APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2003; **17**: 17–22
Environment, Biology and Toxicology AOC
Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/aoc.368

The cholesterol pathway of *Trypanosoma congolense* could be a target for triphenyltinsalicylate and triphenylsiliconsalicylate inhibition

Andrew J. Nok[1]*, Ishaya H. Nock[2] and Josiah J. Bonire[3]

[1]Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria
[2]Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria
[3]Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria

Received 3 April 2002; Accepted 31 July 2002

The organometallic compounds triphenyltinsalicylate (TPTS) and triphenylsiliconsalicylate (TPSS) were found to be trypanocidal against culture forms of *Trypanosoma congolense*. Both compounds at 0.4–5 μmol ml^{-1} completely killed the parasites *in vitro* within 3-8 min after incubation. A dosage of 1.5 μmol ml^{-1} TPTS killed at least 50% of the parasite population, which was preceded by a cluster effect as observed under phase contrast microscopy. Also, 3.5 μg ml^{-1} of TPSS was required to kill 50% of the *T. congolense* cells. At a low dosage of 2–10 μg ml^{-1}, it was feasible to monitor the effect and mode of action of the organometallic compounds. There was a 50% reduction in the amount of synthesized cholesterols in the presence of 6 μg ml^{-1} and 10 μg ml^{-1} of TPTS and TPSS respectively. TPTS and TPSS also non-competitively inhibited pyrophosphatase from lysed *T. congolense* with K_i values of 3.6 μM and 8.5 μM respectively. In the *in vivo* experiments, TPTS cured *T. congolense* infected mice at a dosage of 2–10 mg kg day^{-1} for 4 days. TPSS was, however, completely inactive *in vivo*. The use of organometallic compounds in the design of trypanocides is discussed. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: sterol pathway; trypanocidal; triphenyltin; triphenylsilicon; cholesterol

INTRODUCTION

African trypanosomiasis remains a disease with little effective medical control. Some 35 million people and 25 million cattle are at risk of infection with pathogenic trypanosomes.[1] The life cycle of trypanosomes involves multiplication in body fluids of the mammalian host and in the gut and salivary glands of the insect vector of the tsetse fly. In the mammalian host, the variable surface glycoprotein (VSG) represents parasite adaptation in the mammalian host, providing it with an escape mechanism from the immune system.[2] This has been the problem besetting vaccine discovery.

The chemotherapy of trypanosomiasis is beset with several problems associated with the treatment, ranging from a limited repertoire to protracted treatment protocols.[3] On account of the above, there is a pressing need for research into better and cheaper trypanocides. Organotin compounds are widely used in agriculture and industry. They possess one or more direct tin–carbon covalent bonds that are responsible for the specific properties of the molecule.[4] The antitumor, microcidal, and trypanocidal properties of organotin compounds are well documented.[5–9] As potential leads to drug development, understanding the mechanism of action of organic compounds may provide possible modifications to make them more effective. Salicylates are usually employed in the manufacture of analgesic-based drugs. They bind easily to the plasma protein albumin to make it a potential ligand carrier. In the present work, we have explored the effect of organometallic derivatives of salicylates on parasites. Herein, we show that a compromised cholesterol pathway of the trypanosomes is the mode of action of triphenyltinsalicylate (TPTS).

*Correspondence to: A. J. Nok, Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.
E-mail: jandrew@skannet.com

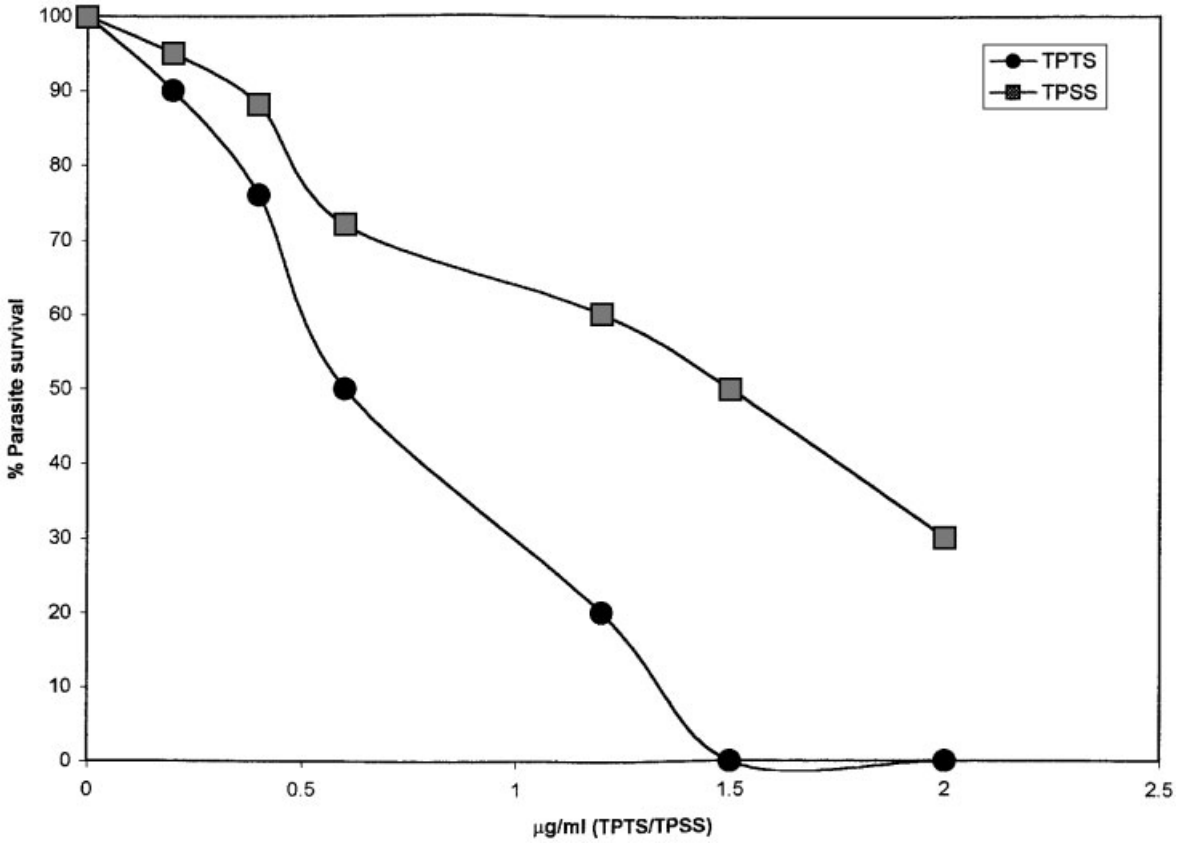

Figure 1. Effect of TPTS and TPSS on the survival of *T. congolense*. The effect of the compounds on the parasites was measured by monitoring motility under a phase contrast microscope.

EXPERIMENTAL

All reagents were purchased from Sigma Chemical Company, St Louis, USA. TPTS and triphenylsilanesalicylate (TPSS) were prepared as described in the literature.[10]

Trypanosoma congolense stb 212 was supplied by the National Institute for Trypanosomiasis Research, Vom, Nigeria.

Cultivation of bloodstream *T. congolense*

T. congolense parasites were cultivated as described in the literature.[11] This was done by collecting the trypanosomes from infected Balb C mice and diluting with Dulbecco medium (GIBCO BRL) supplemented with 100 µM hypoxanthine, 30 µM thymidine, 40 µM adenosine, 1 µM sodium pyruvate, 50 µM L-glutamine, 100 µM 2-mercaptoethanol, and 20% fetal calf serum. About 0.25 ml of the heparinized blood containing 10^6 trypanosomes was dispensed into each well of the 24-well tissue culture plates and incubated at 27 °C under 5% CO_2 and 95% air. After 24 h, about 200 µl of fresh medium was added to each well. Subsequently, 250 µl of fresh medium was added daily, along with the removal of the same amount of spent medium. After 4 weeks the cultured, long, slender bloodstream forms were transferred into 25 cm^3 tissue culture flasks containing 5 ml of the culture medium.

In vitro sensitivity test

About 10 mg of TPTS was dissolved in 200 µl 5% dimethylsulfoxide (DMSO) and gradually made up to 10 ml using phosphate-buffered saline, pH 6.8. About 100 µl of the constituted TPTS was dispensed into the 24 wells of a tissue culture plate in dilutions of 0.2–2 mg ml^{-1}. To each well, 500 µl of the culture bloodstream forms of *T. congolense* (10^6 cells/ml) was added and incubated at 37 °C under 5% CO_2 and 95% air. The control was incubated with phosphate-buffered saline containing 1% DMSO. At the end of 60 min incubation, parasite survival was checked using a hemocytometer. The TPSS treatment procedure was the same as that for TPTS.

In vivo infection

Twenty-four mice were divided into six groups (A–F) of four each. Members of five groups were infected by intraper-

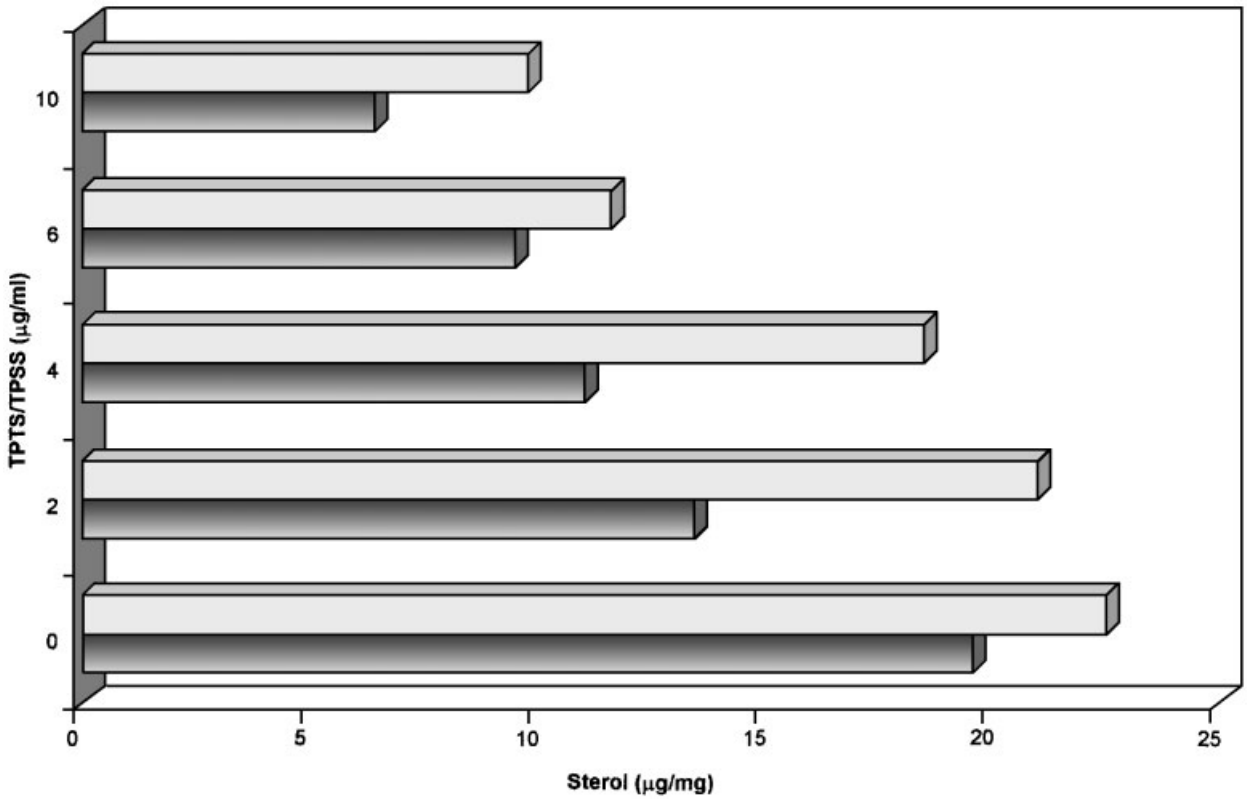

Figure 2. Effect of TPTS and TPSS on the total sterol content of cultured *T. congolense*. Exactly 5 ml of the cultured parasites at 10^6 cells/ml is spanned to collect the cells. The amount of sterol is measured in micrograms per milligram of total parasite proteins.

itoneal (i.p.) inoculation with 10^4 trypanosomes. Group D (uninfected) served as a control. Parasitemia started to rise after 72 h, and treatment was commenced by i.p. inoculation of 10 mg kg^{-1} day^{-1} and 2 mg kg^{-1} day^{-1} of TPTS to groups B and E respectively. Groups C and F were also administered i.p. 10 mg kg^{-1} day^{-1} and 2 mg kg^{-1} day^{-1} respectively. The group A members were left untreated but given 1% DMSO in phosphate-buffered saline. Parasitemia was checked daily, as done previously.

Effect of TPTS and TPSS on total cholesterol in *T. congolense*

About 10^6 parasites/ml of the *T. congolense* cells were grown in the presence of 2–10 μg ml^{-1} TPTS and TPSS prepared from the stock solution. After 5 days of culture, the parasites were harvested by centrifuging 5 ml of the culture medium at 5000*g* for 10 min and lysed by sonication in presence of 5 μM leupeptin, 5 μM pepstatin, and 2 μM trasylol. The lysed material was suspended in 500 μl of 60 mM phosphate buffer, pH 6.8, and analyzed for total cholesterol.[12]

Pyrophosphatase assay

The TPTS- and TPSS-treated *T. congolense* (10^7 cells) were lysed by sonication in the presence of 5 μM leupeptin, 5 μM pepstatin, and 2 μM trasylol. The preparation was then centrifuged at 5000*g* for 10 min. The supernatant was assayed for pyrophosphatase.[8]

RESULTS

The trypanocidal effects of TPTS and TPSS on the blood-stream forms of *T. congolense* are as shown in Fig. 1. TPTS at 0.5–5 μg ml^{-1} inhibited the growth of *T. congolense* in a dose-dependent pattern with a minimum growth inhibitory level of 0.2 μg ml^{-1} and ED_{50} of 0.6 μg ml^{-1}. Also, the parasites were comparatively less sensitive to TPSS with a minimum inhibitory dose of about 0.5 μg ml^{-1} and ED_{50} of 1.5 μg ml^{-1}.

Effect of TPST and TPSS on sterol synthesis of *T. congolense*

When TPTS (2–10 μg ml^{-1}) was incubated with *T. congolense*, the parasites were sufficiently sensitive to allow for the determination of its effect on sterol synthesis. As seen in Fig. 2, the total sterol content of *T. congolense* reduced by about 50% in the presence of 6 μg ml^{-1} of TPTS. Similarly, there was an observable fall by 50% in the level of sterol at

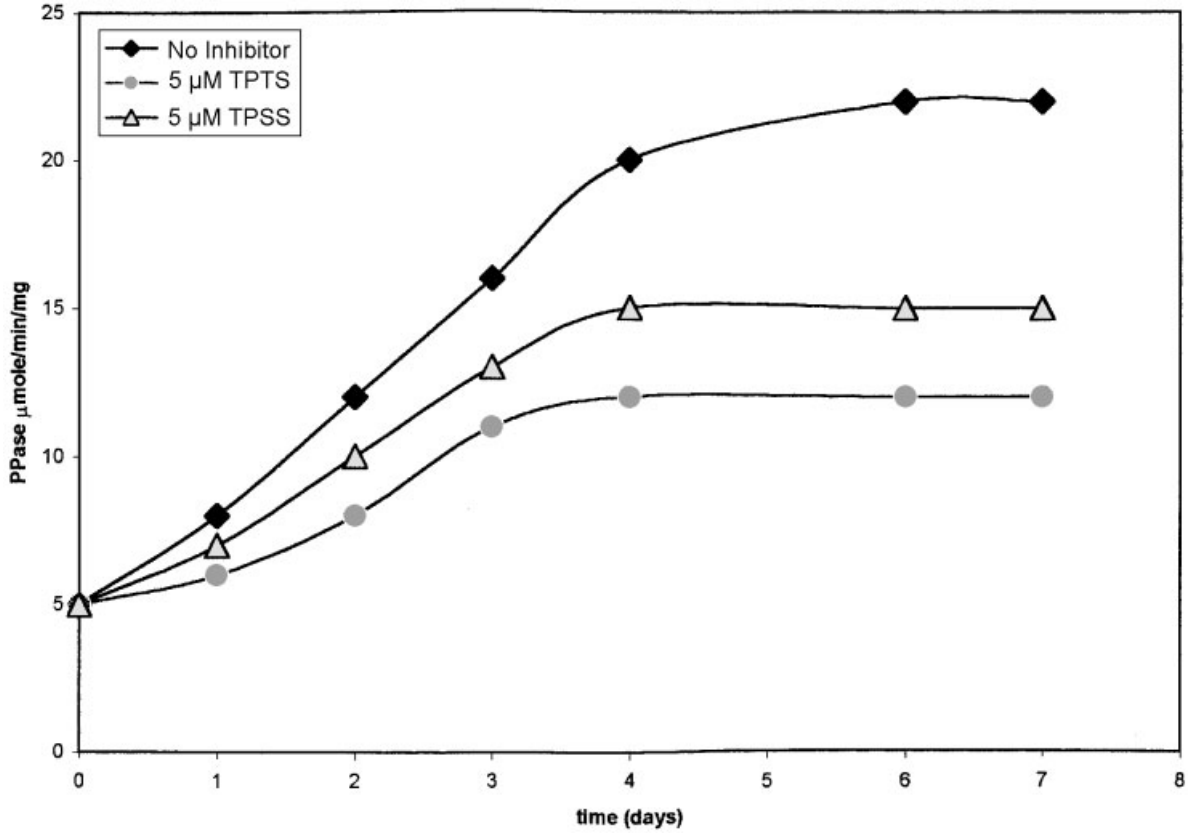

Figure 3. Activity profile of *T. congolense* pyrophosphatase in the presence and absence of 5 μM of TPTS and TPSS with time of cultivation.

10 μg ml^{-1} of TPSS. In both cases, there was diminished flagellar motility.

Effect of TPST and TPSS on *T. congolense* pyrophosphatase

The activity of pyrophosphatase assayed in the culture-grown parasites showed inhibition by about 30–45% in the TPTS- and TPSS-treated parasites. As shown in Fig. 3, it is clear that the V_{max} was not attainable in the presence of either compound. This presupposes that both TPTS and TPSS are non-active-site competitive inhibitors. When the enzyme was solubilized and assayed in the presence of TPTS and TPSS, there was a clear dose-dependent inhibition by the compounds. Kinetic inhibition analysis confirmed non-competitive patterns with binding constants K_i of 3.6 μM and 8.5 μM for TPST and TPSS respectively (Fig. 4).

In vivo experiments

In the *in vivo* experiments, all the infected animals developed parasitemia at 72 h post infection. As observed in Table 1, after the administration of 2 and 10 mg kg^{-1} day^{-1} of TPST and TPSS, for 4 days, there was complete elimination of the parasites in the TPTS-treated group. However, members of the TPSS-treated group (C and F) failed to respond to the treatment. Groups B and E, which responded to TPTS treatment, were monitored for a 6 week period for any relapsing parasitemia.[13] There was, however, no resurgence of any parasites. The LD_{50} of the TPTS was found to be 120 mg kg^{-1}, which falls far below the dosage required to cure the infected mice of groups B and E completely.

Histopathological studies

Representatives of the infected but untreated mice (group A) and the TPSS-treated C and F groups revealed prominent Kupffer cells in the liver, and erythrophagia of some Kupffer cells in their livers. There was also necrosis of the epithelial cells of the proximal and convoluted tubules of their kidneys. Their spleens were also abnormally enlarged. However, none of the cured mice (i.e. treated with TPTS) revealed any pathology that connotes organ damage, except for a fairly enlarged spleen.

DISCUSSION

In this report, we demonstrate the trypanocidal effect of TPTS and TPSS *in vitro*. Also, we show that although TPTS

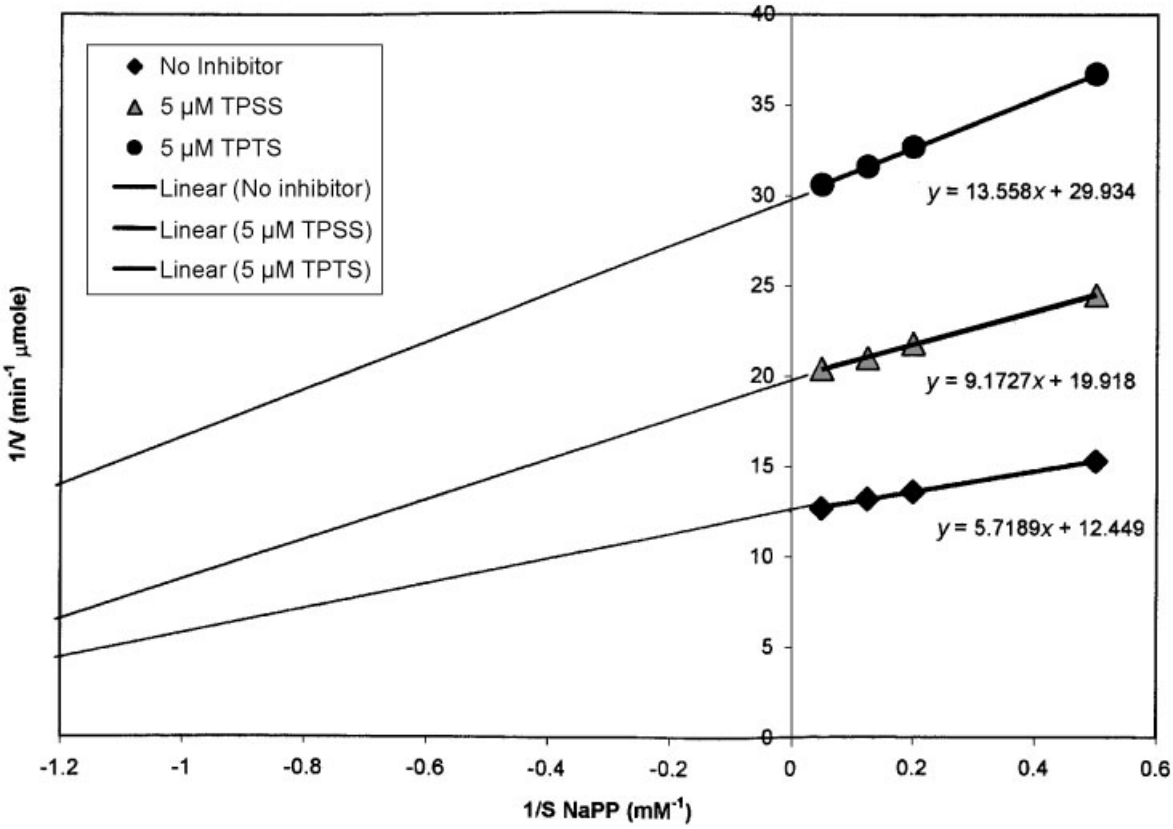

Figure 4. Lineweaver–Burk plots of initial velocity data of *T. congolense* pyrophosphatase catalyzed hydrolysis of NaPPi in the presence and absence of 5 μM of TPTS and TPSS.

eliminates the parasites *in vivo*, TPSS was completely ineffective. The potential application of organotin compounds as trypanocidal agents is only a recent development.

Table 1. Effect of the administration of TPTS and TPSS on mice infected with *T. congolense* at various time. (A) Control (0.1% DMSO in PBS). (B) 10 mg kg^{-1} TPTS. (C) 10 mg kg^{-1} TPSS. (D) Control–uninfected mice. (E) 2 mg kg^{-1} TPTS. Results are expressed as averages of three experiments. Trypanosome motility was observed by inspection of at least 100 trypanosmes under phase contrast microscopy using a hemocytometer

Group	Parasites in peripheral circulation					
	24 h	48 h	72 h	96 h	120 h	144 h
A	10^4	10^4	10^6	5×10^6	10^7	dead
B	10^4	10^4	10^2	0	0	0
C	10^4	5×10^4	10^6	10^6	10^7	dead
D	0	0	0	0	0	0
E	10^4	10^5	10^6	10^4	10^2	10^2
F	10^4	10^5	10^6	5×10^6	10^7	dead

In order to develop more effective anti-trypanosome agents, the identification of precise targets is vital. Herein, we show that cholesterol metabolism could be a target of TPTS- and TPSS-mediated killing of *T. congolense*.

T. congolense pyrophosphatase, like microbial pyrophosphatases, was strongly inhibited by TPTS and TPSS.[8] The foregoing observation suggests that the mechanism of prokaryotic and eukaryotic cell-response to organotins is associated with the enzyme pyrophosphatase. In the etiology of trypanosomiasis, the metabolism of host lipid components is essential, to enable the parasite to build its membrane components. The enzyme pyrophosphatase is involved in driving the β-oxidation pathway from the activation stage by hydrolysis of pyrophosphate (ppi) to generate an equivalent of two adenosine triphosphates. The inhibition of pyrophosphatase will prematurely terminate this pathway, thereby affecting the mevalonate pathway that leads to cholesterol synthesis in *T. congolense*.[14–16]

Both TPTS and TPSS inhibited the uptake of cholesterol in the *T. congolense* cells, thus implicating the sterol pathway as a target of the compounds. African trypanosomes are known to acquire lipids by hydrolysing host membrane lipid, e.g. lecithin, by the release of phospholipases to release; free fatty

acid and lysolecithin.[17,18] The products are then enzymatically incorporated onto the parasites.

Since the acquisition of cholesterol by trypanosomes involves cell-mediated endocytosis by trypanosomal receptors, the observed TPTS/TPSS inhibition of cholesterol uptake could be due to masking of these receptors by the organometallic compounds. Whether such blockage could be as a result of receptor-associated interaction remains to be elucidated. In the *in vivo* experiments, although TPTS remained trypanocidal, TPSS was ineffective. The contrasting observation could be linked to the possible inactivation of the TPSS by host enzymes or interaction with serum proteins like albumin. Moreover, that silicon is higher than tin in group IV of the periodic table makes it more likely for covalent bond formation than tin.

Histopathological results of tissue fixed sections of the liver and kidney of the treated animals did not reveal any abnormality that connotes damage. In contrast, those TSST-treated mice that subsequently died showed clear degeneration of the convoluted tubules and microtubules of the kidney. The potential of organotin derivatives of myristic and cleic acids for anti-trypanosome activity are currently being explored.

REFERENCES

1. Turner M. *J. Adv. Parasitol.* 1982; **21**: 69.
2. Vickerman K. *Br. Med. Bull.* 1985; **41**: 105.
3. Gutteridge WE. *Br. Med. Bull.* 1985; **41**: 162.
4. Pain G and Cooney JJ. *Arch. Environ. Contan. Toxicol.* 1998; **35**: 412.
5. Atassi G. *Rev. Silicon Germanium Tin Lead Compd.* 1985; **8**: 219.
6. Briddle BN and Gray JS. *Appl. Org. Met. Chem.* 1989; **3**: 537.
7. Nok AJ, Esievo KAN, Adaudi A, Achoba II, Gimba CE, Solomon M and Kagbu JA. *J. Clin. Biochem. Nutr.* 1992; **13**: 81.
8. Nok AJ, Shuaibu MN, Bonire JJ, Dabo A, Wushishi Z and Ado S. *J. Enzym. Inhib.* 2000; **15**: 11.
9. Shuaibu MN, Ameh DA, Bonire JJ, Adaudi A, Ibrahim S and Nok AJ. *Parasite* 2000; **7**: 43.
10. Bonire JJ and Fricker SP. *J. Inorg. Biochem.* 2001; **83**: 217.
11. Yabu Y. *Southeast Asian J. Trop. Med. Public Health* 1993; **24**: 705.
12. Searey RL, Berquist LM and Jung RC. *J. Lipid Res.* 1960; **1**: 349.
13. Bachi CV, Yarlett N, Goldberg B, McCann PP, Bitonti AJ and Sjoerdan A. *Antimicrob. Agents Chemother.* 1992; **36**: 185.
14. Dixon H, Ginger CD and Williams S. *Comp. Biochem. Physiol.* 1971; **398**: 247.
15. Coppens I and Courtoy PS. *Mol. Biochem. Parasitol.* 1995; **73**: 179.
16. Coppens I, Baoudhuin P, Opperdoes FR and Courtoy PJ. *Proc. Natl. Acad. Sci. U.S.A.* 1988; **85**: 6753.
17. Mellors A and Samad A. *Parasitol. Today* 1985; **5**: 239.
18. Nok AJ, Esievo KAN, Ibrahim S, Ukoha AI and Ikediobi CO. *Cell Biochem. Funct.* 1993; **1**: 125.

