Structure-Reactivity Relationships on Substrates and Inhibitors of the Lysine Deacylase Sirtuin 2 from *Schistosoma Mansoni* (SmSirt2)

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The only drug for treatment of Schistosomiasis is Praziquantel, and the possible emergence of resistance makes research on novel therapeutic agents necessary. Targeting of *Schistosoma mansoni* epigenetic enzymes, which regulate the parasitic life cycle, emerged as promising approach. Due to the strong effects of human Sirtuin inhibitors on parasite survival and reproduction, *Schistosoma* sirtuins were postulated as therapeutic targets. In vitro testing of synthetic substrates of *S. mansoni* Sirtuin 2 (SmSirt2) and kinetic experiments on a myristoylated peptide demonstrated lysine long chain deacylation as an intrinsic SmSirt2 activity for the first time. Focused in vitro screening of the GSK Kinetobox library and structure-activity relationships (SAR) of identified hits, led to the first SmSirt2 inhibitors with activity in the low micromolar range. Several SmSirt2 inhibitors showed potency against both larval schistosomes (viability) and adult worms (pairing, egg laying) in culture without general toxicity to human cancer cells.

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Supporting information

Structure-reactivity relationships on substrates and inhibitors of the lysine deacylase Sirtuin 2

from *Schistosoma mansoni* (SmSirt2)

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Table S1. Conversion of ZMAL (1) analogues 3a-g by *SmSirt2*.

<i>SmSirt2</i>	1	3a	3b	3c	3d	3e	3f	3g
Conversion (%)^a	21.2 ± 1.0	63.7 ± 3.0	91.1 ± 1.2	89.5 ± 1.5	75.1 ± 4.0	58.6 ± 2.9	27.2 ± 1.2	13.2 ± 1.0

^a Errors are reported as standard deviation (SD, n = 3).

Table S2. Conversion of ZMAL (1) analogues 3a-3g by *hSirt2*.

<i>hSirt2</i>	1	3a	3b	3c	3d	3e	3f	3g
Conversion (%)^a	28.1 ± 2.0	77.3 ± 1.3	79.5 ± 0.4	28.5 ± 1.3	47.7 ± 4.1	9.5 ± 3.4	12.6 ± 2.2	2.6 ± 1.4

^a Errors are reported as standard deviation (SD, n = 3).

Table S3. IC₅₀ of NA with 1 resp. 3 a-g as substrates.

	1	3a	3b	3c	3d	3e	3f	3g
IC₅₀ (μM)^a	23.1 ± 1.8	16.3 ± 2.0	20.5 ± 0.9	25.8 ± 2.0	36.1 ± 3.5	47.9 ± 2.1	420 ± 4.46 11.6	1600 ± 0.140

^a Errors are represented as standard error of the mean (SEM, n=3).

Table S4. Source of the intermediate compounds 45a-e, r, v, w and 46a-h

Compd	Structure	Reference
45a		commercial
45b		commercial
45c		commercial

45d		commercial
45e		commercial
45r		prepared according to Ref. 1
45v		prepared according to Ref. 2
45w		prepared according to Ref. 3
46a		commercial
46b		commercial
46c		commercial
46d		commercial
46e		commercial

46f		commercial
46g		commercial
46h		commercial

Table S5. Sequence identities and similarities between smSirT2 and hSirT2

	Full-length	Catalytic domain
Identity	37%	44%
Similarity	49%	58%

Phylogeny and sequence similarities between SirT2 from various organisms have already been published.^{4,5} Table X summarizes sequence identities and similarities calculated between human and *S. mansoni* SirT2 sequences. For these calculations, both full length and only catalytic domain (based on PDB structure 5D7O) sequences have been used. Both enzymes show identities/similarities indicative of a common fold but with differences that should enable the design of selective inhibitors for smSirT2.

Table S6. Effects of 7 and its analogues on schistosomula viability.

cpd	10 μM^a		20 μM^a	
	% viability $\pm$ SD	Microscopy image	% viability $\pm$ SD	Microscopy image
DMSO Control	90.5 $\pm$ 1.5 %		89.8 $\pm$ 2.3 %
7	0 $\pm$ 0 %		0 $\pm$ 0 %
26	0 $\pm$ 0 %		0 $\pm$ 0 %
29	0 $\pm$ 0 %		0 $\pm$ 0 %
32	2.4 $\pm$ 0.7 %		0 $\pm$ 0 %
33	0 $\pm$ 0 %		0 $\pm$ 0 %
39	0 $\pm$ 0 %		0 $\pm$ 0 %
41	81.5 $\pm$ 2.5 %		4.5 $\pm$ 2 %

^aResults obtained by microscopy examination. 72 h, 500 schistosomula/well (n = 2); 2 biological replicates.

Table S7. Molecular properties for selected compounds.

<i>Cpd</i>	<i>MW</i>	<i>cLogP</i>	<i>H-Acceptors</i>	<i>H-Donors</i>	<i>TPSA</i>	<i>Druglikeness</i>
4	339.825	3.7126	4	1	48.99	2.4775
5	422.502	4.0942	6	1	71.26	3.0543
6	417.472	2.7956	9	2	125.3	3.5505
7	417.472	2.7956	9	2	125.3	3.5505
29	479.542	4.2247	9	2	125.3	1.633
32	447.498	2.7256	10	2	134.53	3.5505
33	509.568	4.1547	10	2	134.53	1.633
41	463.565	3.1511	9	2	150.6	3.8621
42	525.635	4.5802	9	2	150.6	2.0163

HPLC

3a

Peak	Retention time (min)	Area (%)
1	9.935	1.1476
2	13.994	0.4614
3	14.188	0.2239
4	15.569	0.2563
5	16.089	0.3070
6	16.580	0.1060
7 (cp 3a)	19.496	97.4978

3b

Peak	Retention time (min)	Area (%)
1	6.396	0.2422
2	14.942	0.9783
3 (cp 3b)	22.108	98.3549
4	27.222	0.4246

3c

Peak	Retention time (min)	Area (%)
1 (cp 3c)	24.323	99.8563
2	26.612	0.1437

3d

Peak	Retention time (min)	Area (%)
1	13.225	0.1846
2	21.491	0.3085
3	23.676	0.6814
4 (cp 3d)	26.061	98.8255

3e

Peak	Retention time (min)	Area (%)
1	15.562	1.2470
2	18.984	0.1367
3	23.841	0.2541
4 (cp 3e)	25.175	97.2748
5	26.060	0.3904
6	27.018	0.6970

3f

Peak	Retention time (min)	Area (%)
1	13.828	1.3933
2	26.753	0.5925
3	28.163	0.7803
4	29.295	0.2083
5 (cp 3f)	30.424	95.6177
6	31.339	1.4078

3g

Peak	Retention time (min)	Area (%)
1	23.724	0.3035
2	25.898	0.3135
3(cp 3g)	26.635	99.2683
4	29.020	0.1147

6

Peak	Retention time (min)	Area (%)
1	16.220	0.2287
2 (cp 6)	16.559	99.7486
3	17.914	0.0236

11

Peak	Retention time (min)	Area (%)
1	16.132	4.4102
2 (cp 11)	22.798	95.5898

12

Peak	Retention time (min)	Area (%)
1	13.342	1.1369
2 (cp 12)	23.711	98.8631

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