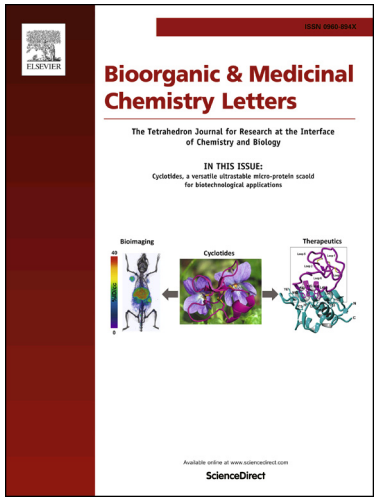

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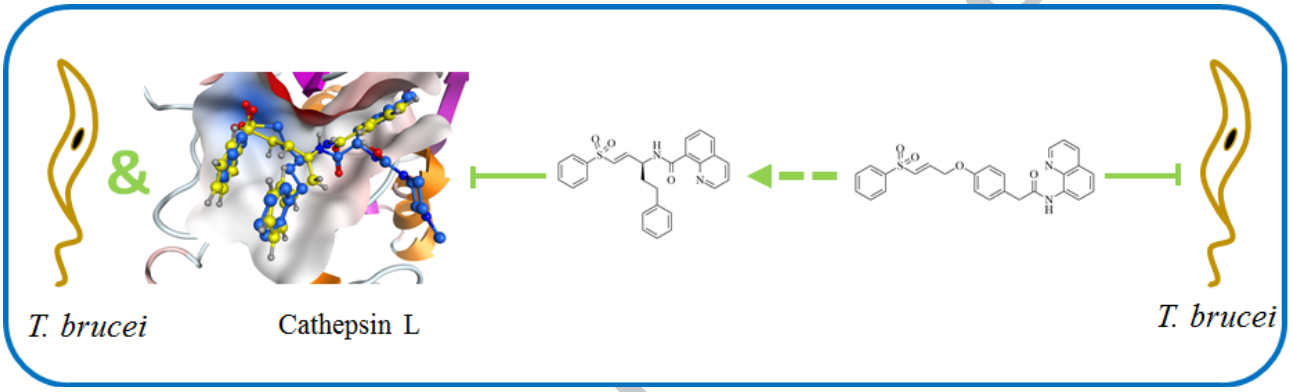

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Discovery of a Quinoline-Based Phenyl Sulfone Derivative as an Antitrypanosomal Agent.

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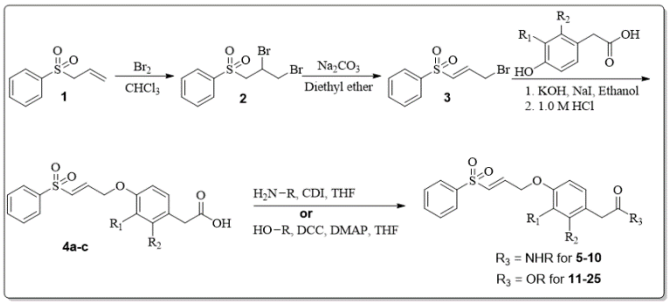
ABSTRACT

A series of natural products-based phenyl sulfone derivative and their property-based analogues were investigated as potential growth inhibitors of *Trypanosoma brucei*. *Trypanosoma brucei* is a kinetoplastid protozoan parasite that causes trypanosomiasis. In this work, we found that nopol- and quinoline-based phenyl sulfone derivative were the most active and selective for *T. brucei*, and they were not reactive towards the active thiol of *T. brucei's* cysteine protease rhodesain. A thiol reactive variant of the quinoline-based phenyl sulfone was subsequently investigated and found to be a moderate inhibitor of rhodesain. The quinoline-based compound that is not reactive towards rhodesain can serve a template for phenotypic-based lead discovery while its thiol-active congener can serve as template for structure-based investigation of new antitrypanosomal agents.

Human African Trypanosomiasis (HAT), one of the neglected tropical diseases (NTDs), caused by protozoan *Trypanosoma brucei* is a declining public health problem on the African continent due to a gradual decrease in the number of reported cases in the past few years. It is most prevalent, at the moment, in the Democratic Republic of the Congo. Historically, the lack of adequate and rapid diagnostic tools as well as lack of effective, safe, and accessible medicines to treat HAT resulted in the death of hundreds of thousands of people. Despite the decrease in reported case, the lack of good network of primary healthcare facilities in most rural and remote places on the continent as well as the possibility of continuous transmission of the parasite from animal reservoirs to humans, make the disease a continuous threat to millions of people.[1-4] Discovery and development of effective oral drugs remains a key objective in combating the disease. In this regard, a promising drug candidate, nitroimidazole fexinidazole, is in the approval stages for the treatment of human African trypanosomiasis. It would be the first approved oral medicine to treat human African trypanosomiasis in several decades. Fexinidazole is also being investigated as a potential treatment for Chagas Disease.[5,6] Despite these recent gains, the drug development pipeline for HAT is sparse and there is need for continued investment and investigation into new chemical entities that can be developed as treatments and/or as prophylactic agents against the disease. Many plant-derived natural products have been reported as antiprotozoal agents. See review by Schmidt and colleagues.[7] In addition, natural products have been widely explored in anti-infective drug discovery. Most anti-infective agents are natural products-based/inspired.[8] However, due to the complexity and scarcity of most active agents, follow-up studies are usually difficult and rarely pursued in NTDs drug discovery.

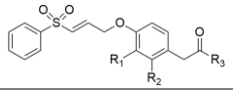
The compounds described in this work were synthesized as outlined in **Schemes 1** and **2**. For compounds **5** to **25**, allyl phenyl sulfone (**1**) was reacted with bromine to obtain the 1,2-dibromide (**2**), in good yield (93%). This was followed by dehalogenation of the vicinal dibromide with sodium carbonate in diethyl ether to obtain (*E*)-((3-bromoprop-1-en-1-yl)sulfonyl)benzene (**3**). Compounds **4a-c** were obtained via etherification reaction between the appropriate 4-hydroxyphenylacetic acids and **3** in ethanol, using potassium hydroxide and sodium iodide. Compounds **4a-c** were then used to synthesize the corresponding amides (**5-10**) and esters (**11-25**) using CDI or DCC and DMAP as coupling reagents.[9-13] Detailed synthesis and compound characterization data are provided as supporting information.

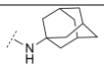
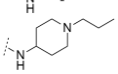
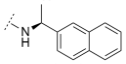
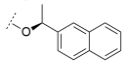
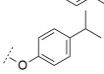
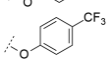
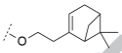
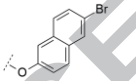
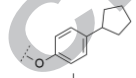
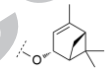
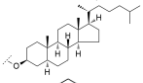
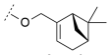
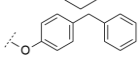
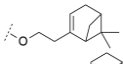
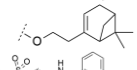
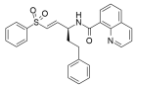
The compounds were subsequently tested for their ability to inhibit the growth of *T. brucei in vitro*.[14] The parasites were exposed to the compounds for 48 hours. Most of the compounds displayed selective but moderate growth inhibitory activity against *T. brucei* when compared with mammalian cells (Hep G2).[15] Compounds derived from 8-aminoquinoline (**9**), (*1R*)-nopol (**15**, **24**), 6-bromo-2-naphthol (**16**), (+) fenchol (**21**) and 4-benzylphenol (**23**) were



Scheme 1. Synthesis of target compounds **5-25**.

Table 1. The antitrypanosomal activities of compounds **5-27**.

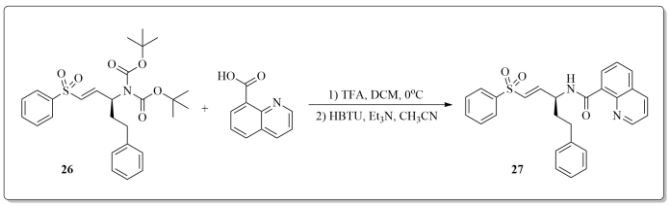


	R_1	R_2	R_3	*T. brucei* IC_{50}	Hep G2 IC_{50}
5	H	H		11.72 ± 0.83	>20
6	H	H		10.77 ± 0.31	>20
7	H	H		>20	>20
8	H	H		>20	>20
9	H	H		0.76 ± 0.11	>80
10	H	H		5.45 ± 0.20	>20
11	H	H		7.16 ± 0.42	>20
12	H	H		5.62 ± 0.65	>20
13	H	H		>20	>20
14	H	H		>20	>20
15	H	H		2.01 ± 0.12	>80
16	H	H		2.18 ± 0.25	>80
17	H	H		6.04 ± 0.03	>20
18	H	H		>20	>20
19	H	H		>20	>20
20	H	H		5.63 ± 0.61	>20
21	H	H		4.04 ± 0.01	11.9 ± 1.03
22	H	H		>20	>20
23	H	H		1.47 ± 0.40	>80
24	Cl	H		3.04 ± 0.07	>80
25	H	F		7.03 ± 0.17	>20
27				5.97 ± 0.12	>80
			Suramin	0.004 ± 0.001	n/a
			Podophyllotoxin	n/a	0.008 ± 0.0003

the most active (**Table 1**). The 8-aminoquinoline-based compound (**9**), being the most selective, was evaluated for *in vivo* antitrypanosomal activity. Two groups of *T. brucei* (STIB795)-infected mice were treated for 4 consecutive days intraperitoneally with 50 mg/kg/day and 100 mg/kg/day of **9**, respectively.[16] The infected mice were positive for parasites 24 hours posttreatment, suggesting that compound **9** lack *in vivo* efficacy. Several generations of aminoquinoline-based compounds have found clinical use in the treatment of malaria but not in the treatment of trypanosomiasis.[17] This is perhaps due to the unique mechanism of action of aminoquinolines in plasmodium-infected cells. However, there are increasing reports of quinoline-based growth inhibitors of trypanosomes, although, the mechanism of action of the quinoline-based compounds have not been deciphered.[18-21]

The presence of the vinyl sulfone moiety in **5-25** suggests that they are potential covalent inhibitors of trypanosoma cysteine proteases. Compounds **5-25** were then tested for inhibitory activity against the major cathepsin L protease in *T. brucei*, rhodesain.

Rhodesain is a validated drug target and it is known to be essential for the survival and infectivity of the parasite. Its role in the ability of the parasite to proliferate has been extensively investigated.[22-24] None of the compounds displayed noteworthy inhibition of the protease at 20 µM. The inactivity of the compounds may be because of the proximity of the vinylic Michael acceptor to the phenoxide oxygen in **5-25**. It is also possible that the compounds are just unable to adopt favorable orientation at the active site of the protease. Nevertheless, a quinoline-based thiol reactive structural variant of **9** was synthesized and tested for protease inhibition, and for trypanocidal activity. Compound **27** was synthesized from boc-protected (*E*)-5-phenyl-1-(phenylsulfonyl)pent-1-en-3-amine (**26**). Compound **26** was a generous gift from Prof. J Love (University of British Columbia), and it was reported by Kiemele and co-workers in 2016.[25] Compound **27** was able to completely inactivate rhodesain at 20 µM after 1 hour of incubation with estimated IC_{50} value of 800 nM, and a K_{inact}/K_i value of 99 $M^{-1}s^{-1}$ (Figure 1).[26]



Scheme 2. Synthesis of target compound **27**.

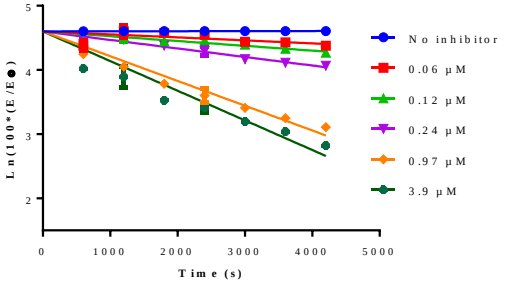


Figure 1. Pseudo-first order inhibition plots for compound **27**.

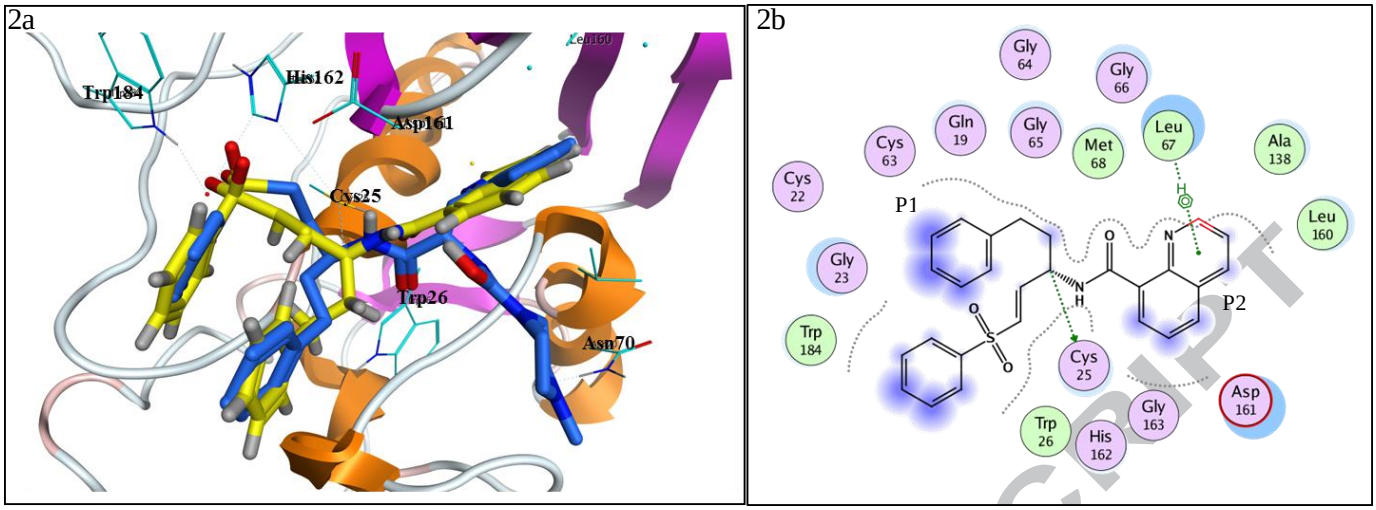


Figure 2. **a**. The superposition of docked compound **27** (Yellow) and **D1R** (Blue) at the active site of rhodesain; **b**. The interaction plot of compound **27** with active site residues.

It has a moderate antitrypanosomal activity with an IC_{50} value of 5.97 uM. Compound **27** was also tested for inhibitory activity against human cathepsin L, but it was inactive (from 0.1 µM to 125 µM).[26] Crystallographic investigation of rhodesain-inhibitor (**27)** complex has been attempted but it has not been successful. However, it is still being pursued. In order to understand the interactions responsible for the inhibitory activity of **27** on rhodesain, template docking was used.[27] Compound **27** was docked in the previously reported crystal structure of rhodesain using the bound ligand (**D1R**) as template.[28] Three of the top five docking poses suggests that the vinyl group is in the vicinity of active thiol (**Cys25**), while the homophenyl moiety occupies the P1 site, and the quinoline moiety occupies the P2 site (**Figure 2a** and **2b**). The phenyl sulfone moiety is predicted to have steric interactions with Gln19 and His162 while the quinolyl motif have steric interactions with Met68. Quite noticeable is the empty P3 site.

In conclusion, a series of phenyl sulfone natural products-based compounds were synthesized and evaluated as potential antitrypanosomal agents. Quinoline- and nopol-based compounds, **9** and **15,** were the most active and selective. The quinoline-based compound (**9**) can serve a template for phenotypic-based lead discovery and the thiol-active compound (**27**) may serve as template for structure-based investigation of new covalent antitrypanosomal agents.

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14. *Trypanosoma brucei* assay: The growth inhibitory activity of the compounds was evaluated using the Alamar blue assay. Bloodstream forms of *T. brucei brucei* (strain 427) cultured in HMI-9-medium supplemented with 10% FBS, 10% Serum plus (SAFC), 0.05 mM bathocuproinesulfonate, 1.5 mM L-cysteine, 1 mM hypoxanthine, 0.2 mM β-mercaptoethanol, 0.16 mM thymidine, 1 mM pyruvate, and 0.0125% Tween 80 were dispensed into sterile 96-well plates at 5 X 10^3 cells/well, and treated with compounds for 48 hours. The compounds were prepared in DMSO and were tested in triplicates with a total assay volume of 100 µL. Next, Alamar blue (20 µL) was added and the plate was incubated at 37°C for 4 hours. Immediately following incubation, fluorescence signals were read (λ_{ex} 530 nm, λ_{em} 590nm). IC_{50} values were determined by testing compounds in a dose range of 0.3 – 50 µM. Suramin was used as positive control.

15. Cytotoxicity Assay: Human hepatocarcinoma cell line (Hep G2, CRL-11997™) was used for cytotoxicity studies. The cells were grown in complete medium (DMEM: F12 containing L-glutamine and sodium bicarbonate, 10% FBS, 1% penicillin/streptomycin, 0.0125% Tween 80) incubated at 37°C in a 5% CO2 environment. Once 80-90% confluent, the cells were washed with phosphate-buffered saline (PBS), treated with 0.25% (w/v) of trypsin/EDTA, counted and suspended in fresh complete media. Into 96-well plates, 198 µL of 5 x 10^5 cells/mL were seeded and incubated for about 24 hours. Cells were treated with the compounds prepared in DMSO for 72 hours. After 72 hours, the old cell medium is removed and fresh DMEM:F12 medium containing MTT (5 mg/mL in PBS) was added to the cells, and incubated for 1.5 hour. The MTT-containing medium was gently removed and replaced with DMSO (200 µL/well). Lyzed cells were then repeatedly resuspended in DMSO using multichannel pipette in order to allow the formazan crystals to dissolve. Plates were incubated for 10 minutes and absorbance were measured at 550 nm. All compounds were tested in triplicates. SDS (10%) was used as assay positive control and podophyllotoxin was used as cytotoxicity control.

16. *In vivo* assay: *T. b. brucei* STIB795-Luc model was used for *in vivo* studies. The studies were conducted at the Swiss Tropical and Public Health Institute (Basel, Switzerland). They were approved by the veterinary office of the Canton Basel-Stadt, Switzerland under license number 2813. Four female NMRI mice were used per experimental group. A control group and two treatment groups (50 mg/kg/day and 100 mg/kg/day). Each mouse was inoculated intraperitoneally (ip) with 1 x 10^4 bloodstream forms of STIB795-Luc. Treatments were administered ip in water (plus 10% DMSO) 3 days post-infection. All mice were monitored for parasitemia by live imaging. Moribund mice were euthanized after detection of parasitemia.

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26. **a**. *T. brucei* cathepsin L (rhodesain) was expressed in *Pichia pastoris.* The inhibition assays were carried out in 50 mM sodium acetate (assay buffer) pH 5.5, containing 2 mM DTT and 0.01% Triton X-100. The enzyme (5 µL, final concentration of 0.8 nM) and test compound (5 µL, final concentration of 20 µM) mixtures were pre-incubated for 1 hour, followed by the addition of 100 µL of the substrate, Z-Phe-Arg-AMC (10 µM), in sodium acetate buffer pH 5.5. Fluorescence (RFU/sec) resulting from proteolytic cleavage of the substrate was monitored at 25 °C (λ_{ex} 355 nm and λ_{em} 460 nm) on a PolarStar Omega plate reader (BMG LABTECH, Germany). E-64 (10 µM) was used as positive control (100% inhibition). For time dependent inhibition studies, rhodesain-compound assay mixture includes 30 µL of compound **27** in DMSO, 30 µL of rhodesain in assay buffer, and 180 µL of assay buffer. Aliquots (10 µL) of rhodesain-**27** mixture were assayed every 10 minutes using Z-Phe-Arg-AMC (10 µM) as substrate. Fluorescence (RFU/sec) resulting from proteolytic cleavage of the substrate was monitored for 7 minutes as described above. Compound **27** was assayed at the following concentrations: 0, 0.06, 0.12, 0.24, 0.97, and 3.9 µM.; **b**. Cathepsin L (CatL) from human liver (Millipore Sigma) was used for protease

selectivity studies. Human CatL (0.58 nM) was assayed in 400 mM sodium acetate (100 µL) pH 5.5, containing 8 mM DTT, 4 mM EDTA, and 0.01% Triton X-100. Human CatL and **27** were pre-incubated for 1 hour in assay buffer, followed by the addition of 100 µL of the substrate, Z-Phe-Arg-AMC (20 µM), in sodium acetate buffer and the plate was read as described above.

27. Molecular structure of compound **27** was built using SPARTAN '10 for Windows. Its geometries were optimized using the MMFF 94 force field. The docking simulations were carried out using the MolDock docking algorithm of the Molegro Virtual Docker in the template docking mode. The X-ray crystal structure (PDB ID: 2P7U) of rhodesain was used for the docking calculation. The bound inhibitor in 2P7U was used as template molecule. A docking sphere (15 Å radius) was placed on the binding site (X = -9.38; Y = 1.66; Z = 10.38) in order to allow different orientations of the ligand to be searched. The docking algorithm was set at maximum iterations of 1500 with a simplex evolution population size of 50, and a minimum of 50 runs. The 2D representations of rhodesain-**27** complex was prepared using MOE 2016.

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