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Identification of Selective Tubulin Inhibitors as Potential Anti-Trypanosomal Agents

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Human African trypanosomiasis, also known as sleeping sick ness, is a vector borne parasitic disease and also a serious health threat to a large number of people living in sub Saharan Africa where health systems are least effective, or even non existent.[1–3] *Trypanosoma brucei gambiense* (*T. b. gambiense*) and *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*) are the etiological parasites of sleeping sickness in humans. In West and Central Africa, *T. b. gambiense* is the major parasite to cause the disease, while in sub Saharan Africa, *T. b. rhodesiense* predominates. These subspe cies of trypanosome are responsible for the West and East African forms of the disease, respectively.[2] The main difference between the two infections is the rate of progression from the blood/lym phatic stage to the cerebral stage. *T. b. gambiense* infection is chronic because it takes months for the disease to progress. By con trast, the infection of *T. b. rhodesiense* is more acute, and could reach the cerebral stage in one to three weeks. For, *T. b. gambiense*, humans are the main hosts. However, wild and domestic animals, especially cattle, are the major reservoirs for *T. b. rhodesiense*.[1] A third closely related subspecies, *Trypanosoma brucei brucei* (*T. b. brucei*), is less infectious to humans, but is responsible for many cases of nagana in cattle. It significantly limits the agricultural development in Africa.[4,5] As *T. b. brucei* shares many features with *T. b. gambiense* and *T. b. rhodesiense* (such as antigenic variation), it is often used as a model for human infections in laboratory and animal studies.

The current chemotherapy of the human trypanosomiasis relies on only five drugs including Suramin, Pentamidine, Melarsoprol, Eflornithine and Nifurtimox Eflornithine combination.[6] The main drawbacks of these drugs are: (1) high toxicity to the hosts, which is mainly due to their poor selectivity to the parasite cells than the mammalian cells; (2) these agents have to be administered via intramuscular or intravenous injections; (3) they have very narrow anti trypanosomiasis spectrum; and (4) treatment using these drugs needs the high cost of hospitalization. Overall, these drugs are not successful in the treatment of the disease, and there is a general lack of effective, inexpensive chemotherapeutic agents for the treatment of human African trypanosomiasis. Clearly, im proved chemotherapeutics with better selectivity to the trypano somes are needed to effectively battle this disease.[5,7,8]

Tubulin containing structures are important for many impor tant cellular functions, including chromosome segregation during cell division, intracellular transport, development and mainte nance of cell shape, cell motility, and distribution of molecules on cell membranes.[9] Tubulin is a very attractive target in anti cancer drug discovery field, and several successful tubulin binders are the first line chemotherapeutic agents in clinic.[10] Tubulin also plays an essential role during trypanosome cell division. The fast population doubling rate of trypanosomes makes them highly dependent on tubulin polymerization/depolymerization.[11] More importantly, tubulin is very critical for the trypanosome locomo tion, which is an essential function for trypanosomes to survive.

The *T. brucei* cell body is roughly cylindrical in shape with tapered anterior and posterior ends. A single flagellum emerges from the basal body near the posterior end of the cell. Within the flagellum is a canonical '9+2' microtubule axoneme that drives flagellar movement.[12] Tubulin inhibitors not only block the *T. brucei* cell division but will also affect the locomotion function of flagellum and lead to cell death.[13] The flagellar pocket is known to be an important structure in the uptake and internalization of molecules for trypanosomes.[14] Such uptake could enhance the binding of the tubulin inhibitors to intracellular tubulin, particularly in the fla gella pocket. Therefore, tubulin inhibitors could be effective agents to suppress flagellar locomotion function.[13] These factors indicate that there are potential advantages of tubulin inhibitors for the treatment of trypanosomiasis. In addition, identification of binding pockets uniquely located on *T. brucei* tubulin would allow develop ment of selective tubulin inhibitors, which could dramatically re duce the toxic effects of the anti parasite drugs to the host cells.

Tubulin is a highly conserved protein. Examination of tubulin sequences from mammalian cells and yeast cells reveals 70 90% identity. However, differences in susceptibility to antimitotic agents are known to exist between tubulins from different organ isms, suggesting that differences of tubulin structures exist among different species.[15] For example, the antifungal compound methyl *N* (benzimidazol 2 yl) carbamate shows high selectivity to yeast tubulin. It has been reported that the compound is at least 300 fold more potent as an inhibitor of yeast tubulin than that of bovine brain tubulin.[16] In addition, oxfendazole and thiabendazole com pounds are also more effective to inhibit nematode tubulin poly merization than mammalian tubulin.[17] The results from these investigations reveal that there are differences in tubulin drug sus ceptibility for different organisms. Based on the differences of tubulin in *T. brucei* and mammalian cells, it is highly expected that selective tubulin inhibitors could be developed. Some microtubule disrupting herbicides such as phosphoric thioamide herbicide amiprophos methyl (APM) and dinitroaniline herbicides exhibit activity against protozoan parasites by aiming tubulin as the molecule target.[15,17–20] Research has been done to optimize these compounds to generate more potent and selective tubulin inhibi tors for *T. brucei*.[15] Werbovetz's group successfully developed sev eral drug candidates showing promising in vitro anti parasite activity and selectivity. However, these compounds did not show good in vivo potency due to the poor stability.[21] However, these investigations demonstrated the feasibility to generate selective tubulin inhibitors as anti trypanosomal agents.

To search for selective tubulin inhibitors as better therapeutic agents to treat sleeping sickness, we firstly examined the inhibitory activity of several tubulin inhibitors that are current clinical drugs or in clinical trials for cancer treatment[10,22] on *T. brucei* (*T. b. brucei* was used as the representative strain) with 3 (4,5 dimethylthiazol 2 yl) 5 (3 carboxymethoxyphenyl) 2 (4 sulfophenyl) 2*H* tetrazo lium (MTS) assay,[23] then on mammalian cell growth (SKBR 3 breast cancer cell line as a model) with MTT assay.[24] Among the few tested drugs, paclitaxel showed very similar activity on both *T. brucei* and SKBR 3 breast cancer cells (Table 1), suggesting that tubulin binding domain of paclitaxel is very similar in the two organisms. However, vinblastine and the colchicine domain binders including colchicine, indibulin,[25] nocodazole and ABT751[22] exhibited strong inhibition to mammalian cells but very weak inhibitory effect on *T. brucei* growth, which is consistent to other studies focusing on tubulin inhibitors with *T. brucei*.[26,27] These results suggest that sig nificant differences exist in the colchicine binding domain between mammalian and *T. brucei* tubulins.

Table 1
Well-defined tubulin inhibitors exhibited growth inhibition to mammalian and *T. brucei* cells

Entry	IC_{50} against SKBR-3 breast cancer cell growth (μM)	IC_{50} against *T. brucei* growth (μM)
Paclitaxel	0.0019 ± 0.0009	0.0046 ± 0.0018
Indibulin	0.033 ± 0.012	114.1 ± 45.5
ABT751	0.74 ± 0.20	82.1 ± 37.0
Colchicine	0.0064 ± 0.0023	14.0 ± 7.2
Vinblastine	0.00091 ± 0.00031	0.41 ± 0.21
Nocodazole	0.084 ± 0.022	44.2 ± 23.5

Due to the very different biological activities of the well defined tubulin inhibitors on mammalian and *T. brucei* cells, we compared the tubulin amino acid sequence of the two organisms (Table 2). Bovine tubulin was listed as a representative of mammalian tubu lin. *T. brucei* tubulin showed an 85% identity to bovine α tubulin and 86% identity to bovine β tubulin when analyzed with SWISS MODEL repository.[28,29] It is hard to estimate whether the binding sites of tubulin inhibitors are very different between mammalian and *T. brucei* cells just based on the protein sequence comparison. However, the difference of certain key amino acids of tubulin is very likely to affect the tubulin inhibitor's binding affinity. It has been reported that Leucine 316 of β Tubulin (Table 2, L316 is marked in blue) is critical for colchicine activity against bovine tubulin polymerization.[30–32] In *T. brucei* β tubulin, residue 316 is changed to Valine, which is expected to greatly decrease the col chicine binding and presumably explains the weak inhibitory activity of colchicine on the growth of *T. brucei* cells (Table 1).

To further illustrate the difference of the colchicine binding do main of bovine and *T. brucei* tubulin, a predicated structure of *T. brucei* tubulin was generated based on the crystal structure of bo vine tubulin (PDB1SA0)[30] using SWISS MODEL Repository program (Fig. 1).[28,29] The model shows great similarity between *T. brucei* and bovine tubulin, since the protein sequence identity is ~85%. How ever, the colchicine binding domain shows clear difference be tween the two types of tubulins. Several β sheets of the bovine and the *T. brucei* tubulin (Fig. 1B, circled area) in the colchicine binding domain do not overlap well. These β sheets form the bind ing pocket for colchicine, and are critical for ligand binding. Other colchicine domain binder including nocodazole, indibulin, and ABT751 also rely on these β sheets to bind to tubulin.[30,33] The dif ference between the effects of these well defined tubulin inhibi tors on *T. brucei* and mammalian cells (Table 1) is also consistent with the predicated structure difference between the two tubulin homologous. This significant docking site difference provides a good foundation for the development of selective colchicine domain binders for the treatment of sleeping sickness.

We previously developed a class of sulfonamide tubulin inhibi tors (Table 3) as anti cancer agents.[24,34] These inhibitors were identified to be colchicine domain binders and some of them exhibited very potent cell cycle arrest and apoptosis inducing activity in mammalian cells.[24] Due to the structural diversity of their benzamide moiety, we hypothesized that some of the analogs might selectively inhibit *T. brucei* growth, since mammalian and *T. brucei* tubulin exhibit differences on colchicine binding domain, particularly at the benzamide moiety binding pocket (Figs. 1 and 2). More specifically, the benzamide moiety of the tubulin inhibi tors interacts with the β sheets of the colchicine binding domain as indicated with blue arrows in Figure 2.[34] The differences of these β sheets in *T. brucei* and mammalian cells will form different bind ing pockets, and highly likely cause different interactions with dif ferent benzamide moieties, which will lead to selectivity.

The compounds were tested with *T. brucei* cell growth assay, and the IC_{50}s are listed in Table 3. The activities against SKBR 3 breast cancer cells from previous studies[24] are listed in the table for comparison. Several compounds, including **3**, **17**, **26**, **38**, and **43**, exhibited very specific inhibitory effect on *T. brucei* growth, with selectivity index (IC_{50} inhibiting human cancer cell growth/ IC_{50} inhibiting *T. brucei* growth) being five or more. Particularly,

Table 2
Comparison of amino acid sequence between bovine and *T. brucei* β tubulin (part of the sequence)

Table 2. Comparison of amino acid sequence between bovine and *T. brucei* β tubulin (part of the sequence)	
Bovine - β tubulin	QAADPRHGRYLTASA**L**FRGRMSTKEVDEQMLNVQNKNSSYFIEWIPNNIKSSVCDIPPKG 300
T. brucei - β tubulin	AACDPRHGRYLTVAA**V**FRGRMSMKEVDEQMLNVQNKNSSYFVEWIPNNVKTAVCDIPPRG 300
Identical residues	.A.DPRHGRYLT..A. FRGRMS.KEVDEQMLNVQNKNSSYF.EWIPNN.K..VCDIPP.G 300

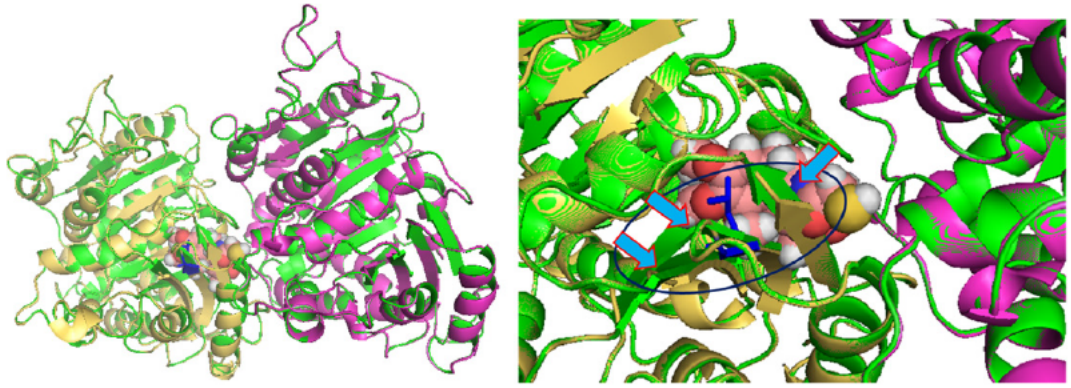


Figure 1. *T. brucei* tubulin protein homology model. (A) The alignment of bovine tubulin crystal structure (green) and the predicted *T. brucei* tubulin model (α tubulin, magenta; β tubulin, yellow). Colchicine is shown in the ball model. Leucine 316 is labeled in blue. (B) The colchicine-binding domain in higher magnification. Several β sheets of the predicated *T. brucei* tubulin structure (circled region) do not overlap with that of the bovine tubulin.

compound **3** with a selective index of eight also showed an a low IC_{50} of 0.42 ± 0.21 μM to inhibit *T. brucei* cell growth, and com pound **26** with a selective index of 34 exhibited an IC_{50} of 1.62 ± 1.23 μM to inhibit *T. brucei* cell growth. Therefore, both the selectivity and potency of these compounds are very promising.

The structure activity relationship (SAR) for anti cancer potency of these agents generated in previous study suggests that the elec tron donating group substituted benzamide moiety enhances the anti cancer activity.[24] The 2,5 dimethyl substituted benzyl moiety is critical for the anti cancer activity as well.[35,36] On the contrary, the electron withdrawing group substituted benzamide moiety enhances the anti parasite activity in our *T. brucei* growth inhibi tion study. Compound **3**, **7**, **8**, **9**, **15**, and **26** all have electron withdrawing group substituted benzamide moiety, and they all exhibited relatively better potency to inhibit *T. brucei* cell growth. The 2,5 dimethyl group on the benzyl moiety appears not very important, since compound **42** and **43** also exhibited potent anti parasite activities even though they lack the 2,5 dimethyl group. We subsequently did a correlation study of the anti mammalian cell and anti *T. brucei* growth activities and found that there was significant differences between the two effects (Fig. 3), which is consistent with the homology analysis results. Although, the col chicine binding domain in bovine and *T. brucei* showed good sim ilarity, there are critical differences that lead to significant different effects of the tubulin inhibitors tested above. As colchicine domain binders, these sulfonamide tubulin inhibitors interfering with a do main showing difference in mammalian and T. brucei cells, there fore, exhibited good selectivity between the two organisms.

Based on the inhibitory effects of the compounds on the *T. bru cei* cell proliferation, a SAR was summarized. The pharmacophore of the tubulin inhibitor promoting the mammalian cancer cell growth inhibition and the structures enhancing the parasite growth inhibition are described in Figure 4. There is a clear differ ence between the SAR generated in the anti cancer studies[24] and the current anti *T. brucei* investigation. For the benzamide moie ties, introducing more electron withdrawing groups may generate more potent inhibitors for *T. brucei* growth. In addition, changing the di methyl benzyl group to other non di methyl substituted benzyl may further diminish the mammalian cell growth inhibi tory effect,[35,36] and this changing is unlikely to harm the anti *T. brucei* activity according to our results. It is therefore expected that more selective and potent tubulin inhibitors for trypanosomal dis ease could be developed based on this discovery.

Furthermore, several sulfonamide tubulin inhibitors including compounds **3**, **4**, **5**, **7**, **8**, **9**, **15**, **21**, **26**, **28**, and **38** with IC_{50}s below 2 μM to inhibit *T. brucei* cell growth were also tested for their ef fects on human primary fibroblast IMR90 cells with MTT cell growth assay.[24] The results can provide the general cytotoxicity information of the compounds. Compound **3**, **26** and **38** with good selectivity to inhibit *T. brucei* cell growth showed no clear growth inhibition activity to IMR 90 cells at 5 μM (Fig. 5), suggesting the three compounds will have low toxicity to the normal mammalian cells. The rest of the compounds exhibited different levels of growth inhibitory activities to the IMR 90 cells at 5 μM. Generally, these compounds are less active to inhibit the growth of IMR 90 cells than SKBR 3 breast cancer cells. The results suggest that sev eral compounds from the anti trypanosomiasis agent library will have mild adverse effects to the hosts at concentrations that can effectively eliminate the trypanosomes.

In brief, our finding provided a unique molecular scaffold that selectively target *T. brucei* tubulin, and opened a new area on trypanosome specific tubulin inhibitor development. The discov ery is based on a class of colchicine domain binders developed in our laboratory recently.[24,34] This is the first study focusing on the specific binding site differences between mammalian and *T. brucei* tubulin to develop selective anti trypanosome tubulin inhibitors. The results suggest that it is very promising to develop selective colchicine domain binders as novel anti trypanosome drugs based on our lead compounds. To prove these agents are tubulin inhibitors in *T. brucei* cells, more studies such as cell cycle arrest and *T. brucei* tubulin polymerization experiments should be performed in the future when more potent and selective analogs are developed. Further lead optimization based on the current

Table 3
Comparison of the growth inhibitory effects of the tubulin inhibitors on mammalian and *T. brucei* cells. The treatments were quadruplicated and repeated three times

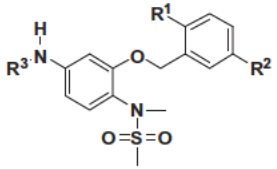
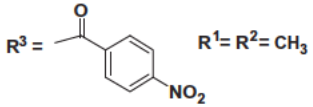
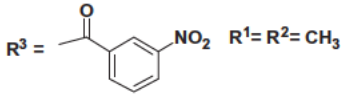
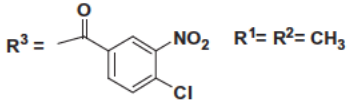
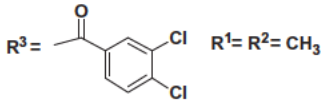
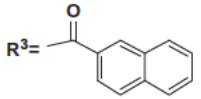
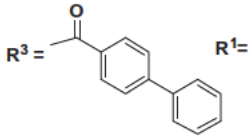
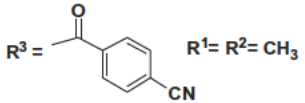
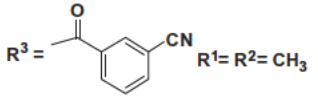
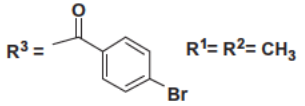
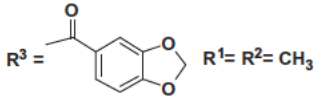
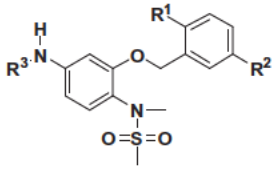
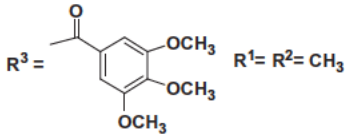
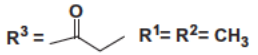
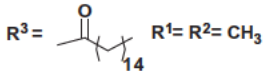
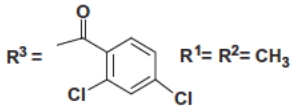
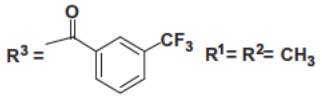
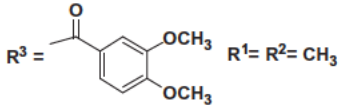
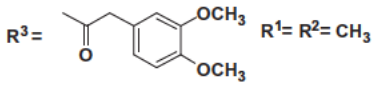
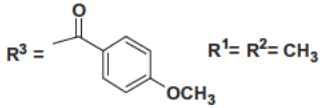
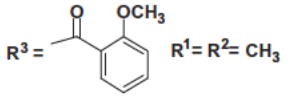
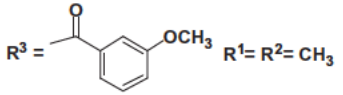
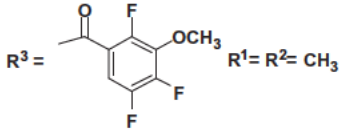
Entry		IC_{50} (±SD) against SKBR-3 breast cancer cell growth (μM)	IC_{50} (±SD) against *T. brucei* cell growth (μM)	IC_{50} of mammalian cells/IC_{50} of *T. brucei*	*CLog P* (calculated with ChemDraw Pro 12.0.1 CambridgeSoft)
1		1.13 ± 0.10	2.14 ± 1.29	0.5	4.04
2		1.97 ± 0.21	2.56 ± 1.36	0.8	4.04
3		3.35 ± 0.40	0.42 ± 0.21	8.0	4.61
4		0.91 ± 0.05	1.36 ± 0.79	0.7	5.61
5		0.21 ± 0.01	1.22 ± 0.45	0.2	5.47
6		2.28 ± 0.09	4.70 ± 2.68	0.5	6.19
7		1.46 ± 0.06	0.69 ± 0.28	2.1	3.73
8		3.01 ± 0.12	1.85 ± 0.99	1.6	3.73
9		0.22 ± 0.01	1.48 ± 0.68	0.1	5.16
10		0.20 ± 0.01	3.22 ± 1.49	0.1	4.27

Table 3 (*continued*)

Entry		IC_{50} (±SD) against SKBR-3 breast cancer cell growth (μM)	IC_{50} (±SD) against *T. brucei* cell growth (μM)	IC_{50} of mammalian cells/IC_{50} of *T. brucei*	*CLog P* (calculated with ChemDraw Pro 12.0.1 CambridgeSoft)
11		0.30 ± 0.02	4.93 ± 2.27	0.1	3.60
12		43.27 ± 7.38	22.29 ± 9.36	1.9	3.39
13		11.05 ± 4.76	118.04 ± 64.29	0.1	10.27
14		0.80 ± 0.01	2.09 ± 1.07	0.4	5.73
15		2.78 ± 0.29	1.58 ± 0.71	1.8	5.18
16		0.19 ± 0.14	2.58 ± 0.92	0.1	3.96
17		34.02 ± 2.01	6.85 ± 3.44	5.0	4.29
18		0.15 ± 0.05	2.06 ± 0.76	0.1	4.22
19		0.68 ± 0.32	6.04 ± 2.29	0.1	4.22
20		6.88 ± 3.18	5.56 ± 2.33	1.2	4.22
21		2.16 ± 1.08	1.88 ± 0.92	1.1	4.38

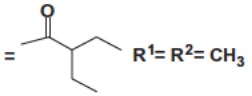
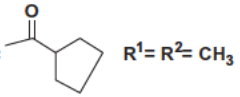
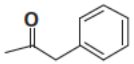
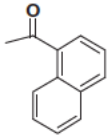
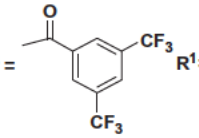
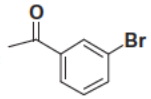
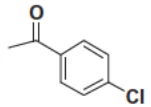
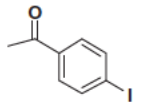
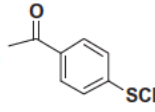
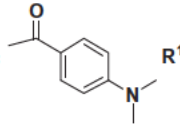
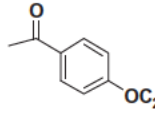
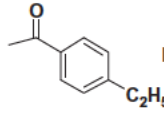
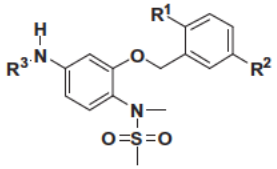
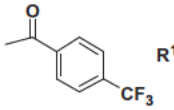
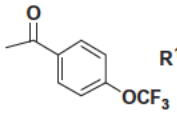
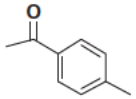
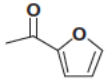
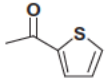
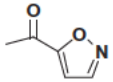
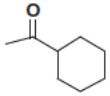
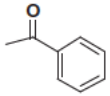
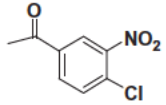
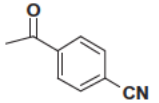
22	R^3 =  $R^1 = R^2 = CH_3$	51.24 ± 4.49	12.93 ± 6.51	4.0	4.76
23	R^3 =  $R^1 = R^2 = CH_3$	11.21 ± 4.47	5.46 ± 1.92	2.1	4.34
24	R^3 =  $R^1 = R^2 = CH_3$	120.8 ± 35.09	51.26 ± 15.02	2.3	4.63
25	R^3 =  $R^1 = R^2 = CH_3$	3.95 ± 2.09	4.94 ± 1.68	0.8	5.47
26	R^3 =  $R^1 = R^2 = CH_3$	55.35 ± 4.11	1.62 ± 1.23	34.2	6.07
27	R^3 =  $R^1 = R^2 = CH_3$	7.34 ± 3.99	2.53 ± 1.47	2.9	5.16
28	R^3 =  $R^1 = R^2 = CH_3$	2.15 ± 0.94	1.95 ± 1.07	1.1	5.01
29	R^3 =  $R^1 = R^2 = CH_3$	0.13 ± 0.07	2.09 ± 1.23	0.1	5.42
30	R^3 =  $R^1 = R^2 = CH_3$	0.66 ± 0.32	3.11 ± 2.16	0.2	4.86
31	R^3 =  $R^1 = R^2 = CH_3$	1.01 ± 0.52	3.93 ± 2.21	0.3	4.47
32	R^3 =  $R^1 = R^2 = CH_3$	0.41 ± 0.03	2.22 ± 1.36	0.2	4.75
33	R^3 =  $R^1 = R^2 = CH_3$	2.48 ± 1.44	2.14 ± 1.26	1.2	5.33

Table 3 (*continued*)

Entry		IC_{50} (±SD) against SKBR-3 breast cancer cell growth (μM)	IC_{50} (±SD) against *T. brucei* cell growth (μM)	IC_{50} of mammalian cells/IC_{50} of *T. brucei*	*CLog P* (calculated with ChemDraw Pro 12.0.1 CambridgeSoft)
34	R^3 =  $R^1 = R^2 = CH_3$	1.20 ± 0.59	2.43 ± 1.56	0.5	5.18
35	R^3 =  $R^1 = R^2 = CH_3$	0.58 ± 0.29	2.18 ± 1.25	0.3	5.33
36	R^3 =  $R^1 = R^2 = CH_3$	2.82 ± 1.51	5.30 ± 2.38	0.5	4.80
37	R^3 =  $R^1 = R^2 = CH_3$	16.65 ± 2.26	10.13 ± 5.71	1.6	3.48
38	R^3 =  $R^1 = R^2 = CH_3$	20.12 ± 5.28	2.69 ± 1.50	7.5	3.95
39	R^3 =  $R^1 = R^2 = CH_3$	21.88 ± 5.08	12.69 ± 7.71	1.7	2.28
40	R^3 =  $R^1 = R^2 = CH_3$	1.38 ± 0.10	10.61 ± 4.37	0.1	4.90
41	R^3 =  $R^1 = R^2 = CH_3$	3.65 ± 0.19	6.44 ± 4.06	0.6	4.30
42	R^3 =  $R^1 = R^2 = H$	9.22 ± 4.11	2.30 ± 1.19	4.0	3.66
43	R^3 =  $R^1 = R^2 = H$	16.88 ± 6.53	2.80 ± 1.12	6.0	2.79

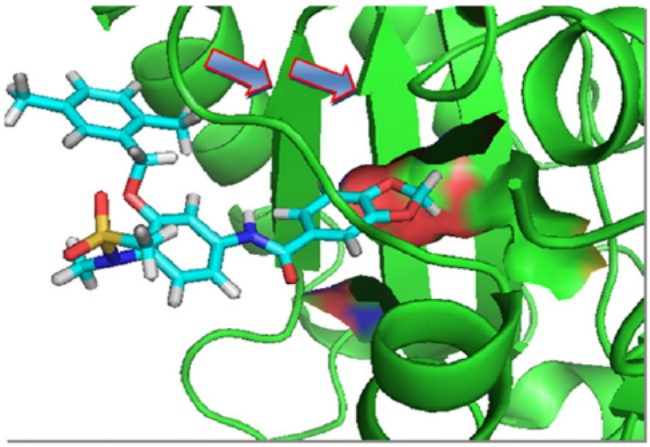


Figure 2. Predicted structure of compound **10** docking in the colchicine-binding domain of bovine tubulin. The Blue arrows indicate the β sheets that interact with the benzamide moiety of the compound. These β sheets show difference in *T. brucei* and bovine tubulin, and form the binding pocket for the benzamide moiety of the compound.

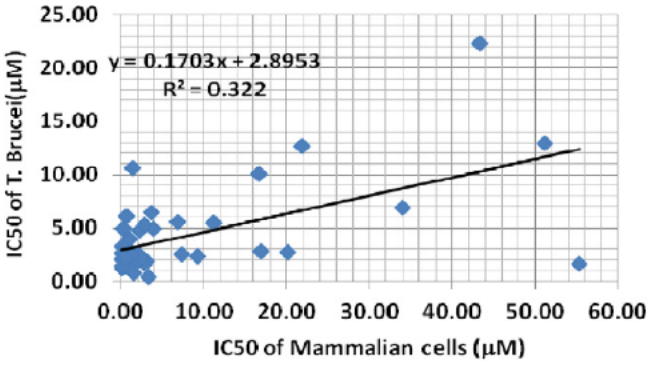


Figure 3. Correlation study of the growth inhibitory effects on mammalian and *T. brucei* cells.

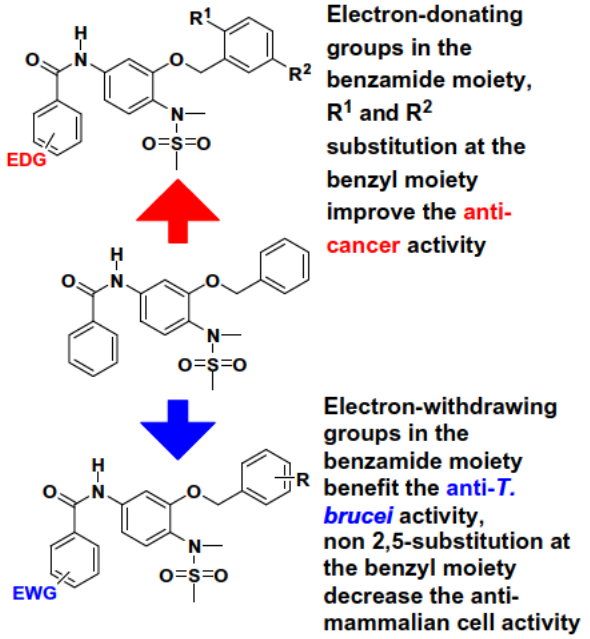


Figure 4. Anti-cancer activity and anti-parasite activity can be enhanced by different substitution groups on the sulfonamide tubulin inhibitors.

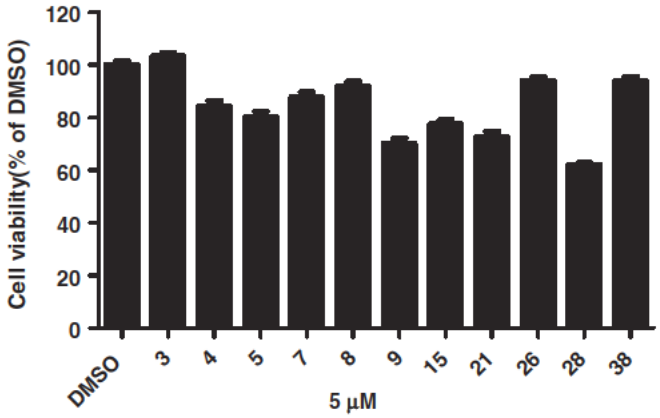


Figure 5. Several Sulfonamide tubulin inhibitors showing potent anti-*T. brucei* activities were tested for their effects on human primary fibroblast IMR-90.

discovery to generate better tubulin inhibitors for trypanosomal disease is currently underway in our laboratory.

Acknowledgments

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four replicates. Cells were grown in RPMI1640 medium in 96-well flat-bottomed plates for 24 h, and were exposed to various concentrations of test compounds dissolved in DMSO (final concentration ⩽0.1%) in medium for 48 h. Controls received DMSO vehicle at a concentration equal to that in drug-treated cells. The medium was removed, replaced by 200 μL of 0.5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide in fresh media, and cells were incubated in the CO_2 incubator at 37 °C for 2 h. Supernatants were removed from the wells, and the reduced 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide dye was solubilized in 200 μL /well DMSO. Absorbance at 570 nm was determined on a SpectraMax Plus384 spectrophotometer (molecular devices).

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