

Accepted Manuscript

Title: Decoquinatone derivatives: A new class of potent antischistosomal agents against *Schistosoma japonicum*

Authors: Wen-Long Wang, Li-Jun Song, Bo-Chun Hu, Li Miao, Xiao-Yu Chen, Wen-Hua Fan, Xu-Ren Yin, Shuang Shen, Zhao-Feng Ding, Chuan-Xin Yu



PII: S1001-8417(17)30124-9
DOI: <http://dx.doi.org/doi:10.1016/j.cclet.2017.03.036>
Reference: CCLET 4031

To appear in: *Chinese Chemical Letters*

Received date: 26-12-2016
Revised date: 27-2-2017
Accepted date: 26-3-2017

Please cite this article as: Wen-Long Wang, Li-Jun Song, Bo-Chun Hu, Li Miao, Xiao-Yu Chen, Wen-Hua Fan, Xu-Ren Yin, Shuang Shen, Zhao-Feng Ding, Chuan-Xin Yu, Decoquinatone derivatives: A new class of potent antischistosomal agents against *Schistosoma japonicum*, Chinese Chemical Letters <http://dx.doi.org/10.1016/j.cclet.2017.03.036>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Original article

Decoquinatone derivatives: A new class of potent antischistosomal agents against *Schistosoma japonicum*

Wen-Long Wang^{a, b, *}, Li-Jun Song^{b, #}, Bo-Chun Hu^{a, #}, Li Miao^a, Xiao-Yu Chen^a, Wen-Hua Fan^a, Xu-Ren Yin^b, Shuang Shen^b, Zhao-Feng Ding^d, Chuan-Xin Yu^{b, c, *}

^a School of Pharmaceutical Science, Jiangnan University, Wuxi 214122, China

^b Key Laboratory of National Health and Family Planning Commission on Parasitic Disease Control and Prevention, Jiangsu Provincial Key Laboratory on Parasite and Vector Control Technology, Jiangsu Institute of Parasitic Diseases, Wuxi 214064, China

^c Medical School, Jiangnan University, Wuxi 214122, China

^d Suzhou Kemu Veterinary Pharmaceutical Co., Ltd, Meiyuan High-technology Development Zone, Suzhou 215225, China

* Corresponding author.

E-mail address: wwenlong2011@163.com (W.-L. Wang), chxnyu@163.com (C.-X. Yu)

#These authors contributed equally to this work.

ARTICLE INFO

Article history:

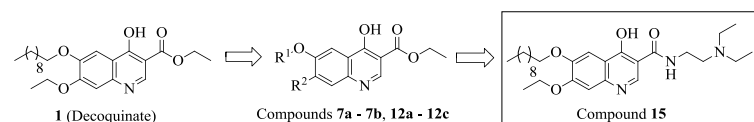
Received 27 December 2016

Received in revised form 27 February 2017

Accepted 8 March 2017

Available online

Graphical abstract



Based on decoquinatone (**1**), a series of decoquinatone derivatives was designed, synthesized, evaluated as a new class of antischistosomal agents. Among them, compound **15** killed 100% of adult *S. japonicum* in 72 hours at the concentration of 10 $\mu\text{mol/L}$ *in vitro*, exhibited stronger worm-killing activity than that of PZQ.

ABSTRACT

Decoquinatone (**1**), an old and inexpensive coccidiostat, exhibited potent antimalarial activity, however, its antischistosomal activity against *Schistosoma japonicum* has not yet been evaluated. Based on decoquinatone, a series of decoquinatone derivatives was designed, synthesized, evaluated as a new class of antischistosomal agents against *S. japonicum* adult worms *in vitro*. Among them, compound **15** killed 100% of adult *S. japonicum* in 72 hours at the concentration of 10 $\mu\text{mol/L}$ *in vitro*, exhibited stronger worm-killing activity than PZQ *in vitro* and could serve as a promising lead compound to develop new antischistosomal agents.

Keywords:

Decoquinatone

Derivatives

Worm-killing activity

Schistosoma japonicum

Structure-activity relationships (SARs)

1. Introduction.

Schistosomiasis is a neglected tropical disease (NTD) caused by blood-dwelling trematodes belonging to the genus *Schistosoma*. *Schistosoma haematobium*, *Schistosoma japonicum*, and *Schistosoma mansoni* are the main species parasitizing humans [1]. It has been estimated that 779 million people are at risk for schistosomiasis transmission, with 207 million infected in 76 countries and territories [1, 2]. In China, the disease caused by *Schistosoma japonicum* remains a major public health concern with more than 280 thousand people infected [3]. In the absence of a vaccine, praziquantel (PZQ) is the only drug recommended by the World Health Organization for the treatment and control of schistosomiasis through mass drug administration (MDA) program for more than three decades [4]. The massive and exclusive use of PZQ has triggered concerns about the emergence of drug resistant parasite, and evidence of drug resistance has been reported [4-6]. Therefore, it is imperative to develop new antischistosomal agents for the treatment of schistosomiasis. Drug repositioning is attractive option in the case of tropical diseases for which the finance and resources to support de-novo drug development are limited [7]. Artemisinin and its derivatives, which are a vital cornerstone in treatment and control of malaria, were now considered as alternative chemotherapies against *S. japonicum* [7]. Decoquinatate (**1**) is an old and inexpensive coccidiostat. Like Artemisinin, Decoquinatate exhibited strong antimalarial activity. [8-10]. We hypothesized that Decoquinatate, acting similarly as Artemisinin, may also possess antischistosomal activity. Using decoquinatate as a template, a series of compounds were designed and synthesized, with the aim to improve aqueous solubility and other drug like properties, and their antischistosomal activities were evaluated against *S. japonicum* adult worms *in vitro*. (Fig 1). Among them, compound **15** killed 100% of adult *S. japonicum* in 72 hours at the concentration of 10 $\mu\text{mol/L}$ *in vitro*, while praziquantel (PZQ) (10 $\mu\text{mol/L}$) caused 37.5% of adult *S. japonicum* death in 72 hours. The result indicated that compound **15** had stronger worm-killing activity than PZQ *in vitro* and could serve as a promising lead compound to develop novel antischistosomal agents (Fig. 1).

2. Results and discussion

2.1 Chemistry

Based on the structure of decoquinatate **1**, compounds **7a-7b**, **12a-12c** and **13-21** were designed and synthesized (Table 1). Phenolic compound **2** was nitrated with 65% nitric acid in the presence of catalytic amounts of iron(III) nitrate nonahydrate to give compound **3** in 52%. After alkylation of hydroxyl group on compound **3**, compound **4a** and **4b** were obtained. Nitro compounds (**4a** and **4b**) were reduced by Fe/NH₄Cl to produce amino compounds (**5a** and **5b**), which reacted with ethoxymethylenemalonate diethyl ester (EMME) to give the intermediates **6a** and **6b** using similar methods reported by wang group (11). The last cycle-closing reaction was carried out by heating the intermediates **6a** and **6b** in diphenyl ether at 260 °C to obtain the key intermediates **7a** and **7b** (Scheme 1) [11]. By removal of ethoxy group from decoquinatate, intermediates **12a-12c** were synthesized using similar procedures to compounds **7a-7b** (Scheme 2). Ester compounds **1**, **7a-7b**, **12a-12c** were treated with various amine to afford the target compounds **13-21** (Scheme 3).

2.2 Worm-killing activities and structure-activity relationships

All the synthesized compounds were evaluated their worm-killing activities against adult *S. japonicum* *in vitro*, using vehicle as negative control and PZQ as positive control [12-16], and the results were detailed in table 1. We initially prepared intermediates **7a-7b** and **12a-12c** by the route outlined in Scheme 1 and Scheme 2, in which we replaced decyl group on **1** with pentyl group (compound **7a**) or pent-4-en-1-yl group (compound **7b**), or removed ethoxy group (compound **12a**) from **1**, or substituted decyl group on compound **12a** with pentyl group (compound **12b**) or pent-4-en-1-yl group (compound **12c**). We noticed that none of them showed worm-killing activity against adult *S. japonicum* *in vitro* at the concentration of 100 $\mu\text{mol/L}$. To explore novel antischistosomal agents and investigate the SARs, amide compounds **13-21** were synthesized (scheme 3). As for compounds **13-16** converted from decoquinatate **1**, all of them obviously exhibited worm-killing activities against adult *S. japonicum* *in vitro*. Among amide compounds with six-membered ring at R³ position, compound **13** with morpholino group showed lower antischistosomal activities than compound **14** with 4-methylpiperazin-1-yl group, which killed 75 % of adult *S. japonicum* at the concentration of 10 $\mu\text{mol/L}$ in 72 hours. Opening the piperazine ring on compound **14** led to compound **15** with (2-(diethylamino)ethyl)amino group, which completely killed worms at the concentration of 10 $\mu\text{mol/L}$ in 72 hours and exhibited stronger worm-killing activity than PZQ *in vitro*. In addition, replacement of morpholino group (compound **13**) with bis(2-hydroxyethyl)amino group (compound **16**) increased worm-killing activity. The results demonstrated that ring-opening strategy at R³ position significantly improved worm-killing activity. Converting decyl group into pentyl group, the antischistosomal activities of compound **17** was obviously decreased compared to compound **15**. Introduction of C=C bond into R¹ position, the activity of compound **18** dropped significantly compared to compound **15** and **17**. These results demonstrated that long saturated alkyl group at R¹ position could have a beneficial effect on worm-killing activity. Among the compounds without ethoxy group (**19-21**), compounds **19-21** showed lower worm-killing activities than corresponding compounds with ethoxy group (**15**, **17-18**). These results demonstrated that ethoxy group on R² position took positive effect on worm-killing activity.

Furthermore, the activities against female or male adult worms were also observed. The mortality rate was 75.0% for male worms or 50.0% for female worms in 24 h at 25 $\mu\text{mol/L}$ of compound **14**. When the concentration was increased to 50 $\mu\text{mol/L}$, compound **14** killed 92.8% of male worms and 50.0% of female worms in 24 h. (Fig. 2A). The mortality rate was 40.0% for male worms or 27.4% for female worms in 24 h at 10 $\mu\text{mol/L}$ of compound **15** (Fig. 2B). However, the result of statistical analysis showed that there were no differences between male and female worms killing activity of compounds **14** or **15**.

The morphological alternations of *Schistosoma japonicum* worms incubated with compound **15** *in vitro* after 48 hours were observed using an inverted microscopy (Leica, Wetzlar, Germany). The surface of worms in negative control was smooth. The head sucker and the ovarian structure in the body were clearly visible. However, the body of worms incubated with compound **15** became dimmed. The head and the structure in the body was blurred. The worms curled up and there were many vacuoles on the surface of worms (Fig. 3).

2.3 Cytotoxicity assay of compounds **14** and **15** on HeLa cells [14]

The cytotoxic assessment of the compounds **14** and **15** on the HeLa cells was observed at different concentrations (5, 10, 20, 40, 80 $\mu\text{mol/L}$) for 48 h using the MTT method [14] and the viability of the HeLa cells treated with compound **14**, **15** and PZQ at the concentration of 20 $\mu\text{mol/L}$ was 99%, 90% and 83% respectively. The results indicated that compounds **14** showed lower cytotoxicity than PZQ, and the difference was statistically significant ($P < 0.05$). Meanwhile, compound **15** has the similar cytotoxicity to PZQ, and the difference was not statistically significant ($P > 0.05$).

3. Conclusion

Given the emergence of PZQ resistant parasites in the near future, it is undoubtedly urgent to develop new chemotherapy for schistosomiasis. In this paper, we synthesized a series of decoquinatate derivatives as novel antischistosomal agents against adult *S. japonicum* *in vitro*, and identified compound **15** as potent antischistosomal agent. The study of these structurally diverse decoquinatate derivatives would likely provide meaningful information for developing new potent antischistosomal agents.

4. Experimental

4.1 General procedure for the synthesis of compounds **7a-7b** (exemplified by **7a**)

Nitric acid (10 mL, 65%) was added dropwisely into a mixture of 2-ethoxyphenol (10.00 g, 72.50 mmol) and iron (III) nitrate nonahydrate (900 mg, 2.23 mmol) in THF (250 mL) through a constant pressure dropping funnel at 0 °C for 30 minutes. Then the mixture was stirred at room temperature for 30 min and quenched by water (100 mL) and dichloromethane (150 mL). The aqueous phase was extracted with dichloromethane (100 mL $\times$ 3), then the combined organic phases were then processed in the usual way and chromatographed to afford compound **3** (6.90 g, 52%) as solid. The mixture of compound **3** (5.00 g, 27.30 mmol), potassium carbonate (15.10 g, 109.20 mmol) and 1-bromopentane (3.57 mL, 28.70 mmol) in MeCN (50 mL) was refluxed overnight, then the mixture was diluted with EtOAc (200 mL), the organic phases were then processed in the usual way and chromatographed to afford compound **4a** (5.10 g, 74%) as solid. A solution of compound **4a** (5.00 g, 19.70 mmol) in ethanol/water (120 mL/120 mL) was treated with iron powder (3.32 g, 59.30 mmol) and ammonium chloride (3.17 g, 59.30 mmol) and the mixture was heated to reflux overnight. The mixture was then filtered and concentrated under reduced pressure. The resulting oil was partitioned between water and ethyl acetate, and the organic phase was then washed with saturated aqueous sodium chloride, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to provide the desired compound **5a** (2.87 g, 65%) as black oil without purification to use for next step. The mixture of Ethoxymethylenemalononic diethyl ester (EMME, 2.42 g, 11.21 mmol) and compound **5a** (2.50 g, 11.21 mmol) in EtOH (100 mL) was refluxed overnight. After removal of EtOH, the mixture was chromatographed to afford compound **6a** (3.01 g, 68%) as yellow oil. Diphenyl ether (10 mL) was heated to 260 °C and compound **6a** (1.00 g, 2.54 mmol) was added. The reaction mixture was stirred at 260 °C for 15 minutes, and then cooled to room temperature. EtOAc (25 mL) was added to the mixture and the obtained solid was filtrated, washed with EtOAc and dried to obtain **7a** (590 mg, 67%) as solid.

4.2 General procedure for the synthesis of compounds **12a - 12c** (exemplified by **12a**)

The mixture of compound **8** (2.78 g, 20.0 mmol), potassium carbonate (11.03 g, 80 mmol) and 1-bromodecane (4.55 mL, 22.0 mmol) in MeCN (50 mL) was refluxed overnight, then the mixture was diluted with EtOAc (200 mL), the organic phases were then processed in the usual way and chromatographed to afford compound **9a** (4.47 g, 80%) as solid. A solution of compound **9a** (4.47 g, 16.0 mmol) in ethanol (100 mL) and water (100 mL) was treated with iron powder (2.69 g, 48.0 mmol) and ammonium chloride (2.54 g, 48.0 mmol) and the mixture was heated to reflux overnight. The mixture was then filtered and concentrated under reduced pressure. The resulting oil was partitioned between water and ethyl acetate, and the organic phase was then washed with saturated aqueous sodium chloride, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to provide the desired compound **10a** (2.59 g, 65%) as black oil without purification to use for next step. The mixture of Ethoxymethylenemalononic diethyl ester (EMME, 2.08 mL, 10.4 mmol) and compound **10a** (2.59 g, 10.4 mmol) in EtOH (100 mL) was refluxed overnight. After removal of EtOH, the mixture was chromatographed to afford compound **11a** (2.97 g, 68%) as yellow oil. Diphenyl ether (10 mL) was heated to 260 °C and compound **11a** (2.97 g, 7.08 mmol) was added. The reaction mixture was stirred at 260 °C for 15 minutes, and then cooled to room temperature. EtOAc (25 mL) was added to the mixture and the obtained solid was filtrated, washed with EtOAc, and dried to obtain **12a** (1.45 g, 55%) as solid.

4.3 General procedure for the synthesis of compounds **13 - 21** (exemplified by **13**)

The mixture of decoquinatate **1** (100 mg, 0.24 mmol) and morpholine (209 μL , 2.4 mmol) was stirred at 100 °C for 12 hours, cooled to room temperature and diluted with dichloromethane (0.5 mL). Then petroleum ester was added to the mixture. The solid was precipitated, filtrated and dried to obtain **13** (60 mg, 55%).

4.4 Worm-killing activity of compounds DQ 1, 7a-7b, 12a-12c, 13-21 on *S. Japonicum* adult worms in vitro

Stock solutions of compounds DQ 1, 7a-7b, 12a-12c, 13-21 and praziquantel were prepared by dissolving the drugs (1 mg) in dimethyl sulfoxide (DMSO, 0.4 mL) and adding RPMI 1640 medium (0.6 mL). *S. japonicum* worms obtained from mice (C57BL/6, female, 22–24 g, each infected with 50 cercariae) were washed in RPMI 1640 medium, kept at pH 7.5 with HEPES (20 mmol/L) and supplemented with penicillin (100 UI/mL), streptomycin (100 mg/mL) and 10% fetal bovine serum (FBS, Gibco). After washing, 8–15 adult worms were transferred to each well of a 24-well culture plate containing 2 mL of the same medium. The worms were cultured for 30 to 60 min at 37 °C in a humid atmosphere containing 5% CO₂, and then different concentrations of compounds DQ 1, 7a-7b, 12a-12c, 13-21 (10, 25, 50, 100 µmol/L) diluted with RPMI 1640 medium were added. Control worms were treated with equal volumes of RPMI 1640 or DMSO, and worms treated with praziquantel (10, 25, 50, 100 µmol/L) were also observed. The worm mobility, tegumental alterations and parasite survival were monitored under an inverted microscope (Leica, Wetzlar, Germany) at 24, 48 and 72 h. Parasite death was defined as having no motor activity during 2 min of continuous observation as well as morphological and tegumental alterations. The tests were repeated two times when compounds showed worm killing activity below the concentration of 100 µmol/L.

4.5 Cytotoxic assay of compounds 14 and 15 on Hela cells

Cytotoxicity assays were assessed using Hela cells. Cells in the exponential growth phase were collected by centrifugation and a concentration of $4-5 \times 10^4$ /mL was created. 100 µL of the cell suspension was added to each well of a 96-well plate and cultured at 37 °C in a humid atmosphere with 5 % CO₂ overnight. Compounds (at 5, 10, 20, 40 and 80 µmol/L) were added. Each concentration was assayed in triplicate, and control wells containing no drug were assayed at the same time. The cells were cultured for 48 h at 37 °C in a humid atmosphere with 5 % CO₂. Then 20 µL of MTT (5 mg/mL) was added to each well and the culture conditions were maintained for 4 h. The medium was removed and DMSO (150 µL) was added into each well. The value of absorbance of each well was measured (at 570 nm) with a microplate reader to assess the toxic effect of the compounds on the vertebrate cells.

4.6 Statistical analysis

Cell survival rates after exposure to compounds 14, 15 and PZQ were determined using chi-square tests. SPSS 13.0 was used for the statistical analyses. Differences between mean values were considered to be significant at the level of 5 %.

Acknowledgment

This work was supported by Laboratory Research Fund funded by Key Laboratory of National Health and Family Planning Commission on Parasitic Disease Control and Prevention, Jiangsu Provincial Key Laboratory on Parasite and Vector Control Technology, Parasitic Disease Prevention and Control Platform (No. WK014-002), fund from the Department of Science and Technology of Jiangsu Province (No. BY2016022-37), fund from the National Natural Science Foundation of China (Nos. 21472069, 30972581, 8120316), fund from the Natural Science Foundation of Jiangsu Province (Nos. BK2012544, BK20151120), fund from the scientific research projects of Jiangsu Provincial Commission of Health and Family Planning (No. H201635), fund from the scientific research projects of Wuxi City Commission of Health and Family Planning (No. Q201656), fund from the Ministry of Education of the People's Republic of China (No. JUSRP51516) and fund from Research project of Jiangsu Provincial Commission of Health and Family Planning (No. H201635) and Jiangsu Science and Technology Department (No. BM2015024).

References

- [1] A. Danso-Appiah A, P. L. Olhario, S. Donegan, D. Sinclair, J. Utzinger, Drugs for treating *Schistosoma mansoni* infection, Cochrane. Database. Syst. Rev. 2 (2013) CD000528.
- [2] B. Gryseels, K. Polman, J. Clerinx, L. Kestens, Human schistosomiasis, Lancet. 368 (2006) 1106 - 1118.
- [3] H. Zheng, L.J. Zhang, R. Zhu, et al., Schistosomiasis situation in People's Republic of China in 2011, Chin. J. Schisto. Control. 24 (2012) 621 - 626.
- [4] C.R. Caffrey, W.E. Secor, Schistosomiasis: from drug deployment to drug development, Curr. Opin. Infect. Dis. 24 (2011) 410 - 417.
- [5] F.F. Stelma, I. Talla, S. Sow, et al., Efficacy and side effects of praziquantel in an epidemic focus of *Schistosoma mansoni*, Am. J. Trop. Med. Hyg. 53 (1995) 167 - 170.
- [6] W. Wang, L. Wang, Y. S. Liang, Susceptibility or resistance of praziquantel in human schistosomiasis: a review, Parasitol. Res. 111 (2012) 1871 - 1877.
- [7] Y. X. Liu, W. Wu, Y. J. Liang, et al., New uses for old drugs: the tale of artemisinin derivatives in the elimination of schistosomiasis japonica in China, Molecules 19 (2014) 15058 - 15074.
- [8] T. G. Nam, C. W. McNamara, S. Bopp, et al., A Chemical Genomic Analysis of Decoquinone, a *Plasmodium falciparum* Cytochrome b Inhibitor, ACS. Chem. Biol. 6 (2011) 1214 - 1222.
- [9] H. Wang, Q. Li, S. Reyes, et al., Nanoparticle formulations of decoquinone increase antimalarial efficacy against liver stage *Plasmodium* infections in mice, Nanomedicine 10 (2014) 57 - 65.
- [10] R. M. Beteck, D. Coertzen, F. J. Smit, et al., Straightforward conversion of decoquinone into inexpensive tractable new derivatives with significant antimalarial activities, Bioorg. Med. Chem. Lett. 26 (2016) 3006 - 3009.
- [11] C.-R. Yan, J. Xu, Y.-B. Weng, et al., A series of ethyl 6-arylmethoxy-7-alkoxy-4-hydroxy-3-quinolinecarboxylates: synthesis and anticoccidial activity against *Eimeria tenella*, Chem. Biol. Drug. Des. 72 (2008) 314 - 319.
- [12] W. -L. Wang, L. J. Song, X. Chen, et al., Synthesis and SAR studies of praziquantel derivatives with activity against *Schistosoma japonicum*, Molecules. 18 (2013) 9163 - 9178.

- [13] W. -L. Wang, L. J. Song, G. P. Wang, et al., Synthesis and evaluation of the antischistosomal activity against *S. japonicum* of 1-methyl-1,2,3,4-tetrahydroisoquinoline derivatives, *Chin. J. Org. Chem.* 33 (2013) 2588 - 2595.
- [14] L. J. Song, H. Luo, W. H. Fan, et al., Oxadiazole-2-oxides may have other functional targets, in addition to SjTGR, through which they cause mortality in *Schistosoma japonicum*, *Parasit. Vectors.* 9 (2016) 26.
- [15] F. F. Yang, L. J. Song, X. R. Yin, et al., Activity of Gossypol against *Schistosoma japonicum*, *Lat. Am. J. Pharm.* 35 (2016) 1660 - 1663.
- [16] W. -L. Wang, L. J. Song, X. Chen., Synthesis of triclabendazole derivatives and biological evaluation against *Schistosoma japonicum*, *Lat. Am. J. Pharm.* 35 (2016) 2274 - 2278.

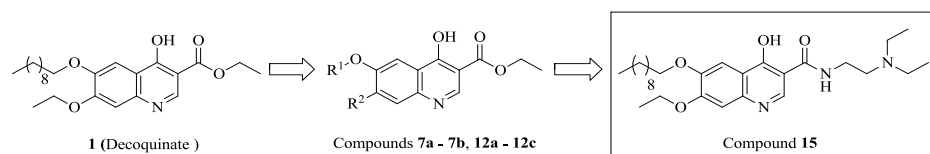


Fig. 1. Structures of novel decoquinone derivatives

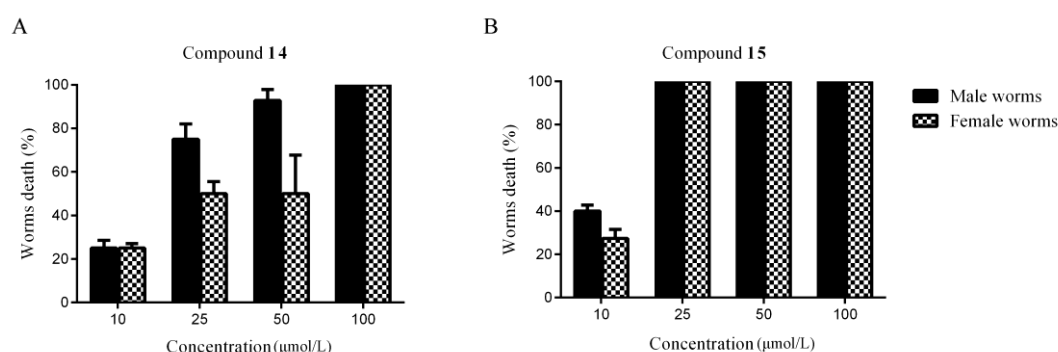


Fig. 2. Killing activity of compounds 14 (A) and 15 (B) against male and female adult *S. japonicum* after 24 h.

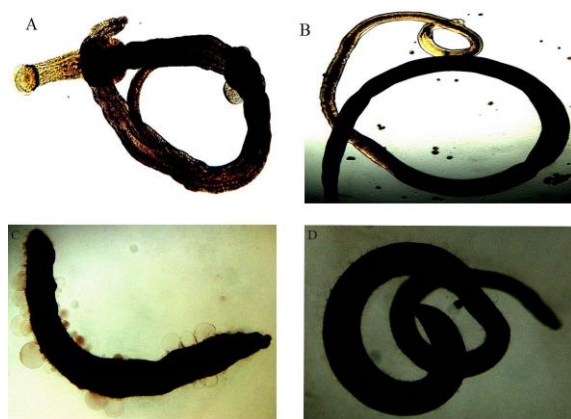


Fig. 3. Morphological investigation of *Schistosoma japonicum* worms after *in vitro* incubation with Compound 15. After 48 h or in the case of death, schistosomes were monitored using an inverted microscopy (Leica, Wetzlar, Germany). (A) Negative control. (B) Vehicle control. (C) 10 μmol/L of Compound 15. (D) 25 μmol/L of Compound 15.

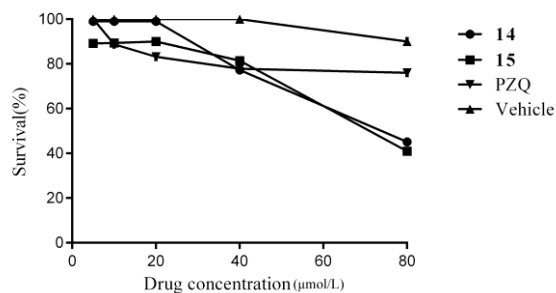
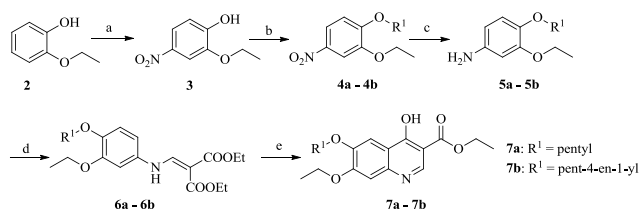
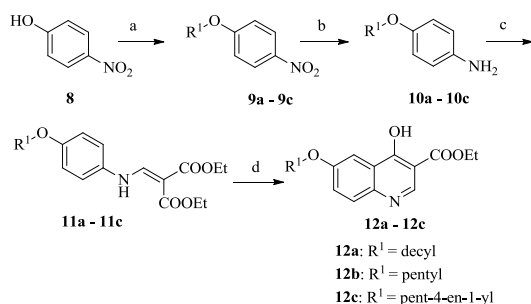


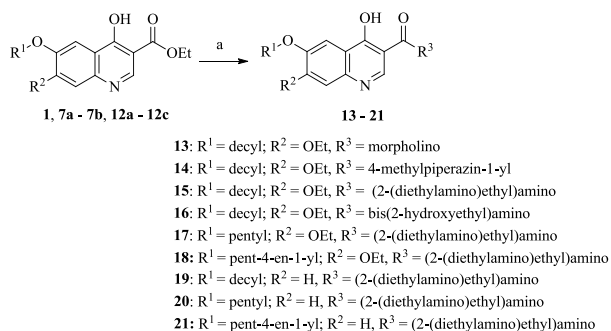
Fig. 4. Cytotoxic assessment of compounds **14**, **15** and PZQ on Hela cells.



Scheme 1. Synthesis of compounds **7a** and **7b**. Reagents and conditions: (a) con HNO_3 (65%), $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, THF, 0 °C to r. t., 52%; (b) R^1Br , K_2CO_3 , CH_3CN , reflux, 74% - 82%; (c) Fe , NH_4Cl , EtOH, reflux, 65% - 70%; (d) ethoxymethylenemalonate diethyl ester (EMME), EtOH, reflux, 68% - 70%; (e) diphenyl ether, 260 °C, 55% - 67%.

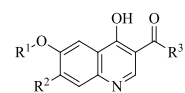


Scheme 2. Synthesis of compounds **12a-12c**. Reagents and conditions: (a) R^1Br , K_2CO_3 , CH_3CN , reflux, 78% - 82%; (b) Fe , NH_4Cl , EtOH, reflux, 65% - 70%; (c) ethoxymethylenemalonate diethyl ester (EMME), EtOH, reflux, 68% - 70%; (d) diphenyl ether, 260 °C, 55% - 66%.

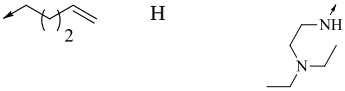


Scheme 3. Synthesis of compounds **13-21**. Reagents and conditions: (a) amine, 100 °C, 40% - 60%.

Table 1 Worm-killing activities against *S. japonicum* adult worms *in vitro* by compounds DQ **1**, **7a-7b**, **12a-12c**, **13-21**.



Comp	R ¹	R ²	R ³	Worm-killing activity ^a			
				Conc. (μmol/L) ^b	24 h	48 h	72 h
Vehicle ^c	-	-	-		n.e	n.e	n.e
PZQ	-	-	-	10	n.e	12.5%	37.5%
				25	37.5%	44.4%	66.7%
				50	44.4%	55.6%	75.0%
				100	55.6%	77.8%	100%
1 ^d		OEt	OEt	100	n.e	n.e	n.e
7a ^d		OEt	OEt	100	n.e	n.e	n.e
7b ^d		OEt	OEt	100	n.e	n.e	n.e
12a ^d		H	OEt	100	n.e	n.e	n.e
12b ^d		H	OEt	100	n.e	n.e	n.e
12c ^d		H	OEt	100	n.e	n.e	n.e
13		OEt		10	n.e	n.e	n.e
				25	n.e	n.e	n.e
				50	25.0%	50.0%	100%
				100	100%	100%	100%
14		OEt		10	25.0%	33.3%	75%
				25	62.5%	100%	100%
				50	71.4%	100%	100%
				100	100%	100%	100%
15		OEt		10	33.7%	50.0%	100%
				25	100%	100%	100%
				50	100%	100%	100%
				100	100%	100%	100%
16		OEt		10	n.e	n.e	37.5%
				25	14.3%	57.1%	71.4%
				50	100%	100%	100%
				100	100%	100%	100%
17		OEt		10	n.e	n.e	n.e
				25	n.e	12.5%	100%
				50	66.7%	100%	100%
				100	100%	100%	100%
18		OEt		10	n.e	n.e	n.e
				25	n.e	n.e	n.e
				50	n.e	66.7%	88.9%
				100	100%	100%	100%
19		H		10	n.e	n.e	37.5%
				25	62.5%	100%	100%
				50	100%	100%	100%
				100	100%	100%	100%
20		H		10	n.e	n.e	n.e

21		25	n.e	n.e	n.e
		50	n.e	66.7%	88.9%
		100	100%	100%	100%
		10	n.e	n.e	n.e
		25	n.e	n.e	n.e
		50	n.e	n.e	62.5%
		100	n.e	100%	100%

^a Data collected by visual examination of worm movement and shape (the values were the averages of two tests); n.e. (no effect): all worms are scored as active in culture with typical appearance; % = number of dead worms/total number of worms observed, and dead worms judged by lack of movement within 2 minutes in addition to morphological and tegumental alterations; ^b The concentration of the chemicals on *S. japonicum* adult worms *in vitro*; ^c RPMI 1640 medium containing 0.5% DMSO; ^d Data only showed the concentration of 100 $\mu\text{mol/L}$ when the chemicals have no effect on the worms at 10, 25 and 50 $\mu\text{mol/L}$.