Synthesis and biological evaluation of a novel series of aryl S,N-ketene acetals as antileishmanial agents

ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY LETTERS · APRIL 2013

Impact Factor: 2.42 · DOI: 10.1016/j.bmcl.2013.04.025 · Source: PubMed

Bioorganic & Medicinal Chemistry Letters 23 (2013) 3979–3982

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of a novel series of aryl *S,N*-ketene acetals as antileishmanial agents ☆

S. N. Suryawanshi [a,*], Santosh Kumar [a], Avinash Tiwari [a], Rahul Shivahare [b], Yashpal Singh Chhonker [c], Susmita Pandey [a], Nishi Shakya [b], Rabi Sankar Bhatta [c], Suman Gupta [b]

[a] Division of Medicinal Chemistry, CSIR-Central Drug Research Institute, Chattar Manzil Palace, P.O. Box 173, Mahatma Gandhi Road, Lucknow 226001, India
[b] Division of Parasitology, CSIR-Central Drug Research Institute, Chattar Manzil Palace, P.O. Box 173, Mahatma Gandhi Road, Lucknow 226001, India
[c] Division of Pharmacokinetics and Metabolism, CSIR-Central Drug Research Institute, Chattar Manzil Palace, P.O. Box 173, Mahatma Gandhi Road, Lucknow 226001, India

ARTICLE INFO

Article history:
Received 28 September 2012
Revised 5 April 2013
Accepted 9 April 2013
Available online 26 April 2013

Keywords:
Aryl *S,N*-ketene acetal
Leishmania donovani
Antileishmanial activity
Hamster
Pharmacokinetic studies

ABSTRACT

A series of aryl *S,N*-ketene acetals **7(a–f)** was synthesized and evaluated for their in vitro and in vivo antileishmanial activity against *Leishmania donovani*. All the **6** compounds exhibited significant in vitro activity against intracellular amastigotes of *L. donovani* with IC_{50} values ranging from 1.2 to 3.5 μM and were found promising as compared with reference drugs, sodium stibogluconate (SSG) and paromomycin. On the basis of good selectivity indices (SI), they were further tested for their in vivo potential against *L. donovani*/hamster model. Two compounds **7a** and **7b** showed significant inhibition of parasite multiplication, 72% and 83%, respectively. These compounds were comparable with SSG and superior to paromomycin. Preliminary in vitro metabolic investigations were also performed to assess the metabolic stability and in vitro hepatic intrinsic clearance (Cl_{int}) of compound **7b** in hamster liver microsomes.

Leishmaniasis is a group of parasitic diseases that affect about twelve million people in tropical and subtropical areas provoking three clinical expressions: visceral leishmaniasis (VL) that is fatal in the absence of treatment,[1] muco-cutaneous leishmaniasis and cutaneous leishmaniasis, the last one often self-curing.[2] Pentavalent antimonials remain the first-line treatment in most parts of the world, but in India, its use is restricted due to the high level of drug resistance. There has been a significant improvement in the number of treatments available for VL during the past decade, with both new drugs and new formulations of old drugs either recently approved or in clinical trial. These new treatments include: AmBisome™, a liposomal amphotericin-B formulation, registered for VL in the USA and Europe in the 1990s with remarkable activity, even at a single dose in India; oral miltefosine, registered in India in 2002 (later in Bangladesh); and a low cost intramuscular formulation of paromomycin (aminosidine), registered in India in 2006 and in phase III trial in East Africa by DNDi. Unfortunately, all of these drugs have significant drawbacks that limit their utilization in disease endemic areas. These include route of administration, length of treatment (21–28 days), toxicity and cost.[3] Thus, the development of new, efficient, and safe drugs for the treatment of this disease is imperative. In this endeavour, diarylheptanoids,[4] oxygenated abietanes,[5] diterpenequinones[6,7] and chalcones[8] showed promising results as antileishmanial agents. Curcumin **1** isolated from *Curcuma longa* Linn., well known as an anticancer agent,[4a] also showed antileishmanial activity in in vitro studies.[4b,9] Exhaustive analoging of curcumin has generated some interesting results.[10] Licochalcone **2** isolated from *Glycerrhiza* sp., first reported for its antibacterial activity[11] has also showed promising antileishmanial activity.[12] Chemical library generated on the basis of licochalcone as a lead molecule was found active in in vitro studies.[8a] Phenolic diketone **3** isolated from *Zingiber officinale*,[13] a structural mimic of **1** and **2** shows radical scavenging activities, quite comparable to curcumin **1**. In continuation of our efforts to generate natural product based novel antileishmanial agents[14] coupled with encouraging results of **1**–**3** (Fig. 1), we have synthesized some novel aryl *S,N*-ketene acetals for their in vitro and in vivo antileishmanial activity profile and preliminary in vitro metabolic studies. The findings are reported in this communication .

α-Oxoketene dithioacetals of aromatic substrates are very useful synthons in the synthesis of variety of heterocyclic and carbocyclic compounds.[15] Various aryl *S,N*-ketene acetals have been synthesized from α-oxoketene dithioacetals.[16a–c] However; they have not been fully exploited for their biological activity profile. 3,4,5-trimethoxy aryl *S,N*-ketene acetals **7**(**a**–**f**) were prepared as shown in Scheme 1. The reaction of 3,4,5-trimethoxybenzaldehyde (tri-*o*-methylgallaldehyde) **4** with acetone based ketene dithioacetal **5** furnished **6** which on further reaction with primary amines

☆ CDRI Communication No. 272/2011/SNS.

* Corresponding author. Tel.: +91 522 221 2411 18; fax: +91 522 222 3405.
E-mail address: shivajins1952@gmail.com (S.N. Suryawanshi).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.04.025

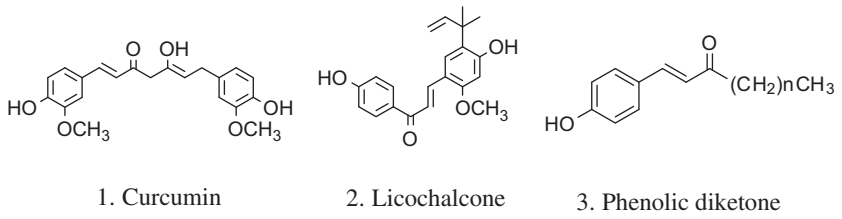
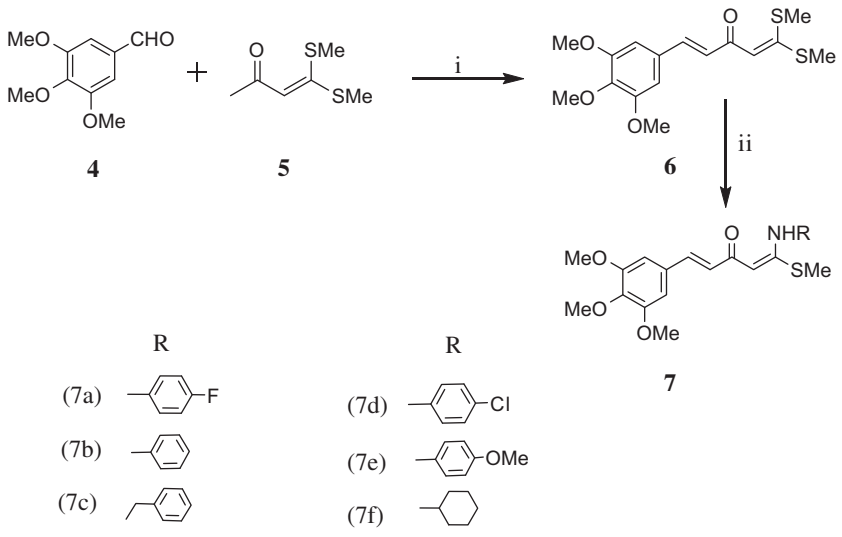


Figure 1. A natural product based approach to synthesized novel aryl *S,N*-ketene acetals.



Scheme 1. Reagents and conditions: (i) aq.KOH, MeOH, stirring, 12 h, rt. (ii) Amines, EtOH, heat in steel bomb, 120 °C, 24 h.

gave **7(a–f)** as a yellow crystalline solids. The structures of **7**(**a**–**f**) were assigned with the help of IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry.

A series of trimethoxy aryl *S,N*-ketene acetals has been synthesized and the compounds were evaluated against extracellular promastigotes and intracellular amastigotes of *Leishmania donovani*. In our earlier studies[17] we have demonstrated compound **6** as antileishmanial agent which showed 66% inhibition of *Leishmania* parasite in hamster model. Therefore, our main objective of present work was to study the effect of substitution on the biological activity profile of compound **6**. Table 1 displays the percent inhibition of these compounds against promastigotes. Interestingly, all the six compounds exhibited high inhibition (96–99%) against promastigotes at 25 μM concentration. These compounds were further screened [19,20] in vitro against intracellular amastigote model and their IC_{50}, CC_{50} and selectivity index (SI) were calculated and presented in Table 1. All the compounds were found highly active with IC_{50} ranging from 1.2 to 3.5 μM. The activity of these compounds was far better than the reference drugs sodium stibogluconate (IC_{50} = 57.3 μg/mL) and paromomycin (IC_{50} = 24.8 μg/mL). Compounds showing SI above 10 **(7a–e)** were tested further for in vivo antileishmanial activity [21] and the results are depicted in Table 1. Amongst them, only two compounds **7a** and **7b** showed significant inhibition of parasite multiplication, 72% and 83%,

Table 1
In vitro and in vivo antileishmanial activity data of synthesized aryl *S,N*-ketene acetals

Compound code no.	In vitro assessment		Cytotoxicity CC_{50} (μM)	Selectivity index (SI) CC_{50}/IC_{50}	In vivo activity (dose-50 mg/kg × 5 days, ip[a]) percent inhibition ± SD
	Anti-promastigote activity percent inhibition (PI) at 25 μM	Anti-amastigote activity IC_{50} (μM)			
7a	97.3–98.5 (97.9)	1.1–1.3 (1.2)	39.7–47.2 (43.4)	36	72 ± 22
7b	95.2–97.2 (96.2)	1.8–2.4 (2.1)	58.8–70.4 (64.6)	31	83 ± 12 64 ± 10 (po[b])
7c	97.5–99.7 (98.6)	1.5–2.5 (2.0)	64.1–79.0 (71.5)	36	NI[c]
7d	97.6–99.8 (98.7)	1.4–1.8 (1.6)	53.6–62.2 (57.9)	36	21 ± 16
7e	98.7–99.5 (99.1)	2.1–3.2 (2.6)	174.8–192.9 (183.8)	69	32 ± 25
7f	98.3–99.5 (98.9)	2.7–4.3 (3.5)	18.7–26.8 (22.7)	7	ND[d]
SSG*	NI	52.6–62.1 (57.3)	397.5–399.2 (398.3)	7	89 ± 8
Paromomycin	NI	22.6–27.1 (24.8)	49.6–57.1 (53.3)	2	46 ± 9

PI, IC_{50} and CC_{50} values are the average of two independent experiments.
[a] ip = intraperitoneal.
[b] po = per oral.
[c] NI = no inhibition.
[d] ND = not done.
* SSG (sodium stibogluconate) and paromomycin-concentration in μg/mL.

respectively against *L. donovani*/hamster model at 50 mg/kg × 5 days dose when administered by intraperitoneal (ip) route. The efficacy of most active compound **7b** was also evaluated by oral route and showed 64% inhibition in parasite growth at same dose regimen. There were no toxic symptoms observed during treatment. The efficacies of these compounds were more or less similar to SSG and superior to paromomycin. Compound with anilino substitution, **7b** showed 83% inhibition, while in case of benzyl amino substitution, the compound **7c** was found to be ineffective indicating that introduction of methylene group made the compound inactive. Similar inhibition was reported in the case of cyclohexylamino substitution **7f**. Among the halogen substituted anilines compound, **7d** having chlorine at *para* position of phenyl ring showed only 21% inhibition of *L. donovani* whereas compound, **7a** with fluorine at *para* position showed 72% inhibition of *L. donovani*. Hence, introduction of more electronegative halogen at *para* position increased the antileishmanial potential of compound **7**. Compound, **7e** having *p*-anisidino group showed 32% inhibition of *L. donovani*. Metabolism is a major contributor of drug clearance and it directly influences the systemic drug exposure. If metabolites are observed in to systemic circulation, they may have pharmacological and/or toxicological effects. Moreover, metabolism also plays a role in drug efficacy by forming metabolites with specific to drug target receptors leading to synergic effects. The metabolic stability of most active compound **7b** at 25 μM was evaluated in pooled hamster liver microsomes (HLM). Testosterone was employed as control compound to evaluate the metabolic stability.

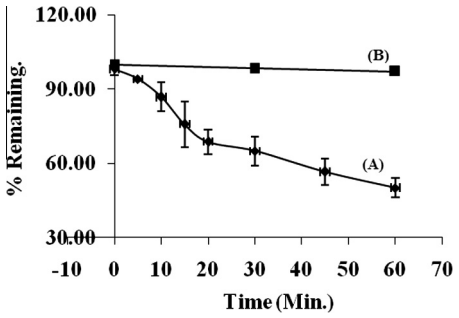


Figure 2. Time-dependent metabolic depletion of **7b** in hamster liver microsomes (HLM). Metabolic elimination profiles (% turnover or amount remaining vs incubation time) for: (A) with NADPH and (B) without NADPH. Data are shown as mean ± SD ($n = 3$).

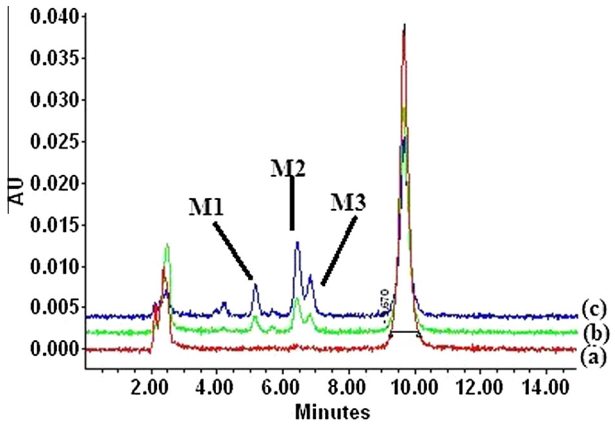


Figure 3. Representative HPLC-PDA chromatograms of (a) compound **7b** incubated without NADPH at 389 nm. (b) Incubated compound **7b** with NADPH at 10 min, metabolites (M1, M2 and M3), and (c) compound **7b** incubated with at 60 min, metabolites (M1, M2 and M3).

The determined intrinsic clearance of these probe substance was comparable to previously reported values.[18] As shown in data presented in Figure 2, compound **7b** was degraded in HLM, with only 56.5% of the original amount left after 45 min incubation. The calculated in vitro half-life for compound **7b** was 60.5 ± 2.7 min and derived intrinsic clearance (CL_i) was 14.3 μL/min × mg of microsomal protein in pooled hamster liver microsomes.

After incubation of compound **7b** with NADPH, three prominent peaks (M1, M2 and M3) were detectable at 4.8, 6.5 and 7.0 min. The M1, M2 and M3 were observed at 389 nm (λ_{max} of compound **7b**). These peaks were not observed in the control samples (Fig. 3). M1, M2 and M3 were completely undetectable on incubation without NADPH and with 1-ABZT (CYP suicidal inhibitor). Hence, it was observed that compound **7b** exhibited moderate metabolic stability with three metabolites (M1, M2 and M3). These metabolites were found to be formed by CYP enzyme. Detection of M1, M2 and M3 will be a gateway for prospective metabolic studies involving structural identification of metabolites (in vitro/in vivo), its efficacy and pharmacokinetic evaluation.

These results clearly indicate that synthesized aryl *S,N*-ketene acetals reported herein, are promising compounds and provide useful model for further structural and biological optimization. The study opens up the possibility of advancing this new class of compounds as novel antileishmanial agents.

Acknowledgments

The authors are thankful to the Division of Sophisticated and Analytical Instrument Facility (SAIF), CSIR-CDRI for providing spectral and elemental analysis data. S.K. is also grateful to CSIR, New Delhi for awarding Senior Research Fellowship. The transgenic *L. donovani* promastigotes were originally procured from Dr. Neena Goyal, Division of Biochemistry, CSIR-CDRI, Lucknow, India.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.04.025.

References and notes

1. Chappuis, F.; Sundar, S.; Hailu, A.; Ghalib, H.; Rijal, S.; Peeling, R. W.; Alvar, J.; Boelaert, M. *Nat. Rev. Microbiol.* **2007**, *5*, 873.
2. Sharma, U.; Singh, S. J. *Vector Borne Dis.* **2008**, *45*, 255.
3. Gupta, S.; Nishi, S. *Indian J. Med. Res.* **2011**, *133*, 27.
4. (a) Alves, L. V.; Do Canto-Cavalheiro, M. M.; Cysne-Finkelstein, L.; Leon, L. *Biol. Pharm. Bull.* **2003**, *26*, 453; (b) Koide, T.; Nose, M.; Ogihara, Y.; Yabu, Y.; Ohta, N. *Biol. Pharma. Bull.* **2002**, *25*, 131; (c) Gomes, D. C.; de Alegrio, L. V.; Freire de Lima, M. E.; Leon, L. L.; Araujo, C. A. *Arzneim.-Farsch. Drug Res.* **2002**, *52*, 120.
5. Tan, N.; Kaloga, M.; Radtke, O. A.; Kiderlen, A. F.; Oksuz, S.; Ulubelen, A.; Kolodziej, H. *Phyto-chemistry* **2002**, *61*, 881.
6. Sairafianpour, M.; Christensen, J.; Staerk, D.; Budnik, B. A.; Kharazmi, A.; Bagherzadeh, K.; Jaroszewski, J. W. *J. Nat. Prod.* **2001**, *64*, 1398.
7. Valderrama, A. J.; Benites, J.; Cortes, M.; Pessoa-Mahana, H.; Prina, E.; Fournet, A. *Bioorg. Med. Chem.* **2003**, *11*, 4713.
8. (a) Liu, M.; Wilairat, P.; Croft, S. L.; Tan, A. L. C.; Go, M. L. *Bioorg. Med. Chem.* **2003**, *11*, 2729; (b) Chowdhury, S. F.; Di Lucrezia, R.; Guerrero, R. H.; Brun, R.; Goodman, J.; Ruiz-Perez, L. M.; Pacanowska, D. G.; Gilbert, I. H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 977; (c) Chowdhury, S. F.; Villamor, V. B.; Guerrero, R. H.; Leal, I.; Brun, R.; Croft, S. L.; Goodman, J. M.; Maes, L.; Ruiz-Perez, L. M.; Pacanowska, D. G.; Gilbert, I. H. *J. Med. Chem.* **1999**, *42*, 4300; (d) Foroumadi, A.; Emami, S.; Sorkhi, M.; Nikhjiri, M.; Nazarian, Z.; Heydari, S.; Ardestani, S. K.; Poorrajab, F.; Shafiee, A. *Chem. Biol. Drug Des.* **2010**, *75*, 590.
9. Saleheen, D.; Ali, S. A.; Ashfaq, K.; Siddiqui, A. A.; Agha, A.; Yasinzai, M. M. *Biol. Pharm. Bull.* **2002**, *25*, 386.
10. (a) Ohtsu, H.; Itokawa, H.; Xiao, Z.; Su, C. Y.; Shih, C. C. Y.; Chiang, T.; Chang, E.; Lee, Y. F.; Chiu, S. Y.; Chang, C.; Lee, K. H. *Bioorg. Med. Chem.* **2003**, *11*, 5083; (b) Ferriera de Gomez, D. C.; Alegrio, L. V.; Leon, L. L.; de Lima, F. *Arzneim.-Forsch. Drug Res.* **2002**, *52*, 695.
11. Tsukiyama, R. I.; Katsura, H.; Tokuriki, N.; Kobayashi, M. *Antimicrob. Agents Chemother.* **2002**, *46*, 1226.
12. Chen, M.; Zhai, L.; Christensen, S. B.; Theander, T. G.; Kharazmi, A. *Antimicrob. Agents Chemother.* **2001**, *45*, 2023.

13. Patro, B. S.; Rele, S.; Chintalwar, G. J.; Chattopadhyay, S.; Adhikari, S.; Mukherjee, T. *ChemBioChem* **2002**, *3*, 364.
14. Pandey, S.; Suryawanshi, S. N.; Gupta, S.; Srivastava, V. M. L. *Eur. J. Med. Chem.* **2004**, *39*, 969.
15. Suresh, J. R.; Kumar, U. K. S.; Ila, H.; Junjappa, H. *Tetrahedron* **2001**, *57*, 781.
16. (a) Kumar, S.; Tiwari, A.; Suryawanshi, S. N.; Mittal, M.; Vishwakarma, P.; Gupta, S. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6728; (b) Kumar, A.; Aggarwal, V.; Ila, H.; Junjappa, H., et al *Synthesis* **1980**, 748; (c) Dong, Y.; Wang, M.; Liu, J.; Ma, W.; Qun, L. *Chem. Commun.* **2011**, *47*, 7380.
17. Pandey, S.; Suryawanshi, S. N.; Gupta, S.; Srivastava, V. M. L. *Eur. J. Med. Chem.* **2005**, *40*, 751.
18. Bogaards, J. J.; Bertrand, M.; Jackson, P.; Oudshoorn, M. J.; Weaver, R. J.; van Bladeren, P. J.; Walther, B. *Xenobiotica* **2000**, *30*, 1131.
19. In vitro antileishmanial assays: The in vitro antileishmanial activity of these compounds against the luciferase-transfected promastigotes of the *L. donovani* was assessed as described previously by Pandey et al. (2007). Briefly, promastigotes ($5 \times 105/100$ μL) were seeded in 96-well plates and incubated with compounds at 40 μM concentration for 72 h. After incubation, 50 μL of promastigote suspension from each well was mixed with an equal volume of steady Glo® reagent (Promega) and luminescence was measured by luminometer. The values are expressed as relative luminescence units (RLU). Data were transformed into graphical program (Excel) and the inhibition of parasitic growth is determined by comparison of the luciferase activity of drug treated parasites with that of untreated controls. In order to assess the activity of compounds against amastigote stage of the parasite, the mouse macrophage cell line (J-774A.1) infected with luciferase-transfected promastigotes was used. Briefly, cells ($4 \times 103/100$ μL) were seeded in 96-well plates. After 24 h incubation in CO_2 incubator, cells were infected with promastigotes ($4 \times 104/100$ μL). Promastigotes phagocytised by the macrophage and transformed in to amastigotes. The test compounds in appropriate concentration (0.62–40 μM) were added and plates were incubated in CO_2 incubator for 72 h. After incubation, the compound-containing medium was aspirated and 50 μL PBS was added in each well and mixed with an equal volume of steady Glo® reagent. After gentle shaking for 1–2 min, the reading was taken in a luminometer (Pandey et al., 2007). The inhibition of parasitic growth was determined as described above. Pandey, S.; Suryawanshi, S. N.; Nishi, S.; Goyal, N.; Gupta, S. *Eur. J. Med. Chem.* **2007**, *42*, 669.
20. Cytotoxicity assay: the cytotoxicity of compounds was determined by following the method of Mosmann (1983). The exponentially growing monkey kidney fibroblast cells (vero cell line) were incubated with test compounds for 72 h and MTT was used as reagent for detection of viable cells. Fifty percent cytotoxic concentration (CC_{50}) values were estimated as described by Huber and Koella (1993). Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55; Huber, W.; Koella, J. C. *Acta Trop.* **1993**, *55*, 257.
21. In vivo antileishmanial assay: the in vivo antileishmanial activity was carried out in golden hamsters infected with *L. donovani* as described by Kumar et al. (2012). Briefly, golden hamsters (inbred strain) of either sex weighing 40–45 g were infected intracardiacally with 1×107 amastigotes per animal. After establishment of infection, drug treatment (50 mg/kg) by either (intraperitoneal) ip or per oral (po) route was initiated for five consecutive days. Sodium stibogluconate (SSG) and paromomycin are used as reference drugs. Post-treatment biopsies were done on day 7 after the last drug administration and amastigote counts are assessed by Giemsa staining. Intensity of infection in both, treated and untreated animals, and also the initial count in treated animals was compared and the efficacy was expressed in terms of percent inhibition (PI). Kumar, S.; Tiwari, A.; Suryawanshi, S. N.; Mittal, M.; Vishwakarma, P.; Gupta, S. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6728.