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## Full Length Article

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

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## ARTICLE INFO

### Article history:

Received 20 June 2015

Received in revised form 10 May 2016

Accepted 24 May 2016

Available online 30 May 2016

### Keywords:

Acetohydrazide analogues

Antileishmanial activity

*Leishmania donovani*

## 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<sub>50</sub> of 48.10 μM, free from cytotoxicity (>153.08 μM).

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## 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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<http://dx.doi.org/10.1016/j.bjbas.2016.05.002>

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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 Shimadzu 8201 PC, FT-IR spectrometer (KBr pellets).  $^1\text{H}$  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)  $\text{cm}^{-1}$ : 3223.16 (NH), 1682.95 (C=O), 1573.97 (C=N), 1097.53 (—O—), 741.65 (C—Cl);  $^1\text{H}$  NMR (DMSO  $d_6$ , 300 MHz)  $\delta$  ppm: 4.94 (s, 2H,  $\text{CH}_2$ ), 6.72–7.74 (m, 11H, ArH), 7.99 (s, 1H,  $\text{CH}=\text{N}$ ), 11.34 (s, 1H, CONH;  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C}$  NMR (DMSO  $d_6$ , 75 MHz)  $\delta$  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 ( $\text{M}^+$ ), 451.41 ( $\text{M}^+ + 2$ ), 453.81 ( $\text{M}^+ + 4$ ). Cal/Ana: [C (56.09) 56.05 H (3.36) 3.38 N (6.23) 6.24].

2.5.2. 2-(2-Chlorophenoxy)-N'-[(4-phenoxyphenyl)methylidene]acetohydrazide (**8b**)

Yield 69%, mp. 135–137 °C,  $R_f$  0.73 [hexane: ethylacetate (1:1)], IR (KBr)  $\text{cm}^{-1}$ : 3186.51 (NH), 1680.05 (C=O), 1487.17 (C=N), 1164.08 (—O—), 743.58 (C—Cl);  $^1\text{H}$  NMR (DMSO  $d_6$ , 300 MHz)  $\delta$  ppm: 5.09 (s, 2H,  $\text{CH}_2$ ), 6.69–7.79 (m, 13H, ArH), 8.17 (s, 1H,  $\text{CH}=\text{N}$ ), 11.04 (s, 1H, CONH $_2$ ;  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C}$  NMR (DMSO  $d_6$ , 75 MHz)  $\delta$  ppm: 68.61, 115.72, 117.21, 117.90, 121.92, 122.56, 122.63, 126.91, 127.90, 128.51, 128.93, 129.92, 143.14, 154.71, 157.01, 159.33, 168.16; EI-MS ( $m/z$ ) 381.41 ( $\text{M}^+$ ), 383.44 ( $\text{M}^+2$ ). Cal/Ana: [C (66.23) 66.19 H (4.50) 4.53 N (7.36) 7.32].

2.5.3. 2-(2-Chlorophenoxy)-N'-[[4-(morpholin-4-yl)phenyl]methylidene]acetohydrazide (**8c**)

Yield 74%, mp. 128–130 °C,  $R_f$  0.76 [hexane: ethylacetate (1:1)], IR (KBr)  $\text{cm}^{-1}$ : 3181.69 (NH), 1682.95 (C=O), 1493.92 (C=N), 1121.64 (—O—, morpholine), 747.44 (C—Cl);  $^1\text{H}$  NMR (DMSO  $d_6$ , 300 MHz)  $\delta$  ppm: 2.40–2.45 (t, 4H, morpholine), 3.71–3.72 (t, 4H, morpholine), 4.84 (s, 2H,  $\text{CH}_2$ ), 6.70–7.36 (m, 8H, ArH), 8.27 (s, 1H,  $\text{CH}=\text{N}$ ), 11.41 (s, 1H, CONH;  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C}$  NMR (DMSO  $d_6$ , 75 MHz)  $\delta$  ppm: 46.31, 66.11, 68.60, 114.41, 115.72, 122.51, 122.68, 123.33, 127.94, 129.92, 130.09, 143.01, 151.92, 154.71, 168.66; EI-MS ( $m/z$ ) 373.44 ( $\text{M}^+$ ), 375.49 ( $\text{M}^+2$ ). Cal/Ana: [C (61.04) 61.01 H (5.39) 5.43 N (11.24) 11.19].

2.5.4. 2-(2-Chlorophenoxy)-N'-[(4-fluorophenyl)methylidene]acetohydrazide (**8d**)

Yield 56%, mp. 122–124 °C,  $R_f$  0.62 [hexane: ethylacetate (1:1)], IR (KBr)  $\text{cm}^{-1}$ : 3200.02 (NH), 1680.12 (C=O), 1457.16 (C=N), 1196.02 (—O—), 687.12 (C—F);  $^1\text{H}$  NMR (DMSO  $d_6$ )  $\delta$  ppm: 4.83 (s, 2H,  $\text{CH}_2$ ), 6.81–7.46 (m, 8H, ArH), 7.99 (s, 1H,  $\text{CH}=\text{N}$ ), 11.02 (s, 1H, CONH;  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C}$  NMR (DMSO  $d_6$ , 75 MHz)  $\delta$  ppm: 68.51, 115.63, 115.71, 122.56, 122.61, 127.91, 129.41, 129.92, 130.82, 143.01, 154.72, 165.22, 168.01; EI-MS ( $m/z$ ) 306.24 ( $\text{M}^+$ ), 308.37 ( $\text{M}^+2$ ). Cal/Ana: [C (58.74) 58.69 H (3.94) 3.89 N (9.13) 9.09].

2.5.5. N'-[[4-(2,4-Dichlorophenoxy)phenyl]methylidene]-2-(2-methyl-phenoxy)acetohydrazide (**8e**)

Yield 84%, mp. 150–152 °C,  $R_f$  0.54 [hexane: ethylacetate (1:1)], IR (KBr)  $\text{cm}^{-1}$ : 3220.27 (NH), 1678.13 (C=O), 1574.93 (C=N), 1099.46 (—O—), 714.65 (C—Cl);  $^1\text{H}$  NMR (DMSO  $d_6$ , 300 MHz)  $\delta$  ppm: 2.23 (s, 3H,  $\text{CH}_3$ ), 4.64 (s, 2H,  $\text{CH}_2$ ), 6.82–7.79 (m, 11H, ArH), 7.98 (s, 1H,  $\text{CH}=\text{N}$ ), 11.45 (s, 1H, CONH;  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C}$  NMR (DMSO  $d_6$ , 75 MHz)  $\delta$  ppm: 14.41, 69.42, 114.21, 117.62, 120.33, 121.01, 124.51, 126.72, 126.81, 126.97, 127.21, 128.83, 128.96, 130.11, 131.01, 143.01, 150.11, 158.77, 159.31, 168.99; EI-MS ( $m/z$ ) 429.45 ( $\text{M}^+$ ), 431.45 ( $\text{M}^+2$ ) 433.44 ( $\text{M}^+4$ ). Cal/Ana: [C (61.55) 61.51 H (4.23) 4.19 N (6.53) 6.49].

2.5.6. 2-(2-Methylphenoxy)-N'-[(4-phenoxyphenyl)methylidene]acetohydrazide (**8f**)

Yield 82%, mp. 145–147 °C,  $R_f$  0.63 [hexane: ethylacetate (1:1)], IR (KBr)  $\text{cm}^{-1}$ : 3237.63 (NH), 1680.05 (C=O), 1497.78 (C=N), 1192.05 (—O—);  $^1\text{H}$  NMR (DMSO  $d_6$ , 300 MHz)  $\delta$  ppm: 2.23 (s, 3H,  $\text{CH}_3$ ), 5.12 (s, 2H,  $\text{CH}_2$ ), 6.79–7.97 (m, 13H, ArH), 8.27 (s, 1H,  $\text{CH}=\text{N}$ ), 11.44 (s, 1H, CONH;  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C}$  NMR (DMSO  $d_6$ , 75 MHz)  $\delta$  ppm: 14.44, 69.92, 114.21, 117.51, 118.63, 121.01, 121.92, 124.51, 126.18, 126.99, 128.55, 129.91, 130.11, 143.12, 157.01, 158.71,

159.93, 168.66; EI-MS ( $m/z$ ) 460.11 ( $\text{M}^+$ ), 461.09 ( $\text{M}^+1$ ). Cal/Ana: [C (73.32) 73.29 H (5.59) 5.55 N (7.77) 7.72].

2.5.7. 2-(2-Methylphenoxy)-N'-[[4-(morpholin-4-yl)phenyl]methylidene]acetohydrazide (**8g**)

Yield 73%, mp. 136–138 °C,  $R_f$  0.65 [hexane: ethylacetate (1:1)], IR (KBr)  $\text{cm}^{-1}$ : 3195.19 (NH), 1670.41 (C=O), 1553.71 (C=N), 1190.12 (—O—), 1123.57 (—O—, morpholine ring);  $^1\text{H}$  NMR (DMSO  $d_6$ , 300 MHz)  $\delta$  ppm: 2.31 (s, 3H,  $\text{CH}_3$ ), 2.96–2.98 (t, 4H, morpholine), 3.62–3.64 (t, 4H, morpholine), 4.82 (s, 2H,  $\text{CH}_2$ ), 6.69–7.62 (m, 8H, ArH), 7.92 (s, 1H,  $\text{CH}=\text{N}$ ), 11.02 (s, 1H, CONH;  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C}$  NMR (DMSO  $d_6$ , 75 MHz)  $\delta$  ppm: 14.45, 46.33, 66.42, 69.62, 114.21, 114.92, 121.01, 123.33, 124.55, 126.81, 130.11, 130.96, 143.09, 151.99, 158.72, 168.72; EI-MS ( $m/z$ ) 353.11 ( $\text{M}^+$ ), 354.41 ( $\text{M}^+1$ ). Cal/Ana: [C (67.97) 67.91 H (6.56) 6.49 N (11.89) 11.86].

2.5.8. N'-[(4-Fluorophenyl)methylidene]-2-(2-methylphenoxy)acetohydrazide (**8h**)

Yield 65%, mp. 164–166 °C,  $R_f$  0.72 [hexane: ethylacetate (1:1)], IR (KBr)  $\text{cm}^{-1}$ : 3185.12 (NH), 1674.46 (C=O), 1453.71 (C=N), 1194.12 (—O—), 697 (C—F);  $^1\text{H}$  NMR (DMSO  $d_6$ , 300 MHz)  $\delta$  ppm: 2.33 (s, 3H,  $\text{CH}_3$ ), 4.92 (s, 2H,  $\text{CH}_2$ ), 6.62–7.32 (m, 8H, ArH), 7.89 (s, 1H,  $\text{CH}=\text{N}$ ), 10.97 (s, 1H, CONH;  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C}$  NMR (DMSO  $d_6$ , 75 MHz)  $\delta$  ppm: 14.46, 69.42, 114.21, 115.56, 121.22, 125.09, 126.81, 129.92, 130.31, 131.11, 143.11, 158.71, 165.22, 168.99; EI-MS ( $m/z$ ) 286.35 ( $\text{M}^+$ ), 287.49 ( $\text{M}^+1$ ). Cal/Ana: [C (67.12) 67.07 H (5.28) 5.29 N (9.78) 9.76].

2.5.9. 2-(2,4-Dichlorophenoxy)-N'-[[4-(2,4-dichlorophenoxy)phenyl]methylidene]acetohydrazide (**8i**)

Yield 62%, mp. 138–140 °C,  $R_f$  0.68 [hexane: ethylacetate (1:1)], IR (KBr)  $\text{cm}^{-1}$ : 3220.27 (NH), 1678.13 (C=O), 1474.63 (C=N), 1099.46 (—O—), 714.65 (C—Cl);  $^1\text{H}$  NMR (DMSO  $d_6$ , 300 MHz)  $\delta$  ppm: 4.54 (s, 2H,  $\text{CH}_2$ ), 6.72–7.71 (m, 10H, ArH), 7.97 (s, 1H,  $\text{CH}=\text{N}$ ), 11.15 (s, 1H, CONH;  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C}$  NMR (DMSO  $d_6$ )  $\delta$  ppm: 66.18, 115.57, 117.76, 117.91, 123.40, 124.97, 126.55, 128.29, 129.41, 129.63, 129.94, 130.74, 143.68, 150.53, 153.29, 158.09, 168.63; EI-MS: ( $m/z$ ) 481.51 ( $\text{M}^+$ ), 483.50 ( $\text{M}^+2$ ), 485.49 ( $\text{M}^+4$ ). Cal/Ana: [C (52.10) 52.05 H (2.91) 2.89 N (5.79) 5.77].

2.5.10. 2-(2,4-Dichlorophenoxy)-N'-[(4-phenoxyphenyl)methylidene]acetohydrazide (**8j**)

Yield 72%, mp. 144–146 °C,  $R_f$  0.69 [hexane: ethylacetate (1:1)], IR (KBr)  $\text{cm}^{-1}$ : 3190.37 (NH), 1680.05 (C=O), 1586.50 (C=N), 1165.04 (—O—), 689.57 (C—Cl);  $^1\text{H}$  NMR (DMSO  $d_6$ , 300 MHz)  $\delta$  ppm: 5.28 (s, 2H,  $\text{CH}_2$ ), 7.02–7.73 (m, 12H, ArH), 7.99 (s, 1H,  $\text{CH}=\text{N}$ ), 11.62 (s, 1H, CONH;  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C}$  NMR (DMSO  $d_6$ , 75 MHz)  $\delta$  ppm: 68.66, 117.71, 118.11, 118.90, 121.01, 124.91, 126.91, 128.01, 128.91, 129.10, 129.94, 131.41, 143.33, 152.82, 157.01, 159.91, 168.62; EI-MS ( $m/z$ ) 415.26 ( $\text{M}^+$ ), 417.43 ( $\text{M}^+2$ ). Cal/Ana: [C (60.74) 60.71 H (3.88) 3.89 N (6.75) 6.73].

2.5.11. 2-(2,4-Dichlorophenoxy)-N'-[[4-(morpholin-4-yl)phenyl]methylidene]acetohydrazide (**8k**)

Yield 71%, mp. 130–132 °C,  $R_f$  0.66 [hexane: ethylacetate (1:1)], IR (KBr)  $\text{cm}^{-1}$ : 3182.65 (NH), 1681.02 (C=O), 1528.65 (C=N), 1185.30 (—O—, morpholine), 724.29 (C—Cl);  $^1\text{H}$  NMR (DMSO  $d_6$ , 300 MHz)  $\delta$  ppm: 4.84 (s, 2H,  $\text{CH}_2$ ), 2.40–2.45 (t, 4H, morpholine), 3.71–3.72 (t, 4H, morpholine), 6.70–7.36 (m, 8H, ArH), 8.27 (s, 1H,

CH=N), 11.41 (s, 1H, CONH; D<sub>2</sub>O exchangeable); <sup>13</sup>C NMR (DMSO *d*<sub>6</sub>, 75 MHz) δ ppm: 46.32, 66.01, 68.16, 114.41, 117.11, 123.01, 124.10, 128.11, 128.94, 130.19, 131.02, 143.12, 151.92, 152.81, 168.72; EI-MS (*m/z*) 408.54 (M<sup>+</sup>), 409.55 (M<sup>+</sup>+2), 411.49 (M<sup>+</sup>+4). Cal/Ana: [C (55.89) 55.85 H (4.69) 4.63 N (10.29) 10.25].

#### 2.5.12. 2-(2,4-Dichlorophenoxy)-N'-(4-fluorophenyl)methylidene] acetohydrazide (8l)

Yield 58%, mp. 172–174 °C, R<sub>f</sub> 0.77 [hexane: ethylacetate (1:1)], IR (KBr) cm<sup>-1</sup>: 3192.61 (NH), 1679.02 (C=O), 1483.21 (C=N), 1198.30 (—O—), 687.29 (C—F); <sup>1</sup>H NMR (DMSO *d*<sub>6</sub>, 300 MHz) δ ppm: 4.91 (s, 2H, CH<sub>2</sub>), 6.72–7.31 (m, 7H, ArH), 7.87 (s, 1H, CH=N), 10.42 (s, 1H, CONH; D<sub>2</sub>O exchangeable); <sup>13</sup>C NMR (DMSO *d*<sub>6</sub>, 75 MHz) δ ppm: 68.12, 115.56, 117.11, 124.01, 128.11, 128.91, 129.44, 130.18, 131.41, 143.12, 152.81, 168.16; EI-MS (*m/z*) 340.11 (M<sup>+</sup>), 341.23 (M<sup>+</sup>+1), 342.54 (M<sup>+</sup>+2). Cal/Ana: [C (52.81) 52.85 H (3.25) 3.23 N (8.21) 8.25].

#### 2.5.13. 2-Phenoxy-N'-(4-phenoxyphenyl)methylidene] acetohydrazide (8m)

Yield 60%, mp. 140–142 °C, R<sub>f</sub> 0.78 [hexane: ethylacetate (1:1)], IR (KBr) cm<sup>-1</sup>: 3083.31 (NH), 1680.05 (C=O), 1562.39 (C=N), 1192.05 (—O—); <sup>1</sup>H NMR (DMSO *d*<sub>6</sub>, 300 MHz) δ ppm: 5.04 (s, 2H, CH<sub>2</sub>), 6.76–7.91 (m, 13H, ArH), 8.02 (s, 1H, CH=N), 11.14 (s, 1H, CONH; D<sub>2</sub>O exchangeable); <sup>13</sup>C NMR (DMSO *d*<sub>6</sub>, 75 MHz) δ ppm: 69.01, 114.31, 117.51, 117.61, 121.09, 121.91, 126.92, 128.50, 128.91, 129.79, 143.11, 157.11, 159.32, 168.19; EI-MS (*m/z*) 346.54 (M<sup>+</sup>), 347.49 (M<sup>+</sup>+1), 348.49 (M<sup>+</sup>+2). Cal/Ana: [C (72.82) 72.78 H (5.24) 5.23 N (8.09) 8.05].

#### 2.5.14. N'-(4-Fluorophenyl)methylidene]-2-phenoxyacetohydrazide (8n)

Yield 62%, mp. 168–170 °C, R<sub>f</sub> 0.74 [hexane: ethylacetate (1:1)], IR (KBr) cm<sup>-1</sup>: 3231.63 (NH), 1679.05 (C=O), 1493.78 (C=N), 1191.05 (—O—), 688 (C—F); <sup>1</sup>H NMR (DMSO *d*<sub>6</sub>, 300 MHz) δ ppm: 4.98 (s, 2H, CH<sub>2</sub>), 6.71–7.27 (m, 8H, ArH), 7.96 (s, 1H, CH=N), 11.12 (s, 1H, CONH; D<sub>2</sub>O exchangeable); <sup>13</sup>C NMR (DMSO *d*<sub>6</sub>, 75 MHz)

δ ppm: 69.11, 114.31, 115.63, 121.11, 129.42, 129.89, 130.83, 143.12, 168.66; EI-MS (*m/z*) 272.11 (M<sup>+</sup>), 273.23 (M<sup>+</sup>+1). Cal/Ana: [C (66.17) 66.11 H (4.81) 4.83 N (10.29) 10.25].

## 2.6. In vitro antileishmanial activity

Promastigotes of Indian *Leishmania donovani* strain MHOM/IN/83/AG83 was obtained from the culture bank of Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna, India. The cryo-cells were revived and grown in RPMI 1640 medium (Sigma-Aldrich) supplemented with 10% foetal calf serum (FCS: Sigma-Aldrich) in BOD incubator at 25 °C.

Specimens were dissolved in dimethyl sulphoxide (DMSO) and further dilutions were made with RPMI 1640 medium (Navarro et al., 2010; Vitale et al., 1989). The concentrations of 500, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, and 1.9 (µg/ml) were prepared from the stock solution of 10 mg/ml in DMSO solution by serial dilution methods. Promastigotes were adapted for growth at 25 °C in RPMI 1640 (Sigma-Aldrich) and supplemented with 20% heat inactivated foetal bovine serum (Miguel et al., 2007). Logarithmic phase cultures were used for experimental purposes, and the *in vitro* susceptibility assay was performed in sterilized 96-well culture micro titre plates (Nunc) (Rolón et al., 2006). A dose of 2.5 × 10<sup>6</sup> parasites was added to each well to a final volume of 200 µl, together with the drug concentration. Growth of promastigotes was monitored after 48 h by counting the number of motile promastigotes microscopically in a Neubauer chambers. The log dose and response were used to calculate the 50% inhibitory concentrations (IC<sub>50</sub>) of compounds. IC<sub>50</sub> was calculated by linear regression analysis with 95% confidence limits. Tests were performed at least in triplicate on three different days in order to verify the results and amphotericin B was used as positive control.

## 2.7. Cytotoxicity

The active compounds were tested for cytotoxicity (IC<sub>50</sub>) in VERO cells by serial double dilution technique. After 72 h exposure,

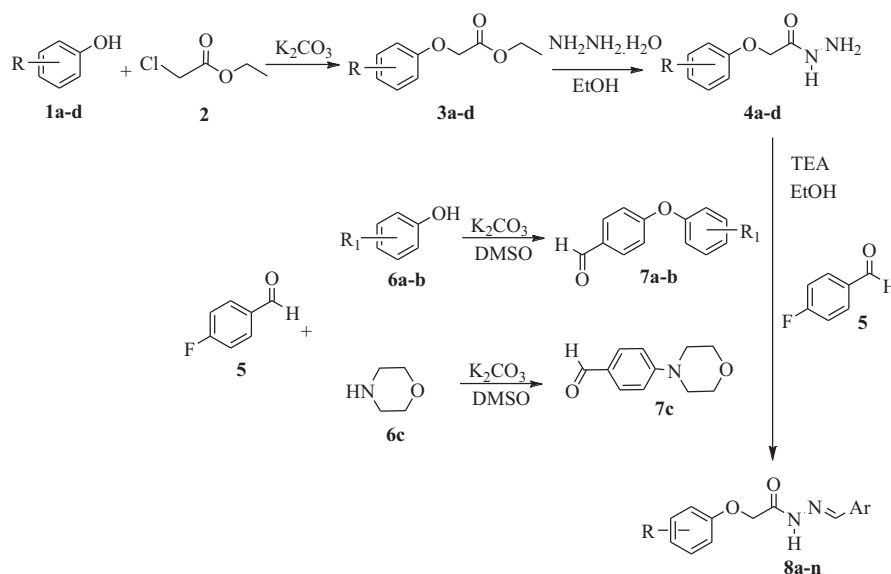
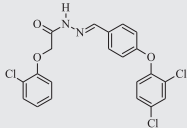
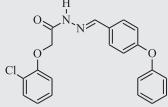
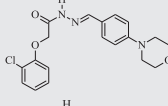
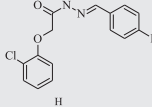
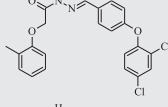
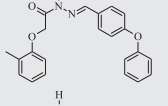
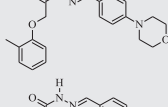
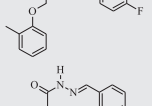
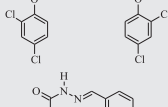
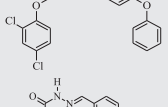
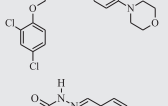
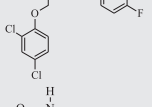
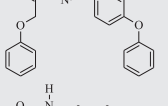
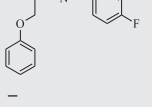


Fig. 1 – Protocol for the synthesis of acetohydrazide analogues (8a-n).



**Table 1 – Antileishmanial activity and cytotoxicity evaluation of the newer acetohydrazone analogues (8a-n).**

S. no.	Compound	Structure	IC <sub>50</sub> (μM)	
			<i>L. donovani</i> <sup>a</sup>	Vero
1	8a		280.05 ± 34.71	ND
2	8b		59.29 ± 25.28	164.11
3	8c		242.06 ± 52.67	ND
4	8d		242.92 ± 37.88	ND
5	8e		183 ± 29.14	>145.59
6	8f		194.03 ± 73.58	>173.42
7	8g		171.44 ± 72.52	ND
8	8h		252.39 ± 32.34	ND
9	8i		110.23 ± 32.74	ND
10	8j		144.60 ± 46.33	150.51
11	8k		48.10 ± 25.86	>153.08
12	8l		270 ± 37.93	ND
13	8m		208.55 ± 27.60	ND
14	8n		466.89 ± 47.49	ND
15	Amphotericin B	–	0.69 ± 0.13	ND

<sup>a</sup> Promastigotes of *Leishmania donovani*; ND: not determined.

viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 Non-Radioactive Cell Proliferation method (Heifets et al., 1989).

### 3. Results and discussion

#### 3.1. Chemistry

In the first step equimolar mixture of substituted phenol and ethyl chloroacetate was suspended in acetone anhydrous potassium carbonate and refluxed for 24 h to ethyl(substitutedphenoxy)acetate analogues (**3a-d**), which was further treated with hydrazine hydrate for 5–6 h in ethanol to obtain 2-(substitutedphenoxy)acetohydrazide analogues (**4a-d**). On the other hand 4-(substitutedphenoxy)benzaldehyde analogues (**7a-c**) were synthesized from 4-fluorobenzaldehyde and substituted phenol and morpholine in DMSO and anhydrous potassium carbonate by refluxing for 14–18 h. We have used different reaction conditions for the synthesis of 4-(substitutedphenoxy)benzaldehyde analogues (**7a-c**) from the earlier reported method (Joshi et al., 2010; Tripathi and Kumar, 2013). In the final step the aromatic aldehydes (**5** and **7a-c**) and 2-(substitutedphenoxy)acetohydrazide analogues (**4a-d**) were refluxed for 10–14 h in ethanol and triethylamine (TEA) to obtain the final product 2-(substitutedphenoxy)-N-[(aryl)methylidene]acetohydrazide analogues (**8a-n**). The reaction was monitored throughout by TLC using chloroform-methanol (9:1) and hexane:ethylacetate (1:1) as mobile phase. The spots were located under iodine vapour/UV light. The reaction sequence is shown in Fig. 1. The synthesized compounds were characterized by spectral analysis and all the compounds were in full harmony with the proposed structures. In general the IR spectra afforded absorption at 3237–3181  $\text{cm}^{-1}$  due to N—H stretching, 1682–1670  $\text{cm}^{-1}$  band due to C=O, 1573–1453  $\text{cm}^{-1}$  band due to C=N and 1056–1198  $\text{cm}^{-1}$  due to ether stretching. In  $^1\text{H}$  NMR the signals of the respective protons of the synthesized title compounds were verified on the basis of their chemical shifts and multiplicities in DMSO  $d_6$ . The spectra showed a singlet at  $\delta$  2.23–2.33 ppm corresponding to  $\text{CH}_3$  (Ar— $\text{CH}_3$ ); a multiplet at  $\delta$  2.40–3.72 ppm corresponding to morpholine protons; a singlet at  $\delta$  4.54–5.28 ppm to  $\text{CH}_2$  (methylene bridge); a singlet at  $\delta$  6.69–7.97 ppm corresponding to aromatic protons; a singlet at  $\delta$  7.79–8.27 ppm corresponding to imine proton (CH=N); and a multiplet at  $\delta$  10.42–11.62 ppm ( $\text{D}_2\text{O}$  exchangeable) corresponding to amide (CONH).

#### 3.2. Antileishmanial activity and cytotoxicity

Compounds were screened for *in vitro* antileishmanial activity against promastigotes of *Leishmania donovani* between 48.11 and 466.88  $\mu\text{M}$ . The most active compounds of the series was **8k** ( $\text{IC}_{50}$  48.11  $\pm$  25.86  $\mu\text{M}$ ) followed by **8b** ( $\text{IC}_{50}$  59.29  $\pm$  25.28  $\mu\text{M}$ ) while rest of the compounds showed moderate to low activity with  $\text{IC}_{50}$  between 110.23  $\pm$  32.74 and 466.88  $\pm$  47.49  $\mu\text{M}$  (Table 1). The antileishmanial activity was carried out in  $\mu\text{g}/\text{ml}$  and later converted in  $\mu\text{M}$ . The SAR explored from the data showed that the terminal aryl group with 4-(4-phenyl)morpholine as in compound **8k** showed maximum

antileishmanial activity and the activity decreased with 4-phenoxybenzene, 4-fluorophenyl and 2,4-dichloro-1-(4-phenoxy)benzene. The *O*-aryl group (acetohydrazide) with 2,4-dichlorophenyl (as in compound **8k**) showed maximum activity followed by 2-chlorophenyl, 2-methylphenyl and unsubstituted phenyl respectively. Two compounds (**8b** and **8k**) showed promising activity against the promastigotes of *L. donovani* and SAR confirmed that the compound **8k** could be considered as lead for further optimization. Further 5 compounds were screened for cytotoxic studies. The results of cytotoxic studies showed that the active compounds (**8b** and **8k**) were found to be non-toxic with  $\text{IC}_{50}$  of 164.11 and >153.08  $\mu\text{M}$  respectively (Table 1).

### 4. Conclusion

All the compounds were synthesized in satisfactory yield. All the compounds were characterized by IR, NMR and mass spectral data. The antileishmanial activity was carried out for all the compounds against the promastigotes of *Leishmania donovani*. Standard protocol was followed for antileishmanial activity. Some of the compounds showed promising antileishmanial activity. The compound **8k** showed the maximum antileishmanial activity with could be considered as lead for antileishmanial activity. The cytotoxic studies also showed that the compound **8k** was found to be non-toxic. The detailed molecular modelling and chemical modifications studies are in progress in our laboratory.

### Acknowledgements

The authors wish to express their gratitude to Maharishi Arvind College of Pharmacy, Jaipur, India, for providing research facilities, and Rajendra Memorial Research Institute of Medical Sciences, Patna, India, for providing facilities to carry out antileishmanial screening of the compounds and leishmanial strain. We are also thankful to the staff of SAIF, Punjab University, Chandigarh, for providing spectral data of synthesized compounds.

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