Heat shock protein 90 as a potential drug target against surra

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SUMMARY

Trypanosomiasis is caused by *Trypanosoma* species which affect both human and animal populations and pose a major threat to developing countries. The incidence of animal trypanosomiasis is on the rise. Surra is a type of animal trypanosomiasis, caused by *Trypanosoma evansi*, and has been included in priority list B of significant diseases by the World Organization of Animal Health (OIE). Control of surra has been a challenge due to the lack of effective drugs and vaccines and emergence of resistance towards existing drugs. Our laboratory has previously implicated Heat shock protein 90 (Hsp90) from protozoan parasites as a potential drug target and successfully demonstrated efficacy of an Hsp90 inhibitor in cell culture as well as a pre-clinical mouse model of trypanosomiasis. This article explores the role of Hsp90 in the *Trypanosoma* life cycle and its potential as a drug target. It appears plausible that the repertoire of Hsp90 inhibitors available in academia and industry may have value for treatment of surra and other animal trypanosomiasis.

Key words: Surra, Hsp90, 17-AAG, Trypanosomiasis, *Trypanosoma evansi*.

INTRODUCTION

The term *Trypanosoma* is derived from ancient Greek, where 'trypanos' means borer and 'soma' means body, because of their corkscrew-like movement. *Trypanosoma* is responsible for causing sleeping sickness and Chagas' disease in humans. Sleeping sickness is caused by subspecies of *Trypanosoma brucei* such as *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodensiense*, whereas Chagas' disease is caused by *Trypanosoma cruzi*. Sleeping sickness affects the central nervous system which causes alteration in circadian rhythms and poses difficulty in walking. The first case of sleeping sickness was recorded by A. R. and J. H. Cook in 1901 (Fevre *et al*. 2004). The outbreak of an epidemic in the Busoga region of Uganda between 1900–1920 marked its emergence by killing 250 000 people (Fevre *et al*. 2004). American trypanosomiasis or Chagas' disease is caused by *T. cruzi* and the infection in humans was first documented in Brazil by Carlos Chagas (Kropf and Sa, 2009). Trypanosomiasis is also widespread in animals and is one of the major diseases resulting in high morbidity and mortality in livestock.

Animal trypanosomiasis is caused by many *Trypanosoma* species like *T. brucei*, *T. congolense*, *T. vivax*, *T. equiperdum*, *T. equinum* and *T. evansi*. *Trypanosoma b. brucei*, *T. congolense* and *T. vivax* are responsible for causing Nagana which is spread by the bite of an infected tsetse fly (*Glossina* spp.) which acts as a vector for transmission. *Trypanosoma vivax* is also transmitted mechanically through other flies such as tabanids and stomoxes. It is mainly found in domestic animals such as pigs, goats, sheep, horses, dogs, cats, water buffaloes and camels. *Trypanosoma equiperdum* causes Dourine, which is mainly transmitted through coitus (OIE, 2013). *Trypanosoma equiperdum* dwells mainly in host tissues rather than blood in equids. The disease is prevalent in South Africa, Northern Africa, South America and Italy (OIE, 2013).

Surra, caused by *T. evansi*, was discovered in India in 1880 by Evans (Desquesnes *et al*. 2013). The infection is prominent in equatorial countries. It infects goats, sheep, camels, dogs, equines, cows and buffaloes. Although *T. evansi* infects only animals, in 2001 the first case of *T. evansi* infection in humans was reported in India, which was due to a deficiency in apolipoprotein L-1 (Joshi *et al*. 2005). The infection is fatal for equines and camels while cows and buffaloes serve as carriers for the disease. If not treated the fatality of infection is 100% (OIE, 2012). The infection is associated with fever, anaemia, weight loss and oedema particularly in the lower parts of the body (OIE, 2012). It is also noted that fever is directly associated with parasite load. However, the intensity of the clinical signs varies among host species. Typical symptoms of surra include dullness and depression, sunken eyes, muscle atrophy, drooping of head and neck and pallor of the mucous membrane (OIE, 2012). There are reported outbreaks of surra in countries such as India, Pakistan, France and Kenya (Njiru *et al*. 2004; Ul Hasan *et al*. 2006; Desquesnes *et al*. 2008). According

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to the World Organization for Animal Health, surra is a serious concern for animal health.

Trypanosomes, being extracellular parasites, are easier to target in comparison to intracellular parasites as the drug does not have to penetrate an extra layer of host membrane. However, being a neglected disease, limited efforts have been made in developing drugs against these infections. Among the currently available drugs, Suramin was introduced in 1916 for clinical use, Isometamidium came to market in 1950 and Diminazene aceturate was first described in 1955 (Peregrine and Mamman, 1993; Morgan *et al*. 2010). The development of melarsomine, an arsenical compound, started in 1947 with melaminophenylarsine melarsoprol. It was completed in the 1990s and marketed as Cymelarsan (Zweygarth and Kaminsky, 1990; Berger and Fairlamb, 1994). Since these drugs, no new molecule has been introduced against surra. As a result, a new drug development strategy is an essential requirement. In this review article, we have focused our attention on limitations of the current treatment regimen and potential of Hsp90 from *T. evansi* as a drug target. We also discuss the possibilities of repositioning the known Hsp90 inhibitors for treatment of surra as mono or combination therapy.

LIMITATIONS OF THE CURRENT TREATMENT REGIMEN FOR SURRA

There is no vaccine currently available for surra. Hence, the control and prevention is supported by drug treatment and eradication of the vector. Most of the drugs used for treatment of surra have limited therapeutic windows, with hypersensitivity and other toxicities as side effects. Further, reports of resistance and use of drugs having unexplained modes of action pose challenges in administration of the required dosage. The information related to the pharmacokinetic (PK) profiles of these drugs is unclear. Currently, there are only five scaffolds available in the market, namely phenanthridine, diminazine, suramin, quinapyramine and melarsomine (Fig. 2). There are nearly 20 generic brands from these five scaffolds, as listed in Table 1, which are used currently for treatment. All these generics have either intramuscular or subcutaneous routes of administration. Phenanthridine compounds are toxic because of their DNA intercalating action (MacGregor and Johnson, 1977). Melarsomine is ineffective against CNS infection in horses and dogs (Desquesnes *et al*. 2013) and the toxic effects of this compound include diarrhoea, fever, panting, restlessness, salivation, vomiting, nephrotoxicity and CNS toxicity. Suramin treatment shows a high relapse rate and has reports of resistance in Sudan (El Rayah *et al*. 1999). Diminazine aceturate is known to have poor efficacy and less tolerance in horses and mules and is toxic to camels (Tuntasuvan *et al*. 2003). Also, there

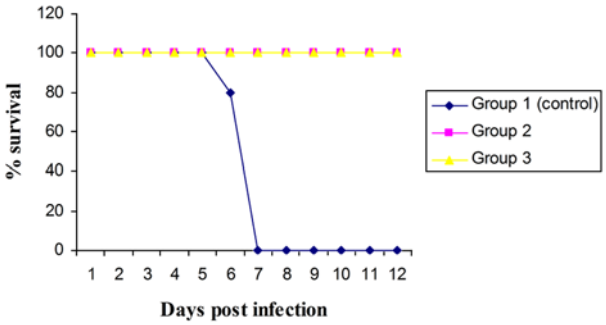

Fig. 1. Treatment of *T. evansi*-infected mice by 17-AAG. Group 1 ($n = 5$) received vehicle alone, Group 2 ($n = 5$) received 17-AAG (30 mg kg^{-1} body weight, for 7 days) after 4 h of injection with parasites. Whereas, Group 3 ($n = 3$) received 17-AAG (50 mg kg^{-1} body weight, for 6 days) upon confirmation of infection. Mice in group 2 and 3 survived with no relapse of infection.

are reports of meningoencephalitis after treatment with diminazene aceturate and quinapyramine sulphate (Ranjithkumar *et al*. 2013). The emergence of resistance to quinapyramine is also reported (Liao and Shen, 2010). Hence, the identification of a new class of drugs is essential for the control of surra.

HEAT SHOCK PROTEIN 90 AS A DRUG TARGET AGAINST SURRA

Hsp90 is a molecular chaperone which helps in supporting cellular dynamics under stress conditions. It belongs to the GHKL family of ATPases, with three distinct functional domains; N-terminal domain (NTD) with ATP binding pocket, which connects to catalytic middle domain via variable linker region and C-terminal domain. It is a dimeric protein and it functions as part of a multi-chaperone complex with accessory co-chaperones. The co-chaperones either regulate ATPase activity of Hsp90 or provide client specificity (Pearl and Prodromou, 2006; Taipale *et al*. 2010).

In biological systems, the unfolded client protein interacts with the ADP-bound complex of Hsp90. To fold an unfolded client protein, Hsp90 works in association with Hsp70 and Hsp40 as a part of a multi-chaperone complex. The dimeric protein forms a closed conformation when ATP is bound to the N-terminal pocket. Further, in the presence of co-chaperones, the multi-chaperone complex performs folding of unfolded client proteins (Siligardi *et al*. 2004). Inhibition of Hsp90 function causes activation of proteosomal degradation of unfolded client proteins (Taipale *et al*. 2010). Hsp90 serves as a regulatory hub of many cellular processes including signal transduction, accumulation of drug resistance and immunity. These properties make Hsp90 an important drug target in both cancer and infectious diseases (Taipale *et al*. 2010; Rochani *et al*. 2013). Hsp90 has been shown to play an essential role in the

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Table 1. List of combination drugs available for the treatment of surra

S.No.	Active pharmaceutical ingredient	Combination ingredient	Brand name	Pharmaceutical manufacturer
1	Quinapyramine	Sulphate and chloride salts	Antrycide prosalt	Virbac
			Gilpol-Q	G. Loucatos
			Lytrip	Lyka
			Suranil	Pranav
			Suravax	Pranav
			Triquin	Vetoquinol
			Triquin-S	Vetoquinol
2	Suramin	–	Naganol	Bayer
3	Diminazene aceturate	Phenazone	Berenil	Intervet SPAH
			Dimaze	Vetoquinol
			Diminagil	G. Loucatos
			Diminat	Vetindia
			Nilbery	Intas
			Percip	Cipla
			Prozzant vet	Neospark
			Prozomin	Virbac
			Sarmorecide	Concept
			Trityl	Lyka
			Trypzin	Vetnex
4	Isometamidium	–	Surral	Alembic
5	Melarsomine		Cymelarsan	–

life cycle of various parasitic protozoa such as *Plasmodium falciparum, Giardia lamblia, Leishmania donovani, Entamoeba histolytica, T. cruzi and T. evansi* (Wiesgigl and Clos, 2001; Graefe *et al*. 2002; Banumathy *et al*. 2003; Pallavi *et al*. 2010; Nageshan *et al*. 2011; Singh *et al*. 2014).

During transmission and manifestation of infection, these parasites experience high levels of stress including heat, osmotic and nutritional stress. Owing to the levels of stress experienced by these parasites, they require a robust stress response machinery. Hsp90 is a key member of this response machinery, which is highlighted by the growth arrest of these protozoa upon Hsp90 inhibition.

Studies on different *Trypanosoma* species show their dependence on Hsp90 function for their growth and survival. For example, it was observed that transcripts of Hsp70 and Hsp90 were abundant in the blood stage of *T. brucei* compared with the insect stages, suggesting that the stress response causes up-regulation of Hsp90 (Van der Ploeg *et al*. 1985). In *T. brucei*, Hsp90 (TbHsp83) inhibition leads to growth arrest of both the procyclic and the bloodstream forms. Compared with humans, *T. brucei* (bloodstream form) shows 50- to 60-fold higher sensitivity to inhibition by GA derivative 17-allylamino 17-demethoxy geldanamycin (17-AAG) (Jones *et al*. 2008). 17-AAG treatment resulted in increased sensitization of *T. brucei* to heat shock, leading to severe morphological abnormalities (Meyer and Shapiro, 2013). Mouse studies using a soluble GA derivative, 17-Dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG), cured mice of *T. brucei* infection (Meyer and Shapiro, 2013).

Studies on *T. cruzi* have shown that Hsp90 plays an important role in stage transition. Inhibition of Hsp90 by GA not only inhibits proliferation but also arrests epimastigotes in the G_1 phase of the life cycle. Although in the presence of GA, trypomastigotes (bloodstream form) converted to spheromastigote-like forms, differentiation into epimastigotes was permanently blocked (Graefe *et al*. 2002).

In the case of *T. evansi*, stress can be attributed to two levels of heat shock experienced by the parasite. First, when *T. evansi* gets transmitted to the host where the host body temperature is higher than the vector; second, during fever experienced by the host on infection with *T. evansi*. Studies on *T. brucei* and *T. cruzi* can be extrapolated to *T. evansi*, as *T. evansi* is thought to be evolved from *T. brucei* by losing maxi-circle DNA from kinetoplastids (Wells, 1984) and thus these species are very closely related. Further, Pallavi *et al*. (2010) demonstrated the *in vivo* efficacy of Hsp90 inhibition for the treatment of surra (Pallavi *et al*. 2010). This study showed that 17-AAG specifically binds to *T. evansi* Hsp90 (TeHsp90) and was effective against surra in the pre-clinical rodent model. Intra-peritoneal administration of 17-AAG at 30 mg kg^{-1} body weight for 4 continuous days was able to cure *T. evansi* infection in mice with a 60% survival rate with no relapse of infection for the 90 days that the mice were monitored (Pallavi *et al*. 2010). Additionally, treatment with an increased dose of 50 mg kg^{-1} body weight showed 100% survival, as shown in Fig. 1 (unpublished data). This pre-clinical study for surra treatment, along with studies on different *Trypanosoma* spp.,

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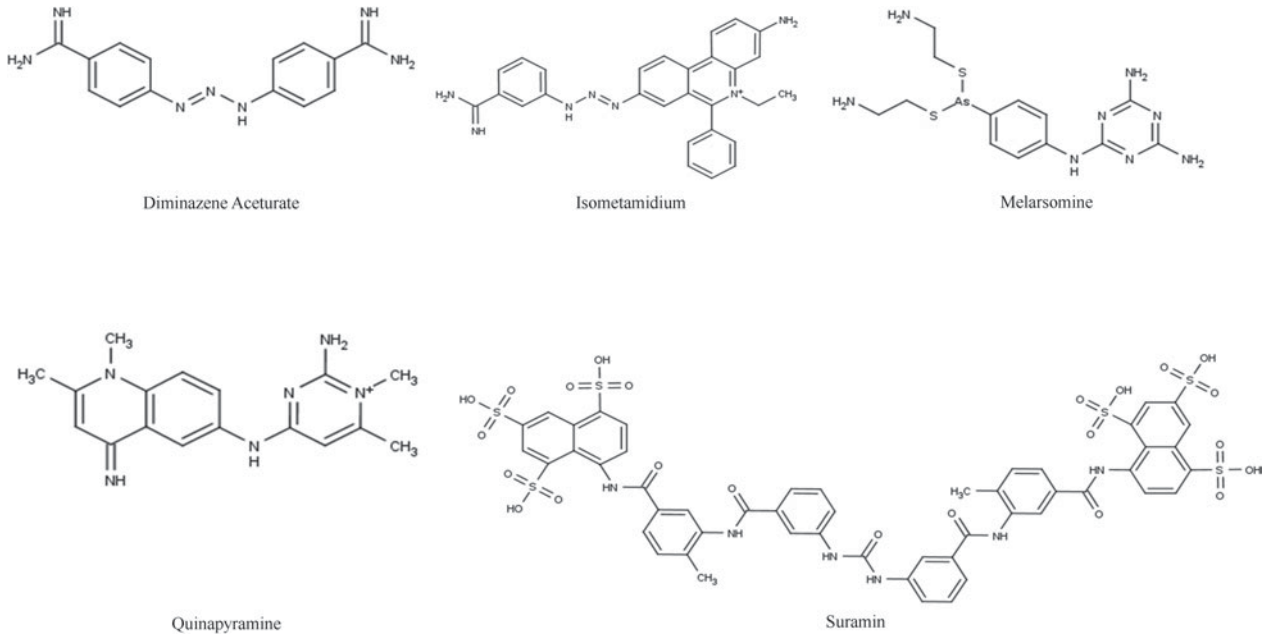

Fig. 2. Drugs currently used for treatment of animal trypanosomiasis.

demonstrates the potential of Hsp90 as a chemotherapeutic target for *T. evansi* infection.

HSP90 TARGETED ANTI-TRYPANOSOMAL COMPOUNDS

Ansamycin scaffolds were the first to be explored for inhibitory activity against Hsp90 and are specific towards the GHKL family of ATPases. They are a class of antibiotics which are extensively used against microbial infections. They are polyketide compounds obtained from natural sources. Ansamycins are categorized as benzoquinone and naphthoquinone derivatives. The benzoquinone ansamycins family includes geldanamycin, the parent molecule of 17-AAG. Geldanamycin was first isolated from the soil bacteria *Streptomyces hygroscopicus* var. *geldanus*. Although being a potent Hsp90 inhibitor, the molecule remained out of clinical reach due to its hepatoxicity (Samuni *et al*. 2010). The relatively safe medicinal profile of 17-AAG enabled it to enter clinical trials for cancer in humans (Rochani *et al*. 2013). It is noted that the binding affinity of the geldanamycin nucleus to Hsp90 from parasites such as *Plasmodium* and *Trypanosoma* is stronger compared with ATP (Pallavi *et al*. 2010). As discussed in the previous section, *Trypanosoma* shows higher sensitivity to Hsp90 inhibitors compared with host cells. Apart from 17-AAG, there are three other molecules, namely IPI-504, IPI-493 and 17-DMAG, from the ansamycin class which are being explored for their Hsp90 inhibitory activities in cancer and can be repositioned to see their effect on *Trypanosoma* infection.

Another important scaffold of Hsp90 inhibitors belongs to the purine family. The purine analogues are known to target the ATP binding site at NTD to inhibit ATPase activity. Some of the purine analogues, like allopurinol, have shown inhibitory activity against *T. cruzi* amastigotes *in vitro* with a 50% growth inhibitory concentration of 3 μM (Nakajima-Shimada *et al*. 1996). 3′-Deoxyinosine and 3′-deoxyadenosine also suppressed *T. cruzi* growth in host cells, although with less sensitivity (Nakajima-Shimada *et al*. 1996). The adenosine analogues PU-H71, Debio 0932, MPC-3100 and CNF 2024 (BIIB021) have entered clinical trials for cancer and are known to have safe profiles. These compounds can be tested against TeHsp90 as a repositioning strategy. The structures of these adenosine analogues are shown in Fig. 3.

Pizarro *et al*. (2013) have shown the binding affinities of purine and benzamide analogues towards NTD of TbHsp83 protein. It is interesting to note that one of the benzamide derivatives (NVP-AUY922) is reported to have a K_d value of 3 nM. This study also reported that GA and its derivatives such as 17-AAG and 17-DMAG, along with the ansamycin compound macbecin, show higher binding affinities to TbHsp83 than human Hsp90. GA derivatives and compounds synthesized by the group showed sub micro-molar EC_{50} for *T. brucei* (Pizarro *et al*. 2013). This study can be extrapolated to *T. evansi* for exploring their effect as anti-surra drugs since TeHsp90 and TbHsp83 are 100% identical (Pallavi *et al*. 2010).

Radicicol is a naturally occurring Hsp90 inhibitor isolated from *Diheterospora chlamydosporia* which has an *in vitro* IC_{50} value of 20–23 nM for cancer cell lines (Sharma *et al*. 1998; Wang *et al*. 2006). Against the bloodstream form of *T. brucei*, radicicol shows potency at the nano-molar scale (Meyer and Shapiro,

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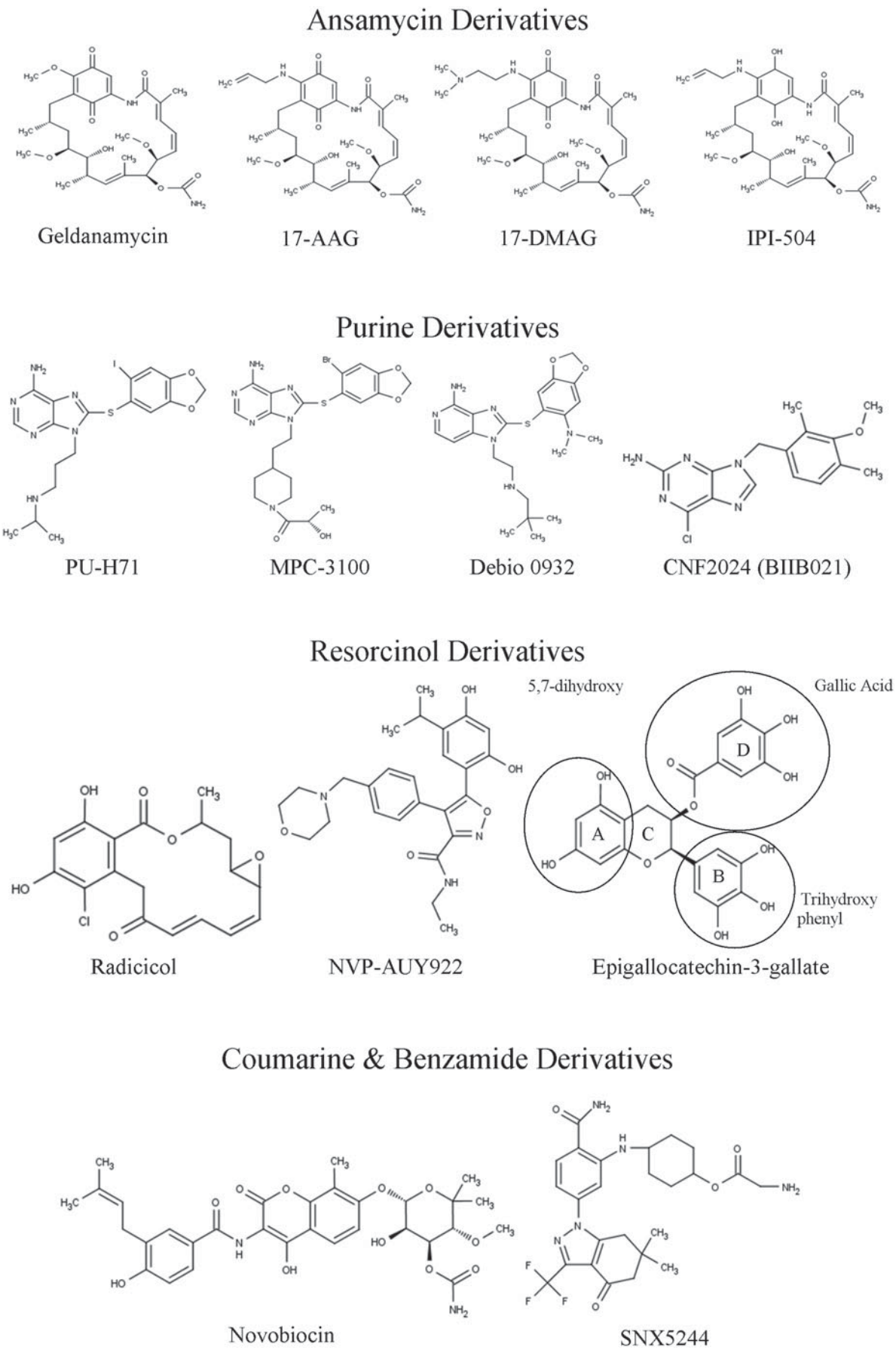

Fig. 3. Structures of Hsp90 inhibitors.

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2013). However, radicicol is not a very good choice of inhibitor owing to its reduced stability *in vivo* but the resorcinol scaffold of radicicol can be used for developing new derivatives which can be explored for their Hsp90 inhibitory activity. Combining resorcinol with triazoles, isoxazoles and benzisoxazoles resulted in increased potency of Hsp90 inhibitors (Janin, 2005; Du *et al*. 2007; Gopalsamy *et al*. 2008; Taldone *et al*. 2009). Further, the lead optimization of a resorcinol heterocyclic hybrid gave rise to STA9090 or Ganetispib and NVP-AUY922. Both the molecules are under clinical trials for use as anticancer drugs (Dutton *et al*. 2014). They have shown promising results for human applications. Their use for the treatment of surra remains unexplored.

The Plant Kingdom has also been explored extensively for newer scaffolds which can be optimized for designing novel Hsp90 inhibitors. A triterpenoid called gedunin, isolated from *Azadirachta indica*, is shown to have affinity towards PfHsp90 (Brandt *et al*. 2008) and the methanolic extract of *A*. *indica* in combination with diminazene aceturate provided anti-trypanosomal activity against mice infection with *T*. *evansi* (Omoja *et al*. 2011). Hence, it will be of interest to find out if the anti-trypanosomal activity of this extract is due to inhibition of Hsp90 from the parasite. Derivatives of coumarin scaffold have emerged as novel options of exploring new Hsp90 inhibitors. Novobiocin was the first coumarin analogue shown to have affinity towards the C-terminal domain of Hsp90 (Marcu *et al*. 2000; Donnelly and Blagg, 2008). Novobiocin shows growth inhibition of *Trypanosoma* at micromolar levels (Meyer and Shapiro, 2013). Epigallocatechin-3-gallate (EGCG) is a polyphenolic coumarin molecule which binds to the C-terminal domain of Hsp90. It is shown to bind to a stretch of 538–728 residues of C-terminal Hsp90 (Yin *et al*. 2009). The exact mechanism of inhibition is still unknown. It has been observed that EGCG is prone to epimerization and auto-oxidation (Khandelwal *et al*. 2013). Hence, attempts were made to develop stable derivatives for increasing the stability of molecule by modifying substitutions on EGCG rings. The structure activity relationship (SAR) study of this molecule showed that the addition of a phenolic group on Ring A and Ring D (Fig. 3) has positive and negative impacts respectively on Hsp90 inhibition. Also, the inclusion of novobiocin-derivative phenyl benzoate in place of gallic acid of EGCG was found to be suitable for Hsp90 inhibition, as shown in Fig. 3. It is also noted that EGCG exhibits inhibition of *T*. *evansi* in a mouse model of infection at $\sim 30\,\mu$M concentration (Vigueira *et al*. 2012). This increased inhibitory concentration can also be attributed towards the instability of the molecule, as stated previously.

These studies highlight the potential of repositioning currently available Hsp90 inhibitors for treatment of surra. In addition to repositioning current Hsp90 inhibitors, there is a need to develop new inhibitors with even higher specificity towards TeHsp90.

CONCLUSION

One of the major advantages of veterinary drug development is the possibility of direct testing in the animal of interest at any point in time (Lathers, 2003). There is also the advantage of repositioning drugs from human to animal application. With this viewpoint, TeHsp90 is being reviewed as a potential drug target against surra. By taking a route of drug crossover we have used 17-AAG for the treatment of surra. As a first attempt towards performing clinical trials in large animals for treatment of surra we experimentally infected ponies with *T*. *evansi* and treated them with 17-AAG. Our preliminary results showed a rapid decline in parasitaemia with recovery of 17-AAG treated animals (unpublished data). The preliminary calculation of PK parameters showed that 17-AAG has a better profile than marketed drugs. This is a first step towards demonstration of an Hsp90 inhibitor being repositioned for a veterinary application. It appears possible that 17-AAG and other Hsp90 inhibitors can be explored as potential drugs against surra.

It will also be interesting to explore the use of Hsp90 inhibitors in combination with other existing anti-surra drugs (Dolgin and Motluk, 2011). The knowledge of molecular scaffolds which work as Hsp90 inhibitors against cancer cell lines and their SAR studies have led to the synthesis of a large number of derivatives which can also be explored as potential anti-infective drugs for veterinary application.

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REFERENCES

Banumathy, G., Singh, V., Pavithra, S. R. and Tatu, U. (2003). Heat shock protein 90 function is essential for *Plasmodium falciparum* growth in human erythrocytes. *Journal of Biological Chemistry* **278**, 18336–18345. doi: 10.1074/jbc.M211309200.

Berger, B. J. and Fairlamb, A. H. (1994). Properties of melarsamine hydrochloride (Cymelarsan) in aqueous solution. *Antimicrobial Agents and Chemotherapy* **38**, 1298–1302. doi: 10.1128/AAC.38.6.1298.

Brandt, G. E., Schmidt, M. D., Prisinzano, T. E. and Blagg, B. S. (2008). Gedunin, a novel hsp90 inhibitor: semisynthesis of derivatives and preliminary structure-activity relationships. *Journal of Medicinal Chemistry* **51**, 6495–6502. doi: 10.1021/jm8007486.

Desquesnes, M., Bossard, G., Patrel, D., Herder, S., Patout, O., Lepetitcolin, E., Thevenon, S., Berthier, D., Pavlovic, D., Brugidou, R., Jacquiet, P., Schelcher, F., Faye, B., Touratier, L. and Cuny, G. (2008). First outbreak of *Trypanosoma evansi* in camels in metropolitan France. *Veterinary Record* **162**, 750–752. doi: 10.1136/vr.162.23.750.

Desquesnes, M., Holzmuller, P., Lai, D. H., Dargantes, A., Lun, Z. R. and Jittaplapong, S. (2013). *Trypanosoma evansi* and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *BioMed Research International* **2013**, 194176. doi: 10.1155/2013/194176.

Dolgin, E. and Motluk, A. (2011). Heat shock and awe. *Nature Medicine* **17**, 646–649. doi: 10.1038/nm0611-646.

Donnelly, A. and Blagg, B. S. (2008). Novobiocin and additional inhibitors of the Hsp90 C-terminal nucleotide-binding pocket. *Current Medicinal Chemistry* **15**, 2702–2717. doi: 10.2174/092986708786242895.

Du, Y., Moulick, K., Rodina, A., Aguirre, J., Felts, S., Dingledine, R., Fu, H. and Chiosis, G. (2007). High-throughput screening fluorescence polarization assay for tumor-specific Hsp90. *Journal of Biomolecular Screening* **12**, 915–924. doi: 10.1177/1087057107306067.

Dutton, B. L., Kitson, R. R., Parry-Morris, S., Roe, S. M., Prodromou, C. and Moody, C. J. (2014). Synthesis of macrolactam analogues of radicicol and their binding to heat shock protein Hsp90. *Organic and Biomolecular Chemistry* **12**, 1328–1340. doi: 10.1039/c3ob42211a.

El Rayah, I. E., Kaminsky, R., Schmid, C. and El Malik, K. H. (1999). Drug resistance in Sudanese *Trypanosoma evansi*. *Veterinary Parasitology* **80**, 281–287. doi: 10.1016/S0304-4017(98)00221-0.

Fevre, E. M., Coleman, P. G., Welburn, S. C. and Maudlin, I. (2004). Reanalyzing the 1900–1920 sleeping sickness epidemic in Uganda. *Emerging Infectious Diseases* **10**, 567–573. doi: 10.3201/eid1004.020626.

Gopalsamy, A., Shi, M., Golas, J., Vogan, E., Jacob, J., Johnson, M., Lee, F., Nilakantan, R., Petersen, R., Svenson, K., Chopra, R., Tam, M. S., Wen, Y., Ellingboe, J., Arndt, K. and Boschelli, F. (2008). Discovery of benzisoxazoles as potent inhibitors of chaperone heat shock protein 90. *Journal of Medicinal Chemistry* **51**, 373–375. doi: 10.1021/jm701385c.

Graefe, S. E., Wiesgigl, M., Gaworski, I., Macdonald, A. and Clos, J. (2002). Inhibition of HSP90 in *Trypanosoma cruzi* induces a stress response but no stage differentiation. *Eukaryotic Cell* **1**, 936–943. doi: 10.1128/EC.1.6.936-943.2002.

Janin, Y. L. (2005). Heat shock protein 90 inhibitors. A text book example of medicinal chemistry? *Journal of Medicinal Chemistry* **48**, 7503–7512. doi: 10.1021/jm050759r.

Jones, C., Anderson, S., Singha, U. K. and Chaudhuri, M. (2008). Protein phosphatase 5 is required for Hsp90 function during proteotoxic stresses in *Trypanosoma brucei*. *Parasitology Research* **102**, 835–844. doi: 10.1007/s00436-007-0817-z.

Joshi, P. P., Shegokar, V. R., Powar, R. M., Herder, S., Katti, R., Salkar, H. R., Dani, V. S., Bhargava, A., Jannin, J. and Truc, P. (2005). Human trypanosomiasis caused by *Trypanosoma evansi* in India: the first case report. *American Journal of Tropical Medicine and Hygiene* **73**, 491–495.

Khandelwal, A., Hall, J. A. and Blagg, B. S. (2013). Synthesis and structure-activity relationships of EGCG analogues, a recently identified Hsp90 inhibitor. *Journal of Organic Chemistry* **78**, 7859–7884. doi: 10.1021/jo401027r.

Kropf, S. P. and Sa, M. R. (2009). The discovery of *Trypanosoma cruzi* and Chagas disease (1908–1909): tropical medicine in Brazil. *História, Ciências, Saúde-Manguinhos* **16** (Suppl. 1), 13–34.

Lathers, C. M. (2003). Challenges and opportunities in animal drug development: a regulatory perspective. *Nature Reviews Drug Discovery* **2**, 915–918. doi: 10.1038/nrd1229.

Liao, D. and Shen, J. (2010). Studies of quinapyramine-resistance of *Trypanosoma brucei evansi* in China. *Acta Tropica* **116**, 173–177. doi: 10.1016/j.actatropica.2010.08.016.

MacGregor, J. T. and Johnson, I. J. (1977). *In vitro* metabolic activation of ethidium bromide and other phenanthridinium compounds: mutagenic activity in *Salmonella typhimurium*. *Mutation Research* **48**, 103–107.

Marcu, M. G., Schulte, T. W. and Neckers, L. (2000). Novobiocin and related coumarins and depletion of heat shock protein 90-dependent signaling proteins. *Journal of the National Cancer Institute* **92**, 242–248. doi: 10.1093/jnci/92.3.242.

Meyer, K. J. and Shapiro, T. A. (2013). Potent antitrypanosomal activities of heat shock protein 90 inhibitors *in vitro* and *in vivo*. *Journal of Infectious Diseases* **208**, 489–499. doi: 10.1093/infdis/jit179.

Morgan, H. P., McNae, I. W., Nowicki, M. W., Zhong, W., Michels, P. A., Auld, D. S., Fothergill-Gilmore, L. A. and Walkinshaw, M. D. (2010). The trypanocidal drug suramin and other trypan blue mimetics are inhibitors of pyruvate kinases and bind to the adenosine site. *Journal of Biological Chemistry* **286**, 31232–31240. doi: 10.1074/jbc.M110.212613.

Nageshan, R. K., Roy, N., Hehl, A. B. and Tatu, U. (2011). Post-transcriptional repair of a split heat shock protein 90 gene by mRNA trans-splicing. *Journal of Biological Chemistry* **286**, 7116–7122. doi: 10.1074/jbc.C110.208389.

Nakajima-Shimada, J., Hirota, Y. and Aoki, T. (1996). Inhibition of *Trypanosoma cruzi* growth in mammalian cells by purine and pyrimidine analogs. *Antimicrobial Agents and Chemotherapy* **40**, 2455–2458.

Njiru, Z. K., Constantine, C. C., Ndung'u, J. M., Robertson, I., Okaye, S., Thompson, R. C. and Reid, S. A. (2004). Detection of *Trypanosoma evansi* in camels using PCR and CATT/*T. evansi* tests in Kenya. *Veterinary Parasitology* **124**, 187–199. doi: 10.1016/j.vetpar.2004.06.029.

OIE, W. O. f. A. H. (2012). OIE Terrestrial Manual 2012, Chapter 2.1.17. "*Trypanosoma evansi* Infections (Including Surra)". http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/.

OIE (2013). Chapter 2.5.3. Dourine. OIE Terrestrial Manual. http://www.oie.int/fileadmin/Home/fr/Health_standards/tahm/2.05.03_DOURINE.pdf.

Omoja, V. U., Anaga, A. O., Obidike, I. R., Ihedioha, T. E., Umeakuana, P. U., Mhomga, L. I., Asuzu, I. U. and Anika, S. M. (2011). The effects of combination of methanolic leaf extract of *Azadirachta indica* and diminazene diaceturate in the treatment of experimental *Trypanosoma brucei brucei* infection in rats. *Asian Pacific Journal of Tropical Medicine* **4**, 337–341. doi: 10.1016/S1995-7645(11)60099-0.

Pallavi, R., Roy, N., Nageshan, R. K., Talukdar, P., Pavithra, S. R., Reddy, R., Venketesh, S., Kumar, R., Gupta, A. K., Singh, R. K., Yadav, S. C. and Tatu, U. (2010). Heat shock protein 90 as a drug target against protozoan infections: biochemical characterization of HSP90 from *Plasmodium falciparum* and *Trypanosoma evansi* and evaluation of its inhibitor as a candidate drug. *Journal of Biological Chemistry* **285**, 37964–37975. doi: 10.1074/jbc.M110.155317.

Pearl, L. H. and Prodromou, C. (2006). Structure and mechanism of the Hsp90 molecular chaperone machinery. *Annual Review of Biochemistry* **75**, 271–294. doi: 10.1146/annurev.biochem.75.103004.142738.

Peregrine, A. S. and Mamman, M. (1993). Pharmacology of diminazene: a review. *Acta Tropica* **54**, 185–203. doi: 10.1016/0001-706X(93)90092-P.

Pizarro, J. C., Hills, T., Senisterra, G., Wernimont, A. K., Mackenzie, C., Norcross, N. R., Ferguson, M. A., Wyatt, P. G., Gilbert, I. H. and Hui, R. (2013). Exploring the *Trypanosoma brucei* Hsp83 potential as a target for structure guided drug design. *PLOS Neglected Tropical Diseases* **7**, e2492. doi: 10.1371/journal.pntd.0002492.

Ranjithkumar, M., Saravanan, B. C., Yadav, S. C., Kumar, R., Singh, R. and Dey, S. (2013). Neurological trypanosomiasis in quinapyramine sulfate-treated horses – a breach of the blood-brain barrier? *Tropical Animal Health and Production* **46**, 371–377. doi: 10.1007/s11250-013-0498-9.

Rochani, A. K., Singh, M. and Tatu, U. (2013). Heat shock protein 90 inhibitors as broad spectrum anti-infectives. *Current Pharmaceutical Design* **19**, 377–386. doi: 10.2174/1381612811306030377.

Samuni, Y., Ishii, H., Hyodo, F., Samuni, U., Krishna, M. C., Goldstein, S. and Mitchell, J. B. (2010). Reactive oxygen species mediate hepatotoxicity induced by the Hsp90 inhibitor geldanamycin and its analogs. *Free Radical Biology and Medicine* **48**, 1559–1563. doi: 10.1016/j.freeradbiomed.2010.03.001.

Sharma, S. V., Agatsuma, T. and Nakano, H. (1998). Targeting of the protein chaperone, Hsp90, by the transformation suppressing agent, radicicol. *Oncogene* **16**, 2639–2645. doi: 10.1038/sj.onc.1201790.

Siligardi, G., Hu, B., Panaretou, B., Piper, P. W., Pearl, L. H. and Prodromou, C. (2004). Co-chaperone regulation of conformational switching in the Hsp90 ATPase cycle. *Journal of Biological Chemistry* **279**, 51989–51998. doi: 10.1074/jbc.M410562200.

Singh, M., Shah, V. and Tatu, U. (2014). A novel C-terminal homologue of Aha1 co-chaperone binds to heat shock protein 90 and stimulates its ATPase activity in *Entamoeba histolytica*. *Journal of Molecular Biology* **426**, 1786–1798. doi: 10.1016/j.jmb.2014.01.008.

Taipale, M., Jarosz, D. F. and Lindquist, S. (2010). Hsp90 at the hub of protein homeostasis: emerging mechanistic insights. *Nature Reviews Molecular Cell Biology* **11**, 515–528. doi: 10.1038/nrm2918.

Taldone, T., Sun, W. and Chiosis, G. (2009). Discovery and development of heat shock protein 90 inhibitors. *Bioorganic and Medicinal Chemistry* **17**, 2225–2235. doi: 10.1016/j.bmc.2008.10.087.

Tuntasuvan, D., Jarabrum, W., Viseshakul, N., Mohkaew, K., Borisutsuwan, S., Theeraphan, A. and Kongkanjana, N. (2003). Chemotherapy of surra in horses and mules with diminazene aceturate. *Veterinary Parasitology* **110**, 227–233. doi: 10.1016/S0304-4017(02)00304-7.

Ul Hasan, M., Muhammad, G., Gutierrez, C., Iqbal, Z., Shakoor, A. and Jabbar, A. (2006). Prevalence of *Trypanosoma evansi* infection in equines and camels in the Punjab region, Pakistan. *Annals of the New York Academy of Sciences* **1081**, 322–324. doi: 10.1196/annals.1373.043.

Van der Ploeg, L. H., Giannini, S. H. and Cantor, C. R. (1985). Heat shock genes: regulatory role for differentiation in parasitic protozoa. *Science* **228**, 1443–1446. doi: 10.1126/science.4012301.

Vigueira, P. A., Ray, S. S., Martin, B. A., Ligon, M. M. and Paul, K. S. (2012). Effects of the green tea catechin (-)-epigallocatechin gallate on *Trypanosoma brucei*. *International Journal for Parasitology – Drugs and Drug Resistance* **2**, 225–229. doi: 10.1016/j.ijpddr.2012.09.001.

Wang, M., Shen, G. and Blagg, B. S. (2006). Radanamycin, a macrocyclic chimera of radicicol and geldanamycin. *Bioorganic and Medicinal Chemistry Letters* **16**, 2459–2462. doi: 10.1016/j.bmcl.2006.01.086.

Wells, E. A. (1984). Animal trypanosomiasis in South America. *Preventive Veterinary Medicine* **2**, 31–41. doi: 10.1016/0167-5877(84)90046-1.

Wiesgigl, M. and Clos, J. (2001). Heat shock protein 90 homeostasis controls stage differentiation in *Leishmania donovani*. *Molecular Biology of the Cell* **12**, 3307–3316. doi: 10.1091/mbc.12.11.3307.

Yin, Z., Henry, E. C. and Gasiewicz, T. A. (2009). (-)-Epigallocatechin-3-gallate is a novel Hsp90 inhibitor. *Biochemistry* **48**, 336–345. doi: 10.1021/bi801637q.

Zweygarth, E. and Kaminsky, R. (1990). Evaluation of an arsenical compound (RM 110, mel Cy, Cymelarsan) against susceptible and drug-resistant *Trypanosoma brucei brucei* and *T. b. evansi*. *Tropical Medicine and Parasitology* **41**, 208–212.