscs and Swiss mice (*Mus musculus*). Mice weighing between 20 and 25 g were infected by exposure to a cercarian suspension of S. *mansoni* with approximately 150 cercarias, using the tail immersion technique (Olivier & Stirewalt 1952).

After seven weeks of infection, the adult *S. mansoni* worms were removed from the mesenteric and portal veins of the infected mice under aseptic conditions (Duvall & Dewitt 1967). The worms were washed in a RPMI1640 (Sigma) medium kept at pH 7.5 with HEPES 20 mM and supplemented with penicillin (100 UI/ml), streptomicin (100 µg/ml) and 10% bovine fetal serum (Cutilab) and transferred to tissue culture plates containing 2 ml of the same medium. Each well received two worms and these were then incubated at 37ºC in a humid atmosphere containing 5% CO_2.

The experiments were approved by the Federal University of Pernambuco's Animal Experiments Ethics Committee, Process no. 185/2004, in accordance with Law 9605 Article 32 Decree 3179 – Art 17.

For the in vitro test with *S. mansoni*, these compounds were dissolved in 1.6% dimethyl sulphoxide (DMSO) and used in concentrations varying from 29 to 640 µM, which were added to the medium containing the worms after a period of 2 h of adaptation to the culture medium. Duplicates were carried out for each concentration used. The parasites were kept for 8 days and monitored every 24 h to evaluate their general condition: motor activity, alterations in the tegument, mortality rate. The control worms were treated with 1.6% of DMSO in an RPMI 1640 medium.

RESULTS

Chemical - In order to obtain the new 3-benzyl-5-benzylidene-1-methyl-2-thioxo-imidazolidin-4-one derivative compounds (**JT**) 5-benzylidene-1-methyl-2-thioxo-imidazolidin-4-one, potassium carbonate as a catalyst and methanol as a solvent were placed in a flask. The reactional mixture was constantly shaken at room temperature for one hour. Subsequently, the substituted benzyl bromide was added. All reactions were accompanied by chromatographic analysis in a thin layer by way of an adequate elution system. The 3-benzyl-5-benzylidene-1-methyl-2-thioxo-imidazolidin-4-one derivatives **JT** were purified by washing with suitable solvents. The fusion points were determined in capillary tubes using a Buchi apparatus. Thin layer chromatography was carried out on Merck 60F254 silicagel chromoplates. The infra-red spectra were produced on KBr tablets at a concentration of 1% using a Perkin Elmer 1310 spectrometer. The magnetic nuclear resonance spectra of ^{1}H NMR protons was carried out using a Bruker AC 200 spectrophotometer in DMSO-d_6 or $CDCl_3$ as a solvent and the mass spectra using a Delsi-Nermag R 1010 C spectrometer on electronic impact.

3-benzyl-5-(3-fluoro-benzylidene)-1-methyl-2-thioxo-imidazolidin-4-one **JT53**

$C_{18}H_{15}FN_2OS$ Yield 41%, M.p. 183-184°C, *Rf* ($CHCl_3$:CH_3OH 8:2) 0,66,. IR (KBr): υ 1680, 1460, 1570 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ3.3 (s, 3H, NCH_3), 4.57 (s, 2H, CH_2), 6.82 (s, 1H, CH), 7.28-7.48 (m, 5H, benzyl), 7.19-7.26 (m, 1H, benzylidene, 4"), 7.28-7.48 (m, 1H, benzylidene, 5"), 7.87 (d, 1H, benzylidene, 2", *J*=7,81), 9.33-9.39 (m, 1H, benzylidene, 6"). MS *m/z* (%) 326(M^+. 100), 327 (M^+.+1 13.7), 328 (M^+.+2 3.38), 293(89), 235(12.5), 148(17.1).

3-benzyl-1-methyl-5-(4-methyl-benzylidene)-2-thioxo-imidazolidin-4-one **JT63**

$C_{19}H_{18}N_2OS$ Yield 57%, M.p. 187-189°C, *Rf* ($CHCl_3$:CH_3OH 6:4) 0,81,. IR (KBr): υ 1770, 1600, 1460 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ2,39(s, 3H, CH_3), 3.28 (s, 3H, NCH_3), 4.62 (s, 2H, CH_2), 6.4 (s, 1H, CH), 7.42-7.46 (m, 2H, benzyl, 2'6'), 7.31-7.35 (m, 3H, benzyl, 3'4'5'), 7.22 (d, 2H, benzylidene, 3"5" *J*=8.1Hz), 8.11 (d, 2H, benzylidene, 2"6" *J*=8.1Hz). MS *m/z* (%) 322(M^+. 44.19), 323 (M^+.+1 10.97), 324 (M^+.+2 1.29), 298(24.2), 145(87.7), 130(58.7), 91(100), 65(43.2).

3-benzyl-1-methyl-5-(4-methoxi-benzylidene)-2-thioxo-imidazolidin-4-one **JT68**

$C_{19}H_{18}N_2O_2S$ Yield 56%, M.p. 178-180°C, *Rf* ($CHCl_3$:CH_3OH 6:4) 0,78,. IR (KBr): υ 1680, 1590, 1460 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ3.29 (s, 3H, NCH_3), 3,81 (s, 3H, OCH_3), 4.58 (s, 2H, CH_2), 6.79 (s, 1H, CH), 7.29-7.48 (m, 3H, benzyl, 3'4'5'), 7.45-7.48 (m, 2H, benzyl, 2'6'), 6.99 (d, 2H, benzylidene, 3"5" *J*=8.6Hz), 8.32 (d, 2H, benzylidene, 2"6" *J*=8.6Hz). MS *m/z* (%) 338(M^+. 21.64), 339 (M^+.+1 3.18), 340 (M^+.+2 0.32), 305(8.6), 161(31.9), 146(100), 91(41.8), 65(17.3).

3-(4-chloro-benzyl)-1-methyl-5-(4-methoxi-benzylidene)-2-thioxo-imidazolidin-4-one **JT69**

$C_{19}H_{17}ClN_2O_2S$ Yield 53%, M.p.. 219-219°C, *Rf* ($CHCl_3$:CH_3OH 6:4) 0,82,. IR (KBr): υ 1670, 1590, 1460 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ3.28 (s, 3H, NCH_3), 3,86 (s, 3H, OCH_3), 4.57 (s, 2H, CH_2), 6.39 (s, 1H, CH), 7.29 (d, 2H, benzyl, 2'6' *J*=8.7Hz), 7.29 (d, 2H, benzyl, 3'5' *J*=8.7Hz), 6.88 (d, 2H, benzylidene, 3"5" *J*=8.8Hz), 8.25 (d, 2H, benzylidene, 2"6" *J*=8.8Hz). MS *m/z* (%) 372(M^+. 13.29), 373 (M^+.+1 3.79), 374 (M^+.+2 6.31), 161(22.1), 146(100), 125(43.4), 90(6.5), 63(17.8).

3-(4-phenyl-benzyl)-1-methyl-5-(4-methoxi-benzylidene)-2-thioxo-imidazolidin-4-one **JT72**

$C_{25}H_{22}N_2O_2S$ Yield 67%, M.p. 157-159°C, *Rf* ($CHCl_3$:CH_3OH 6:4) 0,76,. IR (KBr): υ 1670, 1590, 1450 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ3.21 (s, 3H, NCH_3), 3,84 (s, 3H, OCH_3), 4.6 (s, 2H, CH_2), 6.34 (s, 1H, CH), 7.63-7.65 (m, 1H), 7.25-7.43 (m, 8H, benzyl), 6.92 (d, 2H, benzylidene, 3"5" *J*=8.8Hz), 8.23 (d, 2H, benzylidene, 2"6" *J*=8.8Hz). MS *m/z* (%) 414(M^+. 30.78), 415 (M^+.+1 4.36), 167(75.8), 166(49.2), 165(100), 161(25.6), 152(40.9), 146(93.2), 91(9.6).

Biological - The evaluation of the in vitro susceptibility of *S. mansoni* to the 2-thioxo-imidazolidin-4-one derivatives **JT53**, **JT63**, **JT68**, **JT69**, **JT72** revealed that the worms were sensitive during the first 24 h to the compounds **JT53**, **JT63**, and **JT72,** the highest mortality rate

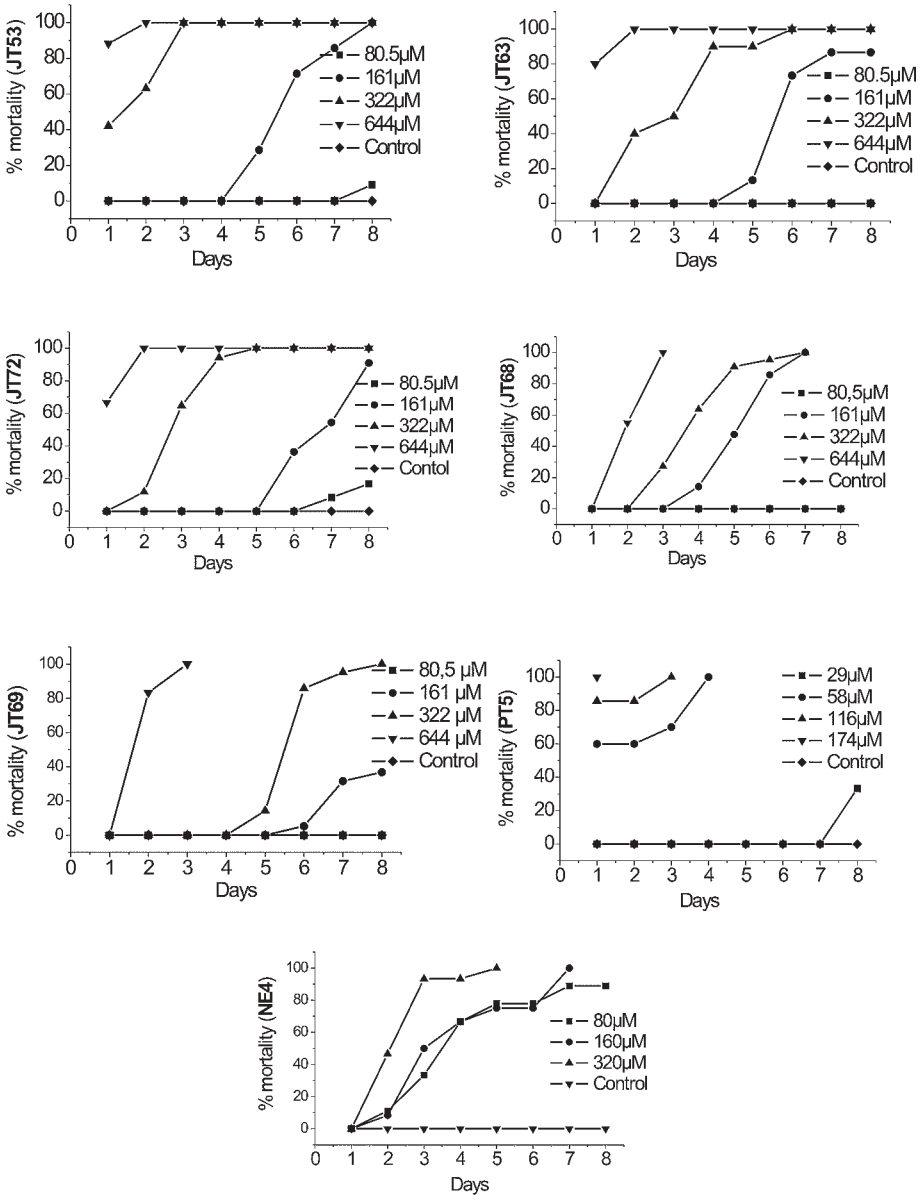
occurring after 48 h of contact at a concentration of 644 µM. The **JT68** and **JT69** compounds, at the same concentration, achieved their maximum response after 72 h. The **JT53** and **JT72** compounds killed around 9 and 16% of the worms respectively with the lower concentration used. Adult male worms, when submitted to the action of derived arylazo imidazolidine **PT5** were shown to be sensitive at all the concentrations used with 100% mortality after 24 h of contact. In the case of female worms, a clear relation was observed between the concentration used and the effect after a period of 24 h. The maximum effect achieved, at concentrations of 58 µM and 116 µM, was after 96 and 72 h, respectively. The sensitivity of the adult *S. mansoni* worms to the imidazolidine-2,4-dione derivative **NE4** could be seen from the first day of treatment onwards. A maximum response of 100% mortality was achieved at the end of the fourth day of exposure to the 320 ìM concentration (Figure).

In all the experiments, the control group remained viable throughout the observation period, and were submitted only to the vehicle used to dissolve the different compounds.

DISCUSSION

The mortality kinetic of adult *S. mansoni* worms in the presence of imidazolidine derivatives was used to evaluate the schistosomacide properties of the imidazolidine derivatives studied.

It can be seen from Table that the compound arylazo imidazolidine **PT5** caused 100% mortality of worms at a concentration of 29 µM in the course of a period of 24 h of contact and was the most active compound. The 2-thioxo-



Mortality of adult *Schistosoma mansoni* worms in the presence of imidazolidine derivatives.

TABLE

Concentration and contact time capable of producing 100% mortality of adult *Schistosoma* worms

Compound	Concentration	Contact time (h)
JT68	640 µM	72h
JT69	640 µM	48h
JT72	640 µM	48h
JT53	640 µM	48h
JT63	640 µM	48h
NE4	320 µM	96h
PT5	174 µM	24h

imidazolidin-4-one derivatives **JT53**, **JT63**, **JT68**, **JT69**, and **JT72** only reached a similar percentage mortality of worms at a concentration of 644 µM, after 48 h. The longest period of time taken to cause the death of all the worms was 96 h of contact at a concentration of 320 µM in the case of the **NE4** derivative.

All the derivatives studied caused alterations in the tegument surface of the worms with the formation of bubbles and peeling, indicating damage to cells, an effect directly related to the duration of exposure. It was also observed that schistosomacide properties followed a dose-response dependent relation.

The mortality rate and the lesions in the teguments suggest that the imidazolidine derivatives are active against *S. mansoni*. The 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-one **PT5** derivative proved to be the most active, probably owing to the participation of the arylazo grouping.

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