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In vitro studies of the antileishmanial activity of the newer 2-(substitutedphenoxy)-N-[(aryl)methylidene]acetohydrazide analogues

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ABSTRACT

A series of new 2-(substitutedphenoxy)-N-[(aryl)methylidene]acetohydrazide analogues (**8a-n**) were synthesized in search of potential therapeutics for leishmaniasis. All the compounds were characterized by infrared (IR), nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry. The compounds were further evaluated for *in vitro* antileishmanial activity against promastigotes of *Leishmania donovani* as per the standard protocol reported elsewhere. 2-(2,4-Dichlorophenoxy)-N′-{[4-(morpholin-4-yl)phenyl]methylidene}acetohydrazide (**8k**) showed the most promising antileishmanial activity with IC_{50} of 48.10 μM, free from cytotoxicity (>153.08 μM).

1. Introduction

Leishmaniasis is a neglected infectious disease caused by protozoa of genus *Leishmania*. More than 20 species are found worldwide causing human leishmaniasis. The vector for the disease is phlebotomine (sand flies) and is manifested in three major clinical forms: cutaneous (CL), mucocutaneous and visceral leishmaniasis (VL). VL, the most severe form of leishmaniasis, is also known as kala-azar in India. The symptoms of VL include fever, hepatosplenomegaly and anaemia that may lead to death. The pathogen is endemic in 88 countries. Nearly 12 million people were infected and 2 million new cases occur every year. An estimated 350 million people are living at risk of contracting leishmaniasis. One of the major threats to control VL is its association with HIV infection (Trouiller and Olliaro, 1999; Alvar et al., 2012; WHO, 2012). Pentavalent antimonials remain the first-line, while polyene antifungal,

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amphotericin B, as a second-line treatment is currently used in many countries. AmBisome (liposomal amphotericin B) and miltefosine were also included in the treatment of VL (Reddy et al., 2007; Sindermann et al., 2004; Thakur et al., 1999). However, the use of these existing drugs is limited due to several complications, such as high cost, toxicity, parenteral administration, emergence and spread of drug resistance, and relapses in HIV-leishmania co-infected patients. Therefore, there is still a need for new efficacious and safe agents against leishmaniasis.

Hydrazides have emerged as biologically and pharmacological promising agents, with antileishmanial (Sagsehetti et al., 2014), antitubercular (Ramamurthy and Bhatt, 1989), anticonvulsant (Kaushik et al., 2010), antibacterial (Sridhar et al., 2002), antifungal (Mallikarjuna et al., 2009), anti-HIV (Vicini et al., 2009), antioxidant (Gurkok et al., 2009), and many more. Hence it was worth to synthesize these compounds.

2. Materials and methods

2.1. *Chemistry*

All the chemicals were supplied by Merck (Germany) and S. D. Fine Chemicals (India). Melting points were determined by open tube capillary method and were uncorrected. Purity of the compounds was checked by elemental analysis and the progress of reactions was monitored throughout by thin layer chromatography (TLC) plates (silica gel G) using mobile phase, hexane:ethylacetate (1:1), and the spots were identified by iodine vapours or UV light. IR spectra were recorded on a Schimadzu 8201 PC, FT-IR spectrometer (KBr pellets). ^{1}H NMR spectra were recorded on a Bruker AC 300 MHz spectrometer using TMS as internal standard in DMSO d_6. Mass spectra were recorded on a Bruker Esquire LCMS using ESI and elemental analyses were performed on Perkin-Elmer 2400 Elemental Analyzer.

2.2. *Synthesis of ethyl(substitutedphenoxy)acetate analogues (3a-d)*

A mixture of equimolar amounts of the substituted phenol and ethyl chloroacetate was taken in a round bottom flask and suspended in 50–60 ml acetone, and anhydrous potassium carbonate (1–2 g) was added in the mixture. The mixture was refluxed for 24 h on the sand bath with vigorous stirring. The completion of the reaction was monitored by TLC using mobile phase hexane:ethylacetate (1:1). Initially, the colour of reaction mixture was colourless in case of phenol, while in other substituted phenols the colour was light yellow and the reaction proceeded until the reaction mixture became dark in colour. The reaction mixture, when cooled, was filtered under vacuum to remove solid potassium carbonate and the filtrate thus obtained was evaporated under vacuum. The residue thus obtained was dissolved into ethylacetate (10–15 ml) and washed with water twice. Ethylacetate layer was separated and dried over anhydrous sodium sulphate. The solvent was evaporated under vacuum and the products thus obtained were used for the next step.

2.3. *Synthesis of 2-(substitutedphenoxy) acetohydrazide analogues (4a-d)*

A solution of ethyl (substitutedphenoxy)acetate (0.01 mol) (**3a-d**) and hydrazine hydrate (0.02 mol) was taken in a round bottom flask and suspended in 50–60 ml ethanol. The mixture was refluxed for 5–6 h on a sand bath with vigorous stirring. The reaction was monitored throughout by TLC. The reaction was continued until the ethyl (substitutedphenoxy)acetate was consumed completely. The reaction mixture was poured in crushed ice filtered under vacuum and washed with water to remove solid 2-(substitutedphenoxy)acetohydrazide analogues (**4a-d**).

2.4. *Synthesis of 4-(substitutedphenoxy) benzaldehyde analogues (7a-c)*

A solution of substituted *p*-fluorobenzaldehyde (0.01 mol) and substituted phenol/morpholine (0.012 mol) was taken in a round bottom flask and suspended in 50–60 ml DMSO and anhydrous potassium carbonate. The mixture was refluxed for 14–18 hrs on a sand bath with vigorous stirring. The reaction was monitored throughout by TLC. Initially, the reaction mixture was colourless in case of phenol, while in other phenols it was light yellow and the reaction became dark in colour as it proceeded and reached to completion. The reaction mixture was cooled, added water and ethylacetate in a separating funnel, and the ethylacetate layer was separated and evaporated under rotatory vacuum evaporator. The solid thus obtained was washed with ethanol, dried and used for the next step.

2.5. *Synthesis of 2-(substitutedphenoxy)-N-[(aryl)methylidene]-acetohydrazide analogues (8a-n)*

A solution of *p*-fluorobenzaldehyde (**5**)/substituted 4-(substitutedphenoxy)benzaldehyde analogues (**7a-c**) (1 mmol) and 2-(substitutedphenoxy)acetohydrazide analogues (**4a-d**) (1 mmol) was taken in a round bottom flask and suspended in 50–60 ml ethanol and TEA (1.5 mol). The mixture was refluxed for 10–14 h on sand bath with vigorous stirring. The reaction was monitored throughout by TLC. The reaction mixture was poured into the cold water and the product was extracted by ethylacetate using a separating funnel. The ethylacetate layer was then separated and evaporated under rotator vacuum evaporator. The solid thus obtained was washed and crystallized with ethanol.

2.5.1. *2-(2-Chlorophenoxy)-N′-{[4-(2,4-dichlorophenoxy) phenyl] methylidene}acetohydrazide (8a)*

Yield 70%, mp. 148–150 °C, R_f 0.63 [hexane: ethylacetate (1:1)], IR (KBr) cm^{-1}: 3223.16 (NH), 1682.95 (C═O), 1573.97 (C═N), 1097.53 (—O—), 741.65 (C—Cl); ^{1}H NMR (DMSO d_6, 300 MHz) δ ppm: 4.94 (s, 2H, CH_2), 6.72–7.74 (m, 11H, ArH), 7.99 (s, 1H, CH═N), 11.34 (s, 1H, CONH; D_2O exchangeable); ^{13}C NMR (DMSO d_6, 75 MHz) δ ppm: 154.83, 146.83, 143.31, 130.74, 129.94, 129.63, 129.40, 126.55, 124.97, 123.40, 121.88, 117.92, 114.20, 91.81; EI-MS (*m*/*z*) 449.75 (M^+), 451.41 (M^++2), 453.81 (M^++4). Cal/Ana: [C (56.09) 56.05 H (3.36) 3.38 N (6.23) 6.24].