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In vitro and in vivo antifilarial activity evaluation of 3,6-epoxy [1,5]dioxocines: A new class of antifilarial agents ☆

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ABSTRACT

A series of 3,6-epoxy [1,5]dioxocines were synthesized and evaluated for their antifilarial activity against adult parasites of human lymphatic filarial parasite *Brugia malayi* (sub-periodic strain) in vitro. Out of these, six compounds (**4a–f**) possessed improved in vitro anti-filarial activity and examples **4d** and **4f** were also found to be active in the in vivo experiments. These results demonstrate that 3,6-epoxy [1,5]dioxocines exhibits potent antifilarial activity and might be developed into a new class of antifilarial drug.

Lymphatic filariasis (LF) is caused by a group of parasitic worms that are transmitted through the bites of infected mosquitoes. It is commonly known as elephantiasis and one of the most prevalent, neglected tropical diseases of the world. According to WHO report more than 1.3 billion people in 81 countries worldwide are threatened by lymphatic filariasis and over 120 million people are currently infected, with about 40 million disfigured and incapacitated by the disease.[1] In India, approximately 25 million people are known to harbor circulating microfilariae (mf) and another 19 million people suffer from filarial manifestations caused by lymph dwelling filariids, *Wuchereria bancrofti* and *Brugia malayi*.[2]

There are only three drugs currently in use for the treatment of LF: albendazole, ivermectin, and diethylcarbamzine (DEC) the chemical structures of which are shown in Figure 1. Albendazole is administered in combination with either ivermectin or DEC through MDA (Mass drug administration) programs. Both DEC and ivermectin kill mf but has no effect on most of the adult filarial species and causes side effects.[3] No drug yet has been found to be effective against adult worms. Also drug resistance to ivermectin appears to be another issue of concern, especially in areas where DEC cannot be administrated. The search for new molecular structures associated with macro filaricidal activity as lead molecules is, therefore, needed.[4,5]

In continuation of our ongoing drug discovery programme, on oxygenated heterocycles,[6] herein we have synthesized 3,6-epoxy [1,5]dioxocines and since a number of molecules from heterocycles are known as good antifilarials, we evaluated our oxygenated heterocycles for their antifilarial activity both in vitro and in vivo. The method of synthesis is simple and efficient and does not involve any special apparatus or reagents.The route followed for the preparation of 3,6-epoxy [1,5]dioxocines and 3,6-epoxy [1,5]dioxocines-chalcone hybrids is illustrated in Scheme 1. Commercially available *ortho* substituted phenols (**1a–c**) underwent the Duff formylation reaction in presence of hexamethylene tetraamine (HMTA) and TFA to furnish dicarbaldehydes (**2a–c**) which on reaction with different acetophenones in refluxing dioxane in the presence of a catalytic amount of conc.HCl give regioselective *para*-condensed chalcones (**3a–l**)[6a,g] In all the chalcones synthesized the *trans* double bond (on the basis of coupling constant) was obtained exclusively. These chalcones (**3a–l**) on reaction with epichlorohydrin in the presence of a catalytic amount of triethylamine furnished 3,6-epoxy [1,5]dioxocine (**4a–l**).[7] The isolated product was found to be a mixture of two enantiomers, in which the chiral centers have *R*,*S* and *S*,*R* configuration.[8] All compounds were characterized using ^{1}H NMR, ^{13}C NMR, 2D NMR, mass spectrometry and IR spectroscopy. The purity of these compounds was ascertained by elemental analysis and spectral analysis. (Please refer to Supplementary data).[9]

For the purpose of biological evaluation of synthesized (racemic 3,6-epoxy [1,5]dioxocine) compounds, initially efficacy of the compounds was assessed in vitro on adult worms and micro-

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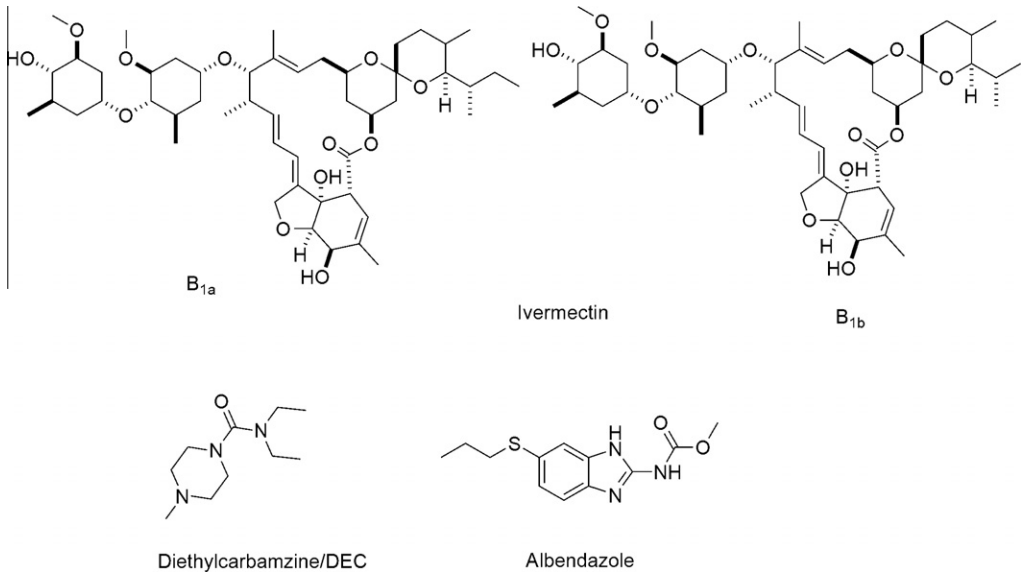
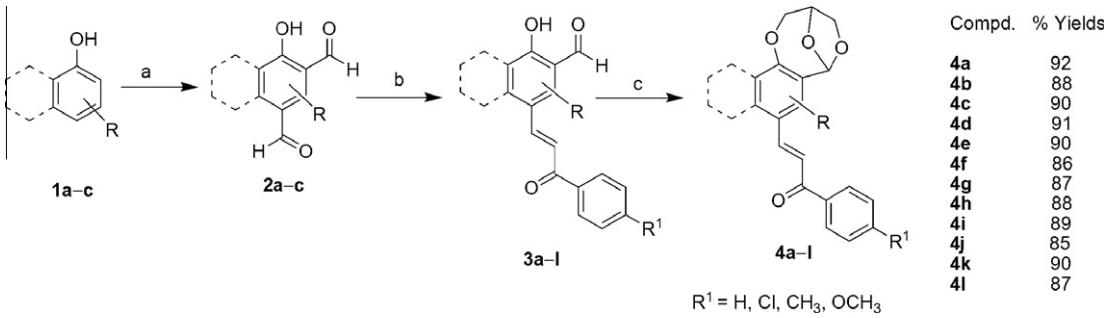


Figure 1. Chemical structure of clinically used antifilarial drugs.



Compd.	% Yields
4a	92
4b	88
4c	90
4d	91
4e	90
4f	86
4g	87
4h	88
4i	89
4j	85
4k	90
4l	87

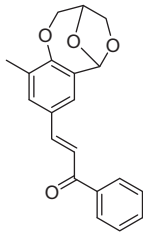
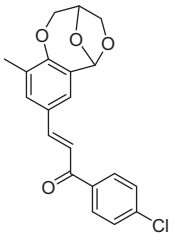
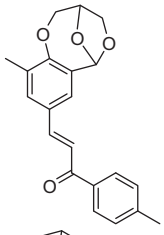
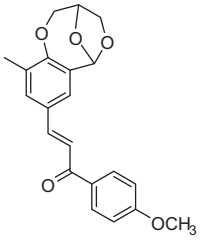
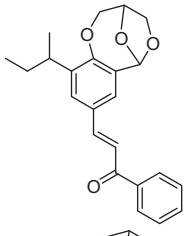
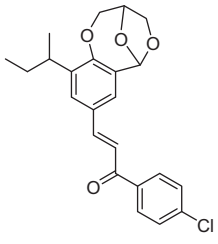
Scheme 1. Synthesis of 3,6-epoxy [1,5]dioxocines derivatives (**4a**–**l**). Reagents and conditions: (a) (1) HMTA/TFA, 120 °C, 3 h; (2) 10% H_2SO_4, 90–100 °C, 2 h; (b) Conc.HCl, *p*-$R^1C_6H_4COCH_3$, dioxane, 80–90 °C, 3.5–4.5 h; (c) epichlorohydrin, Et_3N, reflux, 45–90 min.

filariae (mf) of *B. malayi* using motility assay according to the method of Murthy and Chatterjee.[10] Test compounds and reference drug DEC were dissolved in DMSO. The final concentration of DMSO in the incubation medium was kept below 0.1%. DMSO was used in place of test compounds for controls. The results of the in vitro biological screening have been summarized in Table 1. Among 12 compounds tested, majority of the synthesized compounds exhibited considerable antifilarial activity. As evident from Table 1 compounds **4a**–**c**, **4d**, **4e**, **4f**, and **4g** caused complete inhibition in motility of adult worms at 1.25, 2.5, 5.0 and 10.0 μg/mL (LC_{100}: lethal concentration), respectively, while remaining compounds (**4h**–**l**) required >10 μg/mL concentration for complete inhibition in motility of the worms. The compounds (**4a**–**g**) have shown IC_{50} values in the range of 0.4–1.6 μg/mL. The compounds which had LC_{100} >10 μg/mL were considered inactive and IC_{50} value was not calculated. The compounds **4a**–**d** showed 15–39% inhibition in MTT reduction assays while compound **4e** did not show any inhibition in MTT reduction assay. Further, the effect of these compounds was also assessed on mf and results are shown in Table 1. The compounds **4a**–**b**, **4c**, **4e**, **4f**, **4d** and **4g**–**l** showed LC_{100} 2.5, 5.0, 5.0, 10.0 and more than 10.0 μg/mL. The compounds (**4a**–**f**) have shown IC_{50}/EC_{50} values in the range of 0.9–7.1 μg/mL. The CC_{50} values of compounds (**4a**–**f**) were found in the range of 10–40 μg/mL. After confirming the in vitro antifilarial activities of the compounds, the selectivity index (SI) values of compounds was determined as the ratio of CC_{50} and IC_{50} (SI = CC_{50}/IC_{50}).[11] Generally a compound showing SI value of $\geqslant 10$ is considered safe for in vivo evaluation. The selectivity indexes (SI) of these compounds were reasonably acceptable (Table 1). Therefore, we have selected six compounds (**4a**–**f**) showing better in vitro antifilarial activity profile in comparison to standard drug DEC (Table 1), and these were subjected for further studies in vivo.

Initially, the most potent compounds (**4a**–**f**) were evaluated for their in vivo studies against *B. malayi* in *Meriones unguiculatus* (jird) (primary model). In this model, male jirds of 8–10 weeks old were transplanted intraperitoneally with adult worms isolated from peritoneal cavity of infected jirds.[12] Each animal received 10 female and 5 male adult worms. On day 2 or 3 post-adult worm transplantation (p.a.t.), the peritoneal fluid was aspirated and checked for the presence of mf. The treatment was started on day 7 or 8-p.a.t. The animals were sacrificed on day 42 post initiation of treatment (p.i.t.) and live parasites were collected and counted.[13]

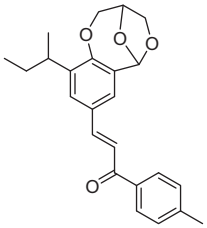
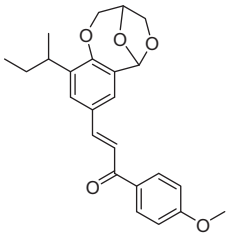
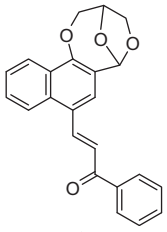
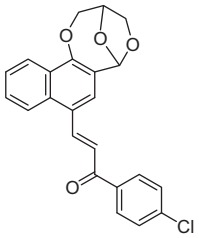
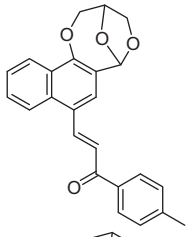
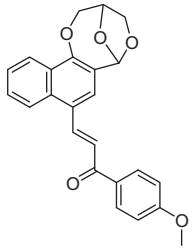
Effect of the compounds on the parasitological parameters is depicted in Table 2. Majority of the synthesized compounds exhibited considerable antifilarial activity. As evident from the table, compounds **4d** & **4f** showed significantly (P <0.001) lesser worm recovery at 200 mg/kg over control. In terms of embryostatic activity, the compounds **4d** & **4f** exerted about 24 & 68% sterility in female worms, respectively, whereas from standard drug DEC-Citrate treated animals 11% sterile female worms were recovered.

Table 1
In vitro activity of 3,6-epoxy [1,5]dioxocines (**4a**–**l**) on adult worms and microfilariae of *Brugia malayi*

Compounds	Effect on female adult worm			Effect on microfilariae (Mf)		CC_{50}[c] (μg/ml)	SI w.r.t. adults motility	SI w.r.t. Mf motility
	LC_{100} (μg/ml) in motility assay [a]	IC_{50} (μg/ml) in motility assay[b]	Mean % inhibition in MTT reduction	LC_{100} (μg/ml) in motility assay	IC_{50} /EC_{50} (μg/ml) in motility assay[b]			
4a 	1.25	1.0	15	2.5	0.9	10	10	11
4b 	1.25	0.6	39	2.5	1.1	10	16.7	9.1
4c 	1.25	0.4	22	5	1.8	40	100	22.2
4d 	10	3.5	22	>10	3.9	33	9.4	8.5
4e 	2.5	0.9	NI	5	0.9	31	34.4	34.4
4f 	5	1.6	NI	10	3.5	37	23.1	10.6

(*continued on next page*)

Table 1 (*continued*)

Compounds	Effect on female adult worm			Effect on microfilariae (Mf)		CC_{50}[c] (μg/ml)	SI w.r.t. adults motility	SI w.r.t. Mf motility
	LC_{100} (μg/ml) in motility assay [a]	IC_{50} (μg/ml) in motility assay[b]	Mean % inhibition in MTT reduction	LC_{100} (μg/ml) in motility assay	IC_{50} /EC_{50} (μg/ml) in motility assay[b]			
4g 	10	0.5	NI	>10	ND	ND	ND	ND
4h 	>10	ND	NI	>10	ND	ND	ND	ND
4i 	>10	ND	34	>10	ND	ND	ND	ND
4j 	>10	ND	41	>10	ND	ND	ND	ND
4k 	>10	ND	33	>10	ND	ND	ND	ND
4l 	>10	ND	35	>10	ND	ND	ND	ND
DEC	800	289	64	500	354	9103	31.5	25.7

[a] 100% reduction in motility indicates death of parasite.
[b] IC_{50} = 50% concentration of the agent at which 50% inhibition in motility is achieved.
[c] CC_{50} = concentration at which 50% of cells are killed; ND = Not Done, SI = Selectivity Index (CC_{50}/IC_{50}).

Nevertheless except compound **4e** the other compounds **4a**–**c** also showed considerable antifilarial activity.

Compounds (**4d** and **4f**) that were found most potent in the *M. unguiculatus* model, were further evaluated against *B. malayi* in *Mastomys coucha* (secondary model) for confirmation of their antifilarial activity. *M. coucha* harboring 5–7 months old *B. malayi* infection and showing progressive rise in microfilaria were selected for the study. Peripheral blood (10-μL tail blood) taken between 12:00

Table 2
Antifilarial efficacy of 3,6-epoxy [1,5]dioxocines (**4a**–**f**) against *Brugia malayi* in jirds (*Meriones unguiculatus*)

Compound No.	Dose mg/kg s.c. × 5 days (n)	Status of microfilaraemia in peritoneal cavity	No. of worms recovered (Mean ± SD)	% Sterilized female worms
4a	200 (4)	Active	6.0 ± 0.0**	29
4b	200 (4)	Active	7.5 ± 0.7*	29
4c	200 (4)	Active	7.5 ± 0.7*	45
4d	200 (4)	Active	5.50 ± 0.7***	24
4e	200 (4)	Active	10.0 ± 2.0	27
4f	200 (4)	Active	5.3 ± 0.6***	63
DEC-Citrate	25 (6)	Active	10.3 ± 2.9	11
Control	Vehicle treated (4)	Active	11 ± 1.53	0

n = number of animals.
* *P* <0.05 (vs control).
** *P* <0.01 (vs control).
*** *P* <0.001 (vs control).

Table 3
Antifilarial activity of compounds **4d** and **4f** and DEC against *Brugia malayi* in *Mastomys coucha* (values are Mean ± SD).

Compound No.	Dosing (mg/kg)[a]	No. Live worms recovered			Sterilized female worms
		Male	Female	Total	Count (%)
Control	Vehicle treated	11.40 ± 3.58	26.60 ± 8.02	38.00 ± 9.06	4.00 ± 2.65 (19.62)
4d	200	8.75 ± 2.63	16.75 ± 2.63	25.50 ± 3.32 (32.89)[b,*]	4.00 ± 2.94 (24.60)
4f	200	11.25 ± 2.63	12.25 ± 6.85	23.50 ± 9.47 (38.16)[b,*]	2.75 ± 3.10 (17.78)
DEC-Citrate	50	6.00 ± 1.79	24.33 ± 4.03	30.33 ± 3.50 (20.18)[b]	3.67 ± 6.09 (20.59)

[a] Each group consisted of 5 animals which received the agent, s.c. × 5 days.
[b] % reduction in worm burden over control; DEC: Diethylcarbamazine.
* *P* <0.05 (vs Control).

noon and 1:00 PM[14] was made into thick smears just before initiation of treatment (day 0), on days 7/8 and 14 and thereafter at fortnightly intervals till day 90 p.i.t. The animals were sacrificed on day 91 p.i.t. Both the compounds, exhibited significant (*P* <0.05) adulticidal activity (**4d**: 33%; **4f**: 38%) compared to control. However, treatment with DEC-Citrate could reduce the adult worm burden by only 20% (Table 3).

A closure look into the structure–activity relationship indicates that compounds containing naphthalene nucleus were found inactive (**4i**–**l**) while compounds containing alkyl group at 10-postion were more promising. Surprisingly, the substitution on the phenyl ring of chalcone seems to have no effect, as the activity was conserved in both.

In conclusion, we report here a series of 3,6-epoxy [1,5]dioxocines (**4a**–**l**) which was first time evaluated for their in vitro as well as in vivo antifilarial activity. One of the compound **4f** displayed potent activity in vitro as well as in vivo and it exerted antifilarial activity better than the standard drug. Thus compound **4f**, found active with no apparent signs of any toxicity in gross health of treated and normal animals, may serve as a prototype lead for further optimization and development of new antifilarial agents. Though the mechanisms underlying this process remain to be fully elucidated, and detailed mechanistic studies and lead optimization of these 3,6-epoxy [1,5]dioxocines are under investigation. Also, the synthesis and bioassay of chiral 3,6-epoxy [1,5]dioxocines which can be synthesized easily from commercially available chiral epichlorohydrins is in progress. It is intended that results from these studies will assist in elucidating their precise mechanisms of action and provide an approach to develop new prototypes for further optimization and development to get new leads for the treatment of filariasis.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.01.009.

References and notes

1. Chauhan, P. M. S. *Drugs Future* **2000**, *25*, 481.
2. Katiyar, S. B.; Bansal, I.; Saxena, J. K.; Chauhan, P. M. S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 47.
3. Sharma, S. *Prog. Drug Res.* **1990**, *35*, 365.
4. Srivastava, S. K.; Chauhan, P. M. S.; Bhaduri, A. P.; Fatima, N.; Chatterjee, R. K. *J. Med. Chem.* **2000**, *43*, 2275.
5. Dhananjeyan, M. R.; Milev, Y. P.; Kron, M. A.; Nair, M. G. *J. Med. Chem.* **2005**, *48*, 2822.
6. (a) Sashidhara, K. V.; Kumar, A.; Kumar, M.; Singh, S.; Jain, M.; Dikshit, M. *Bioorg. Med. Chem. Lett* **2011**, *21*, 7034; (b) Sashidhara, K. V.; Kumar, M.; Modukuri, R. K.; Srivastava, A.; Puri, A. *Bioorg. Med. Chem. Lett* **2011**, *21*, 6709; (c) Sashidhara, K. V.; Kumar, A.; Chatterjee, M.; Rao, K. B.; Singh, S.; Verma, A. K.; Palit, G. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1937; (d) Sashidhara, K. V.; Kumar, A.; Kumar, M.; Sonkar, R.; Bhatia, G.; Khanna, A. K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4248; (e) Sashidhara, K. V.; Rosaiah, J. N.; Kumar, A.; Bhatia, G.; Khanna, A. K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3065; (f) Sashidhara, K. V.; Kumar, A.; Kumar, M.; Srivastava, A.; Puri, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6504; (g) Sashidhara, K. V.; Kumar, A.; Kumar, M.; Sarkar, J.; Sinha, S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7205; (h) Sashidhara, K. V.; Rosaiah, J. N.; Kumar; Gara, R. K.; Nayak, L. V.; Srivastava, K.; Bid, H. K.; Konwar, R. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7127.
7. Sashidhara, K. V.; Kumar, A.; Rao, K. B. *Tetrahedron Lett.* **2011**, *52*, 5659.
8. Janeliunas, D.; Daskeviciene, M.; Malinauskas, T.; Getautis, V. *Tetrahedron* **2009**, *65*, 8407.
9. Representative synthesis of 8-(3-oxo-3-phenylprop-1-enyl),10-methyl-3,4-dihydro-2H,6H–3,6-epoxy-benzo[1,5]dioxocine (**4a**): A solution of (*E*)-2-hydroxy-3-methyl-5-(3-oxo-3-phenylprop-1-enyl)benzaldehyde **3a** (3.0 mmol) in epichlorohydrin containing Et_3N (2.3 mmol) was heated for 65 min, after which excess of epichlorohydrin was removed under reduced pressure. The solid residue was poured in water and extracted threefold with 30 mL of $CHCl_3$. The combined organic layers were dried on Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The crude products were purified over column chromatography (60–120 mesh) to afford pure compound **4a** in 92% yield. Light yellow solid; mp 120–122 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.03 (s, 1H), 8.0 (bs, 1H), 7.73 (d, *J* = 15.7 Hz, 1H), 7.60–7.41

(m, 5H), 7.34 (bs, 1H), 6.06 (s, 1H), 4.68 (d, J = 6.1 Hz, 1H), 4.38 (dd, J = 2.5 and 13.2 Hz, 1H), 4.32 (d, 6.6 Hz, 1H), 4.0–3.96 (m, 3H), 2.28 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 190.4, 156.9, 144.1, 138.4, 133.2, 132.8, 131.7, 131.2, 129.2, 128.7, 128.5, 126.7, 121.2, 106.2, 75.7, 73.9, 66.0, 16.5; IR (KBr): 3074, 1659, 1594, 1017 cm^{-1}; ESI-MS (m/z): 323 $(M+H)^+$. Anal. Calcd for $C_{20}H_{18}O_4$: C, 74.52; H, 5.63; Found: C, 74.61; H, 5.54.

10. (a) Murthy, P. K.; Chatterjee, R. K. *Curr. Sci.* **1999**, *77*, 1084; (b) Lakshmi, V.; Joseph, S. K.; Srivastava, S.; Verma, S. K.; Sahoo, M. K.; Dube, V.; Mishra, S. K.; Murthy, P. K. *Acta Trop.* **2010**, *116*, 127.
11. (a) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55; (b) Page, C.; Page, M.; Noel, C. *Int. J Oncol.* **1993**, *3*, 473; (c) Huber, W.; Koella, J. C. *Acta Trop.* **1993**, *55*, 257.
12. (a) Gaur, R. L.; Dixit, S.; Sahoo, M. K.; Khanna, M.; Singh, S.; Murthy, P. K. *Parasitology* **2007**, *134*, 537; (b) Murthy, P. K.; Murthy, P. S. R.; Tyagi, K.; Chatterjee, R. K. *Folia Parasitol. (Praha)* **1997**, *44*, 302.
13. Chatterjee, R. K.; Fatma, N.; Murthy, P. K.; Sinha, P.; Kulshreshtha, D. K.; Dhawan, B. N. *Drug Dev. Res.* **1992**, *26*, 67.
14. Murthy, P. K.; Tyagi, K.; Roy Chowdhury, T. K.; Sen, A. B. *Indian. J. Med. Res.* **1983**, *77*, 623.