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# Design, synthesis and bioactivities of phenithionate analogues or derivatives for anti-Schistosomiasis

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**Abstract:** A series novel of phenithionate analogues or derivatives were designed and synthesized using phenithionate as the lead compound, and their bioactivities were studied. Their structures were confirmed by $^{1}H$ NMR, $^{13}C$ NMR, HR-ESI-MS, and elemental analysis, respectively. The results of inhibitory activity in *vitro* proved that compounds **5a, 5c, 5g, 5i, 5m** and **5o** had better inhibitory effect on larva and imago schistosoma. Among them, the inhibitory activity of compound **5i** to larva schistosoma was $IC_{50}$=5.21±0.04μg/mL, and to imago schistosoma was $IC_{50}$=6.35±0.08μg/mL. Moreover, the experimental results of anti-Schistosomiasis activity in *vivo* showed that they had good anti-Schistosomiasis activity. Therefore, these compounds had better drugability.

**Keyword:** Phenithionate, design, synthesis, bioactivities

## 1. Introduction

Parasitic diseases spread widely around the world. They are a common disease, especially in tropical and subtropical developing countries. Some parasitic diseases can develop into epidemics in a region[1-5]. When parasitic diseases are prevalent, they have a serious impact on the society and economy of the region. Schistosomiasis is the most prevalent parasitic disease in the world, which is the most harmful to people's health. According to the World Health Organization (WHO), schistosomiasis is endemic in 76 countries and regions, and there are about 200 million schistosomiasis patients, and 500-600 million people are threatened by the infection. Schistosomiasis is a parasitic disease caused by *Schistosoma mansoni* parasitic in human veins[6-10]. There are three species of *Schistosoma mansoni* parasitic on human body, including *Schistosoma haematobium* (*S. haematobium*), *Schistosoma mansoni* (*S. mansoni*) and *Schistosoma japonicum* (*S. japonicum*)[11-14]. Among the three species,

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*Schistosoma japonicum* is the most widely distributed, mainly distributed in China, Japan, Malaysia, Indonesia and other countries[15-17]. *Schistosoma japonicum* not only can cause acute or chronic enteritis, cirrhosis, severe diarrhea, anemia and emaciation, but also can cause great harm to livestock. Chemical treatment of *Schistosoma japonicum* happened in 1918, antimony potassium tartrate injection was used for the treatment of *Schistosoma japonicum*, and achieved good treatment effect, but it had high toxicity. There were adverse reactions to life-threatening by clinical findings if people had the longer course of treatment with this medicine[18-21]. The treatment of *Schistosoma japonicum* with this kind of antimony medicine is very toxic and must be intravenously injected[15-17]. It is rarely used in clinic, and even some countries have banned it. So looking for *Schistosoma japonicum* agent drugs in the treatment of non-antimony is necessary, the first non-antimony drug nithiocyanamine and derivative nitroscanate were shown in Fig. 1. In 1975, nithiocyanamine was designed and synthesized, and it was a broad-spectrum anthelminthics. Nithiocyanamine had a significant role in the killing of *Schistosoma japonicum*. The mechanism was that the body tricarboxylic acid cycle metabolism was disturbed, so, lack of energy supply happened, and this ultimately led to cell death[22-25]. For the treatment of various types of *Schistosoma japonicum* clinically, subsequent clinical manifestations of slow metabolism can cause accumulation of poisoning, about 4%-8% patients showed jaundice, transaminases and other side effects, so it was not used clinically. Nithiocyanamine derivative nitroscanate also has obvious anti-*Schistosoma japonicum* effect and toxicity is slightly lower than nithiocyamine[26]. The study on the structure and activity relationship shows that the isothiocyanate is not only a pharmacophore, but also a toxic group. Therefore, it is also possible to resist the effect of *Schistosoma japonicum* by transforming the cyano group into amino carbamate groups. The modified derivative of diphenyl ester reduced the toxicity greatly. In this research, we modified the structure by replacing the linked nitrogen atom with oxygen atom and sulfur atom with the principle of bioisosteres. The nitro phenithionate benzene ring also was replaced with trifluoromethyl and trichloromethyl groups. The end of benzene ring was modified with hydroxyl and acetylamino groups, while its pharmacophore thioamide was retained (Fig. 2 and Scheme 1). The modified compounds had good anti-*Schistosoma japonicum* effect, and some of them had better insect resistance than phenyl nitrate. According to the structure-activity relationships (SAR) of the compounds, we have retained the basic framework. A new group has been introduced on the basis of phenithionate to change the log*P* and p*Ka*, which affects the activity of drug. When $-CCl_3$ and -OH were introduced to benzene ring, the activity was very high. In addition, the connecting atoms between benzene rings also have certain effects on the activity of drug. When the connecting atoms are N and O, their activity is relatively high, because these atoms can form hydrogen bond with the receptor macromolecule, which can enhance the activity of drug.

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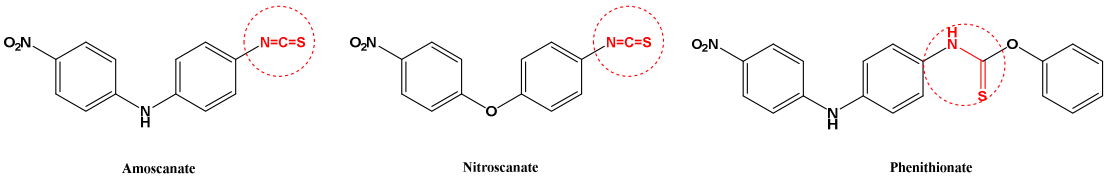



**Fig. 1.** The structures of anti-Schistosomiasis drugs.

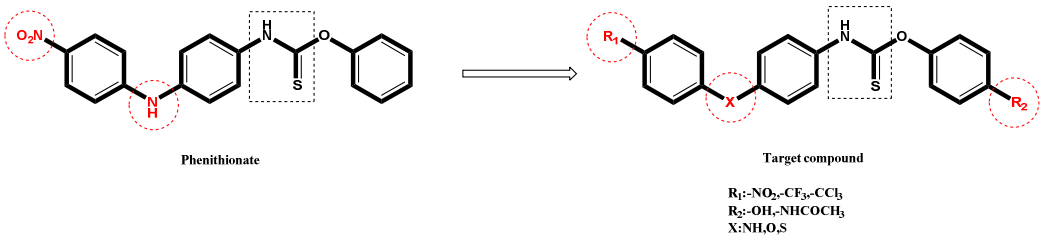
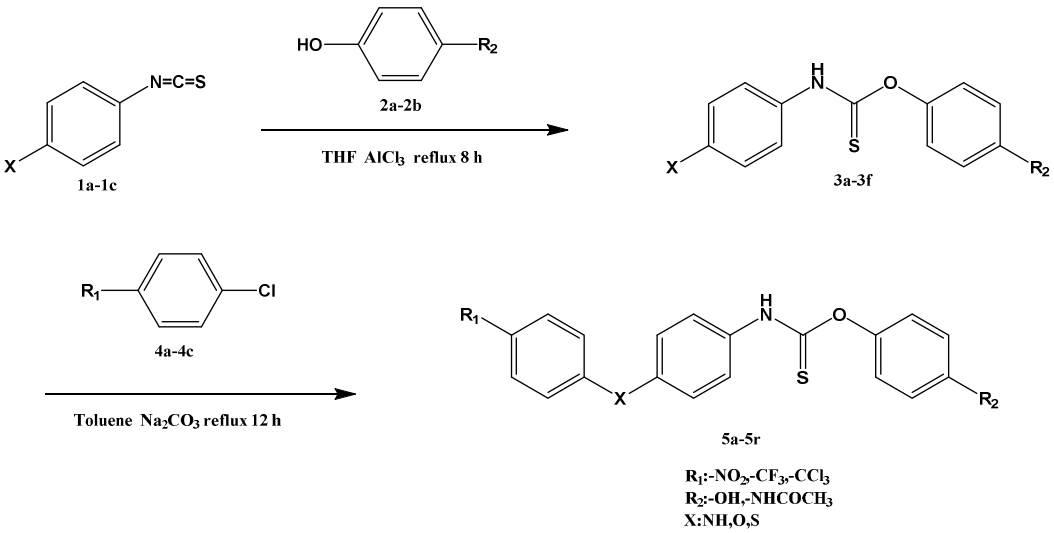



**Fig. 2.** Design of phenithionate analogues or derivatives.




**Scheme 1.** The synthesis of phenithionate analogues or derivatives.

## 2. Results and discussion

*2.1. Design and synthesis of phenithionate analogues or derivatives*

Based on phenithionate esters as a lead compound, a series of novel anti-Schistosomiasis drugs were designed, and their structures were modified by the principle of biological electron exclusion (Fig. 2). In terms of drug structure, these compounds have the same spatial structure as phenithionate esters, so the possibility of drug formation is relatively large. In the structural modification, the basic framework of the compound was retained, and the amino group with the same anti-Schistosomiasis efficacy was retained. At the same time, the substitution of phenyl ester for N atom between benzene rings and substituent on benzene ring have been modified. In the

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process of structural design, N, O, S atoms were selected, and the substituent $R_1$ in the benzene ring increased -$CF_3$ and -$CCl_3$, and $R_2$ selected -OH and -$NHCOCH_3$. Such structural design, mainly taking into account the substituents on the benzene rings and connecting atoms will have an impact on the log*P*, p*Ka* and the interaction between drugs and receptors, so as to achieve the purpose of affecting the efficacy. In the synthesis process, a total of two design schemes (Fig. 3). The target product was obtained by two steps of condensation and addition (Scheme 2). Although the steps of the two schemes are different in sequence, the total yield of target and the difficulty of operation are different. If the synthesis route is first condensed and then added, this route will have many side reactions in the condensation process, the intermediates are difficult to handle, and in addition, the yield is low due to the larger steric hindrance. The specific operation route scheme can be seen (Scheme 1), the addition reaction with THF as solvent, $AlCl_3$ as catalyst in refluxing for 8 hours to complete the first step reaction; condensation reaction with toluene as solvent, anhydrous sodium carbonate as acid binding agent and anti reflux should be 12 h can reach the synthesis of the target product superiority is a simple operation, mild reaction conditions, the target product yield high yield characteristics, 80.5%-93.4%.

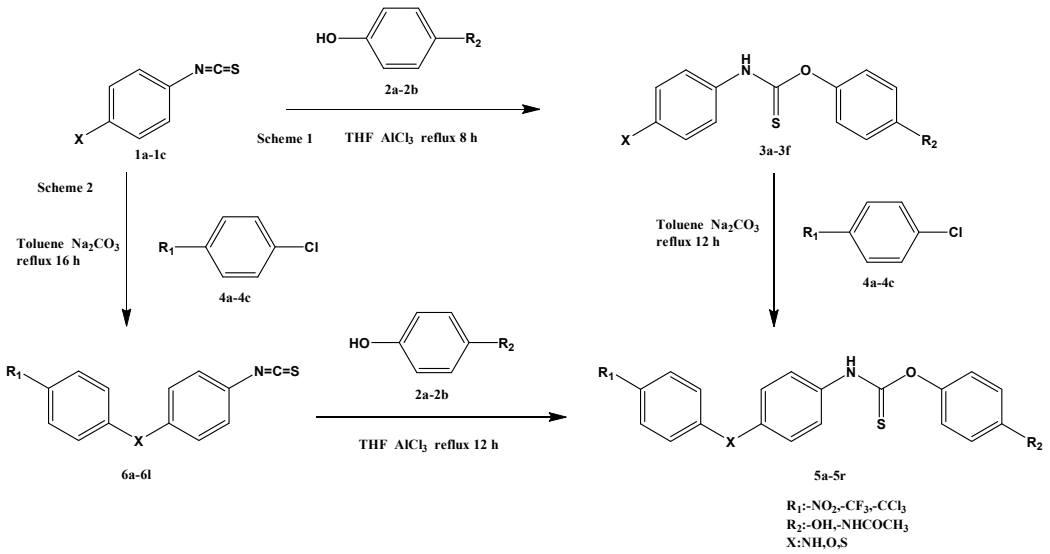


**Fig. 3.** The synthetic route of target compounds.

*2.2. The biological activities*

*2.2.1. The inhibitory activity to anti-Schistosomiasis in vitro*

Before studying the inhibitory activity of target compounds *in vitro*, we first studied the physical and chemical properties of phenithionate analogues or derivatives, namely the lipid/water partition coefficient (log*P*) and dissociation constant (p*Ka*), which might affect their activity (Table 1). As could be seen from Tabel 1, these

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compounds had good fat-soluble,and the log*P* was from 4.32 to 7.43. The higher the partition coefficient of lipid and water was, the better the absorption of drugs was,and the activity was increased.In addition,from the experimental results,it could also be seen that the compounds could be digested and absorbed by the small intestine,and the p*Ka* was from 8.99 to 9.24. The difference in the p*Ka* of drug affected the effective time because the compounds needed to be digested and absorbed by the small intestine,and the effective time would be slightly delayed.After the completion of log*P* and p*Ka* studies, we focused on the anti-Schistosomiasis *in vitro*. In the experiment, Larva and Imago Schistosomiasis were used as the inhibitory targets, and the inhibitory activity was measured by semi inhibitory concentration ($IC_{50}$). Finally, the anti Imago *Schistosoma haematobium* (*S. haematobium*), *Schistosoma mansoni* (*S. mansoni*) and *Schistosoma japonicum* (*S. japonicum*) *in vitro (*Fig. 4). The results showed that the compounds **5a, 5c, 5g, 5i, 5m** and **5o** had high anti-Schistosomiasis activity, especially for *Schistosoma japonicum* (*S. japonicum*).

**Table 1.** The inhibitory activity *in vitro* and some physico-chemical properties.

| Compounds | $R_1$ | $R_2$ | X | log*P* | p$K_a$ | $IC_{50}$±SD（μg/mL） | |
|---|---|---|---|---|---|---|---|
| | | | | | | Larva | Imago |
| **5a** | $-NO_2$ | -OH | NH | 4.54 | 9.22 | 6.42±0.12 | 8.05±0.15 |
| **5b** | $-NO_2$ | $-NHCOCH_3$ | NH | 4.32 | 9.04 | 8.22±0.48 | 15.22±0.37 |
| **5c** | $-NO_2$ | -OH | O | 5.01 | 9.23 | 6.38±0.09 | 8.02±0.12 |
| **5d** | $-NO_2$ | $-NHCOCH_3$ | O | 4.81 | 9.04 | 8.24±0.33 | 14.11±0.51 |
| **5e** | $-NO_2$ | -OH | S | 5.25 | 9.21 | 15.67±0.58 | 30.54±0.39 |
| **5f** | $-NO_2$ | $-NHCOCH_3$ | S | 5.06 | 8.99 | 16.66±0.54 | 31.20±0.38 |
| **5g** | $-CF_3$ | -OH | NH | 6.04 | 9.23 | 5.43±0.11 | 6.68±0.09 |
| **5h** | $-CF_3$ | $-NHCOCH_3$ | NH | 5.32 | 9.05 | 11.20±1.02 | 20.02±1.28 |
| **5i** | $-CF_3$ | -OH | O | 6.11 | 9.23 | 5.21+0.04 | 6.35±0.08 |
| **5j** | $-CF_3$ | $-NHCOCH_3$ | O | 5.41 | 9.03 | 10.37±0.89 | 17.29±1.10 |
| **5k** | $-CF_3$ | -OH | S | 6.66 | 9.22 | 16.56±0.83 | 31.86±0.98 |
| **5l** | $-CF_3$ | $-NHCOCH_3$ | S | 5.96 | 9.02 | 19.01±0.82 | 33.69±0.96 |
| **5m** | $-CCl_3$ | -OH | NH | 6.79 | 9.23 | 7.35±0.26 | 12.39±0.35 |
| **5n** | $-CCl_3$ | $-NHCOCH_3$ | NH | 6.07 | 9.01 | 11.60±1.20 | 20.11±1.34 |
| **5o** | $-CCl_3$ | -OH | O | 6.84 | 9.24 | 7.98±0.41 | 13.55±0.46 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **5p** | $-CCl_3$ | $-NHCOCH_3$ | O | 6.16 | 9.02 | 10.19±1.01 | 18.29±1.21 |
| **5q** | $-CCl_3$ | -OH | S | 7.43 | 9.22 | 21.09±1.15 | 37.28±1.19 |
| **5r** | $-CCl_3$ | $-NHCOCH_3$ | S | 6.71 | 9.01 | 23.54±1.22 | 40.39±1.31 |
| Blank control （DMSO） | | ___ | | ___ | ___ | / | / |
| Phenithionate | | ___ | | ___ | ___ | 6.53±0.13 | 8.21±0.21 |
| Praziquantel | | ___ | | ___ | ___ | 6.33±0.11 | 7.98±0.18 |

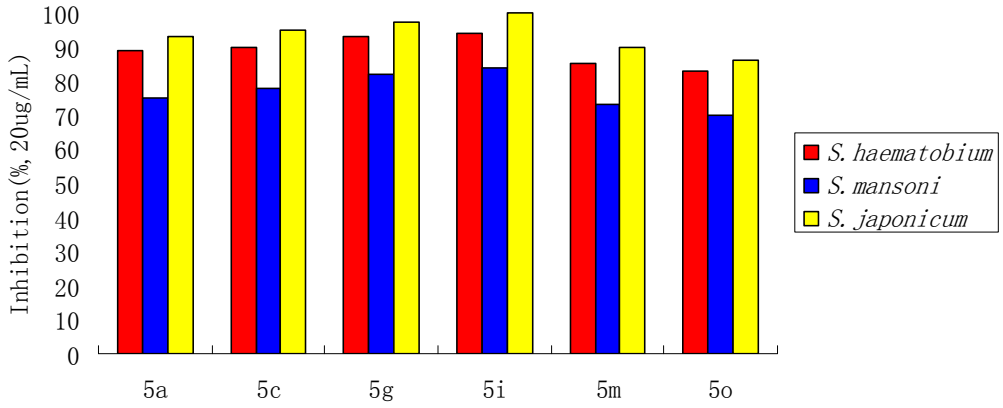


**Fig. 4.** The inhibition rate.

*2.2.2. The anti-Schistosomiasis in vivo*

In the course of the bioactivity study,compounds **5a, 5c, 5g, 5i, 5m** and **5o** with high inhibitory activity and low acute toxicity and tested *in vivo* (Tables 2 and 3). The oral dose were 25mg.kg$^{-1}$.d$^{-1}$ and 50mg.kg$^{-1}$.d$^{-1}$, and the anti-Schistosomiasis was observed in different times. Phenithionate and praziquantel were used as the positive reference substance. The inhibitory activity experimental results showed that these two compounds had fast effective time and good anti-Schistosomiasis activity. And acute toxicity ecperimental results showed that these four compounds had low acute toxicity. In Table 3, for the common anti-Schistosomiasis drugs, phenithionate was $LD_{50}$=310.5±4.1mg/kg and praziquantel was $LD_{50}$>500mg/kg. Compared with commonly used anti-Schistosomiasis drugs, compounds **5a** and **5c**, $LD_{50}$ was smaller (280.1±3.2mg/kg and 275.3±2.6mg/kg), and the acute toxicity was higher. For other compounds **5g**, **5i**, **5m** and **5o**, $LD_{50}$ was greater than 500mg/kg, this compounds belonged to low toxicity or non-toxicity substances.

**Table 2.** The inhibition activity *in vivo*.

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| Compounds | Dosage of drugs($mg.kg^{-1}.d^{-1}$) | Time 1d | 2d | 3d | 4d | 5d | 6d | 7d | 8d | 9d |
|---|---|---|---|---|---|---|---|---|---|---|
| 5a | 25 | +++ | +++ | +++ | ++ | ++ | + | + | — | — |
| | 50 | +++ | ++ | ++ | + | + | — | — | — | — |
| 5c | 25 | +++ | +++ | +++ | ++ | ++ | + | + | — | — |
| | 50 | +++ | ++ | ++ | + | + | — | — | — | — |
| 5g | 25 | +++ | +++ | ++ | ++ | + | — | — | — | — |
| | 50 | +++ | ++ | + | — | — | — | — | — | — |
| 5i | 25 | +++ | +++ | ++ | ++ | + | — | — | — | — |
| | 50 | +++ | ++ | + | — | — | — | — | — | — |
| 5m | 25 | +++ | +++ | +++ | ++ | ++ | + | + | + | + |
| | 50 | — | | | | | | | | |
| 5o | 25 | +++ | +++ | ++ | ++ | + | + | — | — | — |
| | 50 | +++ | +++ | +++ | ++ | ++ | + | + | + | + |
| Blank control （DMSO） | 25 | — | | | | | | | | |
| | | +++ | +++ | ++ | ++ | + | + | — | — | — |
| Phenithionate | 25 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| Praziquantel | | +++ | +++ | +++ | ++ | ++ | + | — | — | — |
| | | +++ | +++ | +++ | ++ | ++ | + | — | — | — |

**Table 3.** The median lethal dose ($LD_{50}$).

| Compounds | $LD_{50}$±SD（mg/kg） |
|---|---|
| **5a** | 280.1±3.2 |
| **5c** | 275.3±2.6 |
| **5g** | >500 |
| **5i** | >500 |
| **5m** | >500 |
| **5o** | >500 |
| Phenithionate | 310.5±4.1 |
| Praziquantel | >500 |

## 3. Conclusion

In general, we reported the design and synthesis of a class of anti-Schistosomiasis compounds, which was simple,

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efficient, and had high yield. All the newly synthesized compounds had good inhibitory activity and low acute toxicity. Among them, it showed by the anti-Schistosomiasis experiments *in vitro* that compounds **5a, 5c, 5g, 5i, 5m** and **5o** had good anti-Schistosomiasis activity, especially for *Schistosoma japonicum* (*S. japonicum*). It also showed by the experiments *in vivo* that compounds **5a, 5c, 5g, 5i, 5m** and **5o** had good anti-Schistosomiasis activity and low acute toxicity, especially the compounds **5g** and **5i**.

# 4. Experimental

*4.1. Synthesis of compounds **3a-3f***

The 4-isothiocyanatoaniline **1a** (0.10 mol) and hydroquinone **2a** (0.10 mol) were place in a 250 mL round bottom flask.100mL of tetrahydrofuran (THF) was added as a solvent.Under constant pressure conditions,0.01mol of aluminium trichloride was addtiion as a reaction catalyst.After completion of addition, the reaction was refluxed for 8 h. After completion of the reflux,the filtrate was filtered while it was hot.The filtrate was collected,cooled,allowed to stand for 12 h, and then filtered and dried in vacuo to give the crude product of compound **3a**. The crude **3a** product was recrystallized in toluene, filtered and dried in vacuo to give pure product as a white crystal. General experimental method was used for the synthesis of compounds **3b-3f**.

*4.2. A general method for all titled analogues or derivatives **5a-5r***

0.10 mol of compound **3a**, 0.06 mol of anhydrous sodium carbonate,and 0.10 mol of compound **4a** were placed in a 500 mL round bottom flask.200 mL toluene was added as solvent. Under magnetic stirring, the reaction was heated and refluxed for 12 h. When the feflux was completed, the filtrate was filtered while it was hot. The filtrate was collected, cooled, allowed to stand for 24 h, and then filtered and dried in vacuo to give the crude product of compound **5a**. The crude **5a** product was recrystallized in toluene, filtered and dried in vacuo to give pure product as a white crystal. General experimental method was used for the synthesis of compounds **5b-5r**.

*O*-(4-hydroxyphenyl) (4-((4-nitrophenyl)amino)phenyl)carbamothioate（**5a**）：yield 90.1%；m.p. 163-165℃；$^{1}$H NMR（300MHz，DMSO）δ：9.46（s,1H,-OH）,8.39(s,1H,-NH-),8.03(m,2H,Ph-H),7.44(m,2H,Ph-H),7.37 (m,2H,Ph-H),7.25(m,2H,Ph-H),6.94(m,2H,Ph-H),6.70(m,2H,Ph-H); $^{13}$C NMR (75MHz, DMSO) δ: 151.1,148.5,148.1,145.0,138.4,137.4,127.4,126.6,124.8,122.0,120.6,117.3;HR-ESI-MS *m/z*: calcd for $C_{19}H_{15}N_3O_4S$ {[M+H]$^{+}$} 381.0785,found 381.4060;Anal.calcd for$C_{19}H_{15}N_3O_4S$:C, 59.83; H, 3.96; N, 11.02; O, 16.78; S, 8.41;found:C, 59.84; H, 3.96; N, 11.01; O, 16.79; S, 8.40%.

*O*-(4-acetamidophenyl) (4-((4-nitrophenyl)amino)phenyl)carbamothioate (**5b**): yield 89.3%；m.p. 171-173℃；$^{1}$H

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NMR（300MHz，DMSO）δ：9.86(s,1H,--NH-);8.39(s,1H,--NH-);8.03(m,2H,Ph-H),7.44(m,2H,Ph-H),7.43 (m,2H,Ph-H),7.37(m,2H,Ph-H),7.25(m,2H,Ph-H),6.86(m,2H,Ph-H),2.06(s,3H,-$CH_3$);$^{13}$C NMR（75MHz，DMSO）δ： 168.9,151.1,148.5,145.0,138.4,137.4,131.1,127.4,126.6,124.8,123.0,122.0,120.6,116.1,24.0;HR-ESI-MS *m/z*: calcd for $C_{21}H_{18}N_4O_4S$ {[M+H]$^+$} 422.1048 ,found 422.4591;Anal.calcd for$C_{21}H_{18}N_4O_4S$:C, 59.71; H, 4.29; N, 13.26; O, 15.15; S, 7.59;found:C, 59.70; H, 4.30; N, 13.26; O, 15.16; S, 7.58%.

*O*-(4-hydroxyphenyl) (4-(4-nitrophenoxy)phenyl)carbamothioate (**5c**): yield 92.6%；m.p. 166-168℃；$^1$H NMR （300MHz，DMSO）δ：9.46（s,1H,-OH）,8.27(m,2H,Ph-H),7.40(m,2H,Ph-H),7.21(m,2H,Ph-H),6.96 (m,2H,Ph-H),6.94(m,2H,Ph-H),6.70(m,2H,Ph-H); $^{13}$C NMR（75MHz，DMSO）δ：163.1,151.1,150.2.148.1,145.0,141.0,130.2,126.2,124.6,117.3,116.2,115.7;HR-ESI-MS *m/z*: calcd for $C_{19}H_{14}N_2O_5S$ {[M+H]$^+$} 382.0622,found 382.3902;Anal.calcd for$C_{19}H_{14}N_2O_5S$:C, 59.68; H, 3.69; N, 7.33; O, 20.92; S, 8.38;found:C, 59.67; H, 3.69; N, 7.34; O, 20.93; S, 8.37%.

*O*-(4-acetamidophenyl) (4-(4-nitrophenoxy)phenyl)carbamothioate (**5d**): yield 89.9%；m.p. 176-178℃；$^1$H NMR （300MHz，DMSO）δ：9.86(s,1H,--NH-);8.27(m,2H,Ph-H),7.43(m,2H,Ph-H),7.40(m,2H,Ph-H),7.21 (m,2H,Ph-H),6.96(m,2H,Ph-H),6.86(m,2H,Ph-H),2.06(s,3H,-$CH_3$);$^{13}$C NMR（75MHz，DMSO）δ：168.9,163.1,151.1,150.2,145.0,141.0,131.1,130.2,126.2,124.6,123.0,116.2,116.1,115.7,24.0;HR-ESI-MS *m/z*: calcd for $C_{21}H_{17}N_3O_5S${[M+H]$^+$}423.0891,found 423.4431;Anal.calcd for$C_{21}H_{17}N_3O_5S$:C, 59.57; H, 4.05; N, 9.92; O, 18.89; S, 7.57;found:C, 59.58; H, 4.05; N, 9.91; O, 18.90; S, 7.56%.

*O*-(4-hydroxyphenyl) (4-((4-nitrophenyl)thio)phenyl)carbamothioat(**5e**): yield 87.3%；m.p. 170-172℃；$^1$H NMR （300MHz，DMSO）δ：9.46（s,1H,-OH）,7.92(m,2H,Ph-H),7.75(m,2H,Ph-H),7.67m,2H,Ph-H),7.29 (m,2H,Ph-H),6.94(m,2H,Ph-H),6.70(m,2H,Ph-H);$^{13}$C NMR（75MHz，DMSO）δ：151.1,148.1,146.4,145.0,141.8,135.6,131.7,131.0,128.3,125.7,123.4,117.3;HR-ESI-MS *m/z*: calcd for $C_{19}H_{14}N_2O_4S_2$ {[M+H]$^+$} 398.0397,found 398.4511;Anal.calcd for$C_{19}H_{14}N_2O_4S_2$: C, 57.27; H, 3.54; N, 7.03; O, 16.06; S, 16.09;found: C, 57.26; H, 3.54; N, 7.04; O, 16.07; S, 16.08%.

*O*-(4-acetamidophenyl) (4-((4-nitrophenyl)thio)phenyl)carbamothioate (**5f**): yield 85.6%；m.p. 184-186℃；$^1$H NMR（300MHz，DMSO）δ：9.86(s,1H,--NH-);7.92(m,2H,Ph-H),7.75(m,2H,Ph-H),7.67(m,2H,Ph-H),7.43 (m,2H,Ph-H),7.29(m,2H,Ph-H),6.86(m,2H,Ph-H),2.06(s,3H,-$CH_3$);$^{13}$C NMR（75MHz，DMSO）δ：168.9,151.1,146.4,145.0,141.8,135.6,131.7,131.1,131.0,128.3,125.7,123.4,123.0,116.1,24.0;HR-ESI-MS *m/z*: calcd for $C_{21}H_{17}N_3O_4S_2$ {[M+H]$^+$} 439.0660,found 439.5042;Anal.calcd for $C_{21}H_{17}N_3O_4S_2$: C, 57.39; H, 3.90; N, 9.56; O, 14.56; S, 14.59;found: C, 57.37; H, 3.91; N, 9.57; O, 14.57; S, 14.58%.

*O*-(4-hydroxyphenyl) (4-((4-(trifluoromethyl)phenyl)amino)phenyl)carbamothioate (**5g**): yield 92.1%； m.p.

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152-154℃；$^{1}$H NMR（300MHz，DMSO）δ：9.46（s,1H,-OH）,7.79(s,1H,-NH-)7.43(m,2H,Ph-H),7.37(m,2H,Ph-H),7.31(m,2H,Ph-H),7.25(m,2H,Ph-H),6.94(m,2H,Ph-H),6.70(m,2H,Ph-H);$^{13}$C NMR（75MHz，DMSO）δ：151.1,148.1,145.7,145.0,138.4,127.4,126.6,126.0,124.1,123.5,120.6,117.3;HR-ESI-MS *m/z*: calcd for $C_{20}H_{15}F_3N_2O_2S${[M+H]$^+$}404.0802,found 404.4071;Anal.calcd for$C_{20}H_{15}F_3N_2O_2S$: C, 59.40; H, 3.74; F, 14.09; N, 6.93; O, 7.91; S, 7.93;found: C, 59.41; H, 3.74; F, 14.08; N, 6.93; O, 7.92; S, 7.92%.

*O*-(4-acetamidophenyl) (4-((4-(trifluoromethyl)phenyl)amino)phenyl)carbamothioate (**5h**): yield 90.4%；m.p. 157-159℃；$^{1}$H NMR（300MHz，DMSO）δ：9.86(s,1H,--NH-);7.79(s,1H,--NH-);7.43(m,4H,Ph-H),7.37 (m,2H,Ph-H),7.31(m,2H,Ph-H),7.25(m,2H,Ph-H),6.86(m,2H,Ph-H),2.06(s,3H,-$CH_3$);$^{13}$C NMR（75MHz，DMSO）δ：168.9,151.1,145.7,145.0,138.4,131.1,127.4,126.6,126.0,124.1,123.5,123.0,120.6,116.1,24.0;HR-ESI-MS *m/z*: calcd for $C_{22}H_{18}F_3N_3O_2S$ {[M+H]$^+$} 445.1073,found 445.4601;Anal.calcd for$C_{22}H_{18}F_3N_3O_2S$:C, 59.32; H, 4.07; F, 12.79; N, 9.43; O, 7.18; S, 7.20;found:C, 59.33; H, 4.06; F, 12.77; N, 9.43; O, 7.19; S, 7.21%.

*O*-(4-hydroxyphenyl) (4-(4-(trifluoromethyl)phenoxy)phenyl)carbamothioate (**5i**): yield 93.4%；m.p. 170-172℃；$^{1}$H NMR（300MHz，DMSO）δ：9.46（s,1H,-OH）,7.43(m,2H,Ph-H),7.40(m,2H,Ph-H),7.26(m,2H,Ph-H),6.96(m,2H,Ph-H),6.94(m,2H,Ph-H),6.70(m,2H,Ph-H);$^{13}$C NMR（75MHz，DMSO）δ：160.3,151.1,150.2,148.1,145.0,130.2,126.9,126.2,124.122.0,117.3,115.7;HR-ESI-MS *m/z*: calcd for $C_{20}H_{14}F_3NO_3S$ {[M+H]$^+$} 405.0645,found 405.3913;Anal.calcd for$C_{20}H_{14}F_3NO_3S$:C, 59.26; H, 3.48; F, 14.06; N, 3.46; O, 11.84; S, 7.91;found:C, 59.27; H, 3.49; F, 14.05; N, 3.46; O, 11.83; S, 7.90%.

*O*-(4-acetamidophenyl) (4-(4-(trifluoromethyl)phenoxy)phenyl)carbamothioate(**5j**): yield 90.2%；m.p. 172-174℃；$^{1}$H NMR（300MHz，DMSO）δ：9.86(s,1H,--NH-);7.43(m,4H,Ph-H),7.40(m,2H,Ph-H),7.26(m,2H,Ph-H),6.96(m,2H,Ph-H),6.86(m,2H,Ph-H),2.06(s,3H,-$CH_3$);$^{13}$C NMR（75MHz，DMSO）δ：168.9,160.3,151.1,150.2,145.0,131.1,130.2,126.9,126.2,124.1,123.0,122.0,116.1,115.7,24.0;HR-ESI-MS *m/z*: calcd for $C_{22}H_{17}F_3N_2O_3S$ {[M+H]$^+$} 446.0913,found 446.4443;Anal.calcd for$C_{22}H_{17}F_3N_2O_3S$:C, 59.19; H, 3.84; F, 12.77; N, 6.27; O, 10.75; S, 7.18;found:C, 59.18; H, 3.85; F, 12.77; N, 6.26; O, 10.75; S, 7.19%.

*O*-(4-hydroxyphenyl) (4-((4-(trifluoromethyl)phenyl)thio)phenyl)carbamothioate(**5k**): yield 90.2%；m.p. 173-175℃；$^{1}$H NMR（300MHz，DMSO）δ：9.46（s,1H,-OH）,7.75(m,2H,Ph-H),7.45(m,2H,Ph-H),7.29 (m,2H,Ph-H),7.19(m,2H,Ph-H),6.94(m,2H,Ph-H),6.70(m,2H,Ph-H);$^{13}$C NMR（75MHz，DMSO）δ：151.1,148.1,145.0,139.0,135.6,131.7,131.5,129.5,128.3,125.7,124.1,117.3;HR-ESI-MS *m/z*: calcd for $C_{20}H_{14}F_3NO_2S_2${[M+H]$^+$} 421.0417,found 421.4523;Anal.calcd for$C_{20}H_{14}F_3NO_2S_2$:C, 57.00; H, 3.35; F, 13.52; N, 3.32; O, 7.59; S, 15.21;found:C, 57.01; H, 3.36; F, 13.50; N, 3.32; O, 7.58; S, 15.22%.

*O*-(4-acetamidophenyl) (4-((4-(trifluoromethyl)phenyl)thio)phenyl)carbamothioate (**5l**): yield 90.2%； m.p.

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173-175℃；$^{1}$H NMR（300MHz，DMSO）δ：9.86(s,1H,--NH-);7.75(m,2H,Ph-H),7.45(m,2H,Ph-H),7.43 (m,2H,Ph-H),7.29(m,2H,Ph-H),7.19(m,2H,Ph-H),6.86(m,2H,Ph-H),2.06(s,3H,-$CH_3$);$^{13}$C NMR（75MHz，DMSO）δ： 168.9,151.1,145.0,139.0,135.6,131.7,131.5,131.1,129.5,128.3,125.7,124.1,123.0,116.124.0;HR-ESI-MS *m/z*: calcd for $C_{22}H_{17}F_3N_2O_2S_2$ {[M+H]$^+$} 462.0685,found 462.5053;Anal.calcd for$C_{22}H_{17}F_3N_2O_2S_2$:C, 57.13; H, 3.71; F, 12.32; N, 6.06; O, 6.92; S, 13.86;found:C, 57.12; H, 3.72; F, 12.32; N, 6.06; O, 6.91; S, 13.87%.

*O*-(4-hydroxyphenyl) (4-((4-(trichloromethyl)phenyl)amino)phenyl)carbamothioate (**5m**): yield 91.3%； m.p. 165-167℃；$^{1}$H NMR（300MHz，DMSO）δ：9.46(s,1H,--OH);7.79(s,1H,--NH-);7.78(m,2H,Ph-H),7.37 (m,2H,Ph-H),7.31(m,2H,Ph-H),7.25(m,2H,Ph-H),6.94(m,2H,Ph-H),6.70m,2H,Ph-H);$^{13}$C NMR（75MHz，DMSO）δ： 151.1,148.1,145.0,144.2,138.4,133.5,127.5,127.4,126.6,123.1,120.6,117.3,97.8;HR-ESI-MS *m/z*: calcd for $C_{20}H_{15}Cl_3N_2O_2S${[M+H]$^+$} 451.9921,found 453.7623;Anal.calcd for $C_{20}H_{15}Cl_3N_2O_2S$: C, 52.94; H, 3.33; Cl, 23.44; N, 6.17; O, 7.05; S, 7.07;found: C, 52.93; H, 3.32; Cl, 23.43; N, 6.17; O, 7.05; S, 7.08%.

*O*-(4-acetamidophenyl) (4-((4-(trichloromethyl)phenyl)amino)phenyl)carbamothioate (**5n**): yield 91.3%； m.p. 165-167℃；$^{1}$H NMR（300MHz，DMSO）δ：9.86(s,1H,--NH-),7.79(s,1H,--NH-),7.78(m,2H,Ph-H),7.43 (m,2H,Ph-H),7.37(m,2H,Ph-H),7.31(m,2H,Ph-H),7.25(m,2H,Ph-H),6.86(m,2H,Ph-H),2.06(s,3H,-$CH_3$);$^{13}$C NMR （75MHz，DMSO）δ：168.9,151.1,145.0,144.2,138.4,133.5,131.1,127.5,127.4,126.6,123.1,123.0,120.6,116.1, 97.8,24.0;HR-ESI-MS *m/z*: calcd for $C_{22}H_{18}Cl_3N_3O_2S$ {[M+H]$^+$} 493.0184,found 494.8152;Anal.calcd for $C_{22}H_{18}Cl_3N_3O_2S$:C, 53.40; H, 3.67; Cl, 21.49; N, 8.49; O, 6.47; S, 6.48;found:C, 53.41; H, 3.66; Cl, 21.49; N, 8.49; O, 6.48; S, 6.47%.

*O*-(4-hydroxyphenyl) (4-((4-(trichloromethyl)phenoxy)phenyl)carbamothioate (**5o**): yield 92.3%；m.p. 177-179℃；$^{1}$H NMR（300MHz，DMSO）δ：9.46(s,1H,--OH);7.78(m,2H,Ph-H),7.40(m,2H,Ph-H),7.26(m,2H,Ph-H), 6.96(m,2H,Ph-H),6.94(m,2H,Ph-H),6.70m,2H,Ph-H);$^{13}$C NMR （75MHz, DMSO）δ： 158.8,151.1,150.2,148.1,145.0,137.1,130.2,126.3,126.2,121.6,117.3,115.7,97.8;HR-ESI-MS *m/z*: calcd for $C_{20}H_{14}Cl_3NO_3S${[M+H]$^+$} 452.9762,found 454.7463;Anal.calcd for $C_{20}H_{14}Cl_3NO_3S$:C, 52.83; H, 3.10; Cl, 23.39; N, 3.08; O, 10.55; S, 7.05;found:C, 52.82; H, 3.10; Cl, 23.38; N, 3.09; O, 10.55; S, 7.06%.

*O*-(4-acetamidophenyl) (4-(4-(trichloromethyl)phenoxy)phenyl)carbamothioate(**5p**):yield 92.3%；m.p. 181-183℃；$^{1}$H NMR（300MHz，DMSO）δ：9.86(s,1H,--NH-),7.78(m,2H,Ph-H),7.43(m,2H,Ph-H),7.40(m,2H,Ph-H), 7.26(m,2H,Ph-H),6.96(m,2H,Ph-H),6.86(m,2H,Ph-H),2.06(s,3H,-$CH_3$);$^{13}$C NMR （75MHz， DMSO）δ： 168.9,158.8,151.1,150.2,145.0,137.1,131.1,130.2,126.3,126.2,123.0,121.6,116.1,115.7,97.8,24.0;HR-ESI-MS *m/z*: calcd for $C_{22}H_{17}Cl_3N_2O_3S$ {[M+H]$^+$} 494.0026,found 495.7992;Anal.calcd for $C_{22}H_{17}Cl_3N_2O_3S$:C, 53.30; H, 3.46; Cl, 21.45; N, 5.65; O, 9.68; S, 6.47;found:C, 53.31; H, 3.45; Cl, 21.45; N, 5.64; O, 9.68; S, 6.48%.

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*O*-(4-hydroxyphenyl) (4-((4-(trichloromethyl)phenyl)thio)phenyl)carbamothioate (**5q**): yield 84.1%； m.p. 175-177℃；$^{1}$H NMR（300MHz，DMSO）δ：9.46(s,1H,--OH);7.75(m,2H,Ph-H),7.63(m,2H,Ph-H),7.45 (m,2H,Ph-H),7.29(m,2H,Ph-H),6.94(m,2H,Ph-H),6.70m,2H,Ph-H);$^{13}$C NMR （75MHz，DMSO）δ：151.1,148.1,145.0,142.5,137.5,135.6,131.7,131.1,128.3,127.2,125.6,117.3,97.8;HR-ESI-MS *m/z*: calcd for $C_{20}H_{14}Cl_3NO_2S_2${[M+H]$^+$} 468.9533,found 470.8072;Anal.calcd for $C_{20}H_{14}Cl_3NO_2S_2$: C, 51.02; H, 3.00; Cl, 22.59; N, 2.98; O, 6.80; S, 13.62;found: C, 51.03; H, 3.00; Cl, 22.59; N, 2.97; O, 6.81; S, 13.61%.

*O*-(4-acetamidophenyl) (4-((4-(trichloromethyl)phenyl)thio)phenyl)carbamothioate (**5r**): yield 80.1%； m.p. 189-191℃；$^{1}$H NMR（300MHz，DMSO）δ：9.86(s,1H,--NH-),7.75(m,2H,Ph-H),7.63(m,2H,Ph-H),7.45 (m,2H,Ph-H),7.43(m,2H,Ph-H),7.25(m,2H,Ph-H),6.86(m,2H,Ph-H),2.06(s,3H,-$CH_3$);$^{13}$C NMR（75MHz，DMSO）δ： 168.9,151.1,145.0,142.5,137.5,135.6,131.7,131.1,128.3,127.2,125.7,123.0,116.1,97.8,24.0;HR-ESI-MS *m/z*: calcd for $C_{22}H_{17}Cl_3N_2O_2S_2${[M+H]$^+$} 509.9798,found 511.8605;Anal.calcd for $C_{22}H_{17}Cl_3N_2O_2S_2$:C, 51.62; H, 3.35; Cl, 20.78; N, 5.47; O, 6.25; S, 12.53;found:C, 51.63; H, 3.36; Cl, 20.78; N, 5.47; O, 6.24; S, 12.52%.

*4.3 Biological activities*

*4.3.1.Screening of biological activity of compounds in vitro*

A total of 100 healthy male mus musculus weighing 20-22g were selected. Each mus musculus was divided into two parts and 50 mice after abdominal shaving. One of them was infected with abdominal skin with 260-280 cercariae of *Schistosoma japonicum*. The mus musculus were killed after 15 days of infection. Low temperature Heinz balanced salt solution containing heparin (HBSS solution) was injected through the thoracic aorta, mesenteric vein and intrahepatic collection of *Schistosoma japonicum*, and washing with HBSS solution of toxoplasma gondii 3-4 after *in vitro* culture experiments for larva. In addition a mouse with 120-140 cercariae infect mus musculus abdominal skin infection, 35 days after the mus musculus were killed, with the same HBSS solution, and the worms collected for imago culture experiments *in vitro*. 10% calf serum -RPMI 1640 culture medium containing penicillin sodium salt (150 IU/mL), streptomycin (150 IU/mL) and amphotericin B (1 μg/mL) was used to culture *Schistosoma japonicum* larva and imago worms *in vitro*. The larva and imago were cultured in 6 hole culture plate, each placed 5 male and female worms, incubated in a constant temperature incubator at 37 ℃ with 5% $CO_2$ for 4 h, and then added different concentrations of compounds, and continued to culture for 72 h. The viability of the insect in 72 h was observed and recorded. When the survival rate was 50%, it was the semi inhibitory concentration ($IC_{50}$) of the compound. Using DMSO as blank control experiment, phenithionate and praziquantel were used as positive control experiment.

*4.3.2.Biological activity of compounds anti-Schistosomiasis in vivo*

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A total of 50 healthy male mus musculus weighing 20-22g were injected with 120-140 cercariae of *Schistosoma japonicum* to infect the abdominal skin of mus musculus. After 35 days of infection, oral dose of 25 $mg.kg^{-1}.d^{-1}$ and $50mg.kg^{-1}.d^{-1}$ compounds and were administered orally for 5 days. After 5 h of oral administration, the mus musculus were collected for venous blood, the blood was coagulated and the serum was centrifuged for 15 min, then the serum was drawn out for 3500 r/min. The obtained serum was detected by triple dot ELISA, and the round spot color was observed. When the round spot showed brown strong positive (+ + +), yellow was positive (+ +), showing slight yellow was weak positive (+), colorless was negative (-). Using DMSO as blank control experiment, phenithionate and praziquantel were used as positive control experiment.

**LIVE SUBJECT STATEMENT:**

The reported experiments were in accordance with the standards set forth in the 8th Edition of *Guide for the Care and Use of Laboratory Animals* published by the National Academy of Sciences, The National Academies Press, Washington DC, United States of America. The academic committee of Chongqing Normal University has approved the experiment.

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## Conflict of Interest

The authors declare no competing interests.

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