Elucidation of the *in vitro* and *in vivo* activity of bridged 1,2,4-trioxolanes, bridged 1,2,4,5-tetraoxanes, tricyclic monoperoxides, silyl peroxides, and hydroxylamines against *Schistosoma mansoni*

Noemi Cowan[a,b], Ivan A. Yaremenko[c], Igor B. Krylov[c], Alexander O. Terent'ev[c] and Jennifer Keiser[a,b]*

[a] *Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, P.O. Box, CH–4002 Basel, Switzerland*

[b] *University of Basel, P.O. Box, CH–4003 Basel, Switzerland*

[c] *N. D. Zelinsky Institute of Organic Chemistry, Russian, Academy of Sciences, 47 Leninsky Prospekt, 119991 Moscow, Russian Federation*

*Corresponding author: Jennifer Keiser, Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Tel.: +41 61 2848218; Fax: +41 61 2848105; E-mail: jennifer.keiser@unibas.ch

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Abstract

Praziquantel is currently the only drug available to treat schistosomiasis. Since drug resistance would be a major barrier for the increasing global attempts to eliminate schistosomiasis as a public health problem, efforts should go hand in hand with the discovery of novel treatment options. Synthetic peroxides might offer a good starting point since their antischistosomal activity has been described in laboratory studies as well as clinical trials. We studied 19 bridged 1,2,4,5-tetraoxanes, 2 tricyclic monoperoxides, 11 bridged 1,2,4-trioxolanes, 12 silyl peroxides, and 4 hydroxylamines against newly transformed schistosomula and adult *Schistosoma mansoni in vitro*. Schistosomicidal compounds were tested for cytotoxicity followed by *in vivo* studies for the most promising compounds. Tricyclic monoperoxides, trioxolanes, and tetraoxanes revealed the highest *in vitro* activity against NTS (IC_{50}s 0.4-20.2 µM) and adult schistosomes (IC_{50}s 1.8-22.8 µM). Tetraoxanes revealed higher cytotoxicity than antischistosomal activity. Selected trioxolane and tricyclic monoperoxides were tested in mice harboring an adult *S. mansoni* infection. Two trioxolanes, compounds **30** and **27,** showed moderate worm burden reductions (WBR) of 44% and 43% (p >0.05), respectively. Compounds of both the trioxolanes and the tricyclic monoperoxides (compounds **21**, **26**, **44**, and **45**) showed low WBRs of 0-27%. Complexation of the compounds with β-cyclodextrin to improve solubility and gastrointestinal absorption did not increase *in vivo* antischistosomal efficacy. The high *in vitro* antischistosomal activity of trioxolanes and tricyclic monoperoxides are a promising basis for future investigations, with the focus on improving *in vivo* efficacy.

1. Introduction

Schistosomiasis is a neglected tropical disease, caused in principal by three human *Schistosoma* species, *S. mansoni, S. haematobium, and S. japonicum.* Chemotherapy using praziquantel is the mainstay of control. Praziquantel is a broad-spectrum antischistosomal agent, and the treatment of choice since its discovery in the 1970s. Every year millions of people are treated with praziquantel in the frame of mass drug administration programs. For example, in 2012, 27.5 million people in 21 countries were treated with praziquantel. In 2018, the World Health Organization aims to treat as many as 235 million people. With increasing drug pressure, the risk for praziquantel resistance or tolerance is rising.[1] Hence, there is a need for new antischistosomal drugs.[2,3]

In the past years, various semisynthetic and synthetic peroxide classes have been studied for their antischistosomal properties in laboratory as well as clinical trials, including the artemisinins,[4] ozonides (or trioxolanes),[5,6] trioxaquines,[7] and dioxolanes.[8] It has been hypothesized that the peroxide moiety interferes with heme polymerization, which is responsible for both the antischistosomal and antimalarial activity.[9,10]

We recently studied the antischistosomal activity of synthetic peroxides (bridged 1,2,4,5-tetraoxanes, alphaperoxides, tricyclic monoperoxides) and identified two promising classes, bridged 1,2,4,5-tetraoxanes and tricyclic monoperoxides, which revealed IC_{50}s of 0.3 and 11.8 µM against adult *S. mansoni in vitro* and WBRs of 75% and 83% in the *S. mansoni* mouse model.[11]

In the present work, we synthesized a new set of bridged 1,2,4,5-tetraoxanes, tricyclic monoperoxides as well as bridged 1,2,4-trioxolanes, silyl peroxides, and hydroxylamines. The latter three substance classes were tested for the first time for their antischistosomal activity. Compounds were first tested against the larval and adult forms of *S. mansoni.* Compounds showing a promising antischistosomal activity and a selectivity index <1 *in vitro* were subsequently tested *in vivo*. Selected compounds were additionally packed into β-cyclodextrin with the aim to improve bioavailability.

2. Material and methods

2.1. Drugs and media

We studied 19 bridged 1,2,4,5-tetraoxanes, 11 bridged 1,2,4-trioxolanes, 12 silyl peroxides, 2 tricyclic monoperoxides, and 4 hydroxylamines, and for comparison 2 hit compounds of the previous study[11] (Table 1). The 50 compounds were prepared based upon methods described in literature.[12,13,14,15,16,17,18, 19,20]

For *in vitro* evaluations, compounds were prepared as 10 mg/ml stock solutions in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Buchs, Switzerland).

Medium 199 and RPMI 1640 were purchased form Life Technologies (Carlsbad, CA), heat inactivated fetal calf serum (FCS), penicillin, and streptomycin from Lubioscience (Lucerne, Switzerland), and L-glutamic acid from Sigma-Aldrich. β-cyclodextrin for drug complexation was purchased from (Acros, Belgium). For oral suspension of *in vivo* testing, compounds not packed in β-cyclodextrin were suspended in Tween 80 (Fluka, Buchs, Switzerland), ethanol, and H_2O (7:3:90), whereas drugs packed in β-cyclodextrin were suspended in polyethylene glycol 300 (Sigma-Aldrich) and H_2O (60:40).

2.2. Mice and parasites

In vivo studies were approved by the veterinary authorities of Canton Basel-Stadt (license no. 2070), based on Swiss cantonal and national regulations.

Three week old female NMRI mice (n=62) were purchased from Charles River (Sulzfeld, Germany), kept at 22°C, 50% humidity, with an artificial 12-hour day/night cycle, and free access to rodent diet and water. Four-week old mice were infected by subcutaneous injection with 100 *S. mansoni* cercariae (Liberian strain), harvested from *S. mansoni*-infected *Biomphalaria glabrata* snails.

2.3. *In vitro* drug assay on newly transformed schistosomula (NTS)

S. mansoni cercariae were mechanically transformed to newly transformed schistosomula (NTS), and stored in Medium 199 supplemented with 5% FCS, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37°C, with 5% CO_2, as described previously.[21]

For the NTS drug assay, NTS were added (100/well) to 12.5 µg/ml compound dilutions in supplemented Medium 199, which were prepared in flat-bottom 96-well plates (BD Falcon, USA). Compounds that killed the NTS after a 72-h incubation period in at least one well were tested at lower concentrations (0.4, 0.8, 1.6, 3.1, 6.3, and 12.5 µg/ml) for IC_{50} determination. NTS exposed to the highest concentration of DMSO (0.13%) served as control. Assays were performed in triplicate, and repeated once. Drug activity was evaluated microscopically (Carl Zeiss, Germany; 80-200x magnification) 72 h post-incubation, using scoring from 3 (normal activity and morphology) to 0 (no motility, impaired morphology, and granularity).[22]

2.4. *In vitro* drug assay on adult *S. mansoni*

Adult schistosomes were harvested by dissection from mesenteric and hepatic portal veins of infected mice, seven to nine weeks post-infection. Schistosomes were stored in RPMI 1640 medium supplemented with 5% FCS, 100 U/ml penicillin, and 100 µg/ml streptomycin, at 37°C with 5% CO_2.[6]

For drug activity assessment, adult schistosomes (three of both sexes) were put into 25.0 µg/ml compound dilutions in supplemented RPMI medium using 24-well flat-bottom plates (BD Falcon). Schistosomes incubated in the highest concentration of DMSO in culture medium (0.25%) served as control. Compounds that killed the worms 72 h post-incubation were subsequently tested at lower concentrations (0.3, 0.9, 2.8, 8.3, and 25.0 µg/ml) for IC_{50} determination, and scored via microscopic readout in the same manner as described above for the NTS. Assays were performed in duplicate, and repeated once.[22]

2.5. L6 cytotoxicity drug assay

Rat skeletal myoblast L6 cells (ATCC, Manassas, VA USA) were seeded (2 x 10^3/well) into 96-well flat-bottom plates (BD Falcon). After a 24-h adherence time, cells were incubated with a 3-fold serial dilution starting at 90 µg/ml. After 70 h, resazurin (Sigma-Aldrich) was added to the wells, and after another 2 h, the fluorescence was read using an excitation wavelength of 536 nm and an emission wavelength of 588 nm (SpectraMax, Molecular Devices; Softmax, version 5.4.1). Cells incubated with a 3-fold serial dilution of podophyllotoxin (Sigma-Aldrich) starting at 100 ng/ml served as positive control. IC_{50} determination was performed in duplicate, and repeated twice.[23]

2.6. Complexation of drugs with β-cyclodextrin

Compound solutions in acetonitrile (2ml) were mixed into a solution of β-cyclodextrin in H_2O and acetonitrile (70:30; 30ml), with a molar ratio of 1:1 β-cyclodextrin to peroxide. The heterogeneous mixture was stirred at 20−25°C for 24 h, and the solvent was subsequently removed in a water jet vacuum pump (Vitlab, Germany). Analytical data are shown in the supplementary file (Supplementary 1).

2.7. Instrumentation and methods

NMR spectra of compounds were recorded on a *Bruker AW-300* (300.13 MHz for ^{1}H, 75.48 MHz for ^{13}C) and *Bruker Avance 400* (400.1 MHz for ^{1}H, 100.6 MHz for ^{13}C) in $CDCl_3$ and DMSO-d6. Thin layer chromatography (TLC) analysis was carried out on standard silica gel chromatography plates. Melting points determinations were carried out on a Kofler hot-stage apparatus. Chromatography was performed using silica gel (63−200 mesh and 5-40 μm). Elemental analysis on carbon, hydrogen, and nitrogen was carried out using a 2400 Perkin-Elmer CHN analyzer. Determination of purity of all compounds was executed by elemental (combustion) analysis. For all peroxides, deviation from the theoretical values for C, H, and N content was less than 0.4%. High-resolution mass spectra (HRMS) were measured by using electrospray ionization (ESI). The measurements were performed in positive-ion mode (interface capillary voltage 4500 V); the spectra were acquired in the m/z range of 50–3000; the external/internal calibration was done with Electrospray Calibrant Solution. Solutions in MeCN were injected with a syringe (flow rate 3 ml/min). Nitrogen was applied as a dry gas; the interface temperature was set at 180°C. These data confirmed >95% purity of all compounds. Structures of all compounds were confirmed using ^{1}H and ^{13}C NMR spectra. Analytical results of the unbound compounds as well as the β-cyclodextrin-compound complexes are shown in supplementary file (Supplementary 1 and 2, respectively).

2.8. *In vivo* drug assay with *S. mansoni*-infected mice

Compound suspensions were orally applied to *S. mansoni*-infected mice (groups of n=4) 49 days post-infection, at a single dose of 400 mg/kg. Untreated infected mice (n=8) served as control. Mice were euthanized and dissected 16-21 days post-treatment to count the worms in the portal and mesenteric veins, and the liver.[6]

2.9. Statistics

Scores of the antischistosomal *in vitro* drug assays were set in relation to the control values. For *in vitro* activities, IC_{50} values were calculated using CompuSyn software (ComboSyn Inc., USA; version 3.0.1, 2007). R-values represent the linear correlation coefficient, which reflects the conformity or goodness of the experimental data.[24] IC_{50} and r^2-values of cytotoxicity determination were calculated by Softmax. IC_{50}s of both antischistosomal activities and cytotoxicity were converted to molarity. Selectivity indices were calculated by dividing the IC_{50} of the mammalian cell line by the IC_{50} of the antischistosomal activity against adult schistosomes. For *in vivo* drug efficacy assessment, WBRs were calculated by comparing worm counts of treated mouse groups to the control group. The Kruskal-Wallis test (StatsDirect Ltd., UK; StatsDirect, version 2.7.2.) was applied for significance determination ($p = 0.05$).

3. RESULTS

3.1. *In vitro* activity against NTS

Of the 48 compounds tested, 24 killed all NTS in at least one well after 72 h at 12.5 µg/ml. Of these, compounds **6** and **21** revealed very high (IC_{50} <1 µM) antischistosomal activities with IC_{50} values of 0.9 and 0.4 µM, respectively. Twenty compounds showed high (IC_{50}s 1-10 µM) activities (9 tetraoxanes, 7 trioxolanes, 2 tricyclic monoperoxides, and 2 silyl peroxides), and 2 compounds were characterized by moderate (IC_{50} >10 µM) antischistosomal activity.

In comparison, in our previous study the most active tetraoxane **20** showed an IC_{50} at 0.1 µM, the most active tricyclic monoperoxide **46** at 14.4 µM, and the gold standard praziquantel at 2.2 µM (Table 2).[11]

3.2. *In vitro* activity against adult *S. mansoni*

All 48 compounds were tested on adult *S. mansoni*. Twenty-six compounds killed the worms following incubation at 25.0 µg/ml for 72 h. Of these, 16 compounds (7 tetraoxanes, 7 trioxolanes, and 2 tricyclic monoperoxides) revealed high (IC_{50} 1-10 µM) antischistosomal activity. Ten compounds showed moderate (IC_{50} >10 µM) activity (6 tetraoxanes, 4 trioxolanes) (Table 2). IC_{50}s of the hit compounds of our previous study were 0.3 µM for tetraoxane **20**, 11.8 µM for tricyclic monoperoxide **46**, and 0.1 µM for praziquantel (Table 2).[11]

3.3. Selectivity of adult *S. mansoni*-active drugs

Compounds exhibiting IC_{50}s ≤10 µM against adult schistosomes were deemed as potent schistosomicidals and therefore tested on a mammalian cell line to determine the compound toxicity and thereof their selectivity (Table 2). Eight compounds indicated selective toxicity towards the parasite (SI >1), namely compounds **21**, **23**, **26**, **27**, **29**, **30**, **44**, and **45**, all representatives of the tricyclic monoperoxide or the trioxolane class. Tetraoxanes were excluded from *in vivo* studies due to unselective toxicity. For comparison, the tetraoxanes of the previous study showed SIs ≥5.7[11]

3.4. In vivo drug efficacy against adult *S. mansoni*

Four trioxolanes (**21**, **26, 27**, **30**) and 2 tricyclic monoperoxides (**44**, **45**) progressed into *in vivo* studies based on antischistosomal activity and selectivity. Compound **29** was not considered for *in vivo* testing because it showed high structural similarity to compound **27**, which had a more promising antischistosomal profile. Furthermore, compound **23** was excluded because of its higher IC_{50} and lower selectivity compared to the other compounds chosen for *in vivo* studies.

Compounds **30** and **27** showed slight, but not significant (p >0.05), worm burden reductions (WBR) of 44% and 43%, respectively. Compounds **26**, **44**, **21**, and **45** showed low WBRs of 0-27%. Compounds **26**, **27**, **30**, and **44** were prepared as β-cyclodextrin complexes with the aim to improve solubility and gastrointestinal wall permeation.[25] For comparison, two lead molecules (**20**, **46**) from our previous study[11] were also packaged. WBRs of the complexes ranged from 23-36% (p >0.05). Compounds **20** and **46** revealed moderate WBRs of 33% and 36%, respectively. Compounds **26**, **27**, **30**, and **44** of the present study showed low WBRs between 0-31%. All *in vivo* results are presented in Table 3.

4. Discussion

Schistosomiasis is a debilitating disease, affecting hundreds of millions of people living in poor, rural areas of the subtropics and tropics. Chemotherapy is the mainstay of control, yet there is no alternative to praziquantel, the gold standard, and no drug is in the clinical pipeline.[26] This is a perilous situation if praziquantel tolerance or resistance should arise.

Given the promising findings obtained with bridged 1,2,4,5-tetraoxanes and tricyclic monoperoxides earlier,[11] in the present study, we tested a new series of peroxidic compounds, including bridged 1,2,4,5-tetraoxanes, tricyclic monoperoxides, bridged 1,2,4-trioxolanes, silyl peroxides, and hydroxylamines. We tested 48 compounds (Table 1) *in vitro* on two stages of *S. mansoni*, the larval (NTS) and the adult, and assessed their cytotoxicity using a mammalian cell line. Subsequently, potent and selective compounds were tested in the *S. mansoni* mouse model.

Of the 48 compounds tested, 24 compounds killed NTS at 33.3 μM of which 22 revealed high activity (IC_{50} ≤10 μM). 26 compounds killed adult *S. mansoni* at 33.3 μM. 16 of these revealed high activity (IC_{50} ≤10 μM). Fourteen compounds showed high activity (IC_{50}s ≤10 μM) against both stages, with NTS being slightly more affected than adult *S. mansoni*. The trend of higher sensitivity of NTS against synthetic peroxides was already observed previously.[11]

Of the 19 tetraoxanes tested, 7 were highly active and resulted in IC_{50}s ≤10 μM against both NTS and adult schistosomes. The 4 adamantyl-containing tetraoxanes were the most potent, with IC_{50} values down to 2 μM on adult flukes. Replacing the adamantyl moiety with small alkyl substituents lowered or annihilated the tetraoxanes activity. Placing aryls at the side position lead to loss of activity as well. For instance, the adamantyl-containing tetraoxane **3** had an IC_{50} of 3.9 μM, whereas replacing the adamantyl substituent with an aryl (compound **10**) or an isobutyl (compound **5**) showed no, or moderate (IC_{50} 20.8 μM) activity, respectively. Therefore, this set of molecules agrees on the supporting but not essential nature of adamantyl, which was noted previously.[11] Due to unselective activity however, no tetraoxane was tested *in vivo.* The toxicity observed with this set of tetraoxanes is in contrast to our previous findings, where the tested tetraoxanes revealed selectivity (SI ≥5.7).

The 2 tricyclic monoperoxides with simple alkyl substituents showed selective antischistosomal activity *in vitro*, but in mice they reduced the *S. mansoni* worm burden inefficaciously. The reason for the differing *in vivo* activity between these two and the previously tested tricyclic monoperoxide derivative remains to be elucidated.

Of the 11 trioxolanes tested, 5 revealed IC_{50} values ≤10 μM against larval and adult schistosomes, which all showed selective schistosomal toxicity. Some trioxolanes were diasteriomers (**21**, **22**; **23**, **24**; **25**, **26**; **27**, **28**; **29**, **30**), but no consistent configuration-dependent activity was noted. Also the role of the electron-drawing residue (e.g. halogen or nitrogen dioxide) could not be determined. Two trioxolanes (**30** and **27**) were tested *in vivo*, and resulted in the highest WBRs of this study with 44% and 43%, respectively, but without significance (p >0.05).

Hydroxylamines were inactive against both NTS and adult *S. mansoni in vitro*. Also the newly synthesized silyl peroxides showed poor to no activity *in vitro*. Only 2 out of 12 silyl peroxides (**32** and **33**) revealed activity against NTS with IC_{50} values ≤10 μM. Poor solubility of these compounds was observed.

Selected compounds were retested *in vivo* after their complexation with β-cyclodextrin, since cyclodextrins are known to improve compound solubility and absorption by biological barrieres, such as mucosas or skin.[25] Nevertheless, observed WBRs of [cyclodextrin-drug] complexes were lower than free drugs. Likewise, two lead compounds from our previous work resulted in low WBRs. In general, cyclodextrins can enhance, but also hamper (e.g. with excess cyclodextrin) drug delivery through biological membranes, hence optimization of the complexation procedure is usually needed.[27]

In conclusion, trioxolanes revealed the most potent *in vitro* schistosomicidal activity and selectivity of all peroxidic drugs investigated in this study, with moderate *in vivo* worm burden reductions. Tetraoxanes and tricyclic monoperoxides, the lead candidates of the previous study, showed high *in vitro* antischistosomal activity, but failed demonstrating selectivity, or *in vivo* efficacy, respectively. Further modifications on the compounds are necessary to improve *in vivo* efficacy.

5. Acknowledgements

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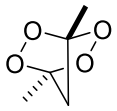
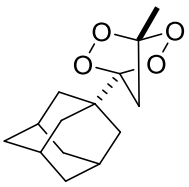
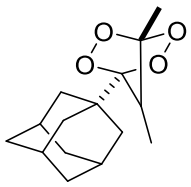
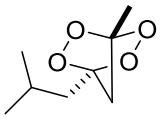
TABLES

Table 1

Chemical structures of investigated compounds.

Bridged 1,2,4,5-tetraoxanes			
			
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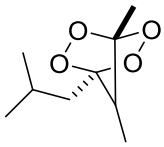	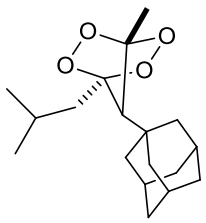	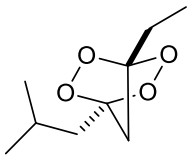	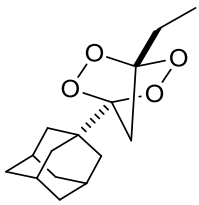
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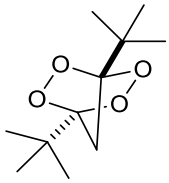	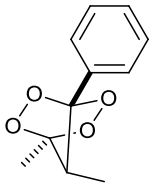	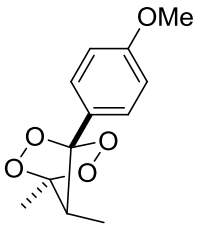	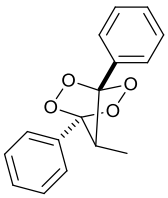
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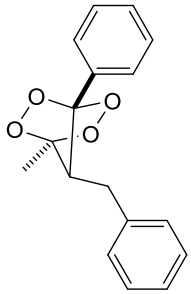	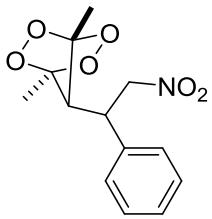	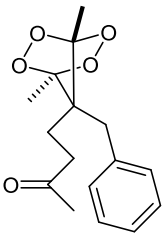	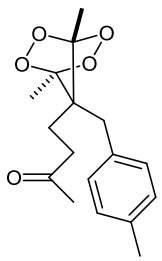
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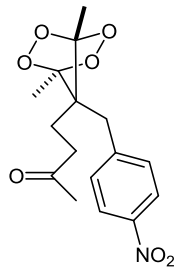	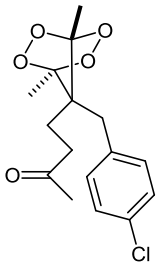	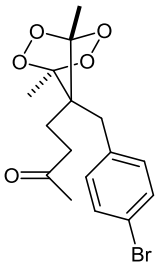	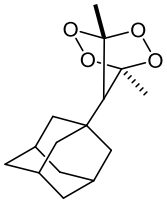
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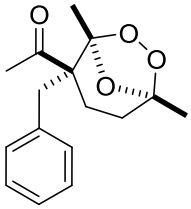	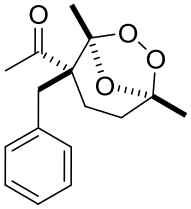	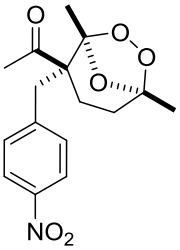	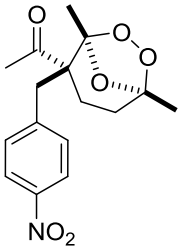
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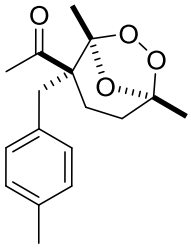	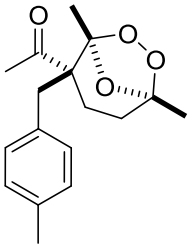	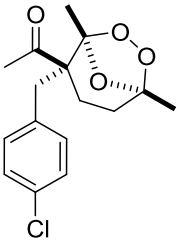	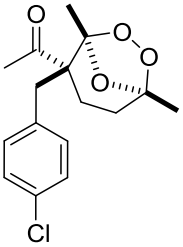
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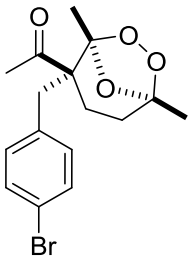	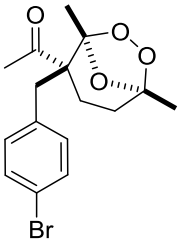	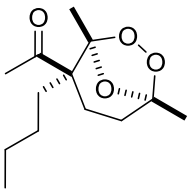	
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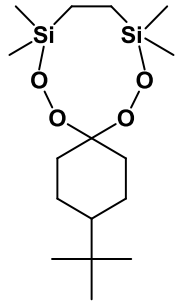
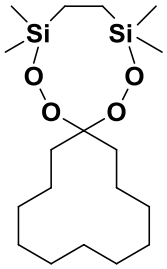
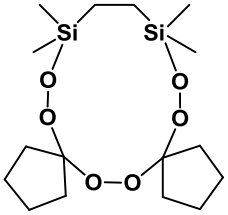
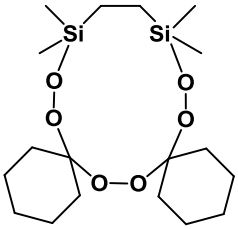
Silyl peroxides			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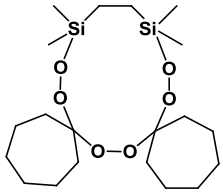
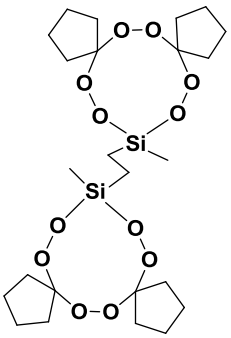
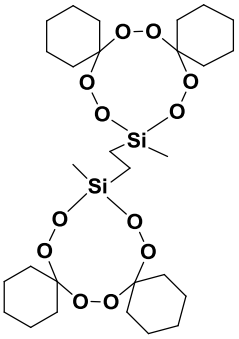
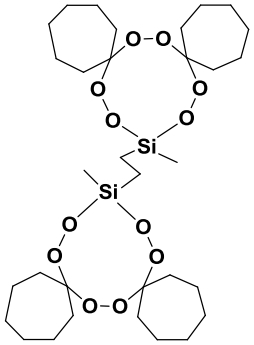
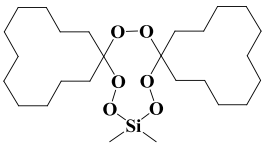
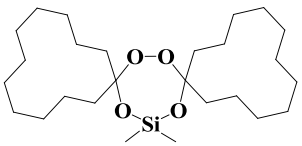
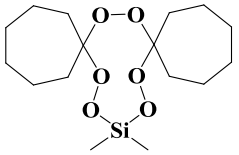
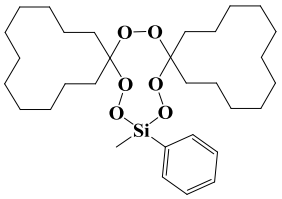
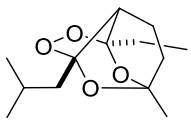
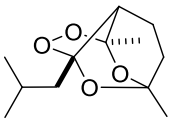
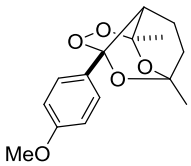
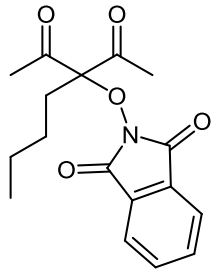
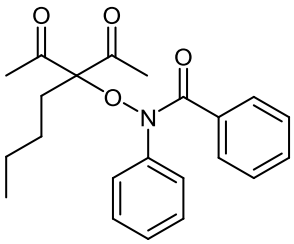
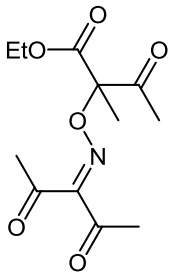
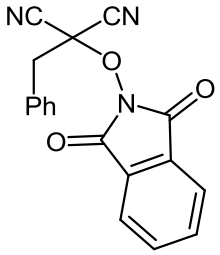
			
36	**37**	**38**	**39**
			
40	**41**	**42**	**43**
Tricyclic monoperoxides			
			
44	**45**	**46***	
Hydroxylamines			

			
47	**48**	**49**	**50**

267 *: Lead compound of previous study (K. Ingram et al, 2012)

268 **Table 2**

269 Compounds showing antischistosomal activity (killing parasite at 33.3 µM), their L6 cytotoxicity, and
270 the resulting selectivity index.

	Compound	NTS		Adult		L6-cells		SI
		IC_{50} [µM]	r-value	IC_{50} [µM]	r-value	IC_{50} [µM]	r-value	
Praziquantel*		2.2	0.9	0.1	1.9	>96	-	>960
Tetraoxane	20*	0.1	0.9	0.3	1.0	1.7	-	5.7
Tricyclic monoperoxide	46*	14.4	0.8	11.8	0.9	8.2	-	4.9
Tetraoxanes	1	20.2	0.98	ND	-	ND	-	-
	4	3.7	0.95	7.4	0.94	4.4	1.00	0.6
	7	4.7	0.86	20.9	0.96	ND	-	-
	9	ND	-	12.1	0.97	ND	-	-
	2	1.3	0.84	2.0	0.90	< 0.4	-	< 0.2
	8	1.8	0.93	2.0	0.90	< 0.4	-	< 0.2
	6	0.9	0.96	1.8	0.96	1.0	1.00	0.5
	15	1.6	0.90	10.9	0.96	ND	-	-
	17	ND	-	23.6	0.90	ND	-	-
	16	5.2	0.98	9.8	0.96	2.5	1.00	0.3
	18	3.5	0.90	15.4	0.97	ND	-	-
	19	1.3	0.88	8.4	0.96	2.5	1.00	0.4
	3	4.0	0.92	3.9	0.96	< 0.4	-	< 0.1
	5	ND	-	20.8	0.99	ND	-	-
Trioxolanes	21	0.4	0.79	1.8	0.89	5.4	0.99	2.9
	22	5.6	1.0	10.3	0.95	ND	-	-
	23	5.7	0.92	7.0	0.92	22.7	0.97	1.7
	24	7.7	0.99	22.8	0.89	ND	-	-
	25	ND	-	10.0	0.97	ND	-	-
	26	12.2	0.98	7.4	0.95	15.3	0.98	7.4
	31	ND	-	12.2	0.89	ND	-	-
	27	2.8	0.81	4.2	0.96	2.7	1.00	1.3
	28	3.2	0.92	11.2	0.95	ND	-	-

	29	6.2	0.98	6.6	0.98	7.3	0.97	1.1
	30	2.2	0.92	4.2	1.00	8.1	0.99	1.6
Tricyclic monoperoxides	44	2.7	0.92	4.4	0.96	24.4	1.00	5.7
	45	2.0	0.88	2.0	0.89	4.9	0.99	3.0
Silyl peroxides	32	4.0	0.94	ND	-	ND	-	-
	33	7.2	0.88	ND	-	ND	-	-

271 ND: not done

272 SI: selectivity index (cytotoxicity IC_{50} divided by adult schistosome IC_{50})

273 *: Lead compound of previous study (K. Ingram et al, 2012)

274

275 **Table 3**

276 *In vivo* worm burden reductions of *S. mansoni*-infected mice after a single oral dose of 400 mg/kg.

Compound	Number of mice tested	Average worm burden (SD)	Worm burden reduction [%]
Control[1]	8	34.1 (10.3)	-
Control[2]	8	23.6 (11.7)	-
20*	6	6.7 (2.5)	75
46*	4	5.3 (5)	83
30[1]	3	19.0 (4.6)	44
27[1]	4	19.5 (12.4)	43
44[1]	3	25.0 (7.0)	27
26[1]	4	30.8 (8.7)	10
21[1]	4	32.0 (3.3)	6
45[1]	4	37.0 (12.5)	0
[CD-44[2]]	3	16.3 (16.6)	31

[CD-27[2]]	4	22.5 (3.3)	5
[CD-26[2]]	4	24.5 (6.6)	0
[CD-30[2]]	3	33.0 (6.7)	0
[CD-20[2]]*	4	33.3 (12.3)	0
[CD-46[2]]*	3	35.7 (18.8)	0

277 CD: complexation with β-cyclodextrin

278 SD: standard deviation

279 1, 2: batch number of *S. mansoni* mouse infection

280 *: Lead compound of previous study (K. Ingram et al, 2012)

281

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