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Chemotherapy of leishmaniasis. Part IX: Synthesis and bioevaluation of aryl substituted ketene dithioacetals as antileishmanial agents ☆

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ABSTRACT

A new series of aryl substituted ketene dithioacetals **6a–h** was synthesized and evaluated for their in vitro and in vivo antileishmanial activity against *Leishmania donovani*. Two compounds exhibited significant in vitro activity against intracellular amastigotes of *L. donovani* with IC_{50} values 3.56 and 5.12 μM and were found promising as compared with reference drug, miltefosine. On the basis of good Selectivity Indices (S.I.), they were further tested for their in vivo response against *L. donovani*/hamster model and showed significant inhibition of parasite multiplication 78% and 83%, respectively. These compounds were better than the existing antileishmanials in respect to IC_{50} and SI values, but were less active than miltefosine in vivo.

Leishmaniasis comprises a spectrum of parasitic illnesses caused by several species of the protozoan kinetoplastid parasites. *Leishmania* spp., affects 12 million people around the world with an annual death rate of approximately 80,000 people.[1] In various clinical manifestations, disseminated visceral infection is most fatal, if left untreated. This form of leishmaniasis is present in 61 countries[2] and having second rank after malaria in the top 10 fatal parasitic diseases worldwide. The first-line therapy includes sodium stibogluconate (Sb^V) which has unfortunately developed resistance in some areas of Bihar, (India) where failure rates up to 65% have been reported and the use of antimony has been abandoned.[3] Pentamidine also went out of favor for treatment of VL.[4] The drawback of the clinical use of pentamidine is the route of administration (injection) and the toxicity of the compound. Administration by injection increases the expense of the treatment and makes the use of the drug less practical in developing nations, where cost is a major factor. The clinical side effects of pentamidine include real and hepatic toxicity, pancreatitis, hypotension; dysglycemia, and cardiac abnormalities.[5,6] The use of liposomal Amphotericin-B is limited due to high market price.[7] A major milestone in chemotherapy of VL is the discovery of miltefosine, an analogue of phosphatidylcholine initially developed as an anticancer agent.[8] It is an effective oral drug but its use in women of child-bearing age is restricted due to teratogenicity. In addition, it has a long half-life, which might encourage the emergence of resistance once its use becomes widespread.[9] Taken together, current armamentarium of leishmaniasis is still limited and alternative therapies are strongly warranted. In this endeavour, diarylheptanoids,[10] oxygenated abietanes,[11] diterpene quinones[12,13] and chalcones[14] are showing promise in the area of in vitro antileishmanial studies. Curcumin **1** isolated from *Curcuma longa* Linn has not only shown promise as anticancer agent[10a] but it is also showing antileishmanial activity profile in in vitro studies.[10b,15] Exhaustive analoging of curcumin has generated some interesting results.[16] Lichochalcone **2** isolated from the root of Chinese licorice plant is also showing promising antileishmanial activity.[17] Chemical Library generated on the basis of Lichochalcone as a lead molecule is showing promise in in vitro antileishmanial studies.[14a] Phenolic diketone **3** isolated from *Zingiber officinale*,[18] a structural mimic of **1** and **2** shows radical scavenging activities, quite comparable to Curcumin **1**. In continuation of our efforts[19a] and others[19b] at CDRI to generate novel antileishmanial agents coupled with encouraging results of **1**, **2**, and **3**, we synthesized some aryl substituted ketene dithioacetals and evaluated for their in vivo antleishmanial activity profile and the results are reported in this Letter Figure 1.

α-Oxoketene dithioacetals of aromatic substrates are very useful synthons in the synthesis of variety of heterocyclic and carbocyclic compounds[20] However, they have been not fully exploited for their biological activity profile. α-Oxoketene dithioacetals **6**(**a**–**h**) were prepared as shown in Scheme 1. The required starting

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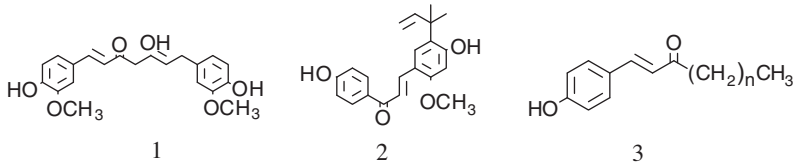
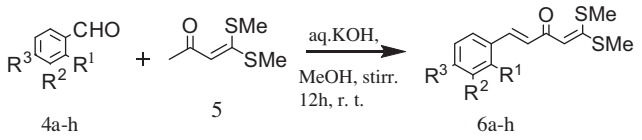


1 2 3

Figure 1. Structures of naturally occurring & synthetic antileishmanial chalcones 1, 2, and 3.



4a, 6a) $R^1 = R^2 = H$, $R^3 = Cl$
4b, 6b) $R^2 = R^3 = H$, $R^1 = Cl$
4c, 6c) $R^1 = R^2 = H$, $R^3 = NO_2$,
4d, 6d) $R^1 = R^3 = H$, $R^2 = NO_2$,
4e, 6e) $R^1 = R^2 = H$, $R^3 = F$
4f, 6f) $R^1 = H$, $R^2 = OCH_3$, $R^3 = OBn$
4g, 6g) $R^1 = R^2 = H$, $R^3 = C_2H_2N_3$
4h, 6h) $R^1 = R^2 = H$, $R^3 = C_3H_3N_2$

Scheme 1. General synthesis of E-5-(4-Substitutedphenyl)-1, 1-bis(methylthio) penta-1,4-dien-3-one (**6a**–**h**).

material 1,1-bis(methylthio)-1-en-3-one **5** was prepared following a known procedure.[21] The reaction of 4-chlorobenzaldehyde **4a** with acetone based ketene dithioacetal **5** furnished **6a** as a yellow crystalline solid, melting at 157–158 °C. The structure of **6a** was assigned on the basis of IR, ^1H, ^{13}C NMR and mass spectrometry.

Similarly the reaction of substituted benzaldehydes **4**(**b**–**h**) with ketene dithioacetal **5** in the presence of methanolic potassium hydroxide furnished ketene dithioacetals **6**(**b**–**h**) in good yield as pale yellow crystalline solids.

For assessing the activity of compounds against the amastigote stage of the parasite, mouse macrophage cell line (J-774A.1) infected with promastigotes expressing luciferase firefly reporter gene was used. Cells were seeded in a 96-well plate (4×10^4 cell/100μL/well) in RPMI-1640 containing 10% foetal calf serum and the plates were incubated at 37 °C in a CO_2 incubator. After 24 h, the medium was replaced with fresh medium containing stationary phase promastigotes (4×10^5 cell/100μL/well). Promastigotes invade the macrophage and are transformed into amastigotes. The test compounds were added at two fold dilutions up to seven points in complete medium starting from 40 μM conc after replacing the previous medium and the plates were incubated at 37 °C in a CO_2 incubator for 72 h. After incubation, the drug containing medium was decanted 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.[22–24] The values are expressed as relative luminescence units (RLU). Data were transformed into a graphic program (Excel). IC_{50} of antileishmanial activity was calculated by nonlinear regression analysis of the concentration response curve using the four parameter Hill equations.

The cell viability was determined using the MTT assay.[25] Exponentially growing cells (KB Cell line) (1×10^5 cells/100μL/well) were incubated with test compounds for 72 h. The test compounds were added at three fold dilutions up to seven points in complete medium starting from 400 μM concentration, and were incubated at 37 °C in a humidified mixture of CO_2 and 95% air in an incubator. Podophyllotoxin was used as a reference drug and control wells containing dimethyl sulfoxide (DMSO) without compounds were also included in the experiment. Stock solutions of compounds were initially dissolved in DMSO and further diluted with fresh complete medium. After incubation, 25 μL of MTT reagent (5 mg/ml) in PBS medium, followed by syringe filtration was added to each well and incubated at 37 °C for 2 h. At the end of the incubation period, the supernatant were removed by inverting the plate completely without disturbing cell layer and 150 μL of pure DMSO are added to each well. After 15 min of shaking the readings were recorded as absorbance at 544 nm on a micro plate reader. The cytotoxic effects were expressed as 50% lethal dose, that is, as the concentration of a compound which provoked a 50% reduction in cell viability compared to cell in culture medium alone. IC_{50} values were estimated as described by Huber et al.[26] The selectivity index (SI) for each compound was calculated as ratio between, cytotoxicity (CC_{50}) and activity (IC_{50}) against Leishmania amastigotes.

The in vivo leishmanicidal activity was determined in golden hamsters (Mesocricetusauratus) infected with MHOM/IN/80/Dd8 strain of *Leishmania donovani* obtained through the courtesy of P.C.C. Garnham, Imperial College, London (UK). The method of Beveridge et al[27] as modified by Bhatnagar et al[28] and Gupta et al[29] was used for in vivo evaluation. Golden hamsters (Inbred strain) of either sex weighing 40–45 g were infected intracardially with 1×10^7amastigotes per animal. The infection is well adapted to the hamster model and establishes itself in 15–20 days. Meanwhile, hamsters gain weight (85–95 g) and can be subjected to repeated spleen biopsies. Pre-treatment spleen biopsy in all the animals was carried out to assess the degree of infection. The animals with +1 infection (5–15 amastigotes/100 spleen cell nuclei) were included in the chemotherapeutic trials. The infected animals were randomized into several groups on the basis of their parasitic burdens. Five to six animals were used for each test sample. Drug treatment by intraperitoneal (ip) route was initiated after 2 days of biopsy and continued for five consecutive days. Post-treatment biopsies were done on day 7 of the last drug administration and amastigote counts were assessed by Giemsa staining. Intensity of infection in both, treated and untreated animals, as also the initial count in treated animals was compared and the efficacy was expressed in terms of percentage inhibition (PI) using the following formula:-

$$PI = 100 - [ANAT \times 100/(INAT \times TIUC)]$$

Where PI is percent inhibition of amastigotes multiplication, ANAT is actual number of amastigotes in treated animals, INAT is initial number of amastigotes in treated animals and TIUC is time increase of parasites in untreated control animals.

The antileishmanial activity of aryl substituted dithioacetals **6(a–h)** has been displayed in Table 1. Of the **6** compounds, **6d** and **6f** have shown interesting antiamastigote activity with IC_{50}'S for *L. donovani* intramacrophagic amastigotes of 5.12 and 3.56 and selectivity indices of 1.92 and 57.26, respectively. The in vitro antileishmanial response of both the compounds were better than the reference drug, miltefosine (IC_{50} = 12.5 μM, S.I. = 0.2). The compounds **6d** and **6f** were also selected for in vivo efficacy evaluation against *L. donovani*/hamster model at the ip dose of 50 mg $kg^{-1} \times 5$ days. Both the compounds exhibited significant inhibition of parasite multiplication (83% and 78%, respectively).

Table 1
Antileishmanial activity of aryl substituted ketene dithioacetals against *L. donovani*

Sr. No.	Compound	In vitro Antiamastigote activity IC_{50} (μM)	CC_{50} (μM)	Selectivity index (S.I.) CC_{50}/IC_{50}	In vivo activity (%inhibition) (50 mg/kg × 5 ip dose)	Remarks
1.	**6a**	>20	ND	—	—	Inactive
2.	**6b**	>40	ND	—	—	Inactive
3.	**6c**	>40	ND	—	—	Inactive
4.	**6d**	5.12	9.83	1.92	82.8 ± 13.00 (n = 9)	Active
5.	**6e**	>40	ND	—	—	Inactive
6.	**6f**	3.56	203.85	57.26	77.97 ± 12.7 (n = 9)	Active
7.	**6g**	>20	ND	—	—	Inactive
8.	**6h**	>40	ND	—	—	Inactive
9.	Miltefosine	12.50	3.23	0.26	95.28 ± 2.49* (n = 8)	Active

* PI at 30 mg/kg × 5 po, Miltefosine: reference drug.

The present study has helped us in identifying a new lead that could be exploited as a potential antileishmanial agent. Although, the compounds showed promising activity, it could not exceed the efficacy of the standard drug miltefosine (Table 1). But due to its merit of easy synthesis and efficacy both in vitro and in vivo against promastigotes and amastigotes, more analogues need to be prepared and screened so as to identify a potential molecule for antileishmanial therapy.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.096.

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