Identification of plumbagin and sanguinarine as effective chemotherapeutic agents for treatment of schistosomiasis

Si-Ming Zhang *, Kristen A. Coultas

Center for Evolutionary and Theoretical Immunology, Department of Biology, MSC03 2020, University of New Mexico, Albuquerque, NM 87131, United States

A R T I C L E   I N F O

Article history:
Received 29 October 2012
Received in revised form 3 December 2012
Accepted 3 December 2012
Available online 29 December 2012

Keywords:
Plumbagin
Sanguinarine
Chemotherapy
Schistosomiasis

A B S T R A C T

Schistosomiasis, a snail-borne parasitic disease, affects more than 200 million people worldwide. Currently the treatment of schistosomiasis relies on a single therapy of praziquantel, a drug developed over 30 years ago. Thus, there is an urgent need to develop alternative antischistosomal drugs. In the pursuit of novel antischistosomal drugs, we examined the antischistosomal activities of 45 compounds that had been reported to exhibit antimicrobial and/or antiparasitic activities. Two plant-derived compounds, plumbagin and sanguinarine, were found to possess potent antischistosomal activities in vitro. For both the compounds, a concentration of 10 μM (equivalent to 1.88 μg/ml for plumbagin and 3.68 μg/ml for sanguinarine) resulted in 100% mortality at 48 h, which meets the World Health Organization’s (WHO) criterion of "hit" compounds for the control of schistosomiasis. Morphological changes and tegumental alterations of the dead worms treated by the two compounds were quite different. The significant morphological changes of worms after treatment by the two compounds suggest the two compounds target different biological pathways, both of which result in parasite’s death. This study provides evidence to suggest plumbagin and sanguinarine have real potential as effective alternative chemotherapeutic agents for the treatment of schistosomiasis.

© 2012 Australian Society for Parasitology. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Schistosomiasis is a chronic, debilitating disease caused by blood-dwelling trematodes of the genus Schistosoma. The global health impact of schistosomiasis is second only to malaria. According to a recent World Health Organization (WHO) report, 239 million people were infected with schistosomiasis (WHO, 2012). In sub-Saharan Africa alone, an estimated 150,000 deaths per year were attributable to schistosomiasis (van der Werf et al., 2003). In addition, people infected with schistosomes may have increased susceptibility to other infectious diseases such as HIV/AIDS (Secor, 2012). It resulted in up to 70 million disability-adjusted life years (DALYs) lost annually. This number exceeds that of malaria and tuberculosis, and nearly equivalent to the DALYs lost from HIV/AIDS (Hotz and Fenwick, 2009). Despite the impacts described, the aggregate health impact of schistosomiasis is often underestimated because of the complexity of evaluating the disease (King, 2010).

Considerable effort has been made in vaccine development, which has been unsuccessful so far (McWilliam et al., 2012). The current treatment of schistosomiasis relies on the drug praziquantel (PZQ), which was developed in the late 1970s (Seubert et al., 1977). PZQ has been widely used as an effective means to control schistosomiasis. However, PZQ does not treat early infection or prevent reinfection (Magnussen, 2003). In addition, available evidence indicates the emergence of PZQ resistance by schistosomes (Cioli et al., 1993; Fallon and Doenhoff, 1994; Ismail et al., 1999). For example, investigations in Egypt, Senegal and Kenya revealed different degrees of PZQ drug resistance in Schistosoma mansoni (Ismail et al., 1999; Melman et al., 2009; van den Enden, 2009). Moreover, recent efforts to expand mass chemotherapy administrations using PZQ (Webster et al., 2009) might accelerate the emergence of drug resistance in schistosomes.

Some antiparasitic drugs like artemether and mefloquine, both antimalarial drugs, as well as miltefosine, an antileishmanial drug, have shown antischistosomal activity (Caffrey and Secor, 2011). Administration of these drugs with or without PZQ is currently under evaluation (Eissa et al., 2011; Keiser et al., 2011; Xiao et al., 2011a). However, it is important to keep in mind the multi-use of antimalarial drugs may put the control of malaria at risk. This is likely due to the co-occurrence of schistosomes and malaria in most endemic areas and also the well-documented ability of Plasmodium to develop drug resistance. An older drug, oxamniquine, is no longer manufactured because it is only effective against one species of schistosome, S. mansoni. Additionally, it has been found to possess undesired side effects and promote drug
resistance (Fallon and Doenhoff, 1994). Some new drug candidates such as the antioxidant inhibitors, oxadiazoles (Sayed et al., 2008), and some protease inhibitors (Abdulla et al., 2007) have promising potentials against a schistosome infection, but have yet to reach clinical trials. Developing drugs for neglected tropical diseases is largely ignored; i.e., out of 1599 new drugs developed worldwide from 1975 to 2004, only 21 were for patients with neglected tropical diseases (Renslo and McKerrow, 2006) despite the large numbers of infected individuals worldwide. Developing a new drug in general is very complicated, only one in ten drug projects at the discovery phase progress to clinical development (Brown and Superti-Furga, 2003). Thus to alleviate these challenges, efforts to explore and discover novel “hit” and “lead” chemotherapeutic compounds are continually undertaken.

In the search of new candidates of alternative antischistosomal drugs, we tested the antischistosomal activities of 45 compounds that had been reported to possess antiparasitic and/or antimicrobial activities. We found that two plant-derived compounds, plumbagin and sanguinarine, possess potent antischistosomal activities. In this paper we describe the antischistosomal activities of the two compounds as well as the morphological changes and tegumental changes observed in the worms after treatment with the two compounds.

2. Materials and methods

2.1. Parasites

NIH strain S. mansoni (NIH-SM-PR2) was maintained in the laboratory using the M line snail Biomphalaria glabrata and mice as intermediate and definitive hosts, respectively (Zhang and Coultas, 2011). All procedures of schistosome infection and mouse perfusion were conducted in accordance with protocols approved by the University of New Mexico Institutional Animal Care and Use Committee.

2.2. Compounds

If not otherwise stated, all chemical reagents were purchased from Sigma (http://www.sigmaaldrich.com/united-states.html) and are listed below. The number in parenthesis is the catalogue number of the product: albendazole (A4673), amodiaquin dihydrochloride dehydrate (A2799), anacardic acids (A7236), apicidin (A8851), arachidonic acid (A9673), artemether (A9361), atovaquone (A7986), berberine chloride hydrate (14050), bisdemethoxycurcumin (B6938), chelidonine (54274), chloroquine diphosphate salt (C6628), closantel (34093), curcumin (C1386), diclofenac sodium salt (D6899), dipyrndamole hydrochloride (D9766), docosahexaenoic acid (D2534), doxycycline hyclate (D9891), ethacrylic acid, etazolate hydrochloride (E1896), halofantrine hydrochloride (H46805), ibuprofen (I4883), lapachol (I2905), levamisol hydrochloride (L4680), macrolactone (M3761), mebendazole (M2523), mefloquine hydrochloride (M2319), methylene blue (M9140), metronidazole (M3761), miltefosine (M5571), 2-methoxy-1,4-naphthoquinine (189162), 1,2-naphthoquinone (346616), oxantel pamoate (O4755), oxefndazole (34176), praziquantel (P4668), pyrantel pamoate (P6210), piperazine (80621), plumbagin (P7262), pyrimethamine (46706), primaqquine bisphosphate (160393), quine (22620), sanguinarine (55890), sulfadoxine (S7821), sulfadiazine (S8626), and trichostatin A (T8552).

2.3. Culture of schistosome worms with compounds

All adult schistosome worms were collected from mice after 42–45 days post exposure to S. mansoni. Stock solutions of individual chemical reagents were prepared in dimethylsulfoxide (DMSO) (EMD Chemicals) and stored in –30 °C. The final concentration of DMSO in all treatments, including control, was 1% (v/v). The enriched RPMI (eRPMI) 1640 culture medium contained 25 mM HEPES, 10% fetal bovine serum (FBS), 125 units/ml each penicillin and streptomycin (all from Gibco) (Coultas and Zhang, 2012). The worms were washed with eRPMI three times and placed into a 6-well plate. In each well, 20–50 worms were cultured in 5 ml culture medium. For the control, the worms were treated with equal amounts of DMSO alone. The worms were cultured at 37 °C in an atmosphere of 5% CO₂. The criterion for establishing dead worms was made according to Issa et al. (2011) and Manneck et al. (2010) with a more stringent modification as described below. The plate was agitated slightly to ensure better parasitic visibility and then placed under a stereomicroscope. Worms that did not exhibit motility, particularly in the mouth region, for two minutes were considered dead.

The measurement of length of worms was conducted under a Zeiss Axioskop 2 Plus Mot Plus microscope. A high-resolution microscopy camera, AxioCam Hrc, was used to photograph the worms. After photography, the curve line in the middle of worm body was drawn to represent the actual length of the worm and exact length of the worm was calculated by a computer connected to the microscope.

With regard to effect of compounds on mortality of cercariae, 50–120 cercariae were placed into a 24-well plate. For a given compound, triplicates at each concentration were performed. Live cercariae show very active movement, thus if no movement was observed, cercariae exhibiting no movement for one minute were considered dead under the Zeiss stereo microscope (Discovery V8). In addition, the death of cercariae as described above was also verified by the propidium iodide staining method (Coultas and Zhang, 2012).

2.4. Scanning electron microscopy (SEM) study

After the adult worms were incubated in the medium that contained either compounds to be tested or a DMSO control, worm samples were collected and washed with fresh eRPMI two times. Worm samples were then washed two more times with phosphate buffer saline (PBS) for 10 min. each at room temperature (RT) to remove any residual compounds and/or media before fixing. Next, samples were fixed in 2.5% glutaraldehyde in PBS buffer (0.1 mol/L, pH 7.4) overnight at 4 °C. After fixing for 24 h, samples were washed with PBS four times for 10 min. each at RT. Samples were dehydrated in a series of ethanol washes (25%, 50%, 70%, 75%, 85%, 95% and 100% ethanol) for 10 min. each at room temperature. The 100% ethanol wash was performed two times. Samples were critically point dried, mounted on carbon paste and coated with gold/palladium before examining with the JEOL 5800LV scanning electron microscope.

3. Results

3.1. Plumbagin and sanguinarine exhibit potent antischistosomal activities in vitro

The compounds that resulted in 100% mortality at a concentration of 50 µmol/L after four days were further investigated. Among the 45 compounds tested (as listed in the Section 2), six compounds (PZQ, plumbagin, sanguinarine, curcumin, mefloquine and miltefosine) met the criterion and received further investigation. Among the six compounds depicted in Fig. 1, the antischistosomal activity of PZQ was the most effective. In addition, the efficacy of plumbagin and sanguinarine were better than that of curcumin, mefloquine or miltefosine.
Given the relatively minor antischistosomal effect of curcumin, mefloquine and miltefosine as compared to plumbagin and sanguinarine, as well as the data published for the antischistosomal activity of the compounds, we focused in the subsequent studies on the effect of different concentrations of plumbagin and sanguinarine on the mortality of the worms (Fig. 2). Investigation revealed that all worms were killed by either of the two compounds within 6 h at concentrations of DMSO was observed within 4 days of worm culture or 4 h of cercariae culture. The final concentration of DMSO is 1% (v/v) for all experiments. Note that the intervals on the X-axis are not equal.

Fig. 2. Effect of various concentrations of plumbagin and sanguinarine on the survival of worms. The average percent of live worms is shown. The average percentages were generated from three independent experiments and the bars show the range of live worm percentages in the experiments. Note that the intervals on the X-axis are not equal.

In addition to the profound in vitro schistosomicidal effects described above, we also noted that the morphological appearance of the dead adult worms treated by the two compounds was quite different (Fig. 4). Adult worms treated with plumbagin became contracted, immobile and at times, appeared tightly coiled. The length of dead male and female worms after treatment with plumbagin was respectively about 54% and 70% of the length of dead adult worms treated by the two compounds was quite consistent with that of the worm experiments described above. We found that sanguinarine displayed the strongest anticercarial effect amongst the compounds investigated. Notable still, plumbagin possessed similar schistosomicidal effects to that of PZQ. Moreover, we found the plumbagin caused a high percentage of separation of cercariae heads from tails whereas sanguinarine resulted in fewer separations. While plumbagin caused muscle contractions, which lead to the splitting of the head from the tail, sanguinarine seemed to simply paralyze the cercariae (Fig. 3B).

3.2. Plumbagin leads to worm contraction, but sanguinarine does not

In addition to the profound in vitro schistosomicidal effects described above, we also noted that the morphological appearance of the dead adult worms treated by the two compounds was quite different (Fig. 4). Adult worms treated with plumbagin became contracted, immobile and at times, appeared tightly coiled. The length of dead male and female worms after treatment with plumbagin was respectively about 54% and 70% of the length of worms incubated with DMSO (P < 0.01). Conversely, no such morphological changes were observed in the sanguinarine-treated worms in both sexes, as compared to the control group (Fig. 4A and B).

3.3. Both compounds can cause tegumental alterations, but each compound exhibits different effects on the tegument

To better understand the effects of the compounds on the worm tegument, the interface between host and parasite, we used SEM to examine the surface membrane of worms under different treatments. We found that both compounds significantly damaged the worm’s tegument (Fig. 5). Interestingly, in most cases, plumbagin showed prominent alterations of the tegumental surfaces, usually with disintegration of tubercles and often times accompanied by a decrease in the number of spines. Emergence of holes on the surface was also observed (Fig. 5C and D). Sanguinarine treatment resulted in severe erosion and disintegration of the tegumental surface between tubercles, however the tubercles and spines were relatively intact. In fact, some tubercles did not display spine loss while other tubercles simply had fewer spines (Fig. 5E and F). For both compounds, the tegument of female worms after treatment are shown to varying degrees of swelling and cracking ranging...
drug, miltefosine (Eissa et al., 2011) have also recently been tested (Keiser et al., 2011; Xiao et al., 2011a,b) and the antileishmanial et al., 2009; Luz et al., 2012). The antimalarial drug, mefloquine, as a potential antischistosomal drug (Allam, 2009; Magalhães the three compounds known to possess schistosomicidal activity. were better than that of curcumin, mefloquine and miltefosine, the most profound amongst all compounds tested and the efficacy of 50 \text{ l} of both compounds resulted 100% mortality at 48 h. WHO’s definition and activity criteria for hits and leads sets inhibition of 100% motility of adult worms at a concentration of 5 \text{ l/ml} (in vitro) and 80% worm reduction after five injections at a dosage 100 mg/kg body-weight/day (in vivo) (Hwaka and Hudson, 2006). Clearly, plumbagin and sanguinarine meet WHO’s criterion for “hit” compounds and are effective against schistosomes. Moreover, our comparative studies revealed that the in vitro antischistosomal effects of the two compounds is better than other antischistosomal drugs such as artemether, mefloquine, miltefosine and curcumin that are under current investigation as alternative therapies (Allam, 2009; Sissoko et al., 2009; Eissa et al., 2011; Keiser et al., 2011; Xiao et al., 2011a,b; Luz et al., 2012). These exciting findings provide a strong impetus to investigate the in vivo efficacy of plumbagin and sanguinarine on schistosomes in the future.

In addition to the antischistosomal activity described, it is intriguing that the morphological appearance of the dead worms after treatment with plumbagin and sanguinarine is quite different. Worms treated with plumbagin become withered while worms treated with sanguinarine show no apparent changes in appearance after death. It seems that plumbagin causes muscle contraction, but sanguinarine does not. This change is similarly observed in experiments of cercariae, where muscle contraction caused by plumbagin may be responsible for the high rate of head separation observed (Fig. 3B). Taken together, these differences imply there are at least two different mechanisms involved in the parasite’s death. Based on the observations, it seems that plumbagin affects muscle function because of the muscle contraction observed. In the case of sanguinarine, it paralyses the worms, potentially through the nervous system. Due to the complexity of drug mechanisms and their mode of action, comparative investigation of these differences using molecular approaches may reveal the fundamental mechanism(s) of killing the parasites, which in turn, can facilitate the design of new drugs to combat schistosomiasis.

Another intriguing phenomenon we observed is that plumbagin and sanguinarine, although possessing different effects on schistosomes, can both alter the tegumental structure of the worms; i.e., both compounds damage the tegument, thus implying their antischistosomal effect may increase in the host. This is due to the fact that the tegument is the interface between the host and parasite and harbors large amounts of molecules that constitute very com-
It is well recognized that the parasite’s surface membrane and tegumental integrity play a vital role in immune evasion, modulation and nutrient uptake and thus ensure worm survival in the host. Profound damage caused by the two compounds observed here could alter tegumental structure and stability. In addition to the direct effect on the survival of the worms, tegumental alteration might result in exposing the typically unexposed parasite antigens to the host’s immune system. As a result, treatment with plumbagin and sanguinarine might increase the vulnerability of parasites in the host and thus further...
enhance the efficiency of the compounds at killing schistosomes in vivo.

Pluumbagin and sanguinarine are plant-derived products (Reuter et al., 2011). Exploring natural products has long been considered an ideal alternative for the development of new drugs (Geary et al., 2012). According to data of all approved agents from 1981 to 2006, about 50% of new drugs were directly or indirectly derived from natural products (Newman and Cragg, 2007). With respect to parasitic diseases, many compounds isolated from plants have proven to be the mainstay in anthelmintics and antimalarial therapy. For example, there are notable plant-derived antiparasitic drugs such as quinoline alkaloids against Leishmania amazonensis and indole alkaloids against Plasmodium and Entamoeba (Kayser et al., 2003). Three major antimalarial drugs, chloroquine, atovaquone and artemisinin, are derived from plants (Oliveira et al., 2009). Chloroquine was synthesized based on the structure of quinine, a natural compound of the tree bark Cinchona spp. that was used to treat malaria 400 years ago (Achan et al., 2011). Atovaquone was structurally derived from lapachol, originally purified from Tabebuia spp. Artemisinin was extracted from Artemisia annua and many more effective drugs such as artemether were designed based on the artemisinin (Miller and Su, 2011). In light of these discoveries, similar efforts on schistosomiasis should be emphasized (Ribeiro-dos-Santos et al., 2006). Although a few plant products have been screened for activity against schistosomes (Cichewicz et al., 2002; Sanderson et al., 2002; Allam, 2009; Magalhães et al., 2010), presently no antischistosomal compounds have been purified and reported.

Plumbagin is one of the simplest secondary plant metabolites of the three major families, Plumbaginaceae, Droseraceae and Ebenaceae. It exhibits many highly potent biological activities including activation of apoptosis, induction of redox cycling and modification of chromatin structure. Plumbagin and its derivatives can act as an antioxidant, anti-inflammatory, anticancer, antibacterial and antifungal agents (Padhye et al., 2012). Additionally, plumbagin is capable of inhibiting the drug efflux mechanism in drug-resistant bacteria, thereby allowing intracellular accumulation of potent drug molecules. With regard to an antiparasitic role, evidence showed that plumbagin possesses antifilarial activity by inhibiting trypanothione and glutathione S-transferases (Srinivasan et al., 2009; Sharma et al., 2012).

During the process of submission of our manuscript, we noted that a paper describing the antischistosomal role of plumbagin was published (Lorsuwannarat et al., 2012). Although the effect of plumbagin on mortality of larvae (cercariae) and the morphology of adult worms was not described in the paper, the published paper and ours provide independent evidence supporting an antischistosomal role of plumbagin on adult worms. The slight differences observed between the two reports in terms of efficacy in vitro and tegumental changes may be due to the different strains of S. mansoni used in each investigation.

Sanguinarine is derived from the root of Sanguinaria spp and possesses a wide spectrum of biological assets including antimicrobial, antioxidant and anti-inflammatory properties. Sanguinarine can induce apoptosis in malignant cell types and interact with chromatin and modulate it epigenetically (Selvi et al., 2009). Active efforts have been made to develop sanguinarine and its derivatives as anticancer agents (Sun et al., 2010; Pica et al., 2012). A recent study has also revealed sanguinarine’s role in killing the fish parasite Ichthyophthirius multifilis (Yao et al., 2010). Our data presented here provides the first evidence suggesting an antischistosomal role of sanguinarine.

Previous studies have shown that plumbagin and sanguinarine have a wide array of medical and health applications, hence it would not be surprising if they additionally possess potent antischistosomal activities. Both compounds belong to naphthoquinones, a large family of chemical compounds. A recent study suggested that a slight modification of some naphthoquinone compounds could increase antileishmanial activity (Ali et al., 2011). This might imply that the compounds, if designed properly, may be an effective antischistosomal therapy. In fact, we have already confirmed that some analogs of the two compounds have a similar antischistosomal activity (data not shown), implying the two chemicals identified in this study can serve as basic compounds for development of novel chemotherapeutics to control schistosomiasis in the future. Given the broad spectrum of documented pharmacological activities exhibited by the compounds, they continue to be the subject of extensive studies to evaluate possible therapeutic applications in human health. This will make future development of the two compounds as antischistosomal therapy relatively easy.

References
Abdul-Ghani, R., Lourfy, N., Sheta, M., Hassan, A., 2011. Artemether shows promising female schistosomicidal and ovidial effects on the Egyptian strain of Schistosoma mansoni after maturity of infection. Parasitol. Res. 108, 1199–1205.
Abdulla, M.H., Lim, K.C., Sajid, M., McKerrow, J.H., Caffrey, C.R., 2007. Schistosomiasis mansoni: novel chemotherapy using a cysteine protease inhibitor. PLoS Med. 4, e14.
Achan, J., Talisuna, A.O., Erhart, A., Yeka, A., Tibeferanada, J.K., Baliraine, F.N., Rosenthal, P.J., D’Alessandro, U., 2011. Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. Malar. J. 10, 144.
Ali, A., Assimopoulou, A.N., Papageorgiou, V.P., Kolodziej, H., 2011. Structure/antileishmanial activity relationship study of naphthoquinones and dependency of the mode of action on the substitution patterns. Planta Med. 77, 2003–2012.
Allam, G., 2009. Immunomodulatory effects of curcumin treatment on murine Schistosomiasis mansoni. Immunobiology 214, 712–727.
Beech, R.N., Skuce, P., Bartley, D.J., Martin, R.J., Prichard, R.K., Gilles, J.D., 2011. Anthelmintic resistance: markers for resistance, or susceptibility? Parasitology 138, 169–174.
Brown, D., Superti-Furg, G., 2003. Rediscovering the sweet spot in drug discovery. Drug Discov. Today 8, 106–1077.
Caffrey, C.R., Secor, W.E., 2011. Schistosomiasis: from drug deployment to drug development. Curr. Opin. Infect. Dis. 24, 410–417.
Cichewicz, R.H, Lim, K.V., McKerrow, J.H., Nair, M.G., 2002. Kwanzoquinones A-G and other constituents of Hemerocallis fulva ‘Kwanzo’ roots and their activity against the human pathogenic trematode Schistosoma mansoni. Tetrahedron 58, 8575–8586.
Cioli, D., Pica-Mattoccia, L., Archer, S., 1993. Drug resistance in schistosomes. Parasitol. Today 9, 162–166.
Coulitas, K.A., Zhang, S.-M., 2012. In vitro cercariae transformation: comparison of morphological and nonmorphological methods and observation of morphological changes of detached cercariae tails. J. Parasitol. http://dx.doi.org/10.1645/GE-30721.1.
Eissa, M.M., El-Azzouni, M.Z., Amer, E.I., Baddour, N.M., 2011. Mitelfosine, a promising novel agent for Schistosomiasis mansoni. Int. J. Parasitol. 41, 235–242.
Fallon, P.G., Doenhoff, M.J., 1994. Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in Schistosoma mansoni in mice is drug specific. Am. J. Trop. Med. Hyg. 51, 83–88.
Geary, T.G., Chihále, K., Abezaz, B., Andrae-Marobela, K., Labajboro, E., 2012. A new approach for anthelmintic discovery for human. Trends Parasitol. 28, 176–181.
Hotz, P.J., Fenwick, A., 2009. Schistosomiasis in Africa: an emerging tragedy in our new global health decade. PLoS Negl. Trop. Dis. 3, e485.
Hwaka, S., Hudson, A., 2006. Innovative lead discovery strategies for tropical diseases. Nat. Rev. Drug Discov. 5, 941–955.
Ismail, M., Botros, S., Metwally, A., William, S., Farghally, A., Tao, L.F., Day, T.A., Bennett, J.L., 1999. Resistance to praziquantel: direct evidence from Schistosoma mansoni isolated from Egyptian villagers. Am. J. Trop. Med. Hyg. 60, 932–935.
Kayser, O., Olbrich, C., Croft, S.L., Kiderlen, A.F., 2003. Formulation and biopharmaceutical issues in the development of drug delivery systems for antiparasitic drugs. Parasitol. Res. 2, 563–570.
Kesarvardi, T., Vaneechoutte, M., 2011. Interactions of melphalan with praziquantel in the Schistosoma mansoni mouse model and in vitro. J. Antimicrob. Chemother. 66, 1791–1797.
King, C.H., 2010. Parasites and poverty: the case of schistosomiasis. Acta Trop. 113, 95–104.
Lorsuwannarat, N., Saowakon, N., Ramasoota, P., Wanichanon, C., Sobhon, P., 2012. The anthelmintic effect of plumbagin on Schistosoma mansoni. Exp. Parasitol. http://dx.doi.org/10.1016/j.exppara.2012.10.003.
Ribeiro-dos-Santos, G., Verjovski-Almeida, S., Leite, L.C., 2006. Schistosomiasis – a Renslo, A.R., McKerrow, J.U., 2006. Drug discovery and development for neglected Pica, F., Balestrieri, E., Serafino, A., Sorrentino, R., Gaziano, R., Moroni, G., Moroni, N., Oliveira, A.B., Dolabela, M.F., Braga, F.C., Jácome, R.L., Varotti, F.P., Póvoa, M.M., 2009. Newman, D.J., Cragg, G.M., 2007. Natural products as sources of new drugs over the Müllner, A., Helfer, A., Kotlyar, D., Oswald, J., Efferth, T., 2011. Chemistry and Miller, L.H., Su, X., 2011. Artemisinin: discovery from the Chinese herbal garden. Melman, S.D., Steinauer, M.L., Cunningham, C., Kubatko, L.S., Mwangi, I.N., Wynn, N.B., Mutuku, M.W., Karanja, D.M., Colley, D.G., Black, C.L., Secor, W.E., Mkoji, G.M., Loker, E.S., 2009. Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of Schistosoma mansoni. PLoS Negl. Trop. Dis. 3, e504. Miller, L.H., Su, X. 2011. Artemisinin: discovery from the Chinese herbal garden. Cell Host Microbe 6, 855–858. Müllner, A., Helfer, A., Kotlyar, D., Oswald, J., Effrth, T., 2011. Chemistry and pharmacology of neglected helminthic diseases. Curr. Med. Chem. 18, 767–789. Newman, D.J., Cragg, G.M. 2007. Natural products as sources of new drugs over the last 25 years. J. Nat. Prod. 70, 461–477. Oliveira, A.B., Dolabela, M.F., Braga, F.C., Jácome, R.L., Varotti, F.P., Póvoa, M.M., 2009. Plant-derived antimalarial agents: new leads and efficient phytomedicines. Part I. Alkaloids. An. Acad. Bras. Cienc. 81, 715–740. Padhye, S., Dandawate, P., Yusu, M., Ahmad, A., Sarkar, F.H., 2012. Perspective on medicine properties of plumbagin and its analogs. Med. Res. Rev. 32, 1131–1158. Pica, F., Balestrieri, E., Serafino, A., Sorrentino, R., Gazzano, R., Moroni, G., Moroni, N., Palmieri, C., Mattei, M., Garaci, E., Sinibaldi-Vallebona, P., 2012. Antitumor effects of the benzophenanthridine alkaloid sanguinarine in a rat syngeneic model of colorectal cancer. Anticancer Drugs 23, 32–42. Renslo, A.R., McKerrow, J.U., 2006. Drug discovery and development for neglected parasitic diseases. Nat. Chem. Biol. 2, 701–710. Reuter, S., Gupta, S.C., Park, B., Goel, A., Aggarwal, B.B., 2011. Epigenetic changes induced by curcumin and other natural compounds. Genes Nutr. 6, 93–108. Ribeiro-dos-Santos, G., Verjovski-Almeida, S., Leite, L.C., 2006. Schistosomiasis – a century searching for chemotherapeutic drugs. Parasitol. Res. 99, 505–521. Sanderson, L., Bartlett, A., Whiffen, P.J., 2002. In vitro and in vivo studies on the bioactivity of a ginger (Zingiber officinale) extract towards adult schistosomes and their egg production. J. Helminthol. 76, 241–247. Sayed, A.A., Simeonov, A., Thomas, C.J., Inglese, J., Austin, C.P., Williams, D.L., 2008. Identification of oxadiazoles as new drug leads for the control of schistosomiasis. Nat. Med. 14, 407–412. Secor, W.E., 2012. The effects of schistosomiasis on HIV/AIDS infection, progression and transmission. Curr. Opin. HIV AIDS 7, 254–259. Selvi, B.R., Pradhana, S.K., Shankilya, J., Das, C., Sailaja, B.S., Shankar, G.N., Gadad, S.S., Reddy, A., Dasgupta, D., Kundu, T.K., 2009. Sanguinarine interacts with chromatin, modulates epigenetic modifications, and transcription in the context of chromatin. Chem. Biol. 16, 203–216. Seubert, J., Pohle, R., Loebisch, F., 1977. Synthesis and properties of Praziquantel, a novel broad spectrum anthelmintic with excellent activity against Schistosomes and Cestodes. Experientia 33, 1036–1037. Sharma, N., Shukla, A.K., Das, M., Dubey, V.K., 2012. Evaluation of plumbagin and its derivative as potential modulators of redox thiol metabolism of Leishmania parasite. Parasitol. Res. 110, 341–348. Sissoko, M.S., Dabo, A., Traore, H., Diallo, M., Traore, B., Konate, N., Diakite, K., Kamate, B., Traore, A., Bathily, B., Tapily, A., Toure, O.B., Cauwenbergh, S., Jansen, H.F., Doumbo, O.K., 2009. Efficacy of artesunate + sulfamethoxypyrazine/pyrimethamine versus praziquantel in the treatment of Schistosoma haematobium in children. PLoS One, e6732. Srinivasan, L., Mathew, N., Muthuswamy, K., 2009. In vitro antifilarial activity of glutathione S-transferase inhibitors. Parasitol. Res. 105, 1179–1183. Sui, M., Lou, W., Chun, J.Y., Cho, D.S., Namdinty, N., Evans, C.P., Chen, J., Yue, J., Zhou, Q., Gao, A.C., 2010. Sanguinarine suppresses prostate tumor growth and inhibits survivin expression. Genes Cancer 1, 283–292. van den Enden, E., 2009. Pharmacotherapy of helminth infection. Expert Opin Pharmacother. 10, 435–451. van der Werf, M.J., de Vlas, S.J., Brooker, S., Looman, C.W., Nagelkerke, N.J., Habbema, J.D., Engels, D., 2003. Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. Acta Trop. 86, 125–139. Webster, J.P., Koukounari, A., Lamberton, P.H., Stothard, J.R., Fenwick, A., 2009. Evaluation and application of potential schistosome-associated morbidity markers within large-scale mass chemotherapy programmes. Parasitology 136, 1789–1799. WHO, 2012. Accelerating work to overcome the global impact of neglected tropical disease: a roadmap for implementation. <http://www.who.int/neglected_diseases/NTD_RoadMap_2012_FullVersion.pdf>. Xiao, S.H., Mei, J.Y., Jiao, P.Y., 2011a. Effect of mefloquine administered orally at single, multiple, or combined with arteether, artesunate, or praziquantel in treatment of mice infected with Schistosoma japonicum. Parasitol. Res. 108, 399–406. Xiao, S.H., Xue, J., Zhang, H.B., 2011b. Further studies on mefloquine and praziquantel alone or interaction of both drugs against Schistosoma japonicum in vitro. Parasitol. Res. 110, 1239–1248. Yao, J.Y., Shen, J.Y., Li, X.L., Xu, Y., Hao, G.J., Pan, X.Y., Wang, G.X., 2010. Effect of sanguinarine from the leaves of Macleaya cordata against Ichthyophthirius multifiliis in grass carp (Ctenopharyngodon idella). Parasitol. Res. 107, 1035–1042. Zhang, S.-M., Coulats, K.A., 2011. Identification and characterization of five transcription factors that are associated with evolutionarily conserved immune signaling pathways in the schistosome-transmitting snail Biomphalaria glabrata. Mol. Immunol. 48, 1868–1818.