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New 1,3-benzodioxole derivatives: Synthesis, evaluation of *in vitro* schistosomicidal activity and ultrastructural analysis

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ABSTRACT

Schistosomiasis is considered a serious public health problem in 78 countries and territories located in Africa, Asia and America and it is estimated in more than 249 million people infected by any of the species of *Schistosoma*. The exclusive use of praziquantel (PZQ), effective drug against all species of *Schistosoma*, has been the basis of the development of a possible resistance against the strains of this parasite. In addition, PZQ is not effective against young forms of worms. Thus, there is a need for the development of new drugs with schistosomicidal activity. The objective of this work was to synthesize and to evaluate the therapeutic potential of new benzodioxole derivatives (**3–14**) candidates for schistosomicidal drugs. All compounds synthesized showed *in vitro* schistosomicidal activity. The derivative **12** was considered the best compound, since it took 100% of worms to mortality in the first 72 h of exposure at the concentration of 100 μM and 83.3% at the concentration of 50 μM. Furthermore, male and female adult worms, incubated for 24 h with the compound **12** showed tegument damages characterized by extensive desquamation and edema, tuber destruction, bubble formation and exposure of the muscle layer. This compound has a restricted structure, where the thiazolidinone is attached to the 4-position of the 1,3-benzodioxol ring. The structural conformation of derivative **12** was probably responsible for the promising schistosomicidal activity, where the presence of an electron/conformational restriction of the thiazolidine ring, as well as the action of bromine as a bulk substitute, favored an increase in biological activity. In addition, tegumentary changes caused by derivative **12** may also have been responsible for the death of adult worms of *Schistosoma mansoni*. Therefore, we verified that the results obtained in this study make benzodioxole derivatives possible candidates for prototypes of new schistosomicidal drugs.

1. Introduction

Schistosomiasis is considered one of the most important neglected diseases. It is endemic in 78 countries and territories located in Africa, Asia and the Americas and it is estimated to be more than 249 million people infected with any of the *Schistosoma* species: *S. haematobium*, *S. guineensis*, *S. intercalatum*, *S. mansoni*, *S. japonicum* and *S. mekongi*. Approximately 92% of the parasitized population inhabit sub-Saharan Africa, a region where schistosomiasis kills about 200,000 people annually. Estimates show that at least 218 million people needed preventive treatment in 2015 as ways to reduce and prevent severe morbidity in highly endemic areas [1].

Praziquantel (PZQ), a pyrazinoisoquinolinic derivative, is the only alternative treatment for schistosomiasis and it is effective against all *Schistosoma* species, safe and with low cost [2–4]. However, PZQ does not act on immature forms of worms, and there are reports of resistant strains [5,6]. At the same time, there is no availability of other schistosomicidal drugs on the market with comparable efficacy to that of PZQ [7].

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Thus, the search for new drugs that may present activity against *Schistosoma* species becomes fundamental. Our group has explored the schistosomicidal potential of synthetic molecules on *S. mansoni*, among them benzodioxole derivatives, obtained by molecular hybridization of 1,3-benzodioxole, thiosemicarbazone and thiazolidinone nuclei.

1,3-benzodioxole is present in several drugs and has been used as an important intermediary in the organic synthesis both in the preparation of several biologically active natural products and in the preparation of synthetic substances originating from planned drug development programs [8,9]. Studies show that the thiosemicarbazones portion has antiparasitic activity [10,11]. Similarly, the thiazolidinone nucleus has also shown proven schistosomicidal activity [12]. Thus, the objective of this work was to evaluate the *in vitro* schistosomicidal activity of benzodioxole derivatives against adult worms of *Schistosoma mansoni*.

2. Material and methods

2.1. Chemistry

IR spectra were recorded on potassium bromide pellets with a Bruker IFS-66 IR spectrophotometer (Bruker, USA). ^{1}H NMR spectra were recorded at 400 MHz on an Varian 400-MR spectrometer. ^{13}C NMR spectra were recorded at 100 MHz on an Varian, USA 400-MR spectrometer with tetramethylsilane as an internal standard and DMSO-d_6 as the solvent. Silica gel 60-N (spherical and neutral, 100–210 m, 37560-79), supplied by Kanto Chemical Co., was used for column chromatography. All melting points were measured on a Quimis-340.27 apparatus (Quimis, Diadema, SP, Brazil) and are uncorrected.

2.1.1. Synthesis and characterization of benzo[d][1,3]dioxole-5-carbaldehyde (2)

A solution of 3,4-dihydroxybenzaldehyde (5 g, 0.011 mol) in DMF (150 mL) was added dropwise to a suspension of CH_2Br_2 (31,4 mL, 16.7 mol) and K_2CO_3 (10 g, 0.0362 mol) in DMF (30 mL) according protocol to Wang et al. (2010) [13] with minor modifications. The mixture was stirred and heated at reflux for 24 h then cooled and filtered. The filtrate was concentrated, diluted with water, and extracted with ethyl acetate (3 × 100 mL). The filter cake was with ethyl acetate (25 mL). The organic layer was washed with 10% NaOH (25 mL), water (25 mL), dried (Na_2SO_4), and evaporated to afford 2 g (38%) as a yellow oil. The aldehyde was also obtained starting from the optimized piperonyl alcohol [14]. The reaction consists of the oxidation of alcohol (1 g, 0.0065 mol) with 15 equivalents of manganese IV oxide (8.55 g) in dry dichloromethane (15 mL) for 2 h at 25 °C. After 2 h, the obtained filtrate was evaporated under reduced pressure and taken to the freezer obtaining a crystalline precipitate in a yield of 85%. The benzo[*d*][1,3] dioxole-4-carbaldehyde was obtained commercially.

2.1.2. Synthesis and characterization of thiosemicarbazones (3–9)

Then an glacial acetic acid (1,5 mL) was added to a solution of benzo[*d*][1,3]dioxole-4-carbaldehyde (**1**) or benzo[*d*][1,3]dioxole-5-carbaldehyde (0.5 g, 0.0043 mol) (**2**) and the appropriate *N*-aryl-thiosemicarbazides (0.0043 mol) in absolute ethanol (40 mL). The condensation reaction was stirred under reflux for the appropriate time (TLC controlled). The solvent was evaporated under reduced pressure to afford the respective crude. After drying, the product was recrystallized from toluene [15].

2.1.2.1. 2-(benzo[d][1,3] dioxol-4-ylmethylene)-N-phenyl-thiosemicarbazone (3). Compound **3** was obtained as white powder (63%): R_f = 0.55 (*n*-Hex/AcOEt 8:2); mp: 192–193 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.00 (1H, s, NH); 10.12 (1H, s, NH); 8.26 (1H, s, −HC =); 7.71 (1H, d, Ar, J = 8 Hz); 7.61 (3H, m, Ar); 7.42 (2H, d, Ar, J = 8.8 Hz); 6.95 (1H, d, Ar, J = 8.8 Hz); 6.86 (1H, t, Ar, J = 8 Hz); 6.11 (2H, s) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 101.54 (1C, CH_2); 109.3 (1C, Ar), 116.13 (1C, Ar), 118.4 (1C, Ar), 121.57 (1C, Ar), 121.57 (1C, Ar), 125.3 (2C, Ar), 128 (2C, Ar), 136.2 (1C, Ar), 138.9 (1C, Ar), 146.53 (1C, Ar), 147.56 (1C, C═N), 175.86 (1C, C═S) ppm; IR (KBr, cm^{-1}) 3307 (NH); 1543 (C═N); 1511 (C═S).

2.1.2.2. 2-(benzo[d][1,3]dioxol-4-ylmethylene)-N-(4-chlorophenyl)-thiosemicarbazone (4). Compound **4** was obtained as white powder (50%): R_f = 0.60 (*n*-Hex/AcOEt 8:2); mp: 220–221 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.01 (1H, s, NH); 10.12 (1H, s, NH); 8.26 (1H, s, −HC =); 7.7 (1H, d, Ar, J = 8 Hz); 7.61 (2H, d, Ar, J = 6,4 Hz); 7.4 (2H, d, Ar, J = 6.4 Hz); 6,94 (1H, d, Ar, J = 7.6 Hz); 6,87 (1H, t, Ar, J = 8 Hz); 6.11 (2H, s) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 101.547 (1C, CH_2); 109.37 (1C, Ar), 116.01 (1C, Ar), 118.33 (1C, Ar), 121.57 (1C, Ar), 121.57 (1C, Ar), 127.31 (2C, Ar), 127.94 (2C, Ar), 129.30 (1C, Ar), 136.54 (1C, Ar), 146.63 (1C, Ar), 147.58 (1C, C═N), 175.87 (1C, C═S) ppm. IR (KBr, cm^{-1}) 3310 (NH); 1541 (C═N); 1506 (C═S).

2.1.2.3. 2-(benzo[d][1,3]dioxol-4-ylmethylene)-N-(4-bromophenyl)-thiosemicarbazone (5). Compound **5** was obtained as white powder (70%): R_f = 0.60 (*n*-Hex/AcOEt 7:3); mp: 216 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.00 (1H, s, NH); 10.10 (1H, s, NH); 8.26 (1H, s, −HC =); 7.70 (1H, d, Ar, J = 8 Hz); 7.55 (4H, s, Ar); 6.95–6.86 (2H, dd, Ar, J = 1.2 Hz, J = 7.6 Hz); 6.11 (2H, d, CH_2) ppm. IR (KBr, cm^{-1}) 3300 (NH); 1541 (C═N); 1502 (C═S).

2.1.2.4. 2-(benzo[d][1,3]dioxol-5-ylmethylene)-N-(4-chlorophenyl)-thiosemicarbazone (6). Compound **6** was obtained as white powder (35%): R_f = 0.60 (*n*-Hex/AcOEt 8:2); mp: 169–171 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.8 (1H, s, NH); 10.1 (1H, s, NH); 8.06 (1H, s, −HC =); 7.4 (2H, d, Ar, J = 8.8 Hz); 7.6 (2H, d, Ar, J = 8.8 Hz); 6.94 (1H, d, Ar, J = 7.6 Hz) 7.1 (1H, d, Ar, J = 6.8 Hz); 7.8 (1H, s, Ar); 6.0 (2H, d, CH_2) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 101.6 (1C, CH_2); 109.37 (1C, Ar), 116.01 (1C, Ar), 118.33 (1C, Ar), 121.57 (1C, Ar), 121.57 (1C, Ar), 127.31 (2C, Ar), 127.94 (2C, Ar), 129.30 (1C, Ar), 136.54 (1C, Ar), 146.63 (1C, Ar), 155.9 (1C, C═N), 175.9 (1C, C═S) ppm. IR (KBr, cm^{-1}) 3294.2(NH); 1548 (C═N); 1497 (C═S).

2.1.2.5. 2-(benzo[d][1,3]dioxol-5-ylmethylene)-N-(4-bromophenyl)-thiosemicarbazone (7). Compound **7** was obtained as white powder (60%): R_f = 0.50 (*n*-Hex/AcOEt 8:2); mp: 187 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.79 (1H, s, NH); 10.09 (1H, s, NH); 8.06 (1H, s, −HC =); 7.81 (1H, s, Ar); 7.55 (2H, d, Ar, J = 8 Hz); 7.54 (2H, d, Ar, J = 8 Hz); 7.15 (1H, d, Ar, J = 8 Hz); 6.94 (1H, d, Ar, J = 8 Hz); 6.08 (2H, s, CH_2) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 101.96 (1C, CH_2); 106.04 (1C, Ar), 108.63 (1C, Ar), 124.89 (1C, Ar), 128.16 (1C, Ar), 128.34 (1C, Ar), 128.93 (1C, Ar), 129.75 (2C, Ar), 138.55 (2C, Ar), 143.43 (1C, Ar), 148.52 (1C, Ar), 149.58 (1C, C═N), 176.16 (1C, C═S) ppm; IR (KBr, cm^{-1}) 3330 (NH); 1543 (C═N); 1498 (C═S).

2.1.2.6. 2-(benzo[d][1,3]dioxol-5-ylmethylene)-N-(4-methoxyphenyl)-thiosemicarbazo- ne (8). Compound **8** was obtained as white powder (60%): R_f = 0.50 (*n*-Hex/AcOEt 8:2); mp: 175–177 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.6 (1H, s, NH); 10.0 (1H, s, NH); 8.04 (1H, s, −HC =); 7.38 (2H, d, Ar, J = 8.8 Hz); 7.13 (2H, d, Ar, J = 9.2 Hz); 7.11 (1H, d, Ar, J = 8 Hz); 6.93 (1H, d, Ar, J = 8 Hz); 7.8 (1H, s, Ar); 6.06 (2H, s, CH_2); 3.34 (3H, s, CH_3) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.2 (1C, CH_3); 101.4 (1C, CH_2); 105.5 (1C, Ar); 108.1 (1C, Ar); 113.1 (1C, Ar) 124.2 (1C, Ar); 127.7 (1C, Ar); 128.6 (2C, Ar); 132 (1C, Ar); 142.3 (1C, Ar); 148.05 (2C, Ar); 148.9 (1C, Ar); 156.9 (1C, C═N); 176.1 (1C, C═S) ppm. IR (KBr, cm^{-1}) 3316.7 (NH); 1542 (C═N); 1499 (C═S).

2.1.2.7. 2-(benzo[d][1,3]dioxol-5-ylmethylene)-N-(naphthalen-1-yl)-thiosemicarbazone (9). Compound **9** was obtained as white powder (42%): R_f = 0.62 (*n*-Hex/AcOEt 8:2); mp: 213–214 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.8 (1H, s, NH); 10.3 (1H, s, NH); 8.12 (1H,