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-one 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-methoxy-benzylidene)-2-thioxo-imidazolidin-4-one JT68; 3-(4-chloro-benzyl)-1-methyl-5-(4-methoxy-benzylidene)-2-thioxo-imidazolidin-4-one JT69; 3-(4-phenyl-benzyl)-1-methyl-5-(4-methoxy-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 imidazolines are a broad class of bioactive compounds that also have schistosomacide properties. Nitidazol, 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 cyanacetate in the presence of piperidine (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...
(Brandão et al. 1997) are described in the literature. Crystalllographic 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 cercarial 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 & Stirewalt 1952).

Cercariae suspension of S. mansoni 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 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 1H NMR protons was carried out 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

C18H15FN1O4S Yield 41%, M.p. 183-184°C, Rf (CHCl3:CH3OH 8:2) 0.66, IR (KBr): v 1770, 1600, 1460 cm⁻¹ 1H NMR (CDCl3): δ 3.21 (s, 3H, NCH3), 3.84 (s, 3H, OCH3), 4.57 (s, 2H, CH2), 6.82 (s, 1H, CH), 7.28-7.48 (m, 3H, 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 23.8), 293 (89), 255 (12.5), 148 (17.1).

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

C19H18N2OS Yield 57%, M.p. 187-189°C, Rf (CHCl3:CH3OH 6:4) 0.81, IR (KBr): v 1770, 1600, 1460 cm⁻¹ 1H NMR (CDCl3): δ 3.29 (s, 3H, NCH3), 3.8 (s, 3H, OCH3), 4.62 (s, 2H, CH2), 6.4 (s, 1H, CH), 7.42-7.46 (m, 2H, benzyl, 2°), 6.71-7.35 (m, 3H, benzyl, 3°), 7.22 (d, 2H, benzylidene, 3°, J=8.6Hz), 8.11 (d, 2H, benzylidene, 2°, J=8.6Hz). 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-methoxy-benzylidene)-2-thioxo-imidazolidin-4-one JT68

C19H17ClN2O2S Yield 67%, M.p. 157-159°C, Rf (CHCl3:CH3OH 6:4) 0.78, IR (KBr): v 1770, 1600, 1460 cm⁻¹ 1H NMR (CDCl3): δ 3.28 (s, 3H, NCH3), 3.86 (s, 3H, OCH3), 4.57 (s, 2H, CH2), 6.39 (s, 1H, CH), 7.29-7.48 (m, 3H, benzyl, 3°), 7.45-7.48 (m, 2H, benzyl, 2°), 6.99 (d, 2H, benzylidene, 3°, J=8.6Hz), 8.32 (d, 2H, benzylidene, 2°, J=8.6Hz). MS m/z (%) 338(M⁺, 21.64), 339 (M⁺+1 13.18), 340 (M⁺+2 0.32), 305 (86), 161 (31.9), 146 (100), 91 (41.8), 65(17.3).

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

C25H22N2O2S Yield 53%, M.p. 219-219°C, Rf (CHCl3:CH3OH 6:4) 0.82, IR (KBr): v 1770, 1600, 1460 cm⁻¹ 1H NMR (CDCl3): δ 3.29 (s, 3H, NCH3), 3.86 (s, 3H, OCH3), 4.57 (s, 2H, CH2), 6.39 (s, 1H, CH), 7.29 (d, 2H, benzyl, 2°, J=8.8Hz), 7.29 (d, 2H, benzyl, 3°, J=8.7Hz), 6.88 (d, 2H, benzylidene, 3°, J=8.6Hz), 8.25 (d, 2H, benzylidene, 2°, J=8.6Hz). MS m/z (%) 372(M⁺, 13.29), 373 (M⁺+1 1.37), 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-methoxy-benzylidene)-2-thioxo-imidazolidin-4-one JT72

C25H22N2O2S Yield 67%, M.p. 157-159°C, Rf (CHCl3:CH3OH 6:4) 0.76, IR (KBr): v 1770, 1590, 1460 cm⁻¹ 1H NMR (CDCl3): δ 3.21 (s, 3H, NCH3), 3.84 (s, 3H, OCH3), 4.6 (s, 2H, CH2), 6.34 (s, 1H, CH), 7.63-7.87 (m, 1H), 7.25-7.43 (m, 8H, benzyl), 6.92 (d, 2H, benzylidene, 3°, J=8.8Hz), 8.23 (d, 2H, benzylidene, 2°, 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 µM 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-
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 imidazoline 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 probable, owing to the participation of the arylazo grouping.

**REFERENCES**


Bahgat MM, Maghraby AS, Heiba ME, Ruppel A, Fatallah OA 2005. Synthesis of new 4-oxo-2-thioxo-1,2,3,4-tetrahydroprymidine derivatives with an incorporated thiazolidine moiety and testing their possible serine protease and cercarial elastase inhibitory effects with a possible prospective to block penetration of *Schistosoma mansoni* cercariae into the mice skin. *Arch Pharm Res* 28:1002-1012.


