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Research Article

# Praziquantel Derivatives with Antischistosomal Activity: Aromatic Ring Modification

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**A series of aromatic ring-modified praziquantel derivatives were prepared and evaluated against juvenile and adult stage of *Schistosoma japonicumin*. Several analogs comparable in activity to the drug praziquantel have been identified based on *in vitro* and *in vivo japonuicum* schistosomes worm viability assay. Structure and activity relationship of these praziquantel aromatic ring-modified compounds was revealed. Specifically, a compound in which a bromine has been introduced in the aromatic ring of praziquantel demonstrated close antischistosomal activity to praziquantel *in vivo*.**

**Key words:** antischistosomal, aromatic ring modification, praziquantel derivatives

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Schistosomiasis is one of the most burdensome nevertheless neglected tropical diseases. The World Health Organization (WHO) estimates that the number of people that treated for schistosomiasis has risen from 12.4 million in 2006 to 33.5 million in 2010.a Over the past 40 years, praziquantel (PZQ, Figure 1) has been used as the only effective drug for schistosomal disease (1,2). There are no backup drugs for the treatment of schistosomiasis should PZQ become less effective. Thus, there is an urgent need to develop replacements of PZQ.

Ever since PZQ was first introduced by Bayer and E. Merck in the 1970s,b much interest has been attracted in the elucidation of the structure–activity relationship (SAR) of PZQ. There are five positions amenable to modification in this molecule (Figure 1). Among these, only the exocyclic amide position R1 was heavily investigated in the original patent and literatures (3,4). In 2007, Mathew Group first reported the modification in the aromatic ring, 10-$NH_2$ and 10-$NO_2$ PZQ derivatives. Unfortunately, these two variants displayed significantly decreased worm killing ability compared with the parent compound (5). We recently reported 10-hydroxy PZQ, with modest capability to decrease worm infection rate *in vivo* (6). Inspired by this discovery, we believe that extensive investigation in the aromatic ring might provide analogs with improved worm killing ability. We are particularly interested in those analogs displaying juvenile worm killing capability *in vivo*. In the course of our investigation, another research group reported several more aromatic ring-modified PZQ analogs (7) (Figure 1). The *in vitro* efficacy study demonstrated that variation of the aromatic ring led to slightly decreased activity, and a smaller thiophene replacement of phenyl ring displayed almost comparable activity with the parent PZQ. Nonetheless, no *in vivo* efficacy was disclosed for these compounds. Herein, we report our continued effort for the SAR study in the aromatic ring of PZQ.

## Experimental Section

### General chemistry

All chemicals (reagent grade) used were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). $^1$H NMR spectra were measured on Varian Unity Inova 300/400 MHz NMR Spectrometer at 25 °C and referenced to TMS. Chemical shifts are reported in ppm using the residual solvent line as internal standard. Splitting patterns are designed as s, singlet; d, doublet; t, triplet; m, multiplet. HRMS spectra were acquired on Bruker Esquire Liquid Chromatography-Ion Trap Mass Spectrometer. Analytical thin-layer chromatography (TLC) was performed on the glass-backed silica gel sheets (silica gel 60 Å GF254). All compounds were detected using UV light (254 or 365 nm). Analytical HPLC was conducted on SHIMADZU LC-20AD. Prior to biological evaluation, all compounds were determined to be >95% pure using appropriate analytical methods (MeOH/$H_2O$ 80% v/v, 5 min; MeOH/$H_2O$ 75% v/v, 15 min; MeOH/$H_2O$ 60% v/v, 10 min; MeOH/$H_2O$ 80% v/v, 5 min) based on the peak area percentage.

### General procedure for synthesis of compounds 2a–h

To 2-phenylethylamine derivatives **1a–h** (1.0 mmol) was added ethylformate (820 mg, 12 mmol, 0.9 mL) at 0 °C and the resulting solution was stirred for 0.5 h. The mixture was then heated at reflux for 12 h. The reaction was quenched by addition of water and extracted with ethyl acetate (3 × 15 mL). The organic layer was washed with brine (2 × 10 mL), dried over $MgSO_4$, and concentrated in



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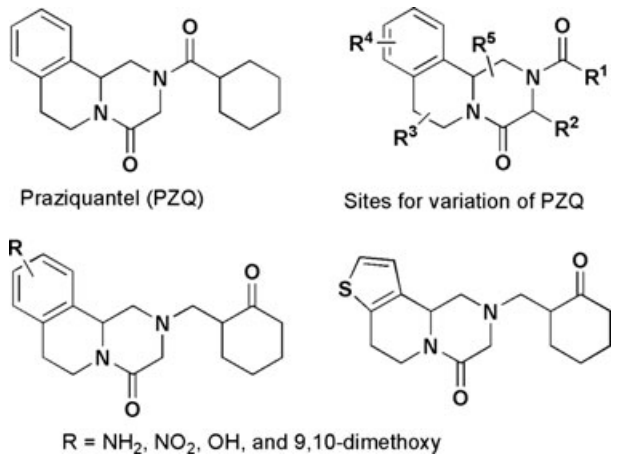



**Figure 1:** Structure of praziquantel and reported derivatives.

vacuum to give the crude product that was purified by flash column chromatography (petroleum ether: ethyl acetate = 2:1) to afford compounds **2a**–**h** as colorless oil.

**2-(2-Methoxyphenyl) ethylformamide 2a**. 93% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.06 (s, 1H), 7.22 (dd, $J$ = 17.7, 10.0 Hz, 1H), 7.10 (dd, $J$ = 15.9, 7.3 Hz, 1H), 6.87 (dd, $J$ = 16.2, 7.8 Hz, 2H), 6.00 (s, 1H), 3.81 (s, 3H), 3.46 (dq, $J$ = 19.5, 6.3 Hz, 2H), 2.87–2.77 (m, 2H).

**2-(4-Bromophenyl) ethylformamide 2b**. 97% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.11 (s, 1H), 7.43 (d, $J$ = 8.0 Hz, 2H), 7.06 (t, $J$ = 9.5 Hz, 2H), 5.67 (s, 1H), 3.56–3.45 (m, 2H), 2.80 (t, $J$ = 6.9 Hz, 2H).

**2-(2-Bromophenyl) ethylformamide 2c**. 92% yield. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.14 (s, 1H), 7.55 (d, $J$ = 7.5 Hz, 1H), 7.25 (s, 2H), 7.12 (d, $J$ = 5.4 Hz, 1H), 5.94 (s, 1H), 3.54 (dd, $J$ = 24.5, 6.5 Hz, 2H), 2.99 (d, $J$ = 6.8 Hz, 2H), 1.99 (s, 1H).

**2-(3-Methoxyphenyl) ethylformamide 2d**. 93% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.14 (s, 1H), 7.23 (d, $J$ = 7.8 Hz, 1H), 6.79 (d, $J$ = 7.4 Hz, 2H), 6.73 (d, $J$ = 15.1 Hz, 1H), 5.54 (s, 1H), 3.80 (s, 3H), 3.58 (dd, $J$ = 12.8, 6.4 Hz, 2H), 2.81 (dd, $J$ = 16.0, 9.4 Hz, 2H).

**2-(3-Bromophenyl) ethylformamide 2e**. 97% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.13 (s, 1H), 7.37 (d, $J$ = 8.2 Hz, 2H), 7.25–7.08 (m, 2H), 5.78 (s, 1H), 3.53 (dt, $J$ = 23.4, 6.1 Hz, 2H), 2.82 (t, $J$ = 6.8 Hz, 2H).

**2-(4-Methylphenyl) ethylformamide 2f**. 84% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.12 (s, 1H), 7.09 (dt, $J$ = 13.8, 7.7 Hz, 4H), 5.57 (s, 1H), 3.59–3.42 (m, 2H), 2.80 (dd, $J$ = 12.5, 5.8 Hz, 2H), 2.33 (s, 3H).

**2-(3-Methylphenyl) ethylformamide 2g**. 98% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.10 (s, 1H), 7.19 (dd, $J$ = 13.8, 6.5 Hz, 1H), 7.07–6.92 (m, 3H), 5.80 (s, 1H), 3.64–3.50 (m, 2H), 2.85–2.71 (m, 2H), 2.39–2.27 (m, 3H).

**2-(2-Methylphenyl) ethylformamide 2h**. 95% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.16 (s, 1H), 7.24–7.08 (m, 4H), 5.60 (s, 1H), 3.54 (dd, $J$ = 13.5, 6.7 Hz, 2H), 2.85 (dd, $J$ = 15.1, 7.9 Hz, 2H), 2.33 (d, $J$ = 8.2 Hz, 3H).

## General procedure for synthesis of 2-phenylethylisocyanide 3a–h

To a solution of 2-phenylethylformamide **2a**–**h** (1.0 mmol) in 15 mL $CH_2Cl_2$ at −10 °C was added TEA (10 mmol) and a solution of $POCl_3$ (1.5 mmol) in $CH_2Cl_2$ (5 mL) dropwise under nitrogen atmosphere. The resulting suspension was stirred for 3 h at −10 °C and quenched by addition of 10 mL saturated $NaHCO_3$ to adjust the pH to 8. The organic layer was separated, washed with water (2 × 20 mL) and brine (2 × 20 mL), dried over sodium sulfate, and evaporated. The crude material was purified by column chromatography (petroleum ether/ethyl acetate = 50:1) to give the isocyanide **3a**–**h** as a yellow oil.

**2-(2-Methoxyphenyl) ethylisocyanide 3a**. 90% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.25 (t, $J$ = 7.7 Hz, 1H), 7.16 (d, $J$ = 7.1 Hz, 1H), 6.91 (t, $J$ = 7.0 Hz, 1H), 6.86 (d, $J$ = 8.0 Hz, 1H), 3.81 (s, 3H), 3.58 (s, 2H), 2.98 (s, 2H).

**2-(4-Bromophenyl) ethylisocyanide 3b**. 80% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.47 (d, $J$ = 8.0 Hz, 2H), 7.11 (d, $J$ = 7.9 Hz, 2H), 3.60 (t, $J$ = 6.3 Hz, 2H), 2.93 (s, 2H).

**2-(2-Bromophenyl) ethylisocyanide 3c**. 83% yield. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.57 (d, $J$ = 7.7 Hz, 1H), 7.30 (s, 2H), 7.17 (s, 1H), 3.67 (s, 2H), 3.13 (s, 2H).

**2-(3-Methoxyphenyl) ethylisocyanide 3d**. 94% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.24 (d, $J$ = 7.8 Hz, 1H), 6.81 (d, $J$ = 7.8 Hz, 2H), 6.76 (s, 1H), 3.80 (s, 3H), 3.60 (t, $J$ = 6.6 Hz, 2H), 2.96 (d, $J$ = 6.3 Hz, 2H).

**2-(3-Bromophenyl) ethylisocyanide 3e**. 81% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.40 (d, $J$ = 13.0 Hz, 2H), 7.19 (s, 2H), 3.62 (s, 2H), 2.96 (s, 2H).

**2-(4-Methylphenyl) ethylisocyanide 3f**. 89% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.14 (q, $J$ = 7.8 Hz, 4H), 3.58 (t, $J$ = 6.1 Hz, 2H), 2.95 (s, 2H), 2.35 (s, 3H).

**2-(3-Methylphenyl) ethylisocyanide 3g**. 76% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.41 (d, $J$ = 8.5 Hz, 1H), 6.38 (d, $J$ = 8.5 Hz, 1H), 4.47–4.24 (m, 2H), 1.58 (d, $J$ = 0.9 Hz, 4H).

**2-(2-Methylphenyl) ethylisocyanide 3h**. 81% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.04 (d, $J$ = 3.5 Hz, 4H), 3.42 (d, $J$ = 7.2 Hz, 2H), 2.87 (d, $J$ = 7.0 Hz, 2H), 2.19 (s, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 136.11 (s), 134.79 (s),

130.67 (s), 129.37 (s), 127.46 (s), 126.43 (s), 41.77 (s), 33.06 (s), 19.33 (s).

## General procedure for synthesis of compounds 4a–h

To a mixture of paraformaldehyde (1.0 mmol), aminoacetaldehyde dimethyl acetal (1.0 mmol), and cyclohexanecarboxylic acid (1.0 mmol, 1.0 equiv) in 10 mL methanol was added (2-isocyanoethyl)benzene derivatives **3a–h** (1.0 mmol) dropwise at 0 °C. The solution was stirred for 48 h at room temperature and concentrated in vacuo. The residue was redissolved in 10 mL $CH_2Cl_2$ and washed with water (2 × 10 mL) and brine (1 × 10 mL) and dried over anhydrous magnesium sulfate. The drying agent was removed by filtration. After concentration, the crude material was purified by column chromatography (petroleum ether/ethyl acetate = 1:1) to give the colorless oil compounds **4a–h.**

***N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-(2-methoxyphenyl) ethyl amino)ethylcyclohexanecarboxamide 4a**. 96% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.22 (d, $J$ = 7.5 Hz, 1H), 7.11 (d, $J$ = 7.1 Hz, 1H), 6.94–6.80 (m, 2H), 6.41 (s, 0.5 H), 4.55 (t, $J$ = 4.9 Hz, 0.5H), 4.40 (t, $J$ = 4.9 Hz, 1H), 3.99 (d, $J$ = 8.6 Hz, 2H), 3.82 (d, $J$ = 3.1 Hz, 3H), 3.51 (dd, $J$ = 12.5, 6.4 Hz, 1H), 3.45 (d, $J$ = 8.8 Hz, 1H), 3.40 (d, $J$ = 9.8 Hz, 3H), 3.35 (s, 3H), 2.88–2.75 (m, 2H), 2.59 (s, 0H), 2.24 (d, $J$ = 11.5 Hz, 1H), 1.76 (s, 2H), 1.66 (s, 2H), 1.61 (s, 3H), 1.47 (s, 2H), 1.22 (s, 2H).

***N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-(4-bromophenyl) ethylamino) ethylcyclohexanecarboxamide 4b**. 92% yield. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.38 (s, 2H), 7.06 (s, 3H), 6.54 (s, 1H), 4.58 (s, 1H), 4.38 (s, 1H), 3.95 (s, 2H), 3.50 (s, 1H), 3.41 (s, 2H), 3.37 (s, 3H), 3.33 (s, 3H), 2.72 (s, 2H), 2.56 (s, 1H), 2.18 (d, $J$ = 11.8 Hz, 1H), 1.75 (s, 2H), 1.62 (d, $J$ = 24.3 Hz, 3H), 1.40 (d, $J$ = 11.8 Hz, 2H), 1.20 (s, 2H), 0.86 (s, 1H).

***N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-(2-bromophenyl) ethylamino) ethylcyclohexanecarboxamide 4c**. 77% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.53 (t, $J$ = 7.1 Hz, 1H), 7.23 (s, 2H), 7.14 (s, 1H), 7.08 (d, $J$ = 5.9 Hz, 1H), 6.53 (s, 1H), 4.61 (t, $J$ = 4.7 Hz, 1H), 4.40 (t, $J$ = 4.9 Hz, 1H), 3.99 (d, $J$ = 9.4 Hz, 2H), 3.55 (dd, $J$ = 13.4, 6.7 Hz, 2H), 3.44 (dd, $J$ = 10.0, 5.1 Hz, 3H), 3.38 (s, 3H), 3.35 (s, 3H), 2.94 (dt, $J$ = 14.4, 7.1 Hz, 2H), 2.59 (t, $J$ = 11.3 Hz, 1H), 2.26 (t, $J$ = 11.3 Hz, 1H), 1.76 (s, 3H), 1.64 (d, $J$ = 12.8 Hz, 4H), 1.54–1.37 (m, 3H), 1.22 (s, 2H).

***N*-(2,2-dimethoxyethyl-*N*-(2-oxo-2-(2-(3-methoxyphenyl) ethylamino) ethylcyclohexanecarboxamide 4d**. 91% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.20 (s, 1H), 6.98 (s, 1H), 6.75 (s, 2H), 6.74–6.70 (m, 1H), 6.47 (s, 1H), 4.57 (s, 1H), 4.39 (s, 1H), 3.98 (d, $J$ = 5.3 Hz, 2H), 3.78 (s, 3H), 3.54 (d, $J$ = 5.8 Hz, 2H), 3.49–3.45 (m, 1H), 3.41 (d, $J$ = 3.4 Hz, 2H), 3.37 (s, 3H), 3.33 (s, 3H), 2.81–2.72 (m, 2H), 2.57 (s, 1H), 2.23 (s, 1H), 2.05 (d, $J$ = 8.6 Hz, 1H), 1.75 (s, 3H), 1.67 (s, 3H), 1.44 (d, $J$ = 9.5 Hz, 3H), 1.24 (d, $J$ = 5.2 Hz, 3H).

***N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-(3-bromophenyl) ethylamino) ethylcyclohexanecarboxamide 4e**. 92% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.35 (d, $J$ = 9.3 Hz, 2H), 7.14 (dd, $J$ = 14.5, 7.3 Hz, 3H), 6.55 (s, 1H), 4.61 (s, 1H), 4.40 (s, 1H), 3.99 (d, $J$ = 7.3 Hz, 2H), 3.54 (dd, $J$ = 12.8, 6.4 Hz, 1H), 3.48 (s, 1H), 3.44 (d, $J$ = 4.4 Hz, 2H), 3.39 (s, 3H), 3.35 (s, 3H), 2.85–2.72 (m, 2H), 2.58 (s, 1H), 2.23 (s, 1H), 1.77 (s, 2H), 1.68 (s, 1H), 1.61 (s, 2H), 1.49–1.40 (m, 2H), 1.23 (s, 1H).

***N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-(4-methylphenyl) ethylamino) ethylcyclohexanecarboxamide 4f**. 74% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.08 (d, $J$ = 6.6 Hz, 4H), 6.93 (s, 1H), 6.43 (s, 1H), 4.58 (s, 1H), 4.39 (s, 1H), 3.98 (d, $J$ = 3.9 Hz, 2H), 3.53 (d, $J$ = 6.3 Hz, 1H), 3.46 (d, $J$ = 6.2 Hz, 1H), 3.41 (s, 2H), 3.38 (s, 3H), 3.34 (s, 3H), 2.81–2.70 (m, 2H), 2.57 (s, 1H), 2.31 (s, 3H), 2.22 (t, $J$ = 11.3 Hz, 1H), 1.75 (s, 2H), 1.67 (s, 1H), 1.61–1.54 (m, 2H), 1.51–1.36 (m, 3H), 1.22 (s, 2H), 0.86 (d, $J$ = 6.7 Hz, 3H).

***N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-(3-methylphenyl) ethylamino) ethylcyclohexanecarboxamide 4g**. 87% yield. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.15 (d, $J$ = 6.7 Hz, 1H), 7.00 (d, $J$ = 10.7 Hz, 3H), 6.45 (s, 1H), 4.55 (s, 1H), 4.37 (s, 1H), 3.97 (s, 2H), 3.57–3.44 (m, 2H), 3.40 (s, 2H), 3.36 (s, 3H), 3.32 (s, 3H), 2.74 (d, $J$ = 9.6 Hz, 2H), 2.54 (d, $J$ = 11.5 Hz, 1H), 2.30 (s, 3H), 2.23 (s, 1H), 2.06 (d, $J$ = 21.2 Hz, 1H), 1.82–1.53 (m, 6H), 1.42 (s, 2H), 1.20 (s, 1H).

***N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-(2-methylphenyl) ethylamino) ethylcyclohexanecarboxamide 4h**. 79% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.12 (d, $J$ = 4.1 Hz, 4H), 7.09–7.03 (m, 1H), 6.53 (s, 1H), 4.60 (s, 1H), 4.40 (s, 1H), 3.99 (d, $J$ = 8.3 Hz, 2H), 3.45 (s, 3H), 3.38 (s, 3H), 3.35 (s, 3H), 2.85–2.74 (m, 2H), 2.59 (s, 1H), 2.32 (d, $J$ = 5.4 Hz, 3H), 2.27 (s, 1H), 1.76 (s, 2H), 1.64 (d, $J$ = 14.9 Hz, 3H), 1.53–1.37 (m, 2H), 1.23 (s, 2H).

## General procedure for the synthesis of compounds 6a–h

*N*-(2,2-Dimethoxyethyl)-*N*-(2-oxo-2-(2-phenethylamino)ethyl) cyclohexanecarboxamide **6a–h** (0.1 mmol) was added portionwise to methanesulfonic acid (1.6 mmol) at 0 °C. After heating to 70 °C for 6 h, the reaction mixture was poured into an ice–water mixture (5.0 mL), and the pH was adjusted to 8 by addition of aqueous solution 2 N NaOH. The solution was extracted with $CH_2Cl_2$ (3 × 10 mL). The combined organic layer was washed with brine (2 × 10 mL) and dried over anhydrous magnesium sulfate. After concentration, the crude material

was purified by column chromatography (petroleum ether/ethyl acetate = 2:1) to afford the final product compounds **6a**–**h**.

**8-Methoxylpraziquantel 6a**. 38% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.22 (s, 1H), 6.89 (d, $J$ = 7.7 Hz, 1H), 6.78 (d, $J$ = 7.9 Hz, 1H), 5.16 (d, $J$ = 12.7 Hz, 1H), 4.88 (d, $J$ = 9.5 Hz, 1H), 4.79 (d, $J$ = 7.0 Hz, 1H), 4.46 (d, $J$ = 17.5 Hz, 1H), 4.05 (d, $J$ = 17.5 Hz, 1H), 3.84 (s, 3H), 2.93 (d, $J$ = 16.3 Hz, 1H), 2.76 (q, $J$ = 11.8 Hz, 2H), 2.66 (d, $J$ = 12.4 Hz, 1H), 2.46 (t, $J$ = 11.0 Hz, 1H), 1.87–1.66 (m, 5H), 1.55 (d, $J$ = 12.9 Hz, 2H), 1.27 (s, 3H).$^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 174.73, 164.25, 157.02, 133.91, 127.46, 123.78, 117.34, 108.46, 55.41, 54.81, 48.96, 45.18, 40.76, 38.49, 29.22, 28.98, 25.69, 22.50. LCMS ($ESI^+$) m/z: ($M + H^+$) (calcd for $C_{20}H_{26}N_2O_3$ 343.2016), found: 343.2018. Error: 0.6 ppm.

**10-Bromopraziquantel 6b**. 40% yield. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.44 (s, 1H), 7.40–7.33 (m, 1H), 7.07 (s, 1H), 5.13 (s, 1H), 4.83 (s, 2H), 4.44 (s, 1H), 4.10 (s, 2H), 2.88 (s, 3H), 2.79–2.69 (m, 2H), 2.53–2.38 (m, 1H), 1.72 (s, 3H), 1.27 (s, 6H), 0.88 (s, 1H). The $^1$H NMR data were consistent with reported literature (8).

**Synthesis of compound 5c**. To a solution of **4c** (48 mg, 0.11 mmol) in 2.0 mL $CH_2Cl_2$ was added *p*-toluenesulfonic acid·$H_2O$ (71 mg, 0.37 mmol) at 0 °C. The reaction was stirred at reflux for 12 h and quenched by addition of 5 mL saturated $NaHCO_3$ to adjust the pH to 8. The organic layer was separated, washed with water (2 × 10 mL) and brine (1 × 10 mL), dried over sodium sulfate, and evaporated. The crude material was purified by column chromatography (petroleum ether/ethyl acetate = 4:1) to give **5c** as a white powder (20 mg, 46%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.53 (d, $J$ = 7.9 Hz, 1H), 7.22 (s, 2H), 7.14–7.08 (m, 1H), 6.10 (d, $J$ = 5.9 Hz, 1H), 5.40 (d, $J$ = 5.9 Hz, 1H), 4.32 (s, 2H), 3.76 (t, $J$ = 7.3 Hz, 2H), 3.05 (t, $J$ = 7.0 Hz, 2H), 2.45 (t, $J$ = 11.5 Hz, 1H), 1.76 (dd, $J$ = 28.4, 14.7 Hz, 6H), 1.55–1.44 (m, 3H), 1.27 (d, $J$ = 9.7 Hz, 4H).

**Synthesis of 8-bromopraziquantel 6c**. A solution of **5c** (577 mg, 1.47 mmol) in concentrated $H_2SO_4$ (2.0 mL) was stirred at 0 °C. After 30 min, the reaction was warmed up to room temperature and the reaction mixture was stirred for 4 h, quenched by addition of 10% aqueous NaOH at 0 °C to adjust the pH to 8. The mixture was extracted with $CH_2Cl_2$ (3 × 30 mL). The combined organic layer was separated and washed with brine (2 × 10 mL), dried over sodium sulfate, and concentrated. The crude material was purified by column chromatography (petroleum ether/ethyl acetate = 4:1) to give compound **6c** as white powder (560 mg, 97%).$^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.52 (d, $J$ = 6.9 Hz, 1H), 7.33–7.23 (m, 1H), 7.16 (s, 1H), 5.13 (d, $J$ = 12.9 Hz, 1H), 4.85 (dd, $J$ = 26.0, 7.6 Hz, 2H), 4.47 (d, $J$ = 17.2 Hz, 1H), 4.06 (d, $J$ = 17.1 Hz, 1H), 2.98 (d, $J$ = 11.1 Hz, 1H), 2.84 (t, $J$ = 10.7 Hz, 3H), 2.47 (s, 1H), 1.76 (d, $J$ = 21.8 Hz, 5H), 1.62–1.41 (m, 2H), 1.28 (s, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 174.71, 164.11, 135.37, 134.51, 131.47, 128.07, 125.60, 124.58, 54.78, 48.91, 45.01, 40.68, 38.67, 29.38, 29.17, 28.97, 25.65. LCMS ($ESI^+$) m/z:391.1022 ($M + H^+$) (calcd for $C_{19}H_{23}BrN_2O_2$ 391.1016) error: 1.5 ppm.

**9-Methoxypraziquantel 6d**. Compound **6d** was obtained using general procedure for preparing compound **6** from compound **4d** in 53% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.20 (d, $J$ = 8.5 Hz, 1H), 6.83 (d, $J$ = 7.6 Hz, 1H), 6.70 (s, 1H), 5.12 (d, $J$ = 12.4 Hz, 1H), 4.82–4.72 (m, 2H), 4.46 (d, $J$ = 17.4 Hz, 1H), 4.07 (d, $J$ = 17.4 Hz, 1H), 3.91 (d, $J$ = 13.3 Hz, 1H), 3.80 (s, 3H), 2.91 (dd, $J$ = 24.1, 12.3 Hz, 3H), 2.76 (t, $J$ = 13.8 Hz, 2H), 2.46 (t, $J$ = 11.2 Hz, 1H), 1.88–1.68 (m, 7H), 1.31 (d, $J$ = 20.4 Hz, 4H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 174.72, 164.38, 158.60, 136.13, 126.57, 124.84, 113.88, 113.03, 108.68, 55.46, 55.29, 54.57, 48.96, 45.22, 40.75, 39.04, 29.21, 28.96, 25.90, 25.69. LCMS ($ESI^+$) m/z:343.2002 ($M + H^+$) (calcd for $C_{20}H_{26}N_2O_3$ 343.2016) error: 4.1 ppm.

**9-Bromopraziquantel 6e**. Compound **6e** was obtained using general procedure for preparing compound **6** from compound **4e** in 50% yield. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.51 (s, 1H), 7.16 (s, 1H), 5.11 (s, 1H), 4.85 (d, $J$ = 23.3 Hz, 2H), 4.47 (d, $J$ = 17.8 Hz, 1H), 4.05 (d, $J$ = 17.8 Hz, 1H), 2.97 (s, 1H), 2.80 (s, 3H), 2.45 (s, 1H), 1.76 (d, $J$ = 21.5 Hz, 6H), 1.59–1.39 (m, 4H).$^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 174.75, 164.31, 137.01, 132.08, 131.78, 130.09, 127.11, 121.35, 54.68, 48.96, 44.78, 40.72, 38.77, 29.18, 28.96, 28.46, 25.65. LCMS ($ESI^+$) m/z: 391.1000 ($M + H^+$) (calcd for $C_{19}H_{23}BrN_2O_2$ 391.1016) error: 4.1 ppm.

**10-Methylpraziquantel 6f**. Compound **6f** was obtained using general procedure for preparing compound **6** from compound **4f** in 63% yield.$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.08 (d, $J$ = 15.6 Hz, 3H), 5.16 (d, $J$ = 13.7 Hz, 1H), 4.82 (d, $J$ = 10.8 Hz, 1H), 4.75 (d, $J$ = 7.4 Hz, 1H), 4.47 (d, $J$ = 17.4 Hz, 1H), 4.08 (d, $J$ = 17.5 Hz, 1H), 2.88 (dd, $J$ = 25.7, 13.2 Hz, 3H), 2.75 (t, $J$ = 12.5 Hz, 2H), 2.51–2.41 (m, 1H), 2.33 (s, 3H), 1.81 (s, 3H), 1.74 (d, $J$ = 9.5 Hz, 3H), 1.30 (d, $J$ = 19.6 Hz, 4H).$^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 174.76, 164.39, 136.63, 132.41, 131.56, 129.11, 128.25, 126.06, 54.97, 48.98, 45.35, 40.77, 39.14, 29.23, 28.97, 28.32, 25.70, 21.11. LCMS ($ESI^+$) m/z: 327.2072 ($M + H^+$) (calcd for $C_{20}H_{26}N_2O_2$ 327.2067) error: 1.5 ppm.

**9-Methylpraziquantel 6g**. Compound **6g** was obtained using general procedure for preparing compound **6** from compound **4g** in 56% yield. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.12 (d, $J$ = 19.1 Hz, 2H), 7.00 (d, $J$ = 11.5 Hz, 1H), 5.13 (d, $J$ = 13.1 Hz, 1H), 4.78 (d, $J$ = 8.8 Hz, 2H), 4.46 (d, $J$ = 17.6 Hz, 1H), 4.06 (d, $J$ = 17.6 Hz, 1H), 2.89

(t, $J$ = 15.4 Hz, 2H), 2.75 (t, $J$ = 13.8 Hz, 2H), 2.47 (d, $J$ = 11.4 Hz, 1H), 2.31 (s, 3H), 1.75 (d, $J$ = 22.1 Hz, 5H), 1.50 (d, $J$ = 11.3 Hz, 1H), 1.24 (s, 4H). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 174.77, 164.44, 137.22, 134.52, 129.77, 127.78, 125.34, 54.82, 48.99, 45.21, 40.78, 39.14, 29.23, 28.98, 28.65, 25.70, 20.97. LCMS (ESI$^+$) m/z: 327.2071 (M + H$^+$) (calcd for $C_{20}H_{26}N_2O_2$ 327.2067) error: 1.2 ppm.

**8-Methylpraziquantel 6h**. Compound **6h** was obtained using general procedure for preparing compound **6** from compound **4h**, in 44% yield. $^1$H NMR (300 MHz,$CDCl_3$) $\delta$ 7.09 (d, $J$ = 39.2 Hz, 3H), 5.15 (d, $J$ = 13.4 Hz, 1H), 4.83 (d, $J$ = 21.9 Hz, 2H), 4.46 (d, $J$ = 17.5 Hz, 1H), 4.04 (d, $J$ = 17.4 Hz, 1H), 2.93–2.64 (m, 4H), 2.46 (s, 1H), 2.27 (s, 3H), 1.75 (d, $J$ = 24.1 Hz, 5H), 1.52 (s, 3H), 1.26 (s, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 174.42, 163.96, 136.49, 132.93, 132.48, 128.46, 126.25, 122.84, 54.79, 48.68, 45.05, 40.47, 38.41, 28.93, 28.70, 25.65, 25.41, 19.08. LCMS (ESI$^+$) m/z: 327.2070 (M + H$^+$) (calcd for $C_{20}H_{26}N_2O_2$ 327.2067) error: 0.9 ppm.

**Synthesis of 10-methoxylpraziquantel 6i**. 10-Hydroxy PZQ (114 mg, 0.35 mmol) in DMF (2.5 mL) was methylated with iodomethane (566 mg, 3.99 mmol) in the presence of potassium carbonate (504 mg, 3.64 mmol) and tetra-butyl ammonium iodide (11.0 mg) at room temperature overnight (9). Analysis by TLC indicated a complete reaction after 48 h. The reaction mixture was quenched with brine and extracted with $Et_2O$ (3 × 5 mL). The organic fractions were washed with brine, combined, dried over $Na_2SO_4$, filtered, and concentrated in vacuum. Purification by flash chromatography (petroleum ether/ethyl acetate = 3:1) gave **6i** as white powder (100 mg, 86%).$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.09 (s, 1H), 6.79 (s, 2H), 5.12 (s, 1H), 4.82 (s, 2H), 4.45 (s, 1H), 4.10 (s, 1H), 3.79 (s, 3H), 2.88 (s, 3H), 2.70 (s, 1H), 2.53–2.41 (m, 1H), 1.82 (s, 3H), 1.72 (s, 3H), 1.28 (s, 4H).$^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 174.37, 163.98, 158.11, 133.36, 129.85, 126.28, 113.60, 109.73, 55.03, 54.64, 48.61, 44.78, 40.34, 38.98, 29.31, 28.86, 28.64, 27.52, 25.37, 25.32. LCMS (ESI$^+$) m/z:343.2015 (M + H$^+$) (calcd for $C_{20}H_{26}N_2O_3$ 343.2016) error: 0.3 ppm.

**Synthesis of 10-acetylpraziquantel 6j**. Acetic anhydride (0.4 mL, 2.44 mmol) was added dropwise to a cooled solution of 10-hydroxy PZQ (200 mg, 0.61 mmol) in pyridine (5 mL) while keeping the reaction temperature below 5 °C (10). After addition, the reaction mixture was then stirred at room temperature for 6 h. The mixture was treated with 15 mL 10% aqueous $NaHCO_3$, washed with water (2 × 10 mL), dried by $MgSO_4$, and concentrated to afford the crude product that was further purified by column chromatography (petroleum ether/EtOAc = 2:1) to give compound **6j** as white powder (190 mg, 84%).$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.19 (d, $J$ = 6.5 Hz, 1H), 7.01 (s, 2H), 5.11 (d, $J$ = 12.9 Hz, 1H), 4.81 (d, $J$ = 17.3 Hz, 2H), 4.47 (d, $J$ = 17.5 Hz, 1H), 4.07 (d, $J$ = 17.8 Hz, 1H), 2.94–2.72 (m, 4H), 2.45 (s, 1H), 2.30 (s, 3H), 1.76 (d, $J$ = 35.0 Hz, 7H), 1.54 (d, $J$ = 11.8 Hz, 2H), 1.26 (d, $J$ = 7.7 Hz, 3H).$^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 174.84, 169.60, 164.47, 149.46, 134.04, 132.37, 130.38, 121.10, 118.82, 55.72, 54.90, 49.09, 44.98, 40.84, 39.14, 29.79, 29.30, 29.07, 28.31, 25.78, 21.13. LCMS (ESI$^+$) m/z: 371.1966 (M + H$^+$) (calcd for $C_{21}H_{26}N_2O_4$ 371.1965) error: 0.3 ppm.

### *Infection and collection of juvenile and adult* Schistosoma japonicum

Female ICR mice were percutaneously infected with 100 *S. japonicum* cercariae via shaved abdominal skin (11). Mice were killed at 14 or 42 days after infection, and then, juvenile and adult *Schistosoma* were collected by perfusion with ice-cold Hank's balanced salt solution (HBSS) from mesenteric vein and livers. Groups of four couples of adult *Schistosoma* were washed and placed in a 12-well Falcon plate containing 4 mL RPMI 1640 medium and stored at 37 °C in an atmosphere of 5% $CO_2$ in air. Praziquantel derivatives were added to achieve a series of diluted concentrations 10–50 $\mu$M, and PZQ was used as reference drug. Compound activity was assessed by survival and vitality rate (%) within 72 h.

### *Schistosome incubation* in vitro

RPMI 1640 medium supplemented with 20% newborn calf serum, 100 U/mL penicillin, 100 U/mL streptomycin, and 0.5 $\mu$g/mL amphotericin B was used to maintain the *Schistosoma in vitro* (6). The volume of medium added to each of the 12 wells of a Falcon plate was 3.98–4.00 mL, and then, four pairs of worms (four male and four female worms) were placed in each well. For juvenile incubation, 50 $\mu$L of 14-day-old schistosomula suspended in the medium was added to each well of the plate. The plate was incubated at 37 °C in 95% air + 5% $CO_2$ overnight before addition of drugs or PZQ at different concentrations. The final volume in each well was 4.0 mL. Control wells contained the worms and medium only or 0.4% dimethyl sulfoxide (DMSO) alone. After addition of the aforementioned drugs, the plates were incubated continuously for 72 h, and each test with juvenile or adult schistosomes was repeated three times. During 72 h, compound activity was assessed by percentage of survival and vitality each 24 h. The score of worm vitality is illustrated as follows: 3 points, male schistosomes oral sucker attached to the plate or worms moved very naturally and actively and the body was transparent just as observed in the control group during the observation period; 2 points, worms acted weakened obviously all over the body, with the body curled or swelling with blebs with various sizes emerged along the tegument; 1 point, worms acted very weak with oral sucker or tail moving occasionally; 0 point, worms were dead without any

movement. The average score of 4 worms was counted for *in vitro* test.

### Schistosoma japonicum *incubation* in vivo

Thirty ICR mice were divided into six groups. Each rat was infected with 100 *S. japonicum* cercariae via shaved abdominal skin (6). Forty-two days after infection, the mice were administered a single 250 mg/kg oral doses of compounds **6a**, **6c**, **6i**, **6j**, and PZQ, respectively. The compounds were prepared as suspensions in 7% (vol/vol) Tween 80 and 3% (vol/vol) ethanol before oral administration. Untreated mice (same amount of solvent only, without compounds) served as controls (Each group include five mice). Animals were killed at 30 day after infection, and *S. japonicum* was perfused with ice-cold HBSS (pH 7.2) from mesenteric vein and liver.

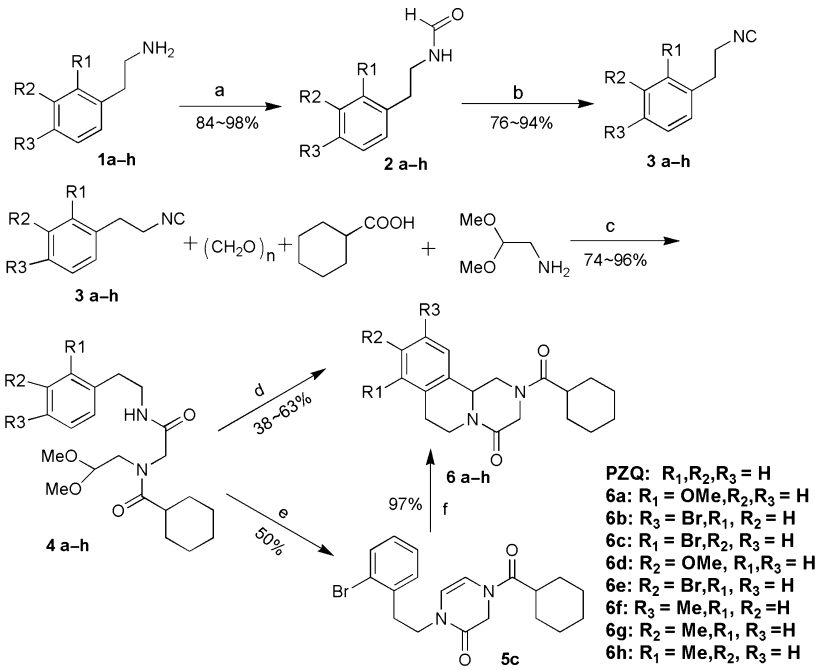
### *Cytotoxicity*

The antiproliferative activities of chalcone thiosemicarbazide derivatives were determined using a standard (MTT)-based colorimetric assay (Sigma, St. Louis, MO, USA) (10). Briefly, cell lines were seeded at a density of $5 \times 10^3$ cells/well in 96-well microtiter plates (Costar, New York, NY, USA). After 24 h, exponentially growing cells were exposed to the indicated compounds at final concentrations of 50 $\mu$M. After 48 h, cell survival was determined by the addition of an MTT solution (20 $\mu$L of 5 mg/mL MTT in PBS). After 6 h, the medium was removed by aspiration. The cells were dissolved in 150 $\mu$L DMSO, and optical absorbance was measured at 570 nm on an Elx800 Bio-Tek microplate reader (Winooski, VT, USA). Survival ratios are expressed in percentages with respect to untreated cells. Values were determined from replicates of three wells from at least three independent experiments.

## Results and Discussions

### *Chemistry*

We initially attempted Kim group reported method to achieve PZQ derivatives (12), which involved a key reaction intermediate amide acetal. This unstable and highly hygroscopic intermediate makes this route far from an efficient way to provide a diversity of PZQ analogs. We then resorted to the Ugi multicomponent reaction (Scheme 1) (7,13). The common reaction intermediate isocyanide **3** was prepared by treating **2** with $POCl_3$ and $Et_3N$ (14). Compounds **2a**–**2e** were readily prepared by a reaction of the corresponding amine with HCOOEt. Then, compounds **3a**–**3e** reacted with paraformaldehyde, cyclohexanecarboxylic acid, and aminoacetaldehyde dimethyl acetal to yield the advanced precursor **4a**–**4e** quantitatively (13). Finally, **4a**–**4b** and **4d**–**4h** undergo the Pictet–Spengler reaction under acidic conditions to yield PZQ derivatives **6a**–**6b** and **6d**–**6h** in high yield (15). Accordingly, **4c** was first converted to **5c** using pyridinium *p*-toluenesulfonate (pTSA) in 50% yield, which undergoes intramolecular cyclization to provide **6c** in concentrated $H_2SO_4$. 10-Hydroxy PZQ was prepared according to the previously reported method (6). It is subsequently treated with $CH_3I$ and $K_2CO_3$, $Bu_4NI$, in DMF (9,16) to yield compound **6i**. Meanwhile, compound **6j** was prepared by treating 10-hydroxy




**Scheme 1:** Synthesis route of praziquantel (PZQ) derivative **6a**–**6j.** a: HCOOEt, reflux; b: $POCl_3$, $Et_3N$, DCM, -10°C; c: MeOH, rt, 48 h; d: MSA, 70 °C; e: PPTS, DCM; f: conc. $H_2SO_4$, rt.

**Table 1:** Effects of compounds **6a**–**6j** on 42-day adult *Schistosoma japonicum in vitro* culture

| Compounds | Concentrations (μM) | Worms | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|---|---|
| | | | Survival (%) | Vitality (%) | Survival (%) | Vitality (%) | Survival (%) | Vitality (%) |
| Control | 25 | 4♂ | 100 | 100 | 100 | 100 | 100 | 100 |
| | | 4♀ | 100 | 100 | 100 | 100 | 100 | 100 |
| **6a** | 25 | 4♂ | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 4♀ | 25 | 8.3 | 25 | 8.3 | 25 | 8.3 |
| | 10 | 6♂ | 100 | 50 | 50 | 16.7 | 40 | 13 |
| | | 4♀ | 100 | 67 | 100 | 67 | 40 | 13 |
| **6b** | 25 | 4♂ | 100 | 56 | 50 | 16.7 | 50 | 16.7 |
| | | 4♀ | 100 | 58 | 100 | 58 | 100 | 42 |
| **6c** | 25 | 4♂ | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 4♀ | 25 | 8.3 | 0 | 0 | 0 | 0 |
| | 10 | 4♂ | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 4♀ | 75 | 25 | 25 | 8.3 | 0 | 0 |
| | 5 | 4♂ | 100 | 33 | 50 | 16.7 | 0 | 0 |
| | | 4♀ | 100 | 67 | 100 | 67 | 100 | 60 |
| **6d** | 25 | 4♂ | 50 | 33 | 50 | 33 | 50 | 33 |
| | | 4♀ | 100 | 50 | 100 | 33 | 100 | 33 |
| **6e** | 25 | 4♂ | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 4♀ | 100 | 58 | 100 | 56 | 100 | 55 |
| | 10 | 4♂ | 100 | 50 | 50 | 25 | 20 | 6.7 |
| | | 4♀ | 100 | 67 | 100 | 58 | 100 | 33 |
| **6f** | 25 | 4♂ | 100 | 33 | 25 | 8.3 | 25 | 8.3 |
| | | 4♀ | 100 | 44 | 100 | 33 | 67 | 22 |
| **6g** | 25 | 4♂ | 33 | 11 | 33 | 11 | 33 | 11 |
| | | 4♀ | 100 | 67 | 100 | 33 | 100 | 33 |
| **6h** | 25 | 4♂ | 73 | 25 | 75 | 25 | 25 | 8.3 |
| | | 4♀ | 100 | 33 | 100 | 33 | 100 | 33 |
| **6i** | 25 | 4♂ | 50 | 17 | 0 | 0 | 0 | 0 |
| | | 4♀ | 75 | 25 | 75 | 25 | 50 | 11 |
| | 10 | 4♂ | 67 | 22 | 33 | 11 | 0 | 0 |
| | | 4♀ | 100 | 42 | 75 | 25 | 25 | 8.3 |
| **6j** | 25 | 4♂ | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 4♀ | 100 | 33 | 50 | 17 | 0 | 0 |
| | 10 | 4♂ | 100 | 50 | 75 | 25 | 0 | 0 |
| | | 4♀ | 100 | 61 | 67 | 33 | 17 | 5.6 |
| **PZQ** | 25 | 4♂ | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 4♀ | 50 | 17 | 25 | 8.3 | 0 | 0 |
| | 10 | 4♂ | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 4♀ | 25 | 8.3 | 0 | 0 | 0 | 0 |
| | 5 | 4♂ | 50 | 16.7 | 25 | 8.3 | 25 | 8.3 |
| | | 4♀ | 75 | 25 | 75 | 25 | 25 | 8.3 |

PZQ, praziquantel.

PZQ with $Ac_2O$ in pyridine (17). All compounds were purified by silica gel column and identified by $^{1}H$ and $^{13}C$ NMR and HRMS.

## In vitro *efficacy of compounds 6a–6j against Schistosoma japonicum*

All prepared analogs were first evaluated for their ability against adult *schistosomes J. in vitro*. According to previously described method (18), four male/female worms obtained from rat infections were distributed in duplicate tissue culture dishes (3.5 cm) in Dulbecco's modified minimum Eagle's medium (bicarbonate buffered) supplemented with 20% newborn calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.5 μg/mL amphotericin B. Cultures were kept at 37 °C in an atmosphere of 5% $CO_2$ in air and were observed under a Leica MZ 12.5 stereomicroscope (Houston, TX, USA). Worms of *S. japonicum* were exposed overnight (16 h) to various compounds, washed, and subsequently cultured in drug-free medium. Juvenile worms were obtained after day 14 infection. In contrast, adult worms were obtained at day 42 infection. Compounds were then added from 5.0 mM DMSO stock solutions to achieve a series of diluted concentrations 10–50 μM. Compound activity was assessed by percentage of survival and vitality within 72 h. The

**Table 2:** Effects of compounds **6a**, **6c**, **6i**, and **6j** on 14-day juvenile *Schistosoma japonicum in vitro* culture

| Compounds | Concentrations ($\mu$M) | Worms | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|---|---|
| | | | Survival (%) | Vitality (%) | Survival (%) | Vitality (%) | Survival (%) | Vitality (%) |
| Control | – | 8 | 100 | 100 | 100 | 100 | 100 | 100 |
| **6a** | 25 | 15 | 73 | 49 | 73 | 42 | 60 | 24 |
| **6c** | 25 | 13 | 46 | 15 | 27 | 9 | 18 | 6 |
| **6i** | 25 | 8 | 69 | 23 | 62 | 21 | 62 | 21 |
| **6j** | 25 | 11 | 0 | 0 | 0 | 0 | 0 | 0 |
| PZQ | 25 | 8 | 37.5 | 12.5 | 25 | 8.3 | 25 | 8.3 |

PZQ, praziquantel.

score of worm vitality is illustrated as follows: 3 scores: the highest score, as observed in the control group during the observation period. Worms moved actively and softly, and the body was transparent. 2 scores: worms acted all over the body, but stiffly and slowly, with the body translucent. 1 score: parasites moved partially with opaque appearance. 0 score: the worm remained contracted, did not resume movements, and we could deem it 'dead'. The average score of 8 worms was counted for *in vitro* test. Data representative of repeated experiments were shown in Table 1 (for adult worm).

For all compounds tested against adult *schistosomiasis*. *J* (Table 1), compounds **6a**, **6i**, and **6j** shown worm killing activity close to PZQ. Compounds **6b**, **6d**, **6f**, **6g**, and **6h** had comparable activity; no worm was killed at concentration up to 25 $\mu$M, but the worm vitality score reduced significantly. The potency of compound **6e** is marginally higher than these five weakest compounds. At 10 $\mu$M, the worm viability is reduced to 6.7% and 33% for male and female worms, respectively, which is obviously less potent compared with PZQ at the same concentration. The result of particular significance is for **6c**. At concentration as low as 10 $\mu$M, this compound killed 100% worm, which is the same as PZQ. At 5 $\mu$M, the efficacy of compound **6c** is slightly better than PZQ against male worm, but much less efficient against female worm compared with PZQ. Notably, all PZQ derivatives demonstrated higher capability against male than female worm. Overall, the *in vitro* effect of these compounds revealed that modification of the 8 and 10 position in the aromatic ring is more tolerated, substitution at position 9 reduced the compound worm killing ability significantly; halogenation of the aromatic ring in position 8 retained the compound capability, bigger sized substitutes, like methoxy, is not favorable to the compound activity.

Based on the adult worm killing ability, compounds displayed significant potency at 25 $\mu$M were further evaluated for their ability against juvenile worm (Table 2) (11). Again, compounds **6c** and **6j** demonstrated significant worm killing activity, reduced worm vitality to <10% in 72 h at 25 $\mu$M. Compounds **6a** and **6i**, although less potent than **6c** and **6j**, also displayed considerable effect against juvenile worm. Compound **6j** displayed highest potency, killed all tested worms within 24 h.

**Table 3:** Effect of single 250 mg/kg oral dose of compounds **6a**, **6c**, **6i**, and **6j** administered to mice harboring 42-day-old adult *Schistosoma japonicum* infection

| Compounds | Number of detected worms/ mice ($\bar{X} \pm s$) | Total worm reduction (%) |
|---|---|---|
| Control | 71.25 ± 1.64 | 0 |
| **6a** | 39.0 ± 4.64 | 44.3 |
| **6c** | 37.75 ± 8.0 | 54.3 |
| **6i** | 59.5 ± 2.06 | 12.8 |
| **6j** | 61.33 ± 1.70 | 13.6 |
| PZQ | 26.33 ± 8.5 | 62.4 |

PZQ, praziquantel.

### *Effect against adult schistosomes in rats*

For the *in vivo* study, female ICR mice were infected with ca. 100 *schistosomiasis*. *J* cercariae on day 0 followed by administration of 250 mg/kg oral doses of test compounds suspended in distilled water to groups of 10 mice on day 42 post-infection (adult stage). At 21 days post-treatment, animals were killed and dissected to assess total worm reduction as described in detail (19,20).

For those compounds shown significant effect against both adult and juvenile worm *in vitro*, the worm reduction capability was evaluated *in vivo*. The result is shown in Table 3. Two of the tested compounds **6a** and **6c** displayed obvious worm killing capability. Especially, compound **6c** demonstrated 54.3% worm reduction ability, which is close to the reference compound. Compound **6j** displayed 13.6% worm reduction capability, which is very close to 10-OH PZQ (6), suggesting **6j** might be hydrolyzed *in vivo* and 10-OH PZQ could be released. Although **6i** exhibited good worm killing activity *in vitro*, the worm reduction capability is not satisfactory *in vivo*. This could be related to the increased lipophilicity by introducing a methoxy group or the intolerance of substitutes in the aromatic ring at the PZQ binding site of the target protein.

**Table 4:** Effect of compounds **6a**, **6c**, **6i**, and **6j** on the morbidity of HL-60 cancer and HL-7702 normal cell lines

| | Percentage of morbidity at 50 $\mu$M (%) | | | | |
|---|---|---|---|---|---|
| | **6a** | **6c** | **6i** | **6j** | PZQ |
| HL-60 | 14.4 ± 6.1 | 18.7 ± 7.9 | 22.3 ± 6.6 | 37.2 ± 3.4 | 4.1 ± 0.1 |
| HL-7702 | −5.7 ± 4.1 | −14.5 ± 6.5 | −14.3 ± 8.4 | −18.5 ± 6.5 | −20.0 ± 10.9 |

PZQ, praziquantel.

### *Determination of compound cytotoxicity*

An excellent antischistosomal drug should be supposed to kill selectively the schistosomes without or to a significantly less extent, being harmful to the host (21,22). The four excellent compounds (**6a**, **6c**, **6i**, **6j**) displaying high activity against adult *S. japonicum in vitro* were tested on HL-60 and a normal liver cell line HL-7702 for their cytotoxic potential at 50 $\mu$M. Praziquantel was used as reference drugs, and data are summarized in Table 4. As PZQ, all tested compounds are not toxic to normal liver cell line and to a small extent, toxic to HL-60 cancer cell.

## Conclusions

In summary, we have explored the structure–activity relationship in PZQ introducing different substitutes to the phenyl ring of the parent compound. We identified that introduction of a bromine substitute at 8- of PZQ leads to compound **6c** with higher potency against adult *S. japonicum* than PZQ *in vitro*, and acetyl 10-OH PZQ displayed significant capability to kill juvenile worm *in vitro*. Our *in vivo* study demonstrated that compound **6c** shown comparable worm reduction ability to PZQ. However, none of these compounds established superior worm killing ability to PZQ. The compound cytotoxicity toward cancer and normal cell line revealed that all prepared PZQ derivatives were not cytotoxic. Nonetheless, we have provided experimental details for the PZQ aromatic ring SAR study.

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## Notes

[a]World Health Organization Media center/Schistosomiasis/fact sheets N15/January 2012/http://www.who.int/mediacentre/factsheets/fs115/en/
[b]http://www.schisto.org.