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# Antischistosomal activity of *N,N′*-arylurea analogs against *Schistosoma japonicum*

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ABSTRACT

Although the antischistosomal activities of *N,N′*-arylurea analogs were reported, systematic structure–activity relationships have not been conducted. In this Letter, we reported the design, synthesis and evaluation of 45 *N,N′*-arylurea analogs. Among these prepared compounds, 13 compounds were urea linker modified and 32 were *N,N′*-arylurea derivatives. The activity evaluation revealed 12 analogs exhibited $IC_{50}$ values lower than 22.6 μM, and 7 of them had $IC_{50}$ less than 10 μM against the juvenile *Schistosoma japonicum* in vitro. Their worm killing potency was even higher against adult worm. Unfortunately, low to moderate worm burden reduction of 0–33.4% was recorded after administration of a single oral dose of 200 mg/kg or 400 mg/kg to mice harboring *S. japonicum*.

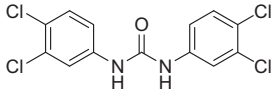


Schistosomiasis is a chronic infectious disease caused by the parasitic trematodes *Schistosomes*, which is also listed as one of the neglected tropical diseases by World Health Organization (WHO).[1] The disease is endemic in 76 countries globally, mainly in South America, South Asia, and mostly Africa. It is estimated that the disease has a prevalence of 230 million cases annually worldwide, and 779 million people live at risk of infection.[2] Depending on the causative species, schistosomiasis presents clinically in three major forms: *Schistosoma mansoni*, *Schistosoma japonicum*, and *Schistosoma haematobium*.[3,4]

As the only drug of choice for the treatment of all forms of schistosomiasis, praziquantel has been implemented over 40 years. Recent identification of field strains with low sensitivity or resistance to this drug highlights the urgent need for the development of novel antischistosomal agents.[5,6] Due to the poor understanding of the disease biology, drug discovery for neglected diseases, including schistosomiasis, relies mainly on whole parasite screening approaches. In this regard, a number of scaffolds identified through phenotype screening have been disclosed.[7–9] Specifically, **MMV665852** (Fig. 1), a *N,N′*-diarylurea analog, is reported to exhibit high schistosome killing activity both in vitro and in vivo.[10] Therefore, **MMV665852** was selected as a lead compound for the discovery of potential antischistosomal agents.

The aim of the present work was to conduct a extensive structural activity relationship investigation of *N,N′*-diarylurea derivatives against *S. japonicum*. To achieve this goal, the urea linker was first replaced by isosteric groups like thiourea, sulfonamide, carbamate, oxalamide, pyrimidine, and triazine. To reduce the linker rigidity and improve the compound drug like property, $sp^3$ hybridization carbons were introduced; Extensive structure research also focused on substitutes in the benzene rings. In total, 45 *N′,N′*-diarylurea derivatives were designed and prepared. The compound activities were evaluated against juvenile and adult worms both in vitro and in vivo.

*Chemistry:* The synthesis of all linker modified compounds were shown in Figure 2. The bissulfonamide **1**, and $N^1$,$N^2$-bis(3,4-dichlorophenyl)oxalamide **2** was prepared from reaction of 3,4-dichloroaniline with sulfuryl chloride and oxalyl chloride with $Et_3N$ as base[11]; reaction of 3,4-dichloroaniline with carbon disulfide and triphosgene subsequently gave **3a**, which was then reacted with 3,4-dichloroaniline to afford compound **3**; compound **4** was prepared from reaction of 3,4-dichlorophenyl isocyanate with *p*-cresol; reaction of oxalyl chloride or glyoxal with 1-(4-chloro-3-(trifluoromethyl)phenyl)-3-(3,4-dichlorophenyl)urea using $Et_3N$ or NaOH as base gave the corresponding compounds **5**




MMV665852

**Figure 1.** Structure of **MMV665852**.

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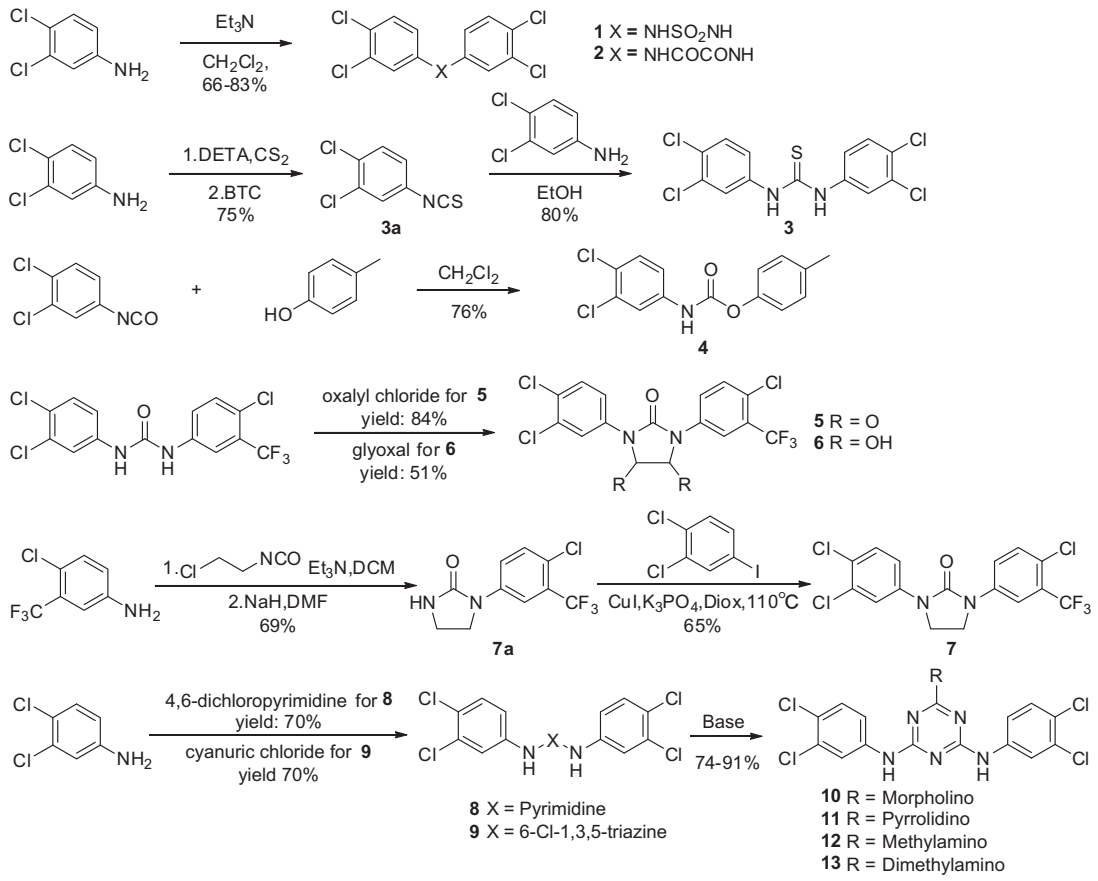



**Figure 2.** Synthetic route for the linker modified urea analogs.

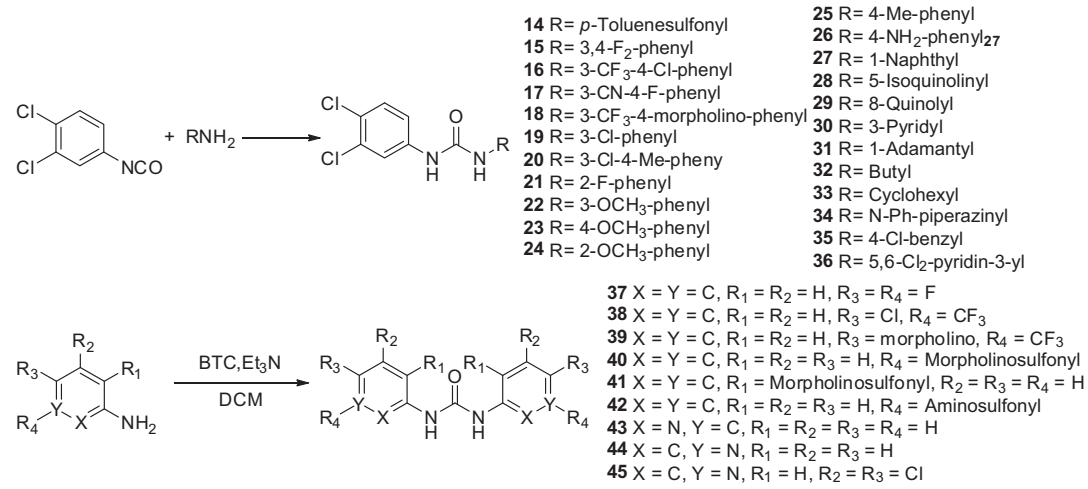


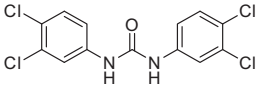
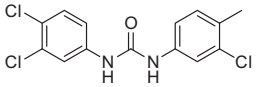
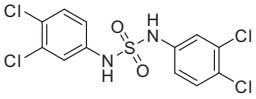
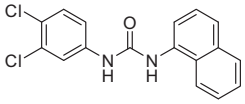
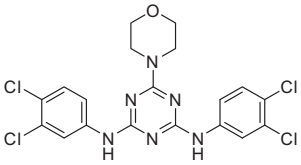
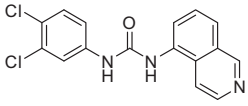
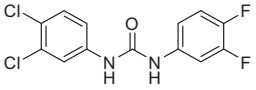
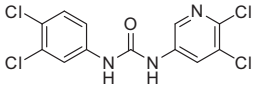
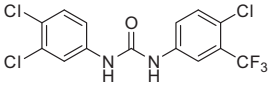
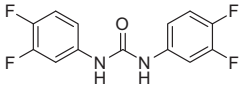
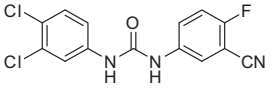
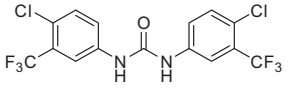
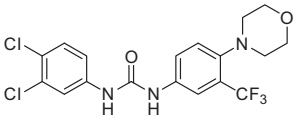
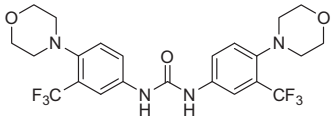
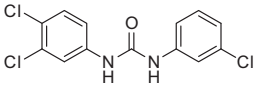
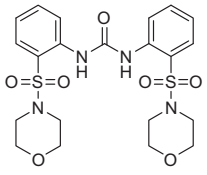

**Figure 3.** Synthetic route for the urea type compounds.

and **6**; reaction of 2-chloroethyl isocyanate with 4-chloro-3-(trifluoromethyl)aniline first gave a crude reaction intermediate, which was treated by sodium hydride to undergo intermolecular cyclization to afford **7a**, subsequent Buchwald–Hartwig reaction furnished compound **7**.[12] Nucleophilic aromatic substitution reaction of 3,4-dichloroaniline with 4,6-dichloropyrimidine or cyanuric chloride would give compounds **8** and **9**, further reaction of **9** with corresponding amines provided compounds **10**–**13**.[13]

The preparation of compounds with urea type linkers were shown in Figure 3. 3,4-Dichlorophenyl isocyanate with different amines would afford compounds **14**–**36**. Finally, compounds **37**–**45** were achieved by reaction of bis(trichloromethyl)carbonate with different substituted phenyl or pyridinyl amines in the presence of triethylamine as base.

*In vitro evaluation of compound activity against S. japonicum:* All prepared compounds were evaluated for their in vitro activities[14] against juvenile *S. japonicum* (Table 1). Of the 45 compounds tested at 32 μM, sixteen compounds killed juvenile worms within 72 h. Among 13 linker modified compounds, only the sulfamide **1** and the triazine linker compound **10** displayed moderate worm killing

**Table 1**
$IC_{50}$s of *N*,*N*′-arylurea analogs against juvenile and adult *S. japonicum*

| Compound | Structure | Juvenile $IC_{50}$ ± SD | Adult $IC_{50}$ ± SD | Compound | Structure | Juvenile $IC_{50}$ ± SD | Adult $IC_{50}$ ± SD |
|---|---|---|---|---|---|---|---|
| **MMV665852** |  | 4.4 ± 0.5 | 2.2 ± 0.6 | **20** |  | 12.7 ± 1.1 | ND |
| **1** |  | 22.6 ± 3.1 | ND | **27** |  | 12.8 ± 0.8 | ND |
| **10** |  | 19.0 ± 2.1 | ND | **28** |  | 22.6 ± 2.4 | ND |
| **15** |  | 4.7 ± 0.4 | 2.2 ± 0.3 | **36** |  | 11.3 ± 1.2 | ND |
| **16** |  | 2.5 ± 0.3 | 1.5 ± 0.1 | **37** |  | 7.0 ± 1.0 | 5.1 ± 1.8 |
| **17** |  | 4.3 ± 0.7 | 2.2 ± 0.3 | **38** |  | 2.8 ± 0.1 | 2.2 ± 0.3 |
| **18** |  | 9.6 ± 0.6 | 8.4 ± 0.5 | **39** |  | 22.6 ± 2.3 | ND |
| **19** |  | 10.1 ± 0.7 | ND | **41** |  | 22.6 ± 2.2 | ND |

Data are presented for compounds showing activity against juvenile worms (lethal at 32 μM after 72 h of incubation). Worms were incubated in 2-fold serial dilutions of compounds, starting at 32 μM, for 72 h, and $IC_{50}$ value was calculated.
ND, not done for compounds with $IC_{50}$s higher than 10 μM against juvenile worms.

activity. Other compounds oxalamide **2**, thiourea **3**, 4,6-pyrimidine **8** and 6-chloro-1,3,5-triazine **9** showed no antischistosomal efficacy. Compounds bearing urea linkers with an alkyl on one side, such as 1-adamantyl **31**, butyl **32**, cyclohexyl **33** and *N*-Ph-piperazinyl **34**, were not active at all. In contrast, most *N*,*N*′-diarylurea compounds were schistosomicidal within 72 h incubation. Compounds that killed the juvenile *S. japonicum* within 72 h were subsequently tested three times in 2-fold serial dilutions from 32 to 2 μM for $IC_{50}$ determinations. Seven compounds, including **19**, **20**, **27**, **28**, **36**, **39** and **44**, were characterized by $IC_{50}$ between 10 and 23 μM; significantly, compounds **15**, **16**, **17**, **18**, **37**, **38** resulted in $IC_{50}$s lower than 10.0 μM, of which compound **16** ($IC_{50}$ 2.5 μM) and **38** ($IC_{50}$ 2.8 μM) exhibited higher activity than the positive control **MMV665852**.

*N*,*N*′-Diphenylureas with electron donating groups at the *N*′-phenyl substructure (e.g., compounds **22**–**26**) demonstrated poor antischistosomal activity (Table 1). Compounds with electron withdrawing groups like halogen, trifluoromethyl and cyano showed excellent activity. The electron withdrawing ability correlated with the compound activity (e.g., compound **16** > compound **MMV665852** > compound **19** > compound **20**; compound **17** > compound **15**, the electron withdrawing ability: $CF_3$ > Cl > H > $CH_3$; CN > F) (Table 1). In addition, the positions of the halogen groups would influence the compound potency, it seemed that the *meta*- and *para*- rather than *ortho*-position were preferred. For example, compound **15** with *meta*-F and *para*-F killed all juvenile worms within 72 h at 32 μM, compounds **21** with an *ortho*-F didn't show any activity at all. Introduction of both $sp^2$ and $sp^3$ carbon to the urea linker would abolish the compound antischistosomal activity (compounds **5**, **6** and **7** were barely active), indicating the urea linker is essential. Finally, substitution of phenyl with pyridyl also decreased the compound potency (activity of **MMV665852** > **36** > **45**). Pyridylurea compounds bearing the chlorine substituent exhibited higher inhibitory potency compared with those without it (compound **36** > compound **30**, compound **43** and compound **44**).

The compounds with $IC_{50} \leqslant 10$ μM against juvenile worms were further determined for their $IC_{50}$ values against the adult worms. The results were shown in Table 1. These seven compounds exhibited $IC_{50}$s ranging from 1.5 to 8.4 μM (Table 1), even lower than those for juvenile worms, suggesting that the adult worms were more sensitive to these compounds treatment. Notably, compound **16** was observed to exhibit the highest potency against both juvenile and adult worms, with $IC_{50}$s both lower than the lead compound **MMV665852**.

*In vivo studies:* The effects of seven most potent compounds on worm burden reductions in 14-day-old juvenile *S. japonicum* harbored in mice were evaluated,[15–18] and the results were

**Table 2**
Worm burden and worm burden reductions of tested compounds in mice harboring a juvenile *S. japonicum* infection

| Compound | Dosage (mg/kg) | No. of mice investigated | Mean number of worms (SD) | Worm burden reduction (%) |
|---|---|---|---|---|
| Control | | 5 | 39.8 (5.2) | – |
| **MMV665852** | 400 | 6 | 27.0 (4.1) | 32.2** |
| **15** | 400 | 5 | 28.8 (7.3) | 27.8* |
| **16** | 200 | 5 | 35.2 (5.4) | 11.6 |
| **17** | 400 | 5 | 34.0 (8.7) | 14.6 |
| **18** | 400 | 6 | 32.3 (2.2) | 18.8 |
| **37** | 400 | 4 | 37.3 (3.1) | 6.4 |
| **38** | 200 | 6 | 26.5 (0.7) | 33.4* |
| Artesunate | 400 | 5 | 11.0 (4.0) | 72.4** |

SD, standard deviation. *T*-test was used to compare each compounds with the control group (*$p$ <0.05, **$p$ <0.01).

**Table 3**
Worm burden and worm burden reductions of tested compounds in mice harboring an adult *S. japonicum* infection

| Compound | Dosage (mg/kg) | No. of mice investigated | Mean number of worms (SD) | Worm burden reduction (%) |
|---|---|---|---|---|
| Control | | 5 | 38.6 (2.3) | – |
| **MMV665852** | 400 | 5 | 35.0 (4.1) | 9.3 |
| **15** | 400 | 5 | 38.8 (5.3) | 0 |
| **16** | 200 | 5 | 28.3 (5.3) | 26.6 |
| **17** | 400 | 5 | 34.2 (3.7) | 11.4 |
| **18** | 400 | 5 | 37.3 (4.0) | 3.4 |
| **37** | 400 | 5 | 37.7 (3.1) | 2.3 |
| **38** | 200 | 6 | 26.0 (3.7) | 32.6** |
| HP-$\beta$-CD-38 | 200 | 5 | 33.0 (4.8) | 14.4 |
| Praziquantel | 400 | 5 | 3.6 (1.1) | 90.7** |

SD, standard deviation. *T*-test was used to compare each compounds with the control group (**$p$ <0.01).

summarized in Table 2. Compound **38** exhibited the highest worm reduction activity, with a worm burden reduction (WBR) of 33.4% ($P$ <0.05) at 200 mg/kg single oral dose. By comparison, the positive control drug artesunate at 400 mg/kg characterized by a WBR of 72.4%. Other compounds resulted in WBRs as follows: compound **MMV665852**, 32.2%; compound **15**, 27.8%; compound **16**, 11.6%; compound **17**, 14.6%; compound **18**, 18.8% and compound **37**, 6.4%. Toxicity was observed for compounds **16** and **38** at 400 mg/kg: five out of six mice died after 1–2 d post-treatment, especially for compound **38**, two out of six tested mice died at day 1 and day 2 post-treatment at the dosage of 200 mg/kg,.

In mice harboring an adult *S. japonicum* infection, compound **38** also revealed the highest activity with worm burden reductions (WBR) of 32.6% ($P$ <0.01) (Table 3). Although compounds **MMV665852**, **15**, **17**, **18** and **37** displayed high efficacy against the adult worms in vitro, these compounds had a low in vivo activity (WBR 0–11.4%) and no statistically significances. Reasons, for example: low compound aqueous solubility, for the in vitro/in vivo discrepancy were suggested. Therefore, hydroxypropyl-$\beta$-cyclodextrin packed compound **38** was prepared and the same dosage as free drug was administrated to the infected mice. Unfortunately, a slight worm burden reduction rate decrease was recorded compared to the free compound.

Schistosomiasis is listed as one of the neglected tropical diseases by WHO. Despite the large number of people that are contaminated or being exposed to circumstance of infection in tropical countries, research efforts toward the development of novel antischistosomal drugs are slow over a half century. The biological knowledge in the parasite research field are far from rich in the science community, resulting in the dependence on phenotype screening as the main strategy for parasite drug discovery. Still, the scope of reference molecules as lead candidates is inadequate.

**MMV665852** was discovered and reported from screening over 400 commercially available malaria-active compounds by the Keiser group. A single oral dose of 400 mg/kg to mice infected by *S. mansoni* revealed high worm burden reduction of 52.5%. Due to the low molecular weight and structural simplicity of **MMV665852**, we figured that further investigation based on this scaffold might reveal candidates with improved antischistosomal activity as well as drug like property.

The **MMV665852** compound is characterized by good coplanarity and high rigidity, resulting in its low aqueous solubility and probably consequent low in vivo activity. Therefore, our initial modification efforts focus on urea linker modification, with the aim of increasing the linker flexibility. Our designed linkers are commonly observed structural units and bioisosteres in medicinal chemistry. Disappointingly, introduction of $sp^3$ hybridized carbon to improve the molecular flexibility failed to achieve the mission of activity enhancement. Only the sulfamide **1** and triazine linker analog **10** displayed moderate in vitro worm killing capability. We then resorted to installation of $sp^3$ flexible carbons to the phenyl ring of the urea analogs (compounds **18** and **39**). Again, decreased worm killing activity was observed for these two compounds in vitro.

An extensive structural activity relationship was investigated on the substituents at the phenyl rings. We revealed that electron withdrawing groups favored the compound antischistosomal activity, while electron donating groups would do the contrary. At the same time, the substitute position at the phenyl ring also influenced the compound activity, with *meta*- or *para*- other than *ortho*-position being helpful, as demonstrated by seven low $IC_{50}$ value compounds: halogen or electron withdrawing trifluoromethyl group at *meta*- or *para*-positions (compounds **15**, **16**, **17**, **18**, **19**, **37** and **38**).

As mentioned by another research group,[19] low aqueous solubility was observed for these urea analogs. Efforts were spent to optimize the solvent system in order to make a homogenous solution. Our tested solvents include: polyethylene glycol with $H_2O$ at different ratio, glycerol–$H_2O$ at different ratio, even high liposolubility corn oil couldn't fully dissolve these compounds. Then, a suspension of compound in corn oil facilitated by ultrasound was administrated to the infected mice. For comparison, a complex of hydroxypropyl-β-cyclodextrin (HP-β-CD) with compound **38** was prepared and solution of this complex was tested in infected mice. Unfortunately, the CD complex resulted in reduced worm burden reduction. The results from corn oil suspensions were provided in Tables 2 and 3.

Finally, the discrepancy for in vivo worm burden reduction between reported data for *S. mansoni* and our tested *S. japonicum* caught our attentions, although there was precedent that certain type of compound would display different activity on *S. mansoni* vs *S. japonicum*.[20] The lead compound **MMV665852** could achieve worm burden reduction of 52.5% at single oral dose 400 mg/kg treatment of mice harboring *S. mansoni*, while this number was only 9.3% when applying to *S. japonicum*. Whether or not this significant difference was related to the drug working mechanism would be worthy of being studied further.

In summary, 45 *N,N′*-arylurea analogs were prepared and evaluated as antischistosomal agents against *S. japonicum*. Although some compounds exhibited strong in vitro worm killing potency, their worm burden reduction activity was not promising in vivo. Interestingly, the observed antischistosomal activity difference between *S. mansoni* and *S. japonicum* might imply these compounds inhibition mechanism being related to worm species specificity.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.01.075.

## References and notes

1. Njoroge, M.; Njuguna, N. M.; Mutai, P. *Chem. Rev.* **2014**, *114*, 11138.
2. Colley, D. G.; Bustinduy, A. L.; Secor, W. E. *Lancet* **2014**, *383*, 2253.
3. Gryseels, B.; Polman, K.; Clerinx, J. *Lancet* **2006**, *368*, 1106.
4. Hotez, P. J.; Bundy, D. A. P.; Beegle, K. *Disease Control Priorities in Developing Countries*, 2nd ed., 2006; pp 467–482.
5. Ismail, M.; Botros, S.; Metwally, A. *Am. J. Trop. Med. Hyg.* **1999**, *60*, 932.
6. Gryseels, B.; Mbaye, A.; de Vlas, S. J. *Trop. Med. Int. Health* **2001**, *6*, 864.
7. Shu-Hua, X.; Jennifer, K.; Jacques, C.; Jurg, U.; Yuxiang, D.; Yvette, E.; Jonathan, L. V.; Marcel, T. *Antimicrob. Agents Chemother.* **2007**, *51*, 1440.
8. Sayed, A. A.; Simeonov, A.; Thomas, C. J.; Inglese, J.; Austin, C. P.; Williams, D. L. *Nat. Med.* **2008**, *14*, 407.
9. Abdulla, M. H.; Lim, K. C.; Sajid, M.; McKerrow, J. H.; Caffrey, C. R. *PLoS Med.* **2007**, *4*, 14.
10. Ingram-Sieber, K.; Cowan, N.; Panic, G. *PLoS Negl. Trop. Dis.* **2014**, *8*, 2610.
11. Merlic, C. A.; You, Y.; McInnes, D. M. *Tetrahedron* **2001**, *57*, 5199.
12. Wilde, R.G.; Takasugi, J.J.; Hwang, S. US2006/167065 A, 2006.
13. Zheng, M.; Xu, C.; Ma, J. *Bioorg. Med. Chem.* **1815**, *2007*, 15.
14. Shuhua, X.; Jennifer, K.; Chollet, J. *Antimicrob. Agents Chemother.* **2007**, *51*, 1440.
15. Duan, W. W.; Qiu, S. J.; Zhao, Y. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1587.
16. Chen, J. L.; Sun, W.; Yang, J. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3785.
17. Wang, Z. X.; Chen, J. L.; Qiao, C. H. *Chem. Biol. Drug Des.* **2013**, *82*, 216.
18. Yang, J. J.; Boissier, J.; Chen, J. L. *Future Med. Chem.* **2015**, *7*, 713.
19. Cowan, N.; Datwyler, P.; Ernst, B. *Antimicrob. Agents Chemother.* **1935**, *2015*, 59.
20. Keiser, J.; Chollet, J.; Xiao, S.-H. *Plos. Neg. Trop. Dis.* **2009**, *3*, e350.