Synthesis and Antitubulin Activity of N^1- and N^4-Substituted 3,5-Dinitro Sulfanilamides against African Trypanosomes and *Leishmania*

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Thirty analogues of N^1-phenyl-3,5-dinitro-N^4,N^4-di-*n*-propylsulfanilamide (GB-II-5, compound **3**), a new antikinetoplastid antimitotic agent, have been synthesized and evaluated. The addition of simple functional groups to the N1 aromatic ring generally decreases antiparasitic and antimitotic potency, but placement of a dibutyl substituent at the N4 nitrogen to give N^1-phenyl-3,5-dinitro-N^4,N^4-di-*n*-butylsulfanilamide (compound **35**) augments antitrypanosomal and antileishmanial activity. Compound **35** possesses IC_{50} values of 0.12 and 2.6 μM against cultured *T. brucei* and *L. donovani* amastigote-like forms, surpassing the activity of compound **3** against these parasites by 3.4- and 1.9-fold, respectively. Compound **35** inhibits the assembly of leishmanial tubulin with an IC_{50} of 6.9 μM and displays antimitotic effects in cultured *T. brucei* as assessed by flow cytometry, but shows little effect on purified mammalian tubulin, and displays 100-fold selectivity for trypanosomes over two mammalian cell lines. Although **3** and **35** were not effective in initial in vivo antitrypanosomal assays, the in vitro potency and selectivity of these compounds make N^1-aryl-3,5-dinitro-N^4,N^4-dialkylsulfanilamides a promising new class of antikinetoplastid agents that act on parasite tubulin.

Introduction

The need for new drugs against leishmaniasis and African trypanosomiasis is clear. These diseases, both caused by protozoan parasites of the order Kinetoplastida, are major public health threats in many developing countries. It is estimated that 1.5−2.0 million new cases of leishmaniasis occur each year, half a million of which are of the potentially fatal visceral manifestation (http://www.who.int/inf-fs/en/fact116.html). Although only about 45 000 cases of African trypanosomiasis are reported annually, the World Health Organization estimates that 300 000 to 500 000 cases of the disease actually occur each year (http://www.who.int/inf-fs/en/fact259.html). Both visceral leishmaniasis and African trypanosomiasis are fatal in the absence of treatment. Unfortunately, the chemotherapeutic mainstays against these diseases are administered by injection, are moderately to extremely toxic, and in some cases are compromised by the development of resistance (see refs 1 and 2 for recent reviews). Rays of hope are provided by the registration of miltefosine in India as the first oral treatment for visceral leishmaniasis[3] and clinical trials of the orally available pentamidine analogue DB289 as a candidate for the treatment of early-stage African trypanosomiasis.[4] The widespread utility of these agents is not yet established, however, and the development of new drug candidates with oral activity against these diseases remains critical.

Tubulin, which plays an indispensable role in eukaryotic cell division, is an established drug target in anticancer chemotherapy[5] and in the treatment of helminth infections.[6] Clearly, selective ligands for kinetoplastid tubulin would be of great interest as drug candidates against leishmaniasis and African trypanosomiasis. Previous work from our group and another laboratory has shown that classical mammalian colchicine-site antimicrotubule agents possess little activity against purified leishmanial tubulin[7] and trypanosomal tubulin,[8] indicating that differences in drug susceptibility exist between the kinetoplastid and mammalian proteins. Over 10 years ago, the antimitotic herbicides trifluralin **1**[9] and oryzalin **2**[10] were suggested as selective lead compounds against leishmanial tubulin. However, no reports of related molecules with improved activity against leishmanial tubulin or with heightened efficacy against the parasite have appeared until recently. In 2002, we showed that simple synthetic modifications to the N1 and N4 positions of oryzalin's sulfonamide core augmented activity compared to the parent compound against purified leishmanial tubulin and against *Leishmania donovani* parasite growth.[11] Subsequent work from our lab indicated that one of these compounds, N^1-phenyl-3,5-dinitro-N^4,N^4-di-*n*-propylsulfanilamide (GB-II-5, compound **3** in this paper), possessed selectivity for leishmanial tubulin compared to mammalian tubulin in vitro and affected cell division in *Leishmania* parasites. Compound **3** also blocked the growth of African trypanosomes in vitro and caused a striking accumulation of *T. brucei* parasites in the G_2M cell cycle phases at 0.5 μM.[12] Compound **3** is thus an exciting lead molecule against kinetoplastid parasites that selectively targets the tubulin of these organisms (see Figure 1 for the structures of compounds **1**−**3**).

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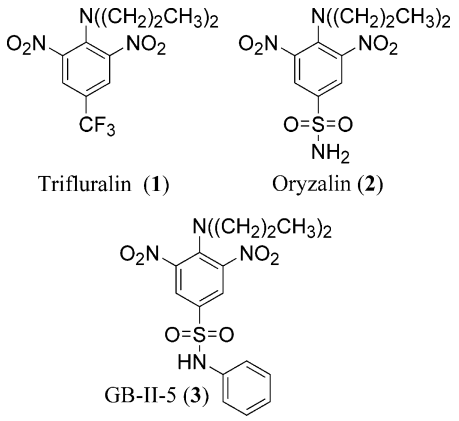
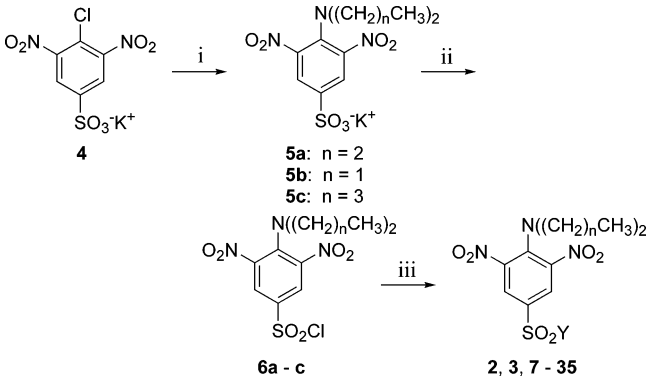


Figure 1. Structures of compounds **1**−**3**.

Scheme 1[a]



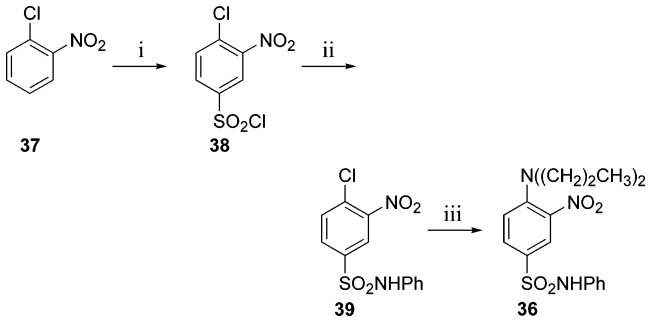
[a] Reagents and conditions: (i) dialkylamine or dialkylamine/MeOH, reflux; (ii) PCl_5/CH_2Cl_2; (iii) method A: 7 N ammonia in MeOH, 40 °C; method B: YH/pyridine, 45 °C; method C: YH; method D, YH in THF; method E, YH in CH_2Cl_2. Note: Y = NHR or NR_1R_2 (see Table 1).

We now report the synthesis and in vitro antileishmanial and antitrypanosomal activity of 30 compounds based on **3**, as well as the antitubulin effects and selectivity of the molecules with the most potent antikinetoplastid activity. Most of these agents possess variations at the N1 position of oryzalin's sulfanilamide core, although we also show that modification at the N4 position can augment antiparasitic potency. In general, the compounds with the highest activity against purified leishmanial tubulin are also the most active against *L. donovani* and *T. brucei* parasites in vitro.

Chemistry

Dinitroanilines were synthesized as outlined in Scheme 1. A modified Ullman condensation of 3,5-dinitro-4-chloro-benzenesulfonate **4**[13] provided intermediates **5a**−**c** in high yield. These N4,N4-disubstituted anilinosulfonate derivatives were then converted to sulfonyl chlorides **6a**−**c** by reaction with PCl_5 in dichloromethane as solvent. Transformation of compounds **6a**−**c** to the target sulfonamides **2**, **3**, and **7**−**35** was accomplished by reaction of the sulfonyl chlorides with ammonia, aliphatic amines, or aromatic amines in pyridine at 45 °C. Treatment of **6a** with methanolic ammonia (method A) provided oryzalin **2** in excellent yield. Conversion of the sulfonyl chlorides to the N^1-aryl compounds **3**, **7**−**17**, and **34**−**36** was accomplished in good yield by heating the aromatic amines with **6a**−**6c** in pyridine at 45 °C

Scheme 2[a]



[a] Reagents and conditions: (i) $ClSO_3H$, 120 °C; (ii) $PhNH_2$/pyridine, 45 °C; (iii) dipropylamine, reflux.

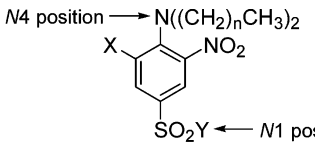
(method B). Alkylamines reacted with **6a** in the amine as solvent (method C), in THF (method D), or in dichloromethane (method E) to form sulfonamides **18**−**33** in good to excellent yield.

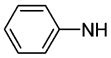
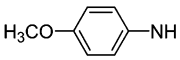
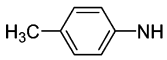
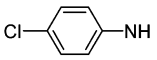
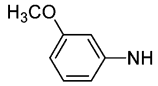
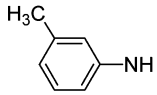
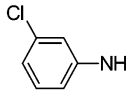
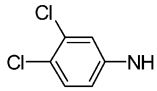
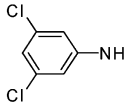
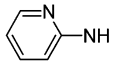
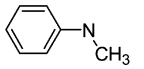
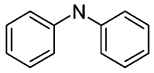
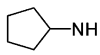
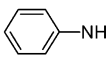
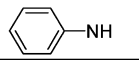
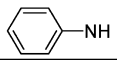
The mononitrosulfonamide derivative **36** was synthesized starting from 1-chloro-2-nitrobenzene **37** as shown in Scheme 2. Thus **37**, when reacted with chlorosulfonic acid at 120 °C, produces the sulfonyl chloride derivative **38**, which when treated with aniline in the presence of pyridine at 45 °C affords sulfonamide **39**. Compound **39** was converted to mononitro N1-substituted sulfonamide **36** through the Ullmann condensation of **39** with dipropylamine.

Biological Results

Table 1 shows the structures of the compounds synthesized, and Table 2 displays the in vitro activities of these agents against axenic amastigote-like *L. donovani*, *T. brucei* variant 221 bloodstream forms, and two mammalian cancer cell lines. Most of these compounds are more active against *T. brucei* than against *L. donovani*, perhaps because of the rapid proliferation rate of trypanosomes in culture. In general, an aryl substituent placed on the sulfonamide nitrogen (the N1 position of the sulfanilamide core) increases activity against kinetoplastid parasites compared to oryzalin **2**. The meta-substituted compounds were more active against *Leishmania* and *T. brucei* than those molecules containing a para substituent, indicating a steric bias against para-substituted compounds. The *m*-chloro compound **12** possesses an activity similar to that of compound **3** against *Leishmania*, while **10** is the most active meta-substituted compound against *T. brucei*. When a dipropyl substituent is present at the N4 position, as is the case with most of the analogues, the 3,5-dichloro-substituted **14** possesses the strongest antileishmanial activity, being slightly more active against *L. donovani* than the mono-chlorinated **12** and the unsubstituted **3**. In vitro antileishmanial activity generally correlates with the inhibition of leishmanial tubulin assembly shown in Table 3, where the activity of the five most potent analogues against the proposed target follows the order **14** > **3** ∼ **35** > **12** > **10**. Only compound **13**, which is a comparatively weak leishmanial tubulin inhibitor, joins the five compounds mentioned above as agents with IC_{50} values below 10 μM against *L. donovani* in vitro. All of the N^1-alkyl compounds are considerably less active than the most potent N^1-aryl analogues, although analogues with N^1-monoalkyl substituents bearing less than three carbons are more

Table 1. N1- and N4-Substituted 3,5-Dinitrosulfanilamides Synthesized



Compound	n	X	Y	Compound	n	X	Y
2	2	NO_2	NH_2	**3**	2	NO_2	
7	2	NO_2		**8**	2	NO_2	
9	2	NO_2		**10**	2	NO_2	
11	2	NO_2		**12**	2	NO_2	
13	2	NO_2		**14**	2	NO_2	
15	2	NO_2		**16**	2	NO_2	
17	2	NO_2		**18**	2	NO_2	$NHCH_3$
19	2	NO_2	$N(CH_3)_2$	**20**	2	NO_2	$NHCH_2CH_3$
21	2	NO_2	$N(CH_2CH_3)_2$	**22**	2	NO_2	$NH(CH_2)_2CH_3$
23	2	NO_2	$N((CH_2)_2CH_3)_2$	**24**	2	NO_2	$NH(CH_2)_3CH_3$
25	2	NO_2	$N((CH_2)_3CH_3)_2$	**26**	2	NO_2	$NH(CH_2)_4CH_3$
27	2	NO_2	$NH(CH_2)_5CH_3$	**28**	2	NO_2	$N((CH_2)_5CH_3)_2$
29	2	NO_2		**30**	2	NO_2	
31	2	NO_2		**32**	2	NO_2	
33	2	NO_2		**34**	1	NO_2	
35	3	NO_2		**36**	2	H	

potent against *T. brucei* than oryzalin **2**. The compound with the most potent antikinetoplastid activity in this series is **35**, which contains a phenyl ring at N1 and a dibutyl substitution at N4. In agreement with our previous observation that **3** was over an order of magnitude more potent against *T. brucei* bloodstream forms than against *L. donovani* axenic amastigotes,[12] **35** is 48 times more potent against African trypanosomes than against *L. donovani* in vitro.

Although the structure−activity relationships for the oryzalin analogues are similar for *L. donovani* and *T. brucei*, there are some intriguing distinctions. In addition to differences in the order of activity for the meta-substituted N^1-aryl compounds mentioned earlier, the 3,5-dichloro compound **14** is substantially less active against trypanosomes than compounds **3**, **10**, **11**, and **12**. Also, the compounds with short N^1-monoalkyl chains (**18**, **20**, **29**, and **30**) are more active than **14** against *T. brucei*. This is despite the fact that compound **14** inhibits leishmanial tubulin assembly with an IC_{50} of 5.8 μM, while compounds **18** and **29** show little activity in this assay when tested at a concentration of 10 μM (see Table 3). These results suggest that differences in drug susceptibility exist between leishmanial and trypanosomal tubulin. Given that leishmanial α- and β-tubulins share 94% and 93% amino acid sequence identity with tubulin from African trypanosomes, these data are a bit surprising. However, even our most potent compounds show little activity against porcine brain tubulin, despite the fact that mammalian tubulins share approximately 80% amino acid identity with leishmanial tubulins. When tested at a concentration of 40 μM, compounds **3**, **7**−**14**, **18**, **29**, and **34**−**36** inhibit the assembly of porcine brain tubulin by less than 20% (in five separate experiments, compound **3** inhibits the assembly of the mammalian protein by 14 ± 10%). Substitutions of one or two amino acids in the target protein are thus likely to have a dramatic influence on the binding affinities of these compounds for tubulins from different species.

Other structural modifications decrease antikinetoplastid activity. Compared to compound **3**, replacement of the N1 hydrogen with a methyl group as in **16** decreases antikinetoplastid activity 2.5- to 4-fold, while exchange of the N1 hydrogen with a phenyl group as in **17** causes a more drastic diminution in activity. Replacement of a nitro group on the sulfanilamide aromatic ring with a hydrogen also had an adverse effect

Table 2. IC_{50} Values for N1- and N4-Substituted 3,5-Dinitrosulfanilamides (μM) against Protozoan Parasites and Mammalian Cell Lines

compd	*L. donovani* axenic amastigotes	*T. b. brucei* variant 221	J774 macrophages	PC3 prostate
2	65 ± 4	12 ± 0	44 ± 8	56 ± 20
3	5.0 ± 1.1	0.41 ± 0.05	28 ± 4	31 ± 8
7	32 ± 15	19 ± 3	15 ± 7	31 ± 5
8	32 ± 13	13 ± 0	11 ± 2	21 ± 2
9	13 ± 3	13 ± 3	10 ± 3	16 ± 5
10	8.1 ± 3.3	1.3 ± 0.2	25 ± 4	36 ± 10
11	23 ± 1	1.9 ± 0.2	16 ± 2	25 ± 0
12	5.5 ± 0.1	2.1 ± 0.2	8.7 ± 0.2	18 ± 1
13	5.0 ± 0.8	7.9 ± 0.6	7.2 ± 0.6	15 ± 4
14	3.7 ± 0.9	8.0 ± 0.6	5.1 ± 0.4	11 ± 5
15	60 ± 6	9.2 ± 1.7	45 ± 16	ND[a]
16	21 ± 3	1.0 ± 0.2	11 ± 3	>100
17	>100	7.0 ± 0.5	>100	>100
18	>100	5.3 ± 0.4	ND[a]	ND[a]
19	>100	5.8 ± 1.0	ND[a]	ND[a]
20	>100	5.4 ± 0.5	ND[a]	ND[a]
21	27 ± 0	8.3 ± 1.8	ND[a]	ND[a]
22	54 ± 9	8.9 ± 0.8	ND[a]	ND[a]
23	55 ± 3	17 ± 1	ND[a]	ND[a]
24	50 ± 24	12 ± 1	ND[a]	ND[a]
25	48 ± 7	38 ± 9	ND[a]	ND[a]
26	43 ± 11	17 ± 3	ND[a]	ND[a]
27	26 ± 5	15 ± 1	ND[a]	ND[a]
28	>100	>50	ND[a]	ND[a]
29	>100	4.1 ± 0.3	ND[a]	ND[a]
30	43 ± 2	3.4 ± 0.5	ND[a]	ND[a]
31	50 ± 7	11 ± 1	ND[a]	ND[a]
32	>100	>100	ND[a]	ND[a]
33	47 ± 4	6.5 ± 0.6	ND[a]	ND[a]
34	11 ± 3	2.8 ± 0.3	48 ± 2	56 ± 14
35	2.6 ± 0.3	0.12 ± 0.07	12 ± 1	13 ± 2
36	43 ± 3	20± 2	31 ± 5	48 ± 15
pentamidine	2.0 ± 0.1	0.013 ± 0.003	ND[a]	ND[a]
suramin	ND[a]	0.21 ± 0.10	ND[a]	ND[a]
ansamitocin P3	ND[a]	ND[a]	0.00047 ± 0.00022	0.000060 ± 0.000042

[a] ND: not determined.

Table 3. Antimicrotubule Activity of Selected Compounds

compd	% inhibition of leishmanial tubulin assembly at 10 μM compound[a]	IC_{50} (μM)[b]
3	89 ± 10[c]	6.7 ± 0.7
7	<20	ND[d]
8	<20	ND[d]
9	<20	ND[d]
10	39 ± 3	ND[d]
11	<20	ND[d]
12	57 ± 3	ND[d]
13	<20	ND[d]
14	102 ± 3	5.8 ± 0.7
16	<20	ND[d]
18	<20	ND[d]
22	<20	ND[d]
29	<20	ND[d]
31	<20	ND[d]
34	<20	ND[d]
35	104 ± 1	6.9 ± 0.0
36	<20	ND[d]

[a] IC_{50} value ± standard deviation of one duplicate experiment unless otherwise noted. [b] IC_{50} value ± standard deviation of two duplicate experiments. [c] IC_{50} value ± standard deviation of five duplicate experiments. [d] ND: not determined.

on antiparasitic activity and potency against leishmanial tubulin (note the 49-fold loss in antitrypanosomal activity in **36** compared to **3**). We earlier reported that restricting the conformation of the N^4-alkylamino chain and changing the sulfonamide group at N1 to another functional group decreased antileishmanial and antitubulin activity.[11]

To assess the selectivity of the more potent N^1-aryl compounds for kinetoplastid parasites, these agents were examined for their effects on the proliferation of murine J774 macrophages and PC3 prostate cancer cells. Many of the compounds, including the lead compound oryzalin **2**, showed little selectivity for kinetoplastid parasites over the tumor cell lines. This is not surprising, given that oryzalin has been investigated as an anticancer candidate.[14] However, striking antitrypanosomal selectivity is observed for compounds **3** and **35**. The selective action of compound **3** against African trypanosomes was recently demonstrated,[12] and compound **35** is 100-fold and 110-fold more active against *T. brucei* than J774 macrophages and PC3 prostate cancer cells in vitro, respectively. Although compounds **3**, **10**, and **35** are clearly more active against *L. donovani* in vitro than against the mammalian cell lines, the selectivity in this case is much less dramatic. These agents are 5.6-fold, 3.1-fold, and 4.6-fold more active against *L. donovani* than against the J774 cells. It is likely that antitubulin activity plays a major factor in selectivity, since compounds **3**, **10**, and **35** all inhibit leishmanial tubulin assembly at low micromolar concentrations (Table 3) and are much less potent against porcine brain tubulin. Given the results in Table 3 and the known effect of oryzalin **2** against intracellular calcium signaling in mammalian cells,[14] the mild to moderate toxicity against the two mammalian cell lines displayed by the N^1-aryl compounds may be due to mechanisms other than interference with microtubule-mediated processes.

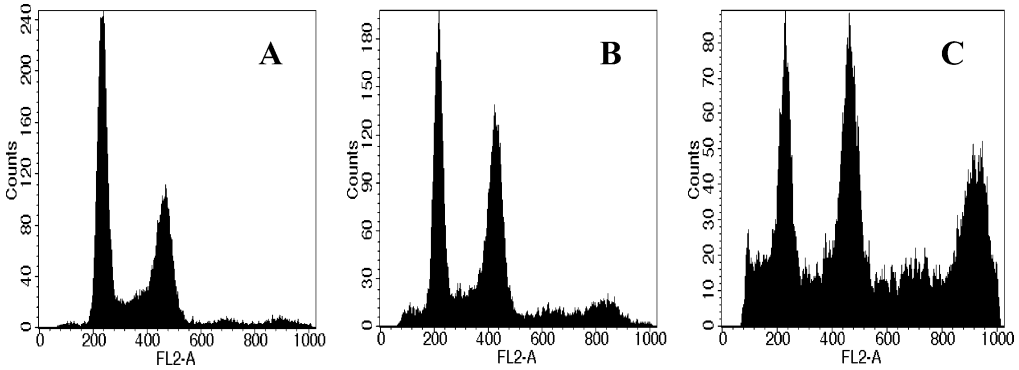


Figure 2. Flow cytometry analysis of *T. brucei* treated with compound **35**. Bloodstream-form *T. brucei* parasites (MITat 1.2, variant 221) were incubated for 24 h at 37 °C in HMI-9 medium in the presence of 1% DMSO (A), 0.2 μM compound **35** (B), or 0.4 μM compound **35** (C). Trypanosomes were fixed, stained with propidium iodide, and analyzed by flow cytometry as described in the Experimental Section. The results shown are from a representative experiment performed on three separate occasions. FL2-A, the *x*-axis label, refers to the fluorescence intensity of a given particle as measured by the flow cytometer.

Table 4. In Vivo Assays with Compounds **3** and **35** against *T. b. brucei* STIB 795

group	dose (mg/kg)	cured/infected	average survival time (day)
control		0/4	6.75
3	4 × 20	0/4	8.0
35	4 × 20	0/4	8.0

Past work showed that exposure of *L. donovani* and *T. brucei* to compound **3** caused clear, dose-dependent effects on the parasite cell cycle, consistent with the hypothesis that this agent exerts its antiparasitic effects through a tubulin mechanism.[12] Similar effects are observed when African trypanosomes are cultured with compound **35** (Figure 2). Trypanosomes incubated for 24 h in the presence of 200 nM compound **35** show an increase in the percentage of cells in the G_2M cell cycle phases compared to those in the G_1 phase. When the appropriate peak areas were quantitated in four independent experiments, the G_2M/G_1 ratio was 0.68 ± 0.08 for control samples and 1.14 ± 0.09 for cultures treated with 200 nM compound **35** (values represent mean ± standard deviation). Treatment with 400 nM compound **35** causes the appearance of cell types possessing 4 times the amount of DNA present in G_1 parasites, indicating a dramatic effect on the trypanosome cell cycle under these conditions. These results, taken together with the effect of compound **35** on purified leishmanial tubulin, provide support for the hypothesis that compound **35** also acts through a tubulin mechanism against kinetoplastid parasites.

To examine the in vivo antitrypanosomal potential of compounds **3** and **35**, these agents were tested for activity against *T. b. brucei* strain STIB 795 in mice (Table 4). When the compounds were given an intraperitoneal dose of 20 mg kg^{-1} day^{-1} for 4 days, no significant increase in survival time was observed compared to control animals. No signs of toxicity were observed when either of these compounds were given at the 20 mg/kg dose.

Conclusions

N^1-Aryl-N^4-dialkyl-3,5-dinitrosulfanilamides are exciting new antimitotic agents with activity against kinetoplastid parasites. Dramatic selectivity has been achieved against African trypanosomes with compounds **3** and **35**, and experiments with purified leishmanial tubulin and cultured organisms indicate that these compounds act by interfering with microtubules in kinetoplastid parasites. Future studies will seek to augment the potency of the N^1-aryl-N^4-dialkyl-3,5-dinitrosulfanilamides against trypanosomes and *Leishmania* in vitro and to determine what pharmacokinetic barriers must be overcome to attain in vivo antikinetoplastid activity with these agents. Since the synthesis of these compounds is straightforward, a wide variety of analogues can be prepared to optimize in vitro and in vivo activity. In addition, the inexpensive large-scale synthesis of appropriate candidates should be possible. Given the economic realities of chemotherapy for trypanosomiasis and leishmaniasis, the simplicity of the N^1-aryl-N^4-dialkyl-3,5-dinitrosulfanilamides bodes well for their future development if in vivo activity can be achieved.

Experimental Section

General Methods. Unless otherwise indicated, all reagents and solvents were from Aldrich and were used without further purification. The melting points were measured on Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker 250, 400, and 600 MHz NMR spectrometers. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA. Thin-layer chromatography was conducted on precoated TLC plates from E. Merck. The yield of sulfonamides is given as the yield of the products after two steps, i.e., after the formation of the sulfonyl chloride and after the subsequent synthesis of the sulfonamide target by reaction of the sulfonyl chloride with the appropriate amine.

3,5-Dinitro-N^4,N^4-di-*n*-propylsulfonate (5a). A suspension of 3,5-dinitro-4-chlorosulfonate[13] (**4**, 2.82 g, 8.8 mmol) in dipropylamine (50 mL) was refluxed for 3 h. By that time almost all the solid had dissolved. After 3 h, the solvent was evaporated, and water was added. The resulting yellow precipitate was filtered and collected. The product was purified by silica gel column chromatography, eluting with MeOH/EtOAc (1:8): yield 2.6 g, 77%; ^{1}H NMR (DMSO-d_6) δ 0.88 (t, 3H, J = 7.25 Hz), 0.90 (t, 3H, J = 7.75 Hz), 1.51 (m, 2H), 1.61 (m, 2H), 2.88 (q, 4H, J = 8.50 Hz), 8.17 (s, 2H); MS *m*/*z* 348 $(M + H)^+$.

3,5-Dinitro-N^4,N^4-di-*n*-ethylsulfonate (5b). A suspension of 3,5-dinitro-4-chlorosulfonate (**4**, 1.41 g, 4.4 mmol) in diethylamine (30 mL) was refluxed for 3 h. After 3 h, the solvent was evaporated, then water was added to the solid. The resulting yellow precipitate was filtered out and collected. The product was purified by silica gel column chromatography, eluting with MeOH/EtOAc (1:8): yield 781 mg, 49%; ^{1}H NMR (DMSO-d_6)

δ 0.96 (t, 6H, J = 7.25 Hz), 2.95 (q, 4H, J = 7.25 Hz), 8.10 (d, 2H, J = 1 Hz).

3,5-Dinitro-N^4,N^4-di-*n*-butylsulfonate (5c). A suspension of 3,5-dinitro-4-chlorosulfonate (**4**, 0.70 g, 2.2 mmol) in dibutylamine (1 mL, 5.98 mmol) in 30 mL of methanol was refluxed for 3 h. After 3 h, the solvent was evaporated. The product was purified by silica gel column chromatography, eluting with MeOH/EtOAc (1:8): yield 689 mg, 76%; ^{1}H NMR ($CDCl_3$) 0.84 (m, 6H), 1.30 (m, 8H), 2.89 (m, 4H), 8.24 (s, 2H).

3,5-Dinitro-N^4,N^4-di-*n*-propylsulfanilamide (2). To a suspension of 3,5-dinitro-N^4,N^4-di-*n*-propylsulfonate (**5a**, 347 mg, 0.90 mmol) in dichloromethane (10 mL), was added PCl_5 (624 mg, 3 mmol), and the reaction mixture was stirred for 2 h. The dichloromethane layer was collected after washing with water and was dried with Na_2SO_4. The product 3,5-dinitro-N^4,N^4-di-*n*-propylsulfonyl chloride (**6a**) was used without further purification. To this product was added 10 mL of 7 N ammonia in methanol (method A), and the mixture was stirred for 3 h at 40 °C. The solvent was evaporated and the product was purified by silica gel column chromatography, eluting with hexane/ethyl acetate (2:1) and crystallized in dichloromethane/hexane: yield 286 mg, 92%; mp = 138−140 °C (lit. 141 °C [15]); ^{1}H NMR (DMSO-d_6) δ 0.78 (t, 6H, J = 7.25 Hz), 1.52 (m, 4H), 2.91 (t, 4H, J = 7.25 Hz), 7.62 (bs, 2H), 8.38 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 11.26, 20.78, 53.80, 127.55, 135.69, 140.24, 145.04; MS (FAB) *m*/*z* calcd 369.0826, measured 369.0830 (M + Na)$^+$. Anal. ($C_{12}H_{18}N_4O_6S$) C, H, N.

General Procedure for the Synthesis of N^1-Aryl-3,5-dinitro-N^4,N^4-di-*n*-propylsulfanilamide Derivatives (3, 7−17). To a suspension of 3,5-dinitro-N^4,N^4-di-*n*-propylsulfonic acid **5a** in dichloromethane was added PCl_5 (2 equiv), and the reaction mixture was stirred for 2 h. The dichloromethane layer was extracted after washing with water and drying with Na_2SO_4. The product 3,5-dinitro-N^4,N^4-di-*n*-propylsulfonyl chloride **6a** was used without further purification. Pyridine was added, the temperature was adjusted to 40 °C, 2−6 equiv of the aniline derivative was added, and the reaction mixture was stirred for 3 h at 40 °C (method B). Pyridine was then evaporated in vacuo, the dark residue was washed with water and extracted with ethyl acetate, and the product was purified by silica gel column chromatography.

N^1-Phenyl-3,5-dinitro-N^4,N^4-di-*n*-propylsulfanilamide (3). 3 was synthesized by the reaction of sulfonyl chloride **6a** (prepared from **5a**, 347 mg, 0.9 mmol) and 547 μL (6 mmol) of aniline in 20 mL of pyridine. The product was isolated by chromatography (hexane/EtOAc (3:1)) and crystallized with dichloromethane/hexane to afford a yellow crystalline solid: yield 342 mg, 90%. Melting point, ^{1}H NMR, ^{13}C NMR, and HRMS data have been reported previously.[11] Anal. ($C_{18}H_{22}N_4O_6S$) C, H, N.

N^1-(4-Methoxy)phenyl-3,5-dinitro-N^4,N^4-di-*n*-propylsulfanilamide (7). 7 was synthesized by the reaction of sulfonyl chloride **6a** (prepared from **5a**, 347 mg, 0.90 mmol) and 369 mg (3 mmol) of 4-methoxyaniline in 20 mL of pyridine. The product was isolated by chromatography (hexane/EtOAc (6:1)) and crystallized using dichloromethane/hexane to obtain a yellow crystalline solid: yield 321 mg, 81%; mp = 121 °C; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 6H, J = 7.25 Hz), 1.61 (m, 4H), 2.91 (m, 4H), 3.79 (s, 3H), 6.58 (bs, 1H), 6.83 (dd, 2H, J = 2.00, 7.00 Hz), 7.30 (dd, 2H, J = 2.00, 7.00 Hz), 8.04 (d, 2H, J = 0.75 Hz); ^{13}C NMR ($CDCl_3$) δ 11.08, 20.65, 53.99, 55.49, 114.85, 125.92, 127.42, 128.60, 129.31, 141.53, 144.40, 158.71; MS (FAB) *m*/*z* calcd for $C_{19}H_{24}N_4O_7S$ (M + Na)$^+$ 475.1263, measured (M + Na)$^+$ 475.1275. Anal. ($C_{19}H_{24}N_4O_7S$) C, H, N.

N^1-(4-Methyl)phenyl-3,5-dinitro-N^4,N^4-di-*n*-propylsulfanilamide (8). 8 was synthesized by the reaction of sulfonyl chloride **6a** (prepared from **5a**, 347 mg, 0.90 mmol) and 321 mg (3 mmol) of 4-methylaniline in 20 mL of pyridine. The product was isolated by chromatography (hexane/EtOAc (6:1)) and crystallized using dichloromethane/hexane to obtain yellow crystals: yield 338 mg, 88%; mp = 107 °C; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 6H, J = 7.25 Hz), 1.60 (m, 4H), 2.31 (s, 3H), 2.91 (t, 4H, J = 7.75 Hz), 6.77 (bs, 1H), 7.10 (d, 2H, J = 8.25 Hz), 7.20 (d, 2H, J = 8.25 Hz), 8.10 (d, 2H, J = 0.75 Hz)); ^{13}C NMR ($CDCl_3$) δ 11.08, 20.65, 20.85, 53.99, 122.87, 128.61, 129.33, 130.27, 132.44, 136.76, 141.61, 144.39; MS (FAB) *m*/*z* calcd for $C_{19}H_{24}N_4O_6S$ (M + Na)$^+$ 459.1314, measured (M + Na)$^+$ 459.1316. Anal. ($C_{16}H_{24}N_4O_6S$) C, H, N.

N^1-(4-Chloro)phenyl-3,5-dinitro-N^4,N^4-di-*n*-propylsulfanilamide (9). 9 was synthesized by the reaction of sulfonyl chloride **6a** (prepared from **5a**, 347 mg, 0.90 mmol) and 351 mg (3 mmol) of 4-chloroaniline in 20 mL of pyridine. The product was isolated by chromatography (hexane/EtOAc (6:1)) and crystallized using dichloromethane/hexane to obtain yellow crystals: yield 258 mg, 63%; mp = 130 °C; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 6H, J = 7.50 Hz), 1.61 (m, 4H), 2.94 (m, 4H), 6.95 (bs, 1H), 7.09 (d, 2H, J = 7.25 Hz), 7.30 (d, 2H, J = 7.25 Hz), 8.14 (d, 2H, J = 0.15 Hz); ^{13}C NMR ($CDCl_3$) δ 11.09, 20.65, 54.03, 123.37, 128.65, 128.77, 129.92, 132.21, 133.75, 141.85, 144.38; MS (FAB) *m*/*z* calcd for $C_{18}H_{21}ClN_4O_6S$ (M + Na)$^+$ 479.0768, measured (M + Na)$^+$ 479.0767. Anal. ($C_{18}H_{21}ClN_4O_6S$) C, H, N.

N^1-(3-Methoxy)phenyl-3,5-dinitro-N^4,N^4-di-*n*-propylsulfanilamide (10). 10 was synthesized by the reaction of sulfonyl chloride **6a** (prepared from **5a**, 201 mg, 0.52 mmol) and 123 mg (1 mmol) of 3-methoxyaniline in 7 mL of pyridine. The product was isolated by chromatography (hexane/EtOAc (6:1)) and crystallized using dichloromethane/hexane to obtain a yellow crystalline solid: yield 137 mg, 58%; mp = 133 °C; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 6H, J = 7.33 Hz), 1.58 (m, 4H), 2.93 (t, 4H, J = 7.40 Hz), 3.78 (s, 3H), 6.49 (bs, 1H), 6.75 (m, 3H), 7.21 (m, 1H), 8.12 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.00, 20.55, 53.89, 55.39, 107.45, 111.70, 113.62, 128.73, 128.89, 130.38, 136.51, 141.72, 144.26, 160.46; MS *m*/*z* 451.1 (M − H)$^-$. Anal. ($C_{19}H_{24}N_4O_7S$) C, H, N.

N^1-(3-Methyl)phenyl-3,5-dinitro-N^4,N^4-di-*n*-propylsulfanilamide (11). 11 was synthesized by the reaction of sulfonyl chloride **6a** (prepared from **5a**, 202 mg, 0.52 mmol) and 107 mg (1 mmol) of 3-methylaniline in 7 mL of pyridine. The product was isolated by chromatography (hexane/EtOAc (6:1)) and crystallized using dichloromethane/hexane to obtain a yellow crystalline solid: yield 125 mg, 55%; mp = 103 °C; ^{1}H NMR ($CDCl_3$) δ 0.86 (m, 6H), 1.57 (m, 4H), 2.31 (s, 3H), 2.93 (t, 4H, J = 7.44 Hz), 6.67 (bs, 1H), 6.90 (m, 2H), 7.03 (d, 1H, J = 7.08 Hz), 7.19 (m, 1H), 8.11 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.03, 20.58, 21.28, 53.93, 119.08, 122.79, 127.25, 128.56, 129.16, 129.47, 135.04, 139.92, 141.57, 144.28; MS *m*/*z* 435.1 (M − H)$^-$. Anal. ($C_{16}H_{24}N_4O_6S$) C, H, N.

N^1-(3-Chloro)phenyl-3,5-dinitro-*N*4,*N*4-di-*n*-propylsulfanilamide (12). 12 was synthesized by the reaction of sulfonyl chloride **6a** (prepared from **5a**, 201 mg, 0.52 mmol) and 117 mg (1 mmol) of 3-chloroaniline in 7 mL of pyridine. The product was isolated by chromatography (hexane/EtOAc (6:1)) and crystallized by dichloromethane/hexane to obtain yellow crystals: yield 125 mg, 52%; mp = 108 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 6H, J = 7.25 Hz), 1.59 (m, 4H), 2.95 (t, 4H, J = 7.43 Hz), 6.74 (s, 1H), 7.17 (m, 4H), 8.16 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.52, 21.06, 54.43, 119.96, 122.17, 126.89, 128.96, 129.15, 131.24, 135.83, 136.90, 142.36, 144.71; MS *m*/*z* 455.1 (M − H)$^-$. Anal. ($C_{18}H_{21}ClN_4O_6S$) C, H, N.

N^1-(3,4-Dichloro)phenyl-3,5-dinitro-*N*4,*N*4-di-*n*-propylsulfanilamide (13). 13 was synthesized by the reaction of sulfonyl chloride **6a** (prepared from **5a**, 347 mg, 0.90 mmol) and 486 mg (3 mmol) of 3,4-dichloroaniline in 20 mL of pyridine. The product was isolated by chromatography (hexane/EtOAc (6:1)) and crystallized using dichloromethane/hexane to obtain a yellow crystalline solid: yield 264 mg, 60%; mp = 111 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (m, 6H), 1.61 (m, 4H), 2.95 (m, 4H), 6.84 (bs, 1H), 7.01 (dd, 1H, J = 2.50, 8.75 Hz)), 7.23 (d, 1H, J = 2.50 Hz), 7.40 (d, 1H, J = 8.75 Hz), 8.16 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.06, 20.63, 54.02, 120.64, 123.27, 128.27, 128.77, 130.16, 131.33, 133.61, 134.84, 142.04, 144.34; MS (FAB) *m*/*z* calcd for $C_{18}H_{20}ClN_4O_6S$ (M + Na)$^+$ 513.0378, measured (M + Na)$^+$ 513.0388. Anal. ($C_{18}H_{22}N_4O_6S$) C, H, N.

N^1-(3,5-Dichloro)phenyl-3,5-dinitro-N^4,N^4-di-*n*-propylsulfanilamide (14). 14 was synthesized by the reaction of sulfonyl chloride **6a** (prepared from **5a**, 347 mg, 0.90 mmol) and 486 mg (3 mmol) of 3,5-dichloroaniline in 20 mL of

pyridine. The product was isolated by chromatography (hexane/EtOAc (6:1)) and crystallized by dichloromethane/hexane to obtain yellow crystals: yield 356 mg, 81%; mp = 144 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 6H, J = 7.50 Hz), 1.63 (m, 4H), 2.95 (q, 4H, J = 6.63 Hz), 6.69 (bs, 1H), 7.06 (d, 1H, J = 0.17 Hz), 7.20 (d, 2H, J = 0.17 Hz), 8.17 (d, 2H, J = 0.25 Hz); ^{13}C NMR ($CDCl_3$) δ 11.01, 20.58, 53.99, 119.28, 126.13, 128.14, 128.64, 136.02, 137.27, 142.02, 144.30; MS m/z 489.1 $(M - H)^-$. Anal. ($C_{18}H_{20}ClN_4O_6S$) C, H, N.

***N*^{1}-(2-Pyridinyl)-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (15). 15** was synthesized by the reaction of sulfonyl chloride **6a** (prepared from **5a**, 347 mg, 0.90 mmol) and 564 mg (6 mmol) of 2-aminopyridine in 20 mL of pyridine. The product was isolated by chromatography (hexane/EtOAc (1:1)) and crystallized using dichloromethane/hexane to afford yellow crystals: yield 326 mg, 86%; mp = 204 °C; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 6H, J = 7.50 Hz), 1.62 (m, 4H), 2.94 (m, 4H), 6.90 (m, 1H), 7.47 (d, 1H, J = 8.00 Hz), 7.85 (m, 1H), 8.24 (m, 1H), 8.31 (s, 2H), 8.65 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.17, 11.25, 20.65, 20.75, 53.67, 54.03, 114.02, 116.12, 127.94, 133.61, 138.62, 141.11, 144.12, 144.83, 155.69; MS m/z 424.0 $(M + H)^+$. Anal. ($C_{18}H_{22}N_4O_6S$) C, H, N.

***N*^{1},*N*^{1}-Phenylmethyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (16). 16** was synthesized by the reaction of sulfonyl chloride **6a** (prepared from **5a**, 347 mg, 0.90 mmol) and 547 μL (6 mmol) of *N*-methylaniline in 20 mL of pyridine. The product was isolated by chromatography (hexane/EtOAc (8:1)) and crystallized by dichloromethane/hexane to afford a yellow crystalline solid: yield 241 mg, 61%; mp = 96 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 (m, 6H), 1.63 (m, 4H), 2.96 (q, 4H, J = 7.00 Hz), 3.24 (s, 3H), 7.15 (m, 2H), 7.35 (m, 3H), 7.87 (d, 2H, J = 0.75 Hz); ^{13}C NMR ($CDCl_3$) δ 11.17, 20.74, 38.46, 54.01, 126.65, 127.37, 128.31, 128.81, 129.41, 140.57, 141.42, 144.52; MS m/z 437.1 $(M + H)^+$. Anal. ($C_{18}H_{22}N_4O_6S$) C, H, N.

***N*^{1}-Diphenyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (17). 17** was synthesized by the reaction of sulfonyl chloride **6a** (prepared from **5a**, 347 mg, 0.90 mmol) and 547 μL (6 mmol) of diphenylamine in 20 mL of pyridine. The product was isolated by chromatography (hexane/EtOAc (16:1)) and crystallized by dichloromethane/hexane to afford a yellow crystalline solid: yield 248 mg, 55%; mp = 132 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (m, 6H), 1.63 (m, 4H), 2.98 (m, 4H), 7.35 (m, 10H), 8.03 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.11, 20.67, 53.96, 117.76, 120.93, 128.17, 128.21, 128.78, 129.28, 129.66, 130.66, 140.57, 141.37, 143.10, 144.45; MS m/z 499.5 $(M + H)^+$. Anal. ($C_{18}H_{22}N_4O_6S$) C, H, N.

Synthesis of *N*^{1}-Alkyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide Derivatives (18−33). To a suspension of 3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfonate (**5a**, 86 mg, 0.22 mmol) in dichloromethane (5 mL) was added PCl_5 (208 mg, 1 mmol), and the reaction mixture was stirred for 2 h. The dichloromethane layer was collected after washing with water, then this layer was dried with Na_2SO_4 and solvent was removed in vacuo. The product 3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfonyl chloride (**6a**) was used without further purification. The sulfonamides were prepared by method C, D, or E. Solvent was evaporated, and the product was purified by silica gel column chromatography.

***N*^{1}-Methyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (18). 18** was synthesized by the reaction of sulfonyl chloride **6a** and 10 mL of 2 M methylamine in THF (method D). The product was isolated by chromatography using hexane/EtOAc (2:1) as eluent and crystallized using dichloromethane/hexane to obtain yellow crystals: yield 88 mg, 97%; mp = 148 °C; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 6H, J = 7.25 Hz), 1.62 (m, 4H), 2.76 (d, 3H, J = 5.25 Hz), 2.97 (m, 4H), 4.38 (bq, 1H), 8.25 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.06, 20.63, 29.31, 53.94, 128.47, 129.55, 141.48, 144.61; MS m/z 361.00 $(M + H)^+$. Anal. ($C_{13}H_{20}N_4O_6S$) C, H, N.

***N*^{1},*N*^{1}-Dimethyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (19). 19** was synthesized by the reaction of sulfonyl chloride **6a** and 10 mL of 2 M dimethylamine in THF (method D). The product was isolated by chromatography (hexane/EtOAc (2:1)) and crystallized using dichloromethane/hexane to obtain yellow crystals: yield 85 mg, 91%; mp = 138 °C; ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 6H, J = 7.25 Hz), 1.63 (m, 4H), 2.81 (s, 6H), 2.98 (m, 4H), 8.15 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.05, 20.62, 37.76, 53.89, 126.88, 128.64, 141.47, 144.65; MS m/z 397.00 $(M + Na)^+$. Anal. ($C_{14}H_{22}N_4O_6S$) C, H, N.

***N*^{1}-Ethyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (20). 20** was synthesized by the reaction of sulfonyl chloride **6a** and 10 mL of 2 M ethylamine in THF (method D). The product was isolated by chromatography (hexane/EtOAc = 3:1) and crystallized using dichloromethane/hexane to obtain yellow crystals: yield 78 mg, 83%; mp = 131 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, J = 7.25 Hz), 1.20 (t, 3H, J = 7.25 Hz), 1.62 (m, 4H), 3.08 (m, 6H), 4.44 (bt, 1H), 8.24 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.06, 15.13, 20.63, 38.41, 53.94, 128.32, 130.64, 141.39, 144.61; MS m/z 372.9 $(M - H)^-$. Anal. ($C_{14}H_{22}N_4O_6S$) C, H, N.

***N*^{1},*N*^{1}-Diethyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (21). 21** was synthesized by the reaction of sulfonyl chloride **6a** and 10 mL (0.79 mol) of diethylamine (method C). The product was isolated by chromatography (hexane/EtOAc (6:1)) and crystallized using dichloromethane/hexane to obtain yellow crystals: yield 73 mg, 73%; mp = 102 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (m, 6H), 1.20 (t, 6H, J = 7.25 Hz), 1.62 (m, 4H), 2.97 (m, 4H), 3.28 (q, 4H, J = 7.00 Hz), 8.18 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.05, 14.27, 20.63, 42.38, 53.92, 128.03, 131.35, 141.07, 144.73; MS m/z 403.1 $(M + H)^+$. Anal. ($C_{16}H_{26}N_4O_6S$) C, H, N.

***N*^{1}-Propyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (22). 22** was synthesized by the reaction of sulfonyl chloride **6a** and 10 mL (0.12 mol) of propylamine (method C). The product was isolated by chromatography (hexane/EtOAc (3:1)) and crystallized using dichloromethane/hexane to obtain yellow crystals: yield 86 mg, 88%; mp = 114 °C; ^{1}H NMR (DMSO-d_6) δ 0.82 (m, 9H), 1.55 (m, 6H), 2.97 (m, 6H), 7.88 (bs, 1H), 8.37 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 11.24, 11.38, 20.74, 22.77, 44.64, 53.71, 128.27, 132.28, 140.59, 145.11; MS m/z 389.3 $(M + H)^+$. Anal. ($C_{15}H_{24}N_4O_6S$) C, H, N.

***N*^{1},*N*^{1}-Dipropyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (23). 23** was synthesized by the reaction of sulfonyl chloride **6a** and 10 mL (0.73 mol) of dipropylamine (method C). The product was isolated by chromatography (hexane/EtOAc (6:1)) to obtain a yellow oil: yield 97 mg, 90%; ^{1}H NMR ($CDCl_3$) δ 0.91 (m, 12H), 1.61 (m, 8H), 2.98 (m, 4H), 3.17 (m, 4H), 8.19 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.01, 20.62, 22.03, 29.56, 50.09, 53.78, 53.91, 128.02, 131.21, 141.00, 144.74; MS m/z 453.2 $(M + Na)^+$.

***N*^{1}-Butyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (24). 24** was synthesized by the reaction of sulfonyl chloride **6a** and 10 mL (0.10 mol) of butylamine (method C). The product was isolated by chromatography (hexane/EtOAc (5:1)) and crystallized using dichloromethane/hexane to obtain yellow crystals: yield 42 mg, 41%; mp = 118 °C; ^{1}H NMR ($CDCl_3$) δ 0.91 (m, 9H), 1.28 (m, 4H), 1.60 (m, 4H), 2.98 (m, 6H), 4.32 (bt, 1H), 8.23 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.06, 13.41, 19.55, 20.63, 31.61, 43.04, 53.95, 128.28, 130.72, 141.35, 144.62; MS m/z 401.0 $(M - H)^-$. Anal. ($C_{16}H_{26}N_4O_6S$) C, H, N.

***N*^{1},*N*^{1}-Dibutyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (25). 25** was synthesized by the reaction of sulfonyl chloride **6a** and 510 μL (3 mmol) of dibutylamine in 10 mL of dichloromethane (method E). The product was isolated by chromatography (hexane/EtOAc (15:1)) to afford a yellow oil that solidified upon refrigeration: yield 81 mg, 71%; mp = 48 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (m, 12H), 1.29 (m, 4H), 1.61 (m, 8H), 2.97 (q, 4H, J = 6.85 Hz), 3.15 (t, 4H, J = 7.50 Hz), 8.16 (d, 2H, J = 1.25 Hz); ^{13}C NMR ($CDCl_3$) δ 11.06, 13.56, 19.78, 20.65, 30.70, 47.96, 53.95, 128.04, 131.22, 140.99, 144.73; MS m/z 459.1 $(M + H)^+$.

***N*^{1}-Pentyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (26). 26** was synthesized by the reaction of sulfonyl chloride **6a** and 450 μL (3 mmol) of amylamine in 10 mL of dichloromethane (method E). The product was isolated by chromatography (hexane/EtOAc (6:1)) and crystallized using dichloromethane/hexane to obtain yellow crystals: yield 91 mg, 87%; mp = 110 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (m, 9H), 1.29 (m, 6H), 1.62 (m, 4H), 2.98 (m, 6H), 4.38 (bt, 1H), 8.23 (d, 2H, J = 0.75

Hz); ^{13}C NMR ($CDCl_3$) δ 11.04, 13.73, 20.63, 22.02, 28.49, 29.21, 43.31, 53.94, 128.29, 130.79, 141.33, 144.63; MS *m/z* 431.1 $(M + H)^+$. Anal. ($C_{17}H_{28}N_4O_6S$) C, H, N.

***N*^{1}-Hexyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (27). 27** was synthesized by the reaction of sulfonyl chloride **6a** and 400 μL (3 mmol) of hexylamine in 10 mL of dichloromethane (method E). The product was isolated by chromatography (hexane/EtOAc (15:1)) and crystallized using dichloromethane/hexane to obtain a yellow crystalline solid: yield 77 mg, 72%; mp = 123 °C; ^{1}H NMR ($CDCl_3$) δ 0.91 (m, 9H), 1.26 (bm, 6H), 1.65 (m, 6H), 3.02 (m, 6H), 4.46 (bt, 1H), 8.23 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.20, 13.88, 20.71, 22.40, 26.12, 29.60, 31.18, 43.42, 54.03, 128.33, 130.91, 141.40, 144.74; MS *m/z* 429.1 $(M - H)^-$. Anal. ($C_{18}H_{30}N_4O_6S$) C, H, N.

***N*^{1},*N*^{1}-Dihexyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (28). 28** was synthesized by the reaction of sulfonyl chloride **6a** and 700 μL of 3 mmol of dihexylamine in 10 mL of dichloromethane (method E). The product was isolated by chromatography (hexane/EtOAc (12:1)) to provide a yellow oil: yield 89 mg, 69%; ^{1}H NMR ($CDCl_3$) δ 0.90 (m, 12H), 1.38 (m, 12H), 1.68 (m, 8H), 2.96 (m, 4H), 3.15 (t, 4H, J = 7.5 Hz), 8.16 (d, 2H, J = 1.5 Hz); ^{13}C NMR ($CDCl_3$) δ 11.03, 13.84, 20.65, 22.39, 26.27, 28.50, 31.27, 48.10, 53.96, 128.00, 131.43, 140.97, 144.77; MS *m/z* 515.3 $(M + H)^+$. Anal. ($C_{24}H_{42}N_4O_6S$) C, H, N.

***N*^{1}-Isopropyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (29). 29** was synthesized by the reaction of sulfonyl chloride **6a** and 10 mL (0.12 mol) of isopropylamine (method C). The product was isolated by chromatography (hexane/EtOAc (6:1)) and crystallized using dichloromethane/hexane to give a yellow crystalline solid: yield 77 mg, 79%; mp = 150 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, J = 7.25 Hz), 1.27 (d, 6H, J = 7.25 Hz), 1.63 (m, 4H), 2.97 (m, 4H), 3.57 (m, 1H), 4.41 (bs, 1H), 8.25 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.11, 20.70, 23.85, 46.68, 54.07, 128.22, 131.99, 141.33, 144.71; MS *m/z* 389.0 $(M + H)^+$. Anal. ($C_{15}H_{24}N_4O_6S$) C, H, N.

***N*^{1}-Cyclopropyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (30). 30** was synthesized by the reaction of sulfonyl chloride **6a** and 10 mL (0.14 mol) of cyclopropylamine (method C). The product was isolated by chromatography (hexane/EtOAc (4:1)) and crystallized by dichloromethane/hexane to afford a yellow crystalline solid: yield 89 mg, 90%; mp = 138 °C; ^{1}H NMR ($CDCl_3$) δ 0.72 (m, 4H), 0.88 (t, 6H, J = 7.5 Hz), 1.63 (m, 4H), 2.55 (m, 1H), 2.98 (m, 4H), 4.94 (bs, 1H), 8.28 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 6.62, 11.04, 20.63, 24.30, 53.97, 128.61, 130.13, 141.47, 144.59; MS *m/z* 387.0 $(M + H)^+$. Anal. ($C_{15}H_{22}N_4O_6S$) C, H, N.

***N*^{1}-Cyclopentyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (31). 31** was synthesized by the reaction of sulfonyl chloride **6a** and 300 μL (3 mmol) of cyclopentylamine in 10 mL of dichloromethane (method E). The product was isolated by chromatography (hexane/EtOAc (5:1)) and crystallized using dichloromethane/hexane to obtain a yellow crystalline solid: yield 81 mg, 78%; mp = 116 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, J = 7.5 Hz), 1.50 (m, 2H), 1.68 (m, 8H), 1.94 (m, 2H), 2.97 (m, 4H), 3.57 (m, 1H), 4.58 (bd, 1H), 8.25 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.07, 20.62, 23.08, 35.57, 53.93, 55.38, 128.31, 131.47, 141.31, 144.35; MS *m/z* 415.11 $(M + H)^+$. Anal. ($C_{17}H_{26}N_4O_6S$) C, H, N.

3,5-Dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfonylmorpholine (32). 32 was synthesized by the reaction of sulfonyl chloride **6a** and 261 μL (3 mmol) of morpholine in 10 mL of dichloromethane (method E). The product was isolated by chromatography (hexane/EtOAc (5:1)) and crystallized by dichloromethane/hexane to obtain yellow crystals: yield 76 mg, 73%; mp = 193 °C; ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 6H, J = 7.25 Hz), 1.65 (m, 4H), 2.98 (t, 4H, J = 7.5 Hz), 3.08 (t, 4H, J = 4.75 Hz), 3.78 (t, 4H, J = 4.75 Hz), 8.12 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.05, 20.61, 45.82, 53.89, 65.86, 125.62, 128.89, 141.68, 144.52; MS *m/z* 417.1 $(M + Na)^+$. Anal. ($C_{15}H_{23}N_4O_6S$) C, H, N.

3,5-Dinitro-*N*^{4},*N*^{4}-di-*n*-propylsulfonylpyrrolidine (33). 33 was synthesized by the reaction of sulfonyl chloride **6a** and 10 mL (0.12 mol) of pyrrolidine (method C). The product was isolated by chromatography (hexane/EtOAc (4:1)) and crystallized by hexane/dichloromethane to obtain yellow crystals: yield 84 mg, 84%; mp = 120 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, J = 7.5 Hz), 1.64 (m, 4H), 1.89 (m, 4H), 2.97 (m, 4H), 3.30 (t, 4H, J = 6.5 Hz), 8.20 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.11, 20.69, 25.36, 48.07, 53.96, 128.43, 128.60, 141.34, 144.80; MS *m/z* 401.0 $(M + H)^+$. Anal. ($C_{16}H_{24}N_4O_6S$) C, H, N.

***N*^{1}-Phenyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-ethylsulfanilamide (34).** The sulfonyl chloride derivative **6b** was prepared as before from the sulfonate **5b** and PCl_5. Then **6b** was treated with 547 μL (6 mmol) of aniline in 20 mL of pyridine. The product was isolated by chromatography (hexane/EtOAc (6:1)) and crystallized using dichloromethane/hexane to afford a yellow crystalline solid: yield 262 mg, 67%; mp = 157 °C. ^{1}H NMR (DMSO-d_6) δ 0.99 (t, 6H, J = 7.00 Hz), 2.99 (q, 4H, J = 7.00 Hz), 7.17 (m, 5H), 8.28 (s, 2H), 10.45 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 12.54, 45.60, 120.82, 124.95, 127.92, 129.43, 130.69, 136.73, 140.63, 144.96; MS *m/z* 392.9 $(M - H)^-$. Anal. ($C_{16}H_{18}N_4O_6S$) H, N. C: calcd, 48.72; found, 48.13.

***N*^{1}-Phenyl-3,5-dinitro-*N*^{4},*N*^{4}-di-*n*-butylsulfanilamide (35).** The sulfonyl chloride derivative **6c** was prepared as before from sulfonate **5c** and PCl_5. It was then treated with 547 μL (6 mmol) of aniline in 20 mL of pyridine. The product was isolated by chromatography (hexane/EtOAc (6:1)) and crystallized by dichloromethane/hexane to afford a yellow crystalline solid. Yield 286 mg, 64%; mp = 156 °C. ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 6H, J = 7.50 Hz), 1.28 (m, 4H), 1.55 (m, 4H), 2.96 (t, 4H, J = 7.50 Hz), 6.57 (bs, 1H), 7.25 (m, 5H), 8.10 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 13.66, 19.85, 29.35, 52.07, 122.18, 126.52, 128.61, 129.09, 129.78, 135.24, 141.66, 144.42. MS *m/z* calcd for $C_{20}H_{26}N_4O_6S$ $(M - H)^-$ 449.0. Anal. ($C_{20}H_{26}N_4O_6S$) C, H, N.

3-Nitro-4-chlorosulfonyl Chloride (38). A solution of 1-chloro-2-nitrobenzene (**37**, 5 g, 31.84 mmol) in 25 mL of chlorosulfonic acid was heated at 120 °C for 16 h. The red solution was poured slowly into a container containing ice. The product was extracted from the aqueous solution using EtOAc, the ethyl acetate layer was dried with sodium sulfate and filtered, and then the ethyl acetate was evaporated to afford a brown viscous liquid which upon refrigeration became solid: yield 6.2 g, 77%; ^{1}H NMR ($CDCl_3$) δ 7.90 (d, 1H, J = 8.50 Hz), 8.21 (dd, 1H, J = 2.25, 8.75 Hz), 8.56 (d, 1H, J = 2 Hz).

Synthesis of *N*^{1}-Phenyl-3-nitro-4-chlorosulfonamide (39). Aniline (1.08 mL, 12 mmol) was added in a stirred solution of 3-nitro-4-chlorosulfonyl chloride (**38**, 762 mg, 3 mmol) in 20 mL of pyridine at 45 °C. Stirring was continued for another 3 h. Then pyridine was evaporated out, and the product was extracted using dichloromethane. It was purified as an oil using silica gel column chromatography eluting with hexane/ethyl acetate (2:1): yield 636 mg, 90%; ^{1}H NMR ($CDCl_3$) δ 6.89 (bs, 1H), 7.10 (m, 1H), 7.27 (m, 4H), 7.81 (dd, 1H, J = 2.00, 8.25 Hz), 7.62 (d, 1H, J = 8.00 Hz), 8.24 (d, 1H, J = 2 Hz), 8.68 (s, 1H).

***N*^{1}-Phenyl-3-nitro-*N*^{4},*N*^{4}-di-*n*-propylsulfanilamide (36).** A solution of *N*^{1}-phenyl-3-nitro-4-chlorosulfonamide (**39**, 636 mg, 2.69 mg) in 20 mL of dipropylamine was refluxed for 3 h. After 3 h the solvent was evaporated under vacuum. The crude product was purified by column chromatography eluting with hexane/EtOAc (3:1) to afford a viscous red oil that was further purified by distillation at 80 °C and 5 mbar pressure: yield 616 mg, 61%; ^{1}H NMR ($CDCl_3$) δ 0.82 (m, 6H), 1.58 (m, 4H), 3.13 (t, 4H, J = 7.25 Hz), 6.68 (bs, 1H), 6.99 (d, 1H, J = 9.25 Hz), 7.20 (m, 5H), 7.61 (dd, 1H, J = 2.75, 9.50 Hz), 8.13 (d, 1H, J = 2.25 Hz); ^{13}C NMR ($CDCl_3$) δ 10.94, 20.35, 53.38, 119.79, 121.23, 125.11, 126.14, 126.96, 129.22, 130.78, 136.29, 137.94, 147.56; MS *m/z* 377.0 $(M - H)^-$. Anal. ($C_{18}H_{22}N_3O_6S$) H, N. C: calcd, 57.28; found, 57.69.

Susceptibility Testing of Parasites and Mammalian Cells. The susceptibility of *Leishmania donovani* amastigote-like parasites (WHO designation: MHOM/SD/62/1S-CL2$_D$) to growth inhibition by compounds of interest was measured in a 3-day assay using the tetrazolium dye-based CellTiter reagent (Promega).[7,16] Compounds were tested for their activity against bloodstream-form *Trypanosoma brucei brucei* (MITat

1.2, variant 221) axenically cultured in HMI-9 medium[17] by the procedure of Ellis et al.[18] with some modifications. Briefly, an amount of 100 μL of *T. brucei* in late log phase was incubated in 96-well plates (Costar) at an initial concentration of 10^5 cells/mL with or without test compounds at 37 °C in a humidified 5% CO_2 atmosphere for 72 h. To each well was then added 25 μL of a 5 mg/mL solution of MTT (prepared in phosphate-buffered saline and filter-sterilized), and plates were reincubated at 37 °C as before for 2 h. One hundred microliters of 10% SDS lysis buffer (prepared in 50% aqueous DMF) was added to each well, and plates were incubated as before for an additional 3−4 h. A SpectraMax Plus microplate reader (Molecular Devices) was then used to measure optical densities at 570 nm. IC_{50} values, the concentration of the compound that inhibited cell growth by 50% compared to untreated control, were determined with the aid of the software program SoftMax Pro (Molecular Devices). This program uses the dose−response equation $y = ((a - d)/(1 + (x/c)^b)) + d$, where x is the drug concentration, y is absorbance at 570 nm, a is the upper asymptote, b is the slope, c is IC_{50}, and d is the lower asymptote.

The effect of compounds on the proliferation of J774 murine macrophages and PC3 prostate cancer cells was measured in a 3-day assay using the CellTiter reagent. J774 macrophages in DMEM medium supplemented with 10% heat-inactivated fetal calf serum, 2.0 mM L-glutamine, 50 units/mL penicillin, and 50 μg/mL streptomycin were added to individual wells of a 96-well plate at a concentration of 10^4 cells/mL and a total volume of 100 μL. Macrophages were allowed to adhere for 24 h, and then the medium was removed and replaced with serial dilutions of the test compounds in the DMEM medium mentioned above without phenol red. After 72 h of incubation with the test compounds at 37 °C in a humidified 5% CO_2 incubator, cell viability was determined using the CellTiter reagent by adding 20 μL of assay solution to each well. After a 5−7 h incubation at 37 °C to allow for color development, absorbance readings in each well were measured at 490 nm using the microplate reader. The effect of compounds on PC3 cells was assessed in the same way as for the macrophages except that the medium used was RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 2.0 mM L-glutamine, 50 units/mL penicillin, and 50 μg/mL streptomycin. IC_{50} values for the J774 and PC3 assays were determined with the software program SoftMax Pro as described earlier.

Tubulin Purification. Porcine brains, freshly obtained from a local slaughterhouse, were sliced into approximately 1 cm sections, frozen on dry ice, and stored at −80 °C until use. Frozen brain (approximately 100 g) was broken into small pieces and thawed in 100 mL PME + DTT buffer (0.1 M PIPES, pH 6.9, 1 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT). This brain tissue was homogenized in a blender at 4 °C using 3 × 5 s bursts at high power. A 150 mL portion of the homogenate was centrifuged in a Ti-70 rotor at 100000*g* for 40 min at 4 °C, and then the supernatant was loaded on an 11 mL column containing DEAE-Sepharose Fast Flow matrix (Amersham Pharmacia) equilibrated with PME + DTT. The DEAE-Sepharose column was washed with two column volumes of PME + DTT, four volumes of PME containing 0.3 M glutamate, and four volumes of PME + DTT containing 1 M glutamate. PME + DTT containing 0.75 M glutamate and 0.3 M KCl was then used to elute tubulin from the column. Tubulin-rich fractions (consisting of approximately 10 mL total) were pooled, and then GTP and DMSO were added slowly with gentle vortexing to bring the final concentrations of these components to 1 mM and 8% (v/v), respectively. This solution was incubated at 37 °C for 40 min. After this 37 °C incubation, the solution became cloudy because of the formation of microtubules, which were collected by centrifugation at 100000*g* at 35 °C for 30 min. The pellet was resuspended in 2.3 mL of PME on ice and sonicated once using a Microson XL2000 sonicator (Misonix, Farmingdale, NY) at an output power of 10 W to completely redissolve the pellet. Following an additional 30 min of incubation on ice, this solution was spun at 80000*g* at 4 °C for 20 min. The supernatant containing porcine tubulin was stored at −80 °C in 80 μL aliquots. Leishmanial tubulin was purified by the method of Werbovetz et al.[7] except that the elution of parasite tubulin from the DEAE-Sepharose Fast Flow column was accomplished using PME containing 0.75 M glutamate and 0.3 M KCl as above for mammalian tubulin rather than with PME containing 0.7 M KCl as reported earlier.

Tubulin Assembly Assays. Assembly reactions (final volume of 50 μL) were conducted in 96-well half-area microplates (Costar). Reactions examining either leishmanial or brain tubulin contained final concentrations of 1.5 mg/mL (15 μM) tubulin, 0.1 mM PIPES (pH 6.9), 1 mM EGTA, 1 mM $MgCl_2$, 10% (v/v) DMSO, and 1 mM GTP with and without compounds of interest. Components of the reaction mixtures were added to the microplate on ice, then assembly was initiated, and components were brought to the final concentrations indicated above by the simultaneous addition of a cold GTP solution (10 μL) to all sample wells with a multichannel pipet. The change in turbidity was measured at 351 nm using the SpectraMax Plus microplate reader immediately after GTP addition, with reaction mixtures allowed to warm to either 30 °C (for leishmanial tubulin) or 37 °C (for brain tubulin) within the plate reader. To ensure maximal solubility of the dinitroanilines at a concentration of 40 μM, compounds were added to reactions containing brain tubulin in 5 μL volumes containing 100% DMSO, and assembly was initiated by adding 10 μL of 5 mM GTP in water. The assembly of leishmanial tubulin occurred too rapidly for accurate measurement under the conditions described for brain tubulin. Since the dinitroaniline sulfonamides were tested at 10 μM concentrations against leishmanial tubulin, solubility was not as much of a concern. Compounds were thus added to reactions containing parasite tubulin in 5 μL volumes containing 50% DMSO, and then assembly was initiated by adding 10 μL of 5 mM GTP in 25% DMSO. Because of the expense of growing large numbers of *Leishmania* parasites for the tubulin isolation, a single duplicate measurement was made to screen compounds of interest for activity against this parasite protein.

Flow Cytometry. *T. b. brucei* bloodstream forms were incubated in T25 flasks (3 mL final volume) at 37 °C in a humidified 5% CO_2 atmosphere in HMI-9 medium for 24 h. Cultures contained 1% DMSO (v/v) in the presence or absence of test compounds. Trypanosomes were counted using a hemacytometer and then were centrifuged at 1000*g* at 4 °C for 10 min. Parasites were resuspended in 150 μL of PBS (0.01 M phosphate, 0.137 M NaCl, and 2.7 mM KCl), then 350 μL of ice-cold methanol was added, and the samples were fixed at −20 °C for 2−3 h. Cells were centrifuged as before and resuspended in PBS containing 0.1% TX-100, 5 μg/mL RNase A, and 10 μg/mL propidium iodide for 20−30 min at 37 °C. Centrifugation was repeated, then cells were resuspended in PBS to a final concentration of $(5-10) \times 10^5$ parasites/mL and stored at 4 °C until analysis. Fluorescence cell sorting was conducted on a Becton-Dickinson FACSCalibur flow cytometer. In each case, gating was performed to exclude doublets and aggregates.

In Vivo Screening against African Trypanosomes. The testing of antimitotic compounds against *T. b. brucei* in vivo was carried out according to Kaminsky and Brun[19] with minor modifications. Female NMRI mice (20−25 g) were infected ip with 10^4 *T. b. brucei* strain STIB 795 bloodstream forms, and the animals were randomly assigned to groups of four. Compounds were first dissolved in DMSO and then diluted with water to provide the desired dose of 20 mg/kg (the percentage of DMSO in the final solution was less than 10%). The test compounds or vehicle was administered ip 4 days after infection for 4 consecutive days. The day of death was then recorded for each animal.

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