



Chemotherapy of leishmaniasis part X: Synthesis and bioevaluation of novel terpenyl heterocycles [☆]

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ARTICLE INFO

Article history:

Received 10 September 2012

Revised 22 October 2012

Accepted 26 October 2012

Available online 2 November 2012

Keywords:

Chalcones

Pyrazolidines

Leishmania donovani

In vitro testing

In vivo trial

Hamster

ABSTRACT

Some novel α and β ionone based chalcones and their dihydropyrazolidines/pyrazolidines have been synthesized and evaluated for their in vitro and in vivo antileishmanial activities against *Leishmania donovani*. Amongst all, one compound (**4d**) exhibited significant in vitro activity against intracellular amastigotes of *Leishmania donovani* with IC₅₀ values of 7.49 μ M and was found promising as compared to reference drug, miltefosine. On the basis of good Selectivity Index (S.I.), the compound was further tested for its in vivo response against *Leishmania donovani*/hamster model and has shown significant inhibition of parasite multiplication (81%). The present study has helped us in identifying a new lead that could be exploited as a potential antileishmanial agent.

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Leishmaniasis is a family of parasitic diseases that affect about 12 million people in tropical and subtropical areas in the form of three clinical expressions: visceral leishmaniasis, which is fatal in the absence of treatment; muco-cutaneous leishmaniasis and cutaneous leishmaniasis, which is often self curing. Classical drugs such as antimonials (Pentostam and Glucantime) are toxic and drug resistance is increasing dangerously in the field.¹ A liposomal amphotericin B formulation (AmBisome) less toxic than amphotericin B deoxycholate is gradually becoming the first-line therapy, especially in immunocompromised patients, but this drug must be administered by a parenteral route.² Miltefosine (Impavido) was the first drug registered against visceral leishmaniasis in the last decade; however, its toxicity and the appearance of drug resistance justify the search for new chemical series in order to find an orally safe and active drug.³ Currently, efforts are being made to search for new molecules from the natural sources and in this endeavor diarylheptanoids,⁴ oxygenated abietanes,⁵ diterpene quinones^{6,7} are showing promise as new lead molecules. Rationally designed heterocyclic ionone like molecules⁸ and some novel terpenyl 2,4-diamino pyrimidines⁹ are showing promising antimicrobial and dihydrofolate reductase inhibitory activities. 2,4-diaminopyrimidines¹⁰ and some de novo-designed molecules¹¹ are

also giving further inputs in the leishmanial dihydrofolatereductase activity. In continuation of our studies on terpenylpyrimidines as novel antileishmanial agents,¹² we designed some novel dihydropyrazolidines/pyrazolidines, having added aryl substitution and evaluated for their in vitro and in vivo antileishmanial activity and the results are reported in this communication.

The synthesis of compounds (**4a–j**) and (**8a–e**) followed the general pathway outlined in Schemes 1 and 2. They were prepared in two steps. Firstly, the chalcones were obtained by direct condensation between the substituted aromatic aldehydes and β -ionone, α -ionone respectively, using phase transfer catalyzed conditions.¹³ Secondly, reaction of different chalcones with phenyl hydrazine in refluxing ethanol leads to the formation of dihydropyrazolidine derivatives. Further, compounds (**8a–e**) were aromatized into compounds (**9a–e**) using Ag₂O in refluxing ethanol. The reaction of β -ionone with substituted benzaldehydes was very facile and furnished chalcones in good to excellent yield.

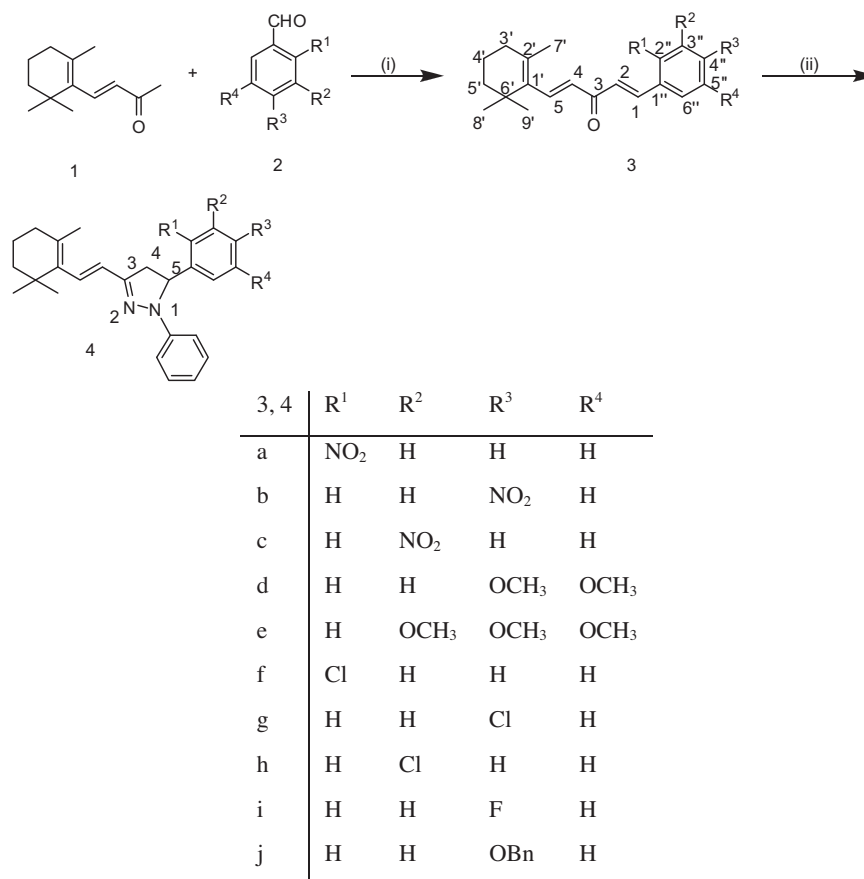
The reaction of phenyl hydrazine with chalcones (**3a–j**) and (**7a–e**) was not only facile but it was also regiospecific in manner. The reaction of chalcone **3a** with phenyl hydrazine in ethanol furnished dihydropyrazolidine **4a** in 40% yield as a crystalline solid melting at 138–140 °C. The structure was assigned on the basis of ¹H and ¹³C NMR spectra.

The ¹H NMR spectrum of **4a** displayed doublet of doublets at 2.87 ppm (*J* = 17, 7 Hz, 1H) & 3.85 ppm (*J* = 17, 12 Hz, 1H) for two geminal protons of the dihydropyrazolidine ring and doublet of

[☆] CSIR-CDRI Communication No: 172/2011/SNS.

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Scheme 1. Reagents and conditions: (i) Cetyl trimethyl ammonium bromide (CTABr), NaOH, H₂O, rt, 24 h; (ii) PhNHNH₂, EtOH, reflux, 8 h.

doublets at 5.69 ($J = 12, 7$ Hz, 1H) for H-5 proton (vicinal to the geminal protons) and it established the assigned structure **4a**. The compound **4d** was synthesized by the reaction of chalcone **3d** with phenyl hydrazine in ethanol (reflux, 8 h). The compound **4d**, Yellow crystalline solid which melted at 90–93 °C. The compound **4d** was characterized by IR, NMR and mass spectrum. The IR spectrum showed a C=N stretching band at 1597 cm⁻¹. The ¹H NMR spectrum of **4d** displayed three doublet of doublets at 2.85 ppm ($J = 17, 7$ Hz, 1H), 3.55 ppm ($J = 17, 12$ Hz, 1H) and 5.01 ($J = 12, 7$ Hz, 1H) for two geminal protons of dihydropyrazolidine ring and one for the proton vicinal to geminal protons respectively. Analysis of ¹³C, DEPT-135 and mass spectra provided the final structural elucidation of compound **4d**. Cyclization in all the synthesized heterocyclic compounds took place near the aromatic ring rather than near the ionone ring. The presence of doublet near 2.25 (δ value) in ¹H NMR spectrum of compounds (**9a–e**) indicated that the cyclization took place near the aromatic ring. If cyclization had taken place near the ionone ring then a singlet would have been obtained in place of doublet in ¹H NMR spectrum of compounds (**9a–e**). Compounds (**4b–j**) and (**8a–e**) were synthesized in good yields under identical reaction conditions as used for the synthesis of compound **4a**. Few dihydropyrazolidines (**8a–e**) were aromatized using Ag₂O in refluxing ethanol and their structures were assigned by ¹H NMR and ¹³C NMR spectra. We used the same reaction conditions, as used for compounds (**8a–e**), for aromatization of compounds (**4a–j**) but we didn't find any aromatized product.

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/

100 μ L/well) in RPMI-1640 containing 10% foetal calf serum and the plates were incubated at 37 °C in a CO₂ incubator. After 24 h, the medium was replaced with fresh medium containing stationary phase promastigotes (4×10^5 /100 μ L/well). Promastigotes invade the macrophage and are transformed into amastigotes. The test compounds were added at two fold dilutions up to 7 points in complete medium starting from 40 μ M conc. after replacing the previous medium and the plates were incubated at 37 °C in a CO₂ incubator for 72 h. At the end of the incubation, the supernatants were removed and 50 μ L 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.^{14–16} The values are expressed as relative luminescence units (RLU). IC₅₀ 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.¹⁷ Exponentially growing cells (KB Cell line) (1×10^5 cells /100 μ L/well) were incubated with test compounds for 72 h. The test compounds were added at three fold dilutions up to 7 points in complete medium starting from 400 μ M concentration, and were incubated at 37 °C in a humidified mixture of CO₂ 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 μ L 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 and 150 μ L of pure DMSO was added to each well. After 15 min. of shaking the

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