Res Chem Intermed
DOI 10.1007/s11164-012-0854-9

In vitro antischistosomal evaluation of some newly synthesized praziquantel derivatives

M. M. Kamel · M. M. Anwar · A. M. Soliman · H. F. Abdel-Hamid

Received: 24 July 2012 / Accepted: 5 October 2012

Abstract Praziquantel, 2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one, was used as the parent starting material to synthesize a new series of praziquantel-3-arylidene derivatives **1a–c** and praziquantel–Mannich bases **2a–d**, hoping to obtain new antischistosomal compounds of more activity and lower adverse effects. The antischistosomal activity of the newly synthesized compounds was evaluated using in vitro *Schistosoma mansoni* worm killing tests. Both compounds **2c** and **2d** exhibited significant in vitro antischistosomal activity and may offer promising use as an antischistosomal drug either alone or in combination with praziquantel.

Keywords Praziquantel · Arylidene derivatives · Mannich bases · Antischistosomal evaluation · *Schistosoma mansoni*

Introduction

Schistosomiasis is the second most prevalent parasitic disease worldwide after malaria, with about 200 million human beings infected in 74 countries. It is estimated that 779 million people are at risk of contracting schistosomiasis and more than 200 million individuals are infected, with more than half of them suffering from disease-associated symptoms [1–3]. Severe disease manifestations are exhibited in about 20 million individuals [4]. The annual mortality rate due to schistosomiasis in sub-Saharan Africa might be as high as 280,000 [5]. Chemotherapeutic measures have been the mainstay for the control of schistosomiasis [6],

M. M. Kamel · M. M. Anwar (✉) · A. M. Soliman
Therapeutical Chemistry Department, National Research Centre, Dokki, Cairo, Egypt
e-mail: Manalhassan232@ymail.com

H. F. Abdel-Hamid
Chemistry of Pesticides Department, National Research Centre, Dokki, Cairo, Egypt

Published online: 19 October 2012

and, since the 1970s, praziquantel (PZQ) has become the drug of choice against the three major human species of schistosomes, *Schistosoma mansoni*, *S. haematobium*, and *S. japonicum* [7, 8]. PZQ is a relatively safe, orally administered drug that leads to reduction in the prevalence of schistosomiasis [9, 10]. Mass drug administration programs currently rely heavily on PZQ for the control of schistosome-induced morbidity. However, with only one drug of choice for treatment and with the possibility of development of parasite resistance, the present situation is dangerous. There is a real and pressing need for discovering alternatives to the only available antischistosomal drug worldwide [11, 12].

In this investigation, we synthesized new praziquantel–arylidene derivatives **1a–c** and praziquantel–Mannich bases **2a–d**, which were evaluated to assess their potential for in vitro antischistosomal activities (Scheme 1).

Experimental

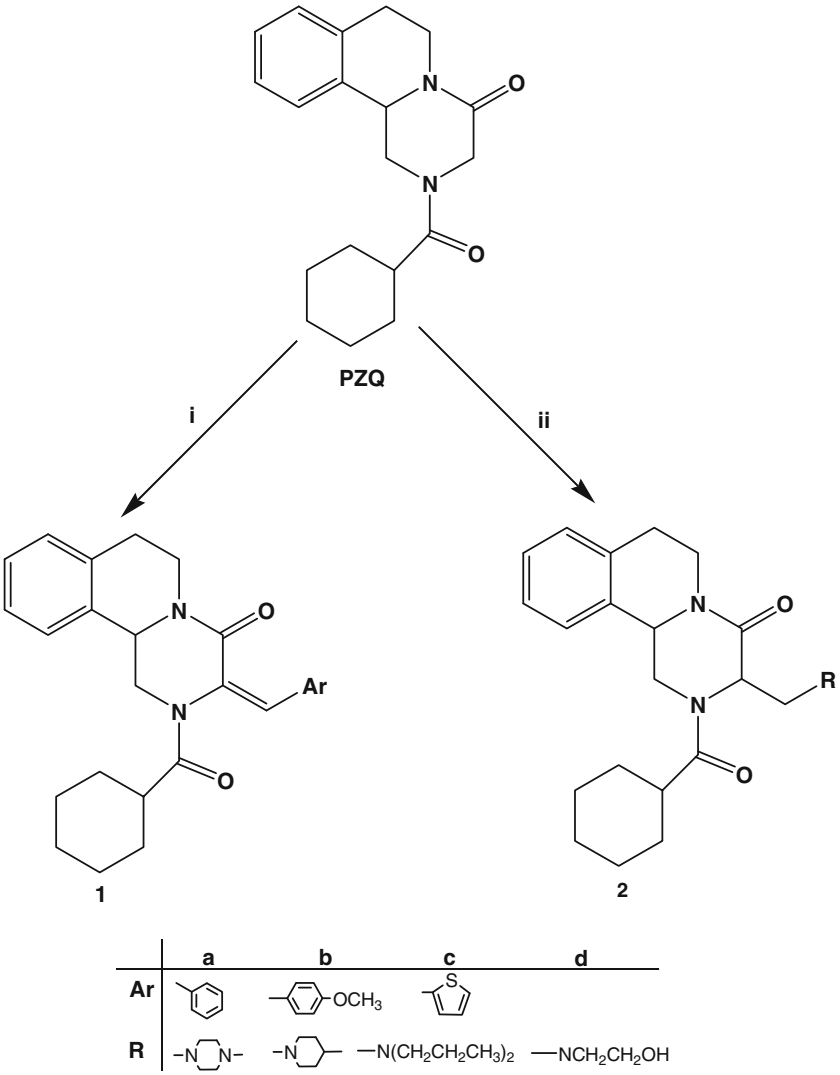
Chemistry

All reagents and solvents were of general reagent grade and used without further purification. Praziquantel is a product of Bayer. All melting points were uncorrected and measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). Microanalytical data were performed by Vario El-Mentar apparatus (Shimadzu, Japan), National Research Centre (NRC), Cairo, Egypt. The found values were within ±0.4 % of the theoretical values. IR spectra (KBr) were recorded on a Perkin-Elmer 1650 spectrophotometer (Japan), NRC, Cairo, Egypt. ^{1}H-NMR and ^{13}C-NMR spectra were determined on a Varian Mercury (300 MHz) spectrometer (Varian, UK) and the chemical shifts were expressed in δ ppm relative to TMS as an internal reference, Faculty of Science, Cairo University, Egypt. Mass spectra were recorded at 70 eV on an EI Ms-QP 1000 EX instrument (Shimadzu, Japan), Faculty of Science, Cairo University, Egypt. TLC was performed on silica gel 60 254F plates (Merck) using a mixture of chloroform and ethanol (15:1, v/v) as an eluent, UV light at λ 254, and iodine accomplished visualization.

Synthesis

*General procedure for preparation of 2-(cyclohexylcarbonyl)-3-substituted arylidene 1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinolin-4-one (**1a–c**)*

A mixture of praziquantel (20 mmol) and different aromatic aldehydes, namely, benzaldehyde, *p*-methoxy benzaldehyde, and thiophene-2-carboxaldehyde (20 mmol), in 5 % ethanolic sodium hydroxide solution (40 mL) was refluxed with continuous stirring for 3 h. The reaction mixture was neutralized with diluted HCl. The obtained solid was filtered and re-crystallized from isopropyl alcohol/petroleum ether to give the arylidene derivatives **1a–c**.



Reaction conditions: **i**: 10 % NaOH, ethanol, appropriate aldehydes, reflux for 3 h.
ii: paraformaldehyde, appropriate amines, absolute ethanol, reflux, 6-9 h.

Scheme 1 Synthesis of praziquantel–arylidene derivatives **1a–c** and praziquantel–Mannich bases **2a–d**

*2-(Cyclohexylcarbonyl)-3-benzylidene-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinolin-4-one (**1a**)*

Yield: 60 %; mp 290 °C; IR (KBr, cm^{-1}): 3012 (CH, aromatic), 1710, 1650 (2CO); ^{1}H-NMR ($CDCl_3$, δ ppm): 1.21 (s, 11H, cyclohexyl ring), 2.75–2.90 (2 m, 4H, C_7–$\underline{H_2}$, C_6–$\underline{H_2}$), 3.10, 3.42 (2d, 2H, C_1–H_2), 5.11 (m, 1H, C_{11b}–$\underline{H}$), 5.62 (s, 1H, –C=C$\underline{H}$–), 7.21–8.00 (m, 9H, aromatic-H); ^{13}C-NMR (DMSO-*d6*, δ ppm): 170.1,

160.5, 143.3, 136.4, 135.2, 128.9, 128.4, 127.6, 127.0, 126.1, 125.3, 122.1, 116.3, 54.2, 52.3, 43.2, 42.3, 29.2, 27.2, 24.4. MS *m/z*: 400 ($M^{+\cdot}$) (10 %). Analysis for $C_{26}H_{28}N_2O_2$ (400.22): Calcd.: C, 77.97; H, 7.05; N, 6.99; Found: C, 77.50; H, 6.78; N, 6.52.

*2-(Cyclohexylcarbonyl)-3-(4-methoxybenzylidene)-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinolin-4-one (**1b**)*

Yield: 75 %; mp 300 °C; IR (KBr, cm^{-1}): 3012 (CH, aromatic), 1700, 1655 (2CO); ^{1}H-NMR ($CDCl_3$, δ ppm): 1.21 (s, 11H, cyclohexyl ring), 2.75–2.90 (2 m, 4H, C_7–$\underline{H_2}$, C_6–$\underline{H_2}$), 3.10, 3.42 (2d, 2H, C_1–H_2), 3.75 (s, 3H, OCH_3), 5.11 (m, 1H, C_{11b}–$\underline{H}$), 5.62 (s, 1H, –C=C$\underline{H}$–), 7.21–8.00 (m, 8H, aromatic-H); ^{13}C-NMR (DMSO-*d6*, δ ppm): 171.2, 160.5, 159.8, 136.2, 135.1, 128.0, 127.4, 127.1, 127.0, 125.8, 121.1, 114.5, 56.6, 53.2, 45.2, 42.0, 40.1, 29.7, 27.3, 26.9, 24.2; MS *m/z*: 430 ($M^{+\cdot}$) (10 %). Analysis for $C_{27}H_{30}N_2O_3$ (430.23): Calcd.: C, 75.32; H, 7.02; N, 6.51; Found: C, 75.84; H, 7.45; N, 6.10.

*2-(Cyclohexylcarbonyl)-3-(thiophen-2-methylene)-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinolin-4-one (**1c**)*

Yield: 70 %, mp 298 °C; IR (KBr, cm^{-1}): 3032 (CH, aromatic), 1700, 1655 (2CO); ^{1}H-NMR ($CDCl_3$, δ ppm): 1.21 (s, 11H, cyclohexyl ring), 2.75–2.90 (2 m, 4H, C_7–$\underline{H_2}$, C_6–$\underline{H_2}$), 3.10, 3.42 (2d, 2H, C_1–H_2), 5.11 (m, 1H, C_{11b}–$\underline{H}$), 5.62 (s, 1H, –C=C$\underline{H}$–), 7.21–8.00 (m, 7H, aromatic-H). ^{13}C-NMR (DMSO-*d6*, δ ppm): 171.2, 160.5, 136.2, 135.3, 135.1, 130.4, 134.0, 128.2, 127.4, 127.1, 127.0, 125.8, 114.2, 54.2, 53.1, 42.2, 41.1, 29.5, 27.6, 26.3, 24.3; MS *m/z*: 405 $(M\text{-}1)^{+\cdot}$ (10 %). Analysis for $C_{24}H_{26}N_2O_2S$ (406.17): Calcd.: C, 70.90; H, 6.45; N, 7.89; S, 7.89; Found: C, 70.46; H, 6.12; N, 8.21; S, 7.40.

*General procedure for preparation of 2-(cyclohexylcarbonyl)-3-alkyl/cycloalkyl aminomethyl 1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinolin-4-one (**2a–d**)*

A solution mixture of *p*-formaldehyde (0.90 g, 10 mmol) and the appropriate amine, namely, 4-methylpiperazine, 4-methylpiperidine, dipropylamine, and 2-aminoethanol (15 mmol), was refluxed in absolute ethanol (20 mL) for 30 min till complete solubilization of *p*-formaldehyde. Then, a solution of praziquantel (4.6 g, 15 mmol) in absolute ethanol (10 mL) was added to the previous mixture and refluxed for 8 h. Upon cooling the reaction mixture, the obtained product was filtered and re-crystallized from dioxane to give the corresponding Mannich bases **2a–d**.

*2-2-(Cyclohexylcarbonyl)-3-((4-methylpiperazin-1-yl)methyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinolin-4-one (**2a**)*

Yield: 60 %; mp 120 °C; IR (KBr, cm^{-1}): 3030 (CH, aromatic), 1710, 1665 (2CO); ^{1}H-NMR ($CDCl_3$, δ ppm): 1.25 (s, 11H, cyclohexyl ring), 2.25 (s, 3H, CH_3 of piperazine), 2.40 (t, $J = 7.2$ Hz, 4H, CH_2 of piperazine), 2.42 (t, $J = 7.2$ Hz, 4H,

CH_2 of piperazine), 2.75–2.90 (2 m, 4H, C_7–$\underline{H_2}$, C_6–$\underline{H_2}$), 4.83–4.93 (dd, 1H, $J = 11.5$ Hz, 5.2 Hz, C_1–$\underline{H}$), 5.11–5.14 (m, 1H, C_{11b}–$\underline{H}$ + 2H, N–CH_2 + 1H, C_3–H), 5.15–5.30 (dd, 1H, $J = 11.0$ Hz, 5.0 Hz, C_1–$\underline{H}$), 7.21–7.81 (m, 4H, aromatic-H); ^{13}C-NMR (DMSO-*d6*, δ ppm): 173.2, 165.1, 136.1, 135.3, 127.5, 127.2, 125.1, 58.4, 55.2, 53.3, 53.0, 52.3, 43.1, 42.9, 41.1, 36.1, 29.3, 27.6, 26.9, 24.3; MS *m/z*: 424 ($M^{+\cdot}$) (15 %). Analysis for $C_{25}H_{36}N_4O_2$ (424.28): Calcd.: C, 70.72; H, 8.55; N, 13.20; Found: C, 70.24; H, 8.00; N, 12.90.

2-(Cyclohexylcarbonyl)-3-((4-methylpiperidin-1-yl)methyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinolin-4-one (2b)

Yield: 65 %, mp 110 °C; IR (KBr, cm^{-1}): 3025 (CH, aromatic), 1698, 1665 (2CO). ^{1}H-NMR ($CDCl_3$, δ ppm): 1.25 (s, 11H, cyclohexyl ring), 2.27 (s, 3H, CH_3), 2.410–2.63 (m, 5H, piperidine protons), 2.71 (t, $J = 5.2$ Hz, 4H, CH_2–N–CH_2 piperidine protons), 2.75–2.90 (2 m, 4H, C_7–$\underline{H_2}$, C_6–$\underline{H_2}$), 4.83–4.93 (dd, 1H, $J = 11.5$ Hz, 5.2 Hz, C_1–$\underline{H}$), 5.11–5.14 (m, 1H, C_{11b} + 2H, N–CH_2 + 1H, C_3–$\underline{H}$), 5.15–5.30 (dd, 1H, $J = 11.0$ Hz, 5.0 Hz, C_1–$\underline{H}$), 7.21–7.81 (m, 4H, aromatic-H); ^{13}C-NMR (DMSO-*d6*, δ ppm): 173.2, 165.1, 136.1, 135.3, 127.5, 127.2, 125.1, 58.4, 53.3, 53.0, 51.4, 42.9, 41.1, 36.1, 32.5, 29.3, 27.6, 26.9, 24.3, 20.4; MS *m/z*: 424 $(M + 1)^{+\cdot}$ (20 %). Analysis for $C_{26}H_{37}N_3O_2$ (423.29): Calcd.: C, 73.72; H, 8.80; N, 9.92; Found: C, 74.12; H, 8.42; N, 9.54.

*2-(Cyclohexylcarbonyl)-3-dipropylaminomethyl-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinolin-4-one (**2c**)*

Yield 60 %, mp 115 °C; IR (KBr, cm^{-1}): 3020 (CH, aromatic), 1708, 1660 (2CO). ^{1}H-NMR ($CDCl_3$, δ ppm): 1.11 (t, 6H, $J = 6.7$ Hz, $2CH_3$, 2 propyl group), 1.25 (s, 11H, cyclohexyl ring), 1.43 (m, 4H, $2CH_2$, 2 propyl group), 2.75–2.90 (2 m, 4H, C_7–$\underline{H_2}$, C_6–$\underline{H_2}$), 4.10 (q, 4H, $J = 7.1$ Hz, $C\underline{H_2}$–N–$C\underline{H_2}$, 2 propyl group), 4.83–4.93 (dd, 1H, $J = 11.5$ Hz, 5.2 Hz, C_1–$\underline{H}$), 5.11–5.14 (m, 1H, C_{11b}–H; 2H, N–CH_2; 1H, C_3–$\underline{H}$), 5.15–5.30 (dd, 1H, $J = 11.0$ Hz, 5.0 Hz, C_1–$\underline{H}$), 7.21–7.81 (m, 4H, aromatic-H); ^{13}C-NMR (DMSO-*d6*, δ ppm): 173.2, 165.1, 136.1, 135.3, 127.5, 127.2, 125.1, 58.4, 56.2, 53.3, 53.1, 42.9, 41.1, 36.1, 29.3, 27.6, 26.9, 24.3, 21.5, 11.8. MS *m/z*: 424 $(M\text{-}1)^{+\cdot}$ (10 %). Analysis for $C_{26}H_{39}N_3O_2$: (425.30): Calcd.: C, 73.37; H, 9.24; N, 9.87; Found: C, 73.373; H, 8.84; N, 9.35.

*2-2-(Cyclohexylcarbonyl)-3-((2-hydroxyethylamino)methyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinolin-4-one (**2d**)*

Yield: 60 %; mp 120 °C; IR (KBr, cm^{-1}): 3020 (CH, aromatic), 1708, 1660 (2CO); ^{1}H-NMR ($CDCl_3$, δ ppm): 1.25 (s, 11H, cyclohexyl ring), 2.75–2.90 (2 m, 4H, C_7–$\underline{H_2}$, C_6–$\underline{H_2}$), 3.32–3.51 (m, 4H, $NC\underline{H_2}C\underline{H_2}O$), 4.83–4.93 (dd, 1H, $J = 11.5$ Hz, 5.2 Hz, C_1–$\underline{H}$), 5.11–5.14 (m, 1H, C_{11b}–H; 2H, N–CH_2; 1H, C_3–$\underline{H}$), 5.15–5.30 (dd, 1H, $J = 11.0$ Hz, 5.0 Hz, C_1–$\underline{H}$), 7.21–7.81 (m, 4H, aromatic-H), 9.11, 11.61 (2 s, 2H, NH, OH, D_2O exchangeable); ^{13}C-NMR (DMSO-*d6*, δ ppm): 173.2, 165.1, 136.1, 135.3, 127.5, 127.2, 125.1, 61.3, 58.4, 53.2, 51.2, 49.3, 42.9, 41.1, 36.1, 29.3,

27.6, 26.9, 24.3. MS *m/z*: 385 ($M^{+\cdot}$) (20 %). Analysis for $C_{22}H_{31}N_3O_3$ (385.24): Calcd.: C, 68.54; H, 8.11; N, 10.90; Found: C, 68.00; H, 8.53; N, 10.54.

Biological evaluation

Antischistosomal activities of the newly synthesized compounds **1a–c** and **2a–d** were evaluated based on the in vitro schistosome worm killing technique [13]: Syrian golden hamsters (*Mesocrietus auratus*) weighing 100–120 g were obtained from the Schistosome Biological Supply Center, Theodor Bilharz Research Institute. *S. mansoni* cercariae (Egyptian strain CD) were used to infect the hamsters with 350 cercariae each by abdominal skin exposure [14]. Praziquantel (Shin Poong Pharmaceutical Co., South Korea) and the respective newly synthesized compounds **1a–c** and **2a–d** were prepared as 5-mM stock solutions in aqueous DMSO. Immediately before use, the stock solutions were diluted with complete medium to the concentrations indicated. *S. mansoni*-infected hamsters were sacrificed and worms harvested from the portomesenteric vessels [15]. 12–16 worms were placed in duplicate Petri dishes, and fresh RPMI 1640 medium (glutamine, 20 % fetal calf serum, and antibiotics [streptomycin, penicillin, and gentamicin]) containing the indicated concentrations (1–100 μM) of test compounds were added. The worms were incubated overnight in a CO_2 incubator, washed thrice with saline, fresh medium without drug was added, and the incubation was continued overnight in the CO_2 incubator. On the second day, worm motility was observed and the medium was again changed and the incubation continued. From day 1 to day 5, the numbers of living and dead worms were recorded. Negative controls using pure medium alone or medium with DMSO and positive control media containing various concentrations of PZQ were similarly evaluated. At the end of the observation period, worms were examined in a laminar flow hood for their motility and appearance by using a stereomicroscope, and the final recording of the percentage worm mortality was assessed (the number of dead worms [contracted and opaque] relative to the total number of worms).

Results and discussion

Chemical studies

Literature survey has proved that the presence of *α, β*-unsaturated carbonyl system of the chalcones is associated with different biological activities, such as antioxidant [16], anticancer [17], bactericidal [18], antimalarial [19], strong antileishmanial activity [20], and antiviral [21]. Thus, it was of interest to prepare new chalcones derivatized from PZQ.

Claisen–Schmidt condensation [22] was carried out by the reaction of equimolar quantities of PZQ in a form of a racemic mixture with different aromatic/heterocyclic aldehydes, namely, benzaldehyde, *p*-methoxybenzaldeyde, and 2-thiophenaldehyde, in the presence of aqueous alcoholic NaOH to give the respective arylidene derivatives **1a–c**. The structures of the resultant products were confirmed depending

upon microanalytical and spectral data. IR spectra represented two characteristic absorption bands at 1710–1650 cm^{-1}, due to the presence of 2CO groups. ^{1}H-NMR spectra of the derivatives exhibited singlet signals δ 1.25 ppm, representing 11H of the cyclohexyl ring, singlet signals δ 5.62 ppm due to the methine proton of the –C=CH group, in addition to the other protons of the formed molecules that appeared in their expected ranges. Mass spectra of the new derivatives exhibited molecular ion peaks at *m/e* 400, 430, and 405, respectively.

Mannich bases have gained wide importance because of their pharmaceutical importance. The presence of basic Mannich side chain has shown a marked antimalarial [23], good anticancer [24], antimycobacterial [25], and remarkable anti-HIV and antitubercular [26] activities. Also, it has been reported that the Mannich side chain may overcome the water insolubility problem through hydrochloride formation [27]. This, in turn, will improve the therapeutic actions of the derivatives. For these reasons, it was interesting to apply the Mannich reaction to PZQ in a form of a racemic mixture by using paraformaldehyde and different alicyclic/alkyl amines, namely, 4-methylpiperazine, 4-methylpiperidine, dipropylamine, and 2-aminoethanol, to furnish the Mannich bases **2a–d**, respectively. The gained structures were elucidated by microanalytical and spectral data. IR spectra represented two characteristic absorption bands at 1710–1650 cm^{-1}, due to the presence of 2CO groups. Mass spectra of the compounds represented molecular ion peaks at their expected regions.

Biological evaluation

S. mansoni killing results

The percentages of in vitro *S. mansoni* worm killing under the influence of the tested compounds at different concentrations versus untreated and DMSO negative controls and positive controls treated with PZQ were determined (Table 1). Controls and DMSO-treated controls had no observed mortality. PZQ was the most effective compound studied, with 100 % worm mortality found between 5 and 10 μM drug concentrations.

The most active compound among those newly synthesized was the Mannich base **2d**, which has 50 % effective concentration (EC50) at 5 μM, 93.3 % worm mortality at 10 μM, and 100 % worm mortality was found at 30 μM. Less activity was achieved by the Mannich base **2c**, which showed 50 % worm mortality at 10 μM, 78.6 % worm mortality at 10 μM, and 100 % worm mortality at 30 μM. On the other hand, compound **1b** was the least active one, with 50 % effective concentration (EC50) at 40 μM and exhibited 93.3 % worm mortality at 60 μM.

Discussion

Discovery of new antischistosomal drugs depends on both in vitro whole parasite screens and *S. mansoni*-infected animal models of the disease. The in vitro worm killing screen is advantageous because it allows the rapid screening of many

Table 1 In vitro mortality (%) of *Schistosoma mansoni* treatment groups **1a–c** and **2a–d** in comparison to praziquantel

Drug concentration (μM)	**PQ**	**1a**	**1b**	**1c**	**2a**	**2b**	**2c**	**2d**
5	93.3	0	0	28.6	28.6	28.6	36.5	50
10	100	12.7	12.7	39.7	39.7	39.7	50	78.6
20	100	30.0	28.6	50	50	50	78.6	93.3
30	100	35.5	39.7	68.4	68.4	78.6	93.3	100
40	100	50	50	82.7	93.3	93.3	100	100
50	100	68.6	78.6	93.3	100	100	100	100
60	100	93.3	93.3	100	100	100	100	100
70	100	100	100	100	100	100	100	100
80	100	100	100	100	100	100	100	100
90	100	100	100	100	100	100	100	100
100	100	100	100	100	100	100	100	100

compounds at several drug concentrations. The tested compounds **2d** and **2c** were previously shown to have greatly increased antischistosomal activity versus PZQ, which might primarily be due to greatly increased cell uptake and conversion to the active metabolite [28]. In the *S. mansoni* worm killing assay, compounds **2d** and **2c** were marginally more active than the rest of the compounds, and the increase in activity was about 2–3-fold more than the other Mannich and arylidene derivatives.

Conclusion

A novel series of praziquantel–arylidene derivatives **1a–c** and praziquantel–Mannich bases **2a–d** were synthesized for antischistosomal activity evaluation. The Mannich base **2d** followed by **2c** exhibited substantial antischistosomal activities, as judged by in vitro worm killing. This result indicated that the presence of alkyl Mannich side chain is more favorable for antischistosomal activity than the alicyclic Mannich side chain and the arylidene substituents at position 3 of the pyrazino[2,1-*a*]isoquinolin ring of PZQ. It would be of interest to examine in further studies the in vivo effects of these compounds in *Schistosoma mansoni*-infected animals, followed by a study of the toxicological and pharmacological activities of these newly synthesized compounds in comparison to PZQ.

Acknowledgments The authors would thank the Microanalytical and Spectral Unit, National Research Centre and Cairo University, Egypt for the microanalytical, IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectral data.

References

1. J. Keiser, J. Utzinger, Advances in the discovery and development of trematocidal drugs. Expert Opin. Drug Discov. **2**(S1), S9–S23 (2007)

2. G.B.F. Carvalho, R.A. da Silva Pereira, L.G.G. Pacífico, C.T. Fonseca, Identification of *Schistosoma mansoni* candidate antigens for diagnosis of schistosomiasis. Mem. Inst. Oswaldo Cruz **106**, 837–843 (2011)
3. L.A.L. Quezada, J.H. McKerrow, *Schistosome* serine protease inhibitors: parasite defense or homeostasis? An. Acad. Bras. Ciênc. **83**, 663–672 (2011)
4. J. Utzinger, J. Keiser, Schistosomiasis and soil-transmitted helminthiasis: common drugs for treatment and control. Expert Opin. Pharmacother. **5**, 263–285 (2004)
5. M.J. van der Werf, S.J. de Vlas, S. Brooker, C.W.N. Looman, N.J.D. Nagelkerke, J.D.F. Habbema, D. Engels, Quantification of clinical morbidity associated with *schistosome* infection in sub-Saharan Africa. Acta Trop. **86**, 125–139 (2003)
6. A. Fenwick, J.P. Webster, Schistosomiasis: challenges for control, treatment and drug resistance. Curr. Opin. Infect. Dis. **19**, 577–582 (2006)
7. M.J. Doenhoff, L. Pica-Mattoccia, Praziquantel for the treatment of schistosomiasis: its use for control in areas with endemic disease and prospects for drug resistance. Expert Rev. Anti Infect. Ther. **4**, 199–210 (2006)
8. R. Gönnert, P. Andrews, Praziquantel, a new broad-spectrum antischistosomal agent. Z. Parasitenk. **52**, 129–150 (1977)
9. B.L. Blas, M.I. Rosales, I.L. Lipayon, K. Yasuraoka, H. Matsuda, M. Hayashi, The schistosomiasis problem in the Philippines: a review. Parasitol. Int. **53**, 127–134 (2004)
10. V.R. Southgate, D. Rollinson, L.A. Tchuem Tchuenté, P. Hagan, Towards control of schistosomiasis in sub-Saharan Africa. J. Helminthol. **79**, 181–185 (2005)
11. C.M.S. Menezes, G. Rivera, M.A. Alves, D.N. do Amaral, J.P.B. Thibaut, F. Noël, E.J. Barreiro, L.M. Lima, Synthesis, biological evaluation, and structure–activity relationship of clonazepam, meclonazepam, and 1,4-benzodiazepine compounds with schistosomicidal activity. Chem. Biol. Drug Des. **79**, 943–949 (2012)
12. S.-H. Xiao, J.-Y. Mei, P-Y Jiao, The in vitro effect of mefloquine and praziquantel against juvenile and adult *Schistosoma japonicum*. Parasitol. Res. **106**(1), 237–246 (2009)
13. S. Botros, J. Bennett, Praziquantel resistance. Expert Opin. Drug Discov. **2**, 535–540 (2007)
14. Y.S. Liang, J.I. Bruce, D.A. Boy, Laboratory cultivation of *schistosome* vector snails and maintenance of *schistosome* life cycle. Proc. First Sino-American Symp. **1**, 34–48 (1987)
15. R.H. Duvall, W.B. DeWitt, An improved perfusion technique for recovering adult schistosomes from laboratory animals. Am. J. Trop. Med. Hyg. **16**, 483–486 (1967)
16. J. Vaya, P.A. Belinky, M. Aviram, Antioxidant constituents from licorice roots: isolation, structure elucidation and antioxidative capacity toward LDL oxidation. Free Radic. Biol. Med. **23**(2), 302–313 (1997)
17. M. Larsen, H. Kromann, A. Kharazmi, S.F. Nielsen, Conformationally restricted anti-plasmodial chalcones. Bioorg. Med. Chem. Lett. **15**(21), 4858–4861 (2005)
18. S.F. Nielsen, T. Boesen, M. Larsen, K. Schønning, H. Kromann, Antibacterial chalcones–bioisosteric replacement of the 4′-hydroxy group. Bioorg. Med. Chem. **12**(11), 3047–3054 (2004)
19. M. Chen, S.B. Christensen, L. Zhai, M. Rasmussen, T.G. Theander, S. Frøkjær, B. Steffansen, J. Davidsen, A. Kharazmi, The novel oxygenated chalcone 2,4 dimethyloxy-4′-butoxychalcone, exhibits potent activity against human malaria parasite *Plasmodium falciparum* in vitro and rodnet parasites *Plasmodium berghei* and *Plasmodium yoelii* in vivo. J. Infect. Dis. **176**, 1327–1333 (1997)
20. L. Zhai, M. Chen, J. Blom, T.G. Theander, S.B. Christensen, A. Kharazmi, The antileishmanial activity of novel oxygenated chalcones and their mechanism of action. J. Antimicrob. Chemother. **43**, 793–803 (1999)
21. D. Binder, C.R. Noe, W. Holzer, B. Rosenwirth, Thiophene as a structural element of physiologically active compounds, XII: thiophene analogues of antiviral chalcones. Arch. Pharm. **318**, 48–59 (1985)
22. J. Shen, H. Wang, H. Liu, Y. Sun, Z. Liu, Brønsted acidic ionic liquids as dual catalyst and solvent for environmentally friendly synthesis of chalcone. J. Mol. Catal. A: Chem. **280**, 24–28 (2008)
23. B.M. Kotecka, G.B. Barlin, M.D. Edstein, K.H. Rieckmann, New quinoline di-mannich base compounds with greater antimalarial activity than chloroquine, amodiaquine, or pyronaridine. Antimicrob. Agents Chemother. **41**(6), 1369–1374 (1997)
24. A.S. Aboraia, H.M. Abdel-Rahman, N.M. Mahfouz, M.A. EL-Gendy, Novel 5-(2-hydroxyphenyl)-3-substituted-2,3-dihydro-1,3,4-oxadiazole-2-thione derivatives: promising anticancer agents. Bioorg. Med. Chem. **14**, 1236–1246 (2006)
25. M.A. Ali, M. Shaharyar, Oxadiazole mannich bases: synthesis and antimycobacterial activity. Bioorg. Med. Chem. **17**, 3314–3316 (2007)

26. D. Sriram, D. Banerjee, P. Yogeeswari, Efavirenz Mannich bases: synthesis, anti-HIV and antitubercular activities. J. Enzyme Inhib. Med. Chem. **24**, 1–5 (2009)
27. M.M. Kamel, M. Nasr, New styrylquinolines of expected antimalarial activity. Pharmazie **34**, 440–441 (1979)
28. M.G.R. Pitta, A.C.A. Silva, J.K.A.L. Neves, P.G. Silva, J.I. Irmão, E. Malagueño, J.V. Santana, M.C.A. Lima, S.L. Galdino, I.R. Pitta, M.C.P.A. Albuquerque, New imidazolidinic bioisosters: potential candidates for antischistosomal drugs. Mem. Inst. Oswaldo Cruz **101**, 313–316 (2006)