EVALUATION OF THE SELECTIVITY OF SULFONAMIDE TUBULIN INHIBITORS AGAINST HUMAN AFRICAN TRYPANOSOMIASIS

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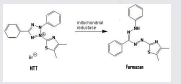
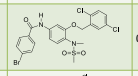
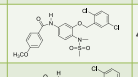
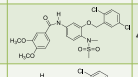
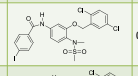
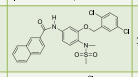
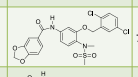
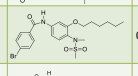
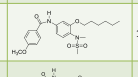
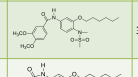
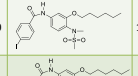
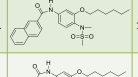
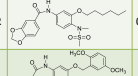
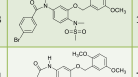
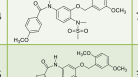
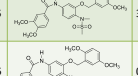
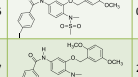
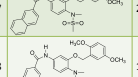
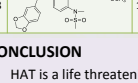
ABSTRACT: Human African Trypanosomiasis (HAT) is an endemic life-threatening disease caused by parasitic protozoan Trypanosoma brucei (T.brucei). The drugs being used for the treatment of HAT exhibit high toxicity to the hosts due to their poor selectivity against the parasite. There is a need for the development of potent drugs with efficient pharmacodynamics and high selectivity against the parasite over mammalian cells. Tubulin plays an important role in T.brucei cell growth because of their rapid rate of cell proliferation. In addition, microtubule within the flagellum of the parasite assists in locomotion, which is vital for their survival. The importance of tubulin in the parasite suggests the potential advantages of tubulin inhibitors against HAT. Based on the differences between mammalian and parasitic tubulin, a library of sulfonamide tubulin inhibitors was screened and evaluated using MTS assay on T. brucei. We propose to compare the anti-parasitic activity of the compounds to their activity against mammalian cells. The compounds with high potency and selectivity will be identified as drug candidates to move further in the drug discovery pipeline. Currently, we completed the proliferation assay with T.brucei cells and, the potent candidates against T. brucei are being screened for toxicity against normal mammalian macrophage cells. Overall, this study provides with the basis for further development of tubulin inhibitors that selectively targets T.brucei for the treatment of HAT.

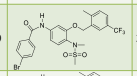
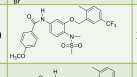
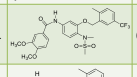
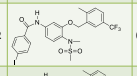
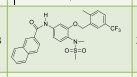
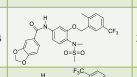
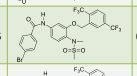
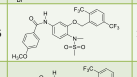
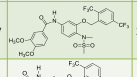
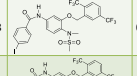
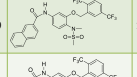
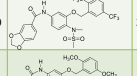
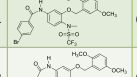
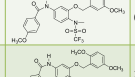
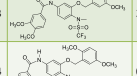
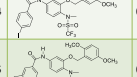
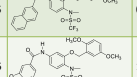
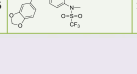
Cell Culture and Cell Viability Assay

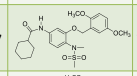
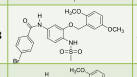
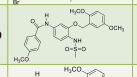
- Mammalian macrophage cells (mouse derived)
- T.B.*brucei* cells
  - Cells grown in 96-well plates for 24 h
  - Exposed to various concentrations of compound analogs in DMSO for 48h
  - MTT Assay assessed for macrophage cell treatment cell viability assay
  - MTS Assay assessed for T. brucei treatment cell-viability assay
  - Absorbance reading at 570 nm
  - Determination of IC_{50} values using Graph-pad prism

Tubulin-β (Bovine)	QAADPRHGRYLTASALFRGRMSTKEVDEQMLNVQNKNSSYFIEWIPNNIKSSVCDIPPKG
Tubulin-β (*T.brucei*)	AACDPRHGRYLTVAAIFRGRMSMKEVDEQMLNVQNKNSSYFVEWIPNNVKTAVCDIPPRG
Identical Residues	.A.DPRHGRYLT.A.FRGRMS.KEVDEQMLNVQNKNSSYF.EWIPNN.K..VCDIPP.G



BZ	Structure	IC_{50} against *T. brucei* μM	IC_{50} against Mammalian Macrophage μM	Selectivity Index
1		0.77 ± 0.29	2.797 ± 1.81	3.6
2		4.08 ± 1.96	1.09 ± 0.75	0.3
3		4.41 ± 2.41	2.601 ± 1.736	0.6
4		0.31 ± 0.15	0.52 ± 0.34	1.7
5		1.64 ± 0.55	1.26 ± 0.63	0.8
6		7.98 ± 3.39	3.31 ± 2.09	0.4
7		0.99 ± 0.28	121.2 ± 58.08	122.0
8		1.09 ± 0.43	10.1 ± 4.65	9.2
9		3.39 ± 1.22	65.9 ± 25.3	19.4
10		1.68 ± 0.84	11.2 ± 7.01	2.0
11		1.16 ± 0.62	>200	172.4
12		0.82 ± 0.41	>200	243.9
13		1.25 ± 0.44	0.0124 ± 0.00781	0.01
14		7.17 ± 3.42	0.00078 ± 0.00048	0.0001
15		3.19 ± 1.42	0.0668 ± 0.0527	0.002
16		0.79 ± 0.32	0.00071 ± 0.00035	0.0008
17		2.49 ± 1.54	0.00861 ± 0.00471	0.003
18		10.0 ± 4.89	0.00375 ± 0.00223	0.0004

BZ	Structure	IC_{50} against *T. brucei* μM	IC_{50} against Mammalian Macrophage μM	Selectivity Index
19		2.37 ± 0.96	3.15 ± 1.97	1.3
20		3.46 ± 1.85	1.21 ± 0.58	0.3
21		6.35 ± 2.30	1.90 ± 0.90	0.3
22		0.80 ± 0.24	1.94 ± 1.12	2.4
23		2.15 ± 0.61	4.98 ± 2.61	2.3
24		2.40 ± 0.96	4.67 ± 2.33	1.9
25		0.47 ± 0.17	8.98 ± 4.65	19.1
26		1.29 ± 0.51	3.79 ± 1.83	2.9
27		3.91 ± 2.27	12.7 ± 6.89	3.2
28		0.73 ± 0.38	2.57 ± 1.53	3.5
29		1.07 ± 0.44	30.76 ± 13.67	28.7
30		2.25 ± 0.77	9.91 ± 4.82	4.4
31		0.37 ± 0.15	0.52 ± 0.32	1.4
32		0.89 ± 0.41	0.168 ± 0.076	0.2
33		2.09 ± 0.98	0.482 ± 0.269	0.2
34		0.34 ± 0.16	0.178 ± 0.086	0.5
35		0.50 ± 0.11	1.246 ± 0.831	2.5
36		1.12 ± 0.39	0.421 ± 0.232	0.4

BZ	Structure	IC_{50} against *T. brucei* μM	IC_{50} against Mammalian Macrophage μM	Selectivity Index
37		7.64 ± 3.34	0.07450 ± 0.03170	0.01
38		8.54 ± 4.26	0.05055 ± 0.02953	0.006
39		72.9 ± 42.2	0.01006 ± 0.005062	0.0001
40		9.13 ± 4.39	0.03879 ± 0.01919	0.004
41		14.0 ± 4.88	0.01814 ± 0.01028	0.001
42		190.9 ± 138.5	0.02146 ± 0.01123	0.0001
43		96.2 ± 56.9	0.01554 ± 0.0757	0.0001
44		8.42 ± 3.79	0.1677 ± 0.1038	0.02
45		3.79 ± 2.08	0.1038 ± 0.05431	0.03
BM	Compounds	-	-	-
1		Did not test	5.1 ± 1.56	Did not test
2		Did not test	8.14 ± 4.08	Did not test
3		Did not test	84.8 ± 37.8	Did not test
4		Did not test	13.9 ± 2.25	Did not test
5		Did not test	1.34 ± 0.66	Did not test
6		Did not test	2.44 ± 1.36	Did not test
7		Did not test	30.9 ± 16.5	Did not test
8		Did not test	1.71 ± 0.58	Did not test

BM	Structure	IC_{50} against *T. brucei* μM	IC_{50} against Mammalian Macrophage μM	Selectivity Index
9		2.69 ± 0.89	0.0159 ± 0.0110	0.006
10		2.04 ± 1.02	0.0432 ± 0.0167	0.02
11		0.92 ± 0.23	0.0546 ± 0.0271	0.06
12		0.32 ± 0.10	0.00680 ± 0.00306	0.02
13		22.6 ± 9.85	0.1083 ± 0.0531	0.0005
14		10.6 ± 4.89	0.23 ± 0.07	0.02
15		1.48 ± 0.59	0.0723 ± 0.0327	0.05
16		1.31 ± 0.52	0.06512 ± 0.0366	0.05
17		9.43 ± 4.42	2.25 ± 0.87	0.2
18		11.9 ± 5.84	10.35 ± 3.82	0.9
19		2.13 ± 0.96	13.1 ± 5.51	6.1
20		1.73 ± 0.68	1.74 ± 0.61	1.0
21		2.29 ± 1.11	0.20 ± 0.09	0.08
22		2.53 ± 1.37	1.99 ± 1.03	0.8
23		0.56 ± 0.21	0.83 ± 0.51	1.5
24		0.36 ± 0.13	0.18 ± 0.09	0.5
25		1.85 ± 1.12	0.39 ± 0.21	0.2
26		1.41 ± 0.75	0.29 ± 0.16	0.2

BM	Structure	IC_{50} against *T. brucei* μM	IC_{50} against Mammalian Macrophage μM	Selectivity Index
27		1.04 ± 0.48	0.0145 ± 0.00872	0.01
28		0.20 ± 0.08	0.00976 ± 0.00435	0.05
29		4.42 ± 1.31	1.25 ± 0.50	0.3
30		2.12 ± 0.76	0.56 ± 0.21	0.3
31		2.33 ± 0.96	0.0642 ± 0.0296	0.02
32		0.54 ± 0.16	0.0596 ± 0.0295	0.03
33		43.5 ± 23.5	0.0439 ± 0.0187	0.001
34		45.9 ± 26.5	0.0760 ± 0.0372	0.002
35		1.34 ± 0.68	0.0845 ± 0.0472	0.06
36		1.19 ± 0.53	0.0366 ± 0.0166	0.03
37		31.5 ± 15.6	0.00650 ± 0.00261	0.0002
38		3.02 ± 2.30	0.0568 ± 0.0303	0.02
39		1.88 ± 1.12	0.0439 ± 0.0233	0.02
40		0.41 ± 0.19	0.00562 ± 0.00236	0.01
41		3.45 ± 1.25	0.21 ± 0.12	0.06
42		1.95 ± 1.16	0.23 ± 0.12	0.1
43		2.43 ± 1.47	0.00975 ± 0.00368	0.004
44		2.01 ± 1.13	0.95 ± 0.66	0.47

CONCLUSION

- HAT is a life threatening disease in Africa
- Current treatments are toxic and expensive
- Sulfonamide Tubulin inhibitors bind to colchicine binding domain
- Further structure optimization and cell viability assays should be performed to identify compounds selective for parasite.

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