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Synthesis and In Vitro Leishmanicidal Activity of Some Hydrazides and Their Analogues

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Abstract—Twenty-one hydrazides were synthesized by treating different esters with hydrazine hydrate. Substituted hydrazides were obtained by treating hydrazides with alkyl/aryl/acyl halides. Some of these compounds exhibit potential in vitro leishmanicidal activity. The structures of all the synthesized compounds were confirmed by spectroscopic analysis.

Introduction

Leishmaniasis is one of the major health problems of tropical, subtropical and Mediterranean regions.[1] It occurs in all continents except Australia. According to WHO technical report about 53 million people all over the world are at risk of acquiring leishmaniasis and it has been estimated that 12 million new cases of leishmaniasis occur each year. It is a parasitic disease caused by a parasite known as leishmania on reticulo-endolethial system of the host.[2] The parasites are transmitted as metacyclic flagellated promastigote forms from host to host by the bite of infected sand flies. The leishmaniasis is classified on the basis of symptomatology as cutaneous, visceral (Kala Azar), mucosal or mucocutaneous and diffused cutaneous leishmaniasis.

The common symptoms of different leishmaniasis are lesions, fever, weight loss, anorexia, discomfort, change in hair colour, enlargement and marked alteration in function of liver, spleen, bone marrow, lymph nodes, ulceration, nasal blockage, swelling of nose and lips with destruction of soft tissues of oronasal cavity, dissemination of skin, thickening in plaques and multiple nodules.

A safe and effective vaccine is not yet available. Some currently used antimonial drugs such as tartar emetic (antimony potassium tartarate), sodium stibogluconate (pentostam),[3] urea stibamine,[4] and meglumine antimoniate (glucantime)[5] cause severe adverse side effects; treatment failure is also common. Various pentamidines and amphotericine B[4,5] are also known for the remedy of leishmaniasis whereas they are toxic at effective therapeutic dose. A number of forms of leishmaniasis are resistant to conventional drug therapy, especially in HIV leishmania co-infected patients. The development of new effective drug is therefore an urgent task. More recently Kevin K. Pitzer et al. reported the synthesis and biological evaluation of 4-chloro-3,5-dinitrobenzotrifluoride analogues as antileishmanial agents.[6] The hydrazides and their analogues are known to have different biological activities such as tuberculostatic activity,[7–9] antibacterial activity,[10,11] antifungal activity[12,13] and monoamine oxidase inhibitory activity.[14–16]

In continuation of our work in search of medicinally important organic molecules,[17–21] we initiated this work by keeping in mind the reports on leishmanicidal activity of nitrogen containing compounds along with the structures of pentamidines, which have four nitrogen atoms in their skeleton. The hydrazides have two nitrogen atoms in their skeleton, which may act as pharmacophore and results of biological activity clearly showed that our hypothesis was worthwhile. Further studies on

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hydrazides and some substituted members of this class are in progress.

Results and Discussion

The hydrazides are readily available from the corresponding esters by the reaction with hydrazine hydrate in very high yields. The different substituted esters required for the synthesis of hydrazides were prepared by the *O*-alkylation of phenolic OH group of the esters by treating these with benzyl bromide in the presence of potassium carbonate.

The alkylation of hydrazides occur at the α-*N* or β-*N* atom depending upon the conditions, in neutral medium, the terminal β-*N* atom is alkylated, whereas in the presence of a strong base like sodium or sodium methoxide, the position of alkylation is strongly dependent upon the nature of solvent. Aprotic solvents like ether and benzene favours α-*N* substitution, while protic solvents like ethanol favours β-*N* substitution.[22] During the reaction it was also observed that the substitution of alkyl groups results in disubstituted product on β-*N* even by using one equivalent of alkylating agent, whereas substitution of acyl group results in monosubstituted product. This behaviour of the hydrazides towards alkylating and acylating agent can be explained on the basis of electronic effect, the substitution by electron-donating alkyl groups results in increase in electron-density at the nitrogen resulting in the formation of *N*,*N*-dialkyl hydrazide. On the other hand substitutions by different acyl groups decrease the electron-density at β-*N* atom resulting in the monoacylated product.

Methyl 4-benzyloxybenzoate (**1a**) and methyl 3-benzyloxybenzoate (**1f**) were synthesized by treating methyl 4-hydroxybenzoate and methyl 3-hydroxybenzoate with benzyl bromide in the presence of potassium carbonate, respectively. The hydrazides **2a** and **2f** were synthesized by treating **1a** and **1f** with hydrazine hydrate, respectively. Compounds **2g** and **2i**–**2u** were synthesized by treating methyl/ethyl esters having aryl, heteroaryl, substituted aryl and alkyl or substituted alkyl groups with hydrazine hydrate (Fig. 1). Compounds **3b**–**3e** were synthesized by treating hydrazide **2a** with acrylonitrile, benzyl bromide, iodoethane and benzoyl chloride, respectively. Compound **3h** was synthesized by treating 4-hydroxybenzohydrazide (**2g**) with methyl chloroformate.

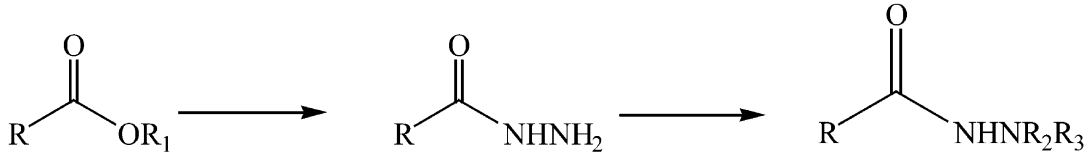
Several reports[2] describing the antileishmanial activity of the nitrogen containing compounds and structures of pentamidines initiated us to test the antileishmanial activity of all the synthetic hydrazides of present series. The activity was done at 100 μg/mL levels according to literature protocol,[23] amphotericin B was used as standard drug having IC_{50} value 50 μg/mL.

Out of the 21 hydrazides tested for their in vitro leishmanicidal activity, five compounds, that is, **2a, 3d, 2f, 2i** and **2s** shown potential in vitro antileishmanial acitivity. The IC_{50} of the 4-benzyloxybenzohydrazide (**2a**) was in the range of 3.13 μg/mL. The compounds **3b, 3c** and **3e** which were derived from compound **2a**, were found to be inactive. This indicates that when one of the hydrogens of β-*N* was replaced by cyanoethyl, benzoyl (compounds **3b** and **3e**) or both hydrogens substituted by benzyl residue (compound **3e**), the hydrazide substantially loses its leishmanicidal activity. However, when both hydrogen atoms were replaced with ethyl groups, it somewhat retains its activity as in case of compound **3d** (IC_{50} 6.25 μg/mL).

Another interesting finding of the present study is that the position of the benzyloxy group, if it is present at *para* or *meta* position of the benzene ring of hydrazide, do not influence the activity as in case of compound **2a** and **2f**, which showed same level of activity (IC_{50} 3.13 μg/mL).

The compound **2g**, which has hydroxyl group on benzene ring and compound **3h** bearing a methoxy carbonyl group on β-*N* atom have not shown very prominent activity. This behaviour indicates that benzyl group may have effect on the activity. The compound **2i** (IC_{50} 6.25 μg/mL) having 2-naphthoyl group and compound **3s** (IC_{50} 6.25 μg/mL) having 3-pyridino group indicate that these groups, present on one side of the carbonyl of hydrazides, may be responsible for activity.

Although compounds **2j**, **2l, 2m, 2q** and **2t** have one benzene ring with carbonyl carbon of the hyrazide, along with both hydrogens on β-*N* atom, these compounds did not show significant activity. The



1a, 1f, 1g, 1i-1u **2a, 2f, 2g, 2i-2u** **3b-3e, 3h**

Figure 1. **a**: R = *p*-$C_6H_5CH_2OC_6H_4$, R^1 = Me; **b**: R = *p*-$C_6H_5CH_2OC_6H_4$, R^1 = Me, R^2 = CH_2CH_2CN; **c**: R = *p*-$C_6H_5CH_2OC_6H_4$, R^1 = Me, R^2 = $CH_2C_6H_5$, R^3 = $CH_2C_6H_5$; **d**: R = *p*-$C_6H_5CH_2OC_6H_4$, R^1 = Me, R^2 = CH_2CH_3, R^3 = CH_2CH_3; **e**: R = *p*-$C_6H_5CH_2OC_6H_4$, R^1 = Me, R^2 = COC_6H_5; **f**: R = *m*-$C_6H_5CH_2OC_6H_4$, R^1 = Me; **g**: R = *p*-HOC_6H_4, R^1 = Me; **h**: R = p-HOC_6H_4, R^1 = Me, R^2 = C000CH_3; **i**: R = 2-$C_{10}H_7$, R^1 = Me; **j**: R = $CH_2C_6H_5$, R^1 = Me; **k**: R = CH_3, R^1 = Et; **l**: R = *o*-$C_6H_4OCH_3$, R^1 = Me; **m**: R = C_6H_5, R^1 = Me; **n**: R = *n*-$CH_2CH_2CH_3$, R^1 = Me; **o**: R = C_2H_5, R^1 = Me; **p**: R = *iso*-C_3H_7, R^1 = Me; **q**: R = *p*-$C_6H_4CH_3$, R^1 = Me; **r**: R = *n*-C_5H_{11}, R^1 = Me; **s**: R = 3-pyridyl, R^1 = Me; **t**: R = *o*-$C_6H_4CH_3$, R^1 = Me; **u**: R = *n*-C_8H_{17}, R^1 = Me.

Table 1. Results of leishmanicidal activity of hydrazides **2a**, **2f**, **2g**, **2i–2u**, **3b–3e**, **3h**

S. No.	Compound	IC_{50} (μg/mL)
1	**2a**	3.13
2	**3b**	> 100
3	**3c**	> 100
4	**3d**	6.25
5	**3e**	> 100
6	**2f**	3.13
7	**2g**	> 100
8	**3h**	> 100
9	**2i**	6.25
10	**2j**	> 100
11	**2k**	> 100
12	**2l**	> 100
13	**2m**	> 100
14	**2n**	> 100
15	**2o**	> 100
16	**2p**	> 100
17	**2q**	> 100
18	**2r**	> 100
19	**2s**	6.25
20	**2t**	> 100
21	**2u**	> 100

compounds **2k, 2n, 2p, 2r** and **2u** having aliphatic substituents with both hydrogens on β-*N* atoms found to be inactive (Table 1).

Conclusion

In the results of the present studies, variously substituted hydrazides were screened and benzyloxy protected hydrazides such as 4-benzyloxybenzohydrazide (**2a**), 4-benzyloxy *N′,N′*-diethylbenzohydrazide (**3d**), 3-benzyloxybenzohydrazide (**2f**), 2-naphthoylhydrazine (**2i**) and nicotinohydrazide (**2s**) were found to be the most potent in vitro leishmanicidal compounds. Conclusively it appeared that benzyloxy group at *meta* or *para* position might be responsible for leishmanicidal activity along with aromatic moiety attached to carbonyl carbon. Extensive mechanism based studies is required to contribute to the better understanding of the mechanism of action of these compounds.

Experimental

$CHCl_3$ and EtOH were dried by standard methods; all other solvents and reagents were of reagent grade and used directly without purification. Melting points were determined on Büchi-535 apparatus and are uncorrected. Column chromatography was performed on silica gel having mesh size (70–230) (E. Merck). IR spectroscopic analysis was done on Shimadzu-IR-460 for KBr pellets and Jasco-A-302 spectrophotometer for $CHCl_3$ solutions and the values are reported in $cm.^{-1}$ ^1H NMR spectroscopic analysis was done on Bruker apparatus; at 300, 400 and 500 MHz and the values are reported in δ (ppm). TMS was taken as internal standard. EI-MS spectroscopic analysis was done on Finnigan-MAT-311-*A* apparatus and the values are reported in *m/z* (rel. abund.%)

General procedure for the preparation of benzoate (1a) and (1f). The solution of potassium carbonate (165 mmol), benzylbromide (56 mmol) and methyl-4-hydroxy benzoate (56 mmol) in acetone was refluxed for 2 h. The reaction mixture was extracted with $CHCl_3$ and crystallized from hexane to afford **1a** and **1f**, respectively.

Methyl 4-benzyloxybenzoate (1a). Yield 15 g (91%); $R_f = 0.35$ (hexane/ethyl acetate, 1:1); mp 94 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 3.88 (s, 3H, OCH_3); 5.11 (s, 2H, $PhCH_2O$), 6.90 (d, 2H, $J = 8.8$ Hz, H-3/H-5), 7.43-7.31 (m, 5H, Ar-H), δ 8.00 (d, 2H, $J = 8.8$ Hz, H-2/H-6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 51.7 (OCH_3), 69.9 ($PhCH_2O$), 114.3 (C-3/C-5), 122.7 (C-1), 128.0 (C-3′/C-5′), 128.4 (C-4′), 128.5 (C-2′/C-6′), 131.5 (C-2/C-6), 136.2 (C-1′), 162.4 (C-4), 166.6 (CO); UV (methanol) λ_{max} (log ε) 257 (4.98); IR (KBr) ν_{max} 3010, 2990, 1715, 1605, 1514, 1250, 1170, 1010 cm^{-1}; MS (*m/z*) 242 (M^+, 30), 211 (9), 123 (5), 91 (100), 65 (53). Anal. calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82. Found: C, 74.27; H, 5.82.

Methyl 3-benzyloxybenzoate (1f). Yield 16 g; (85%); $R_f = 0.40$ (hexane/ethyl acetate, 4:6); mp 70 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 3.89 (s, 3H, OCH_3); 5.05 (s, 2H, $PhCH_2O$), 7.17 (dt, 1H, $J = 8.5$, 1.6 Hz, H-4), 7.29–7.46 (m, 5H, H-2′/H-3′/H-4′/H-5′/H-6′), 7.56 (t, $J = 8.5$ Hz, H-5), 7.61 (t, 1H, $J = 1.6$ Hz), 7.73 (dt, 1H, $J = 8.5$, 1.6 Hz, H-6); ^{13}C NMR (100 MHz, DMSO-d_6) 52.0 (OCH_3). 69.4 ($PhCH_2O$), 114.9 (C-4), 119.9 (C-2), 121.5 (C-5), 127.5 (C-3′/C-5′), 127.7 (C-4′), 128.3 (C-2′/C-6′), 129.7 (C-6), 131.0 (C-1), 136.6 (C-1′), 158.3 (C-3), 165.9 (CO); IR (KBr) ν_{max} 2903, 2705, 1712, 1591, 1485, 1420, 1311, 1273, 1233 cm^{-1}; UV (methanol) λ_{max} (log ε) 293 (5.12). MS (*m/z*) 242 (M^+, 43), 210 (15), 181 (13), 151 (2), 91 (100). Anal. calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82. Found: C, 74.29; H, 5.89.

General procedure for the preparation of hydrazides (2a) and (2f). Methyl 4-(benzyloxy) benzoate (76 mmol) was added in small portions to a solution of hydrazine hydrate (305 mmol) in 6 mL ethanol. After refluxing for 5 h the solid obtained was filtered and washed with hexane to afford hydrazides (**2a**) and (**2f**).

4-Benzyloxybenzohydrazide (2a). Yield 19 g (97%); mp 140 °C; $R_f = 0.58$ (ethyl acetate); ^1H NMR (400 MHz, DMSO-d_6) δ 4.41 (s, 2H, $CONHNH_2$), 5.14 (s, 2 H, $PhCH_2O$), 7.04 (d, 2H, $J = 8.6$ Hz, H-3/H-5), 7.45–7.30 (m, 5H, Ar-H), 7.80 (d, 2H, $J = 8.6$ Hz, H-2/H-6), 9.59 (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6) 69.4 ($PhCH_2O$), 114.3 (C-3/5), 125.8 (C-1), 127.6 (C-3′/C-5′), 127.8 (C-4′), 128.4 (C-2′/C-6′), 128.7 (C-2/C-6), 136.7 (C-1′), 160.5 (C-4), 165.7 (CO); IR (KBr) ν_{max} 3310, 3205, 1663, 1611, 1603, 1511, 1245 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 252 (4.88); MS (*m/z*) 242 (M^+, 51), 210 (28), 181 (2), 121 (13), 91 (100), 65 (59). Anal. calcd for $C_{14}H_{14}N_2O_2$ C, 69.41; H, 5.82; N, 11.56; Found: C, 69.34; H, 5.89; N, 11.49.

3-Benzyloxybenzohydrazide (2f). Yield 21 g (88%); mp 112 °C; $R_f = 0.40$ (ethyl acetate); ^1H NMR (400 MHz, DMSO-d_6) δ 4.51 (bs, 2H, $NHNH_2$), 5.13 (s, 2H, $PhCH_2$), 7.14 (dd, 1H, $J = 8.7$, 1.8 Hz, H-4), 7.46–7.29

(m, 5H, Ar-H), 7.48 (t, $J=8.7$ Hz, H-5), 7.56 (dd, 1H, $J=1.8$ Hz, $J=1.8$ Hz, H-2), 7.74 (dt, 1H, $J=8.7$, 1.8 Hz, H-6), 9.77 (s, 1H, $NHNH_2$); ^{13}C NMR (75 MHz, DMSO-d_6) 69.4 ($PhCH_2O$), 113.3 (C-4), 117.7 (C-2), 119.5 (C-5), 127.5 (C-3′/C-5′), 127.8 (C-4′), 128.4 (C-2′/C-6′), 129.4 (C-6), 134.7 (C-1), 136.8 (C-1′), 158.3 (C-3), 165.7 (CO); IR (KBr) ν_{max} 3313, 1615, 1575, 1255 cm^{-1}; UV (methanol) λ_{max} (log ε) 309 (5.19); MS (FD) m/z 242 (M^+). Anal. calcd for $C_{14}H_{14}N_2O_2$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.33; H, 5.91; N, 11.48.

General procedure for the preparation of hydrazide (3b–3e). To a solution of **2** (29 mmol) in 200 mL of ethanol, acrylonitrile (35 mmol)/benzyl bromide (72 mmol) or benzoyl chloride (33 mmol), iodoethane (87 mmol) was added respectively and reaction was refluxed for 3 days to afford crude solid which was recrystallized from ethanol to furnish **3b–3e**.

4-Benzyloxybenzoyl *N*′-2-cyanoethylbenzohydrazide (3b). Yellow needle like crystals; Yield 12 g (69%); mp 130 °C; $R_f=0.40$ (ethyl acetate); ^1H NMR (400 MHz, DMSO-d_6) δ 2.60 (t, 2H, $J=6.3$ Hz, h-2″); 3.00 (dt, 2H, $J=6.3$, 5.2 Hz, H-1″), 5.15 (s, 2H, $PhCH_2O$), 7.05 (d, 2H, $J=8.7$ Hz, H-3/H-5), 7.30–7.45 (m, 5H, Ar-H), 7.80 (d, 2H, $J=8.7$ Hz, H-2/H-6), 9.90 (d, 1H, $J=5.9$ Hz, NHCO); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.5, (C-2″), 47.0 (C-1″), 69.5 ($PhCH_2O$), 114.4 (C-3/C-5), 119.8 (C-1), 125.5 (C-3″), 127.8 (C-3′/C-5′), 128.3 (C-4′), 129.0 (C-2′/C-6′), 131.4 (C-2/C-6), 136.6 (C-1′), 160.8 (C-4), 165.7 (CO); IR (KBr) ν_{max} 3311, 3053, 2910, 2243, 1645, 1605, 1555, 1505, 1455, 1385, 1355, 1311, 1266, 1179 cm^{-1}; UV (methanol) λ_{max} (log ε) 251 (4.73); MS (m/z) 294 (M^+, 42), 254 (7), 211 (99), 120 (12), 91 (100). Anal. calcd for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found: C, 69.07; H, 5.87; N, 14.16.

***N*′,*N*′-Dibenzyl-4-benzyloxybenzohydrazide (3c).** Yield 21 g (59%); mp 140 °C; $R_f=0.35$ (hexane/ethyl acetate, 1:1); ^1H NMR (400 MHz, DMSO-d_6) δ 4.10 (s, 4H, $PhCH_2N$), 5.11 (s, 2H, $PhCH_2O$), 6.96 (d, 2H, $J=8.7$ Hz, H-3/H-5), 7.18–7.42 (m, 15H, Ar-H), 7.56 (d, 2H, $J=8.7$ Hz, H-2/H-6), 9.27 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 59.3 (NCH_2Ph), 69.2 (OCH_2Ph), 114.1 (C-3/C-5), 126.8 (C-3″/C-5″ and C-3‴/C-5‴), 127.5 (C-3′/C-5′), 127.7 (C-4′), 127.9 (C-4″/C-4‴), 128.1 (C-1), 128.3 (C-2″/C-6″ and C-2‴/C-6‴), 128.5 (C-2′/C-6′), 128.8 (C-2/C-6), 136.6 (C-1′), 138.2 (C-1″/C-1‴), 160.4 (C-4), 164.7 (CO); IR (KBr) ν_{max} 3308, 3258, 3011, 2803, 1642, 1608, 1503, 1253 cm^{-1}; UV (methanol) λ_{max} (log ε) 248 (4.38); MS (m/z) 422 (M^+ 72), 331 (67), 91 (100). Anal. calcd for $C_{28}H_{26}N_2O_2$: C, 79.59; H, 6.20; N, 6.63. Found: C, 79.51; H, 6.28; N, 6.55.

4-Benzyloxy *N*′,*N*′-diethylbenzohydrazide (3d). Yield 14 g (61%); mp 68 °C; $R_f=0.40$ (ethyl acetate); ^1H NMR (400 MHz, DMSO-d_6) δ 1.11 (t, 6H, $J=6.9$ Hz, CH_3), 2.90 (q, 4H, $J=6.9$ Hz, NCH_2), 5.01 (s, 2H, $PhCH_2$), 6.93 (d, 2H, $J=8.6$ Hz, H-3/H-5), 7.25–7.45 (m, 5H, Ar-H), 7.82 (d, 2 H, $J=8.6$ Hz, H-2/H-6); ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.3 (CH_3), 50.2 (NCH_2), 69.3 ($PhCH_2O$), 114.3 (C-3/C-5), 127.8, (C-3′/C-5′), 128.3 (C-1), 128.4 (C-4′), 128.7 (C-2′/C-6′), 128.9 (C-2/C-6), 136.7 (C-1′), 165.0 (C-4), 165.5 (CO); IR (KBr) ν_{max} 3210, 2955, 1655, 1611, 1508, 1263 cm^{-1}; UV (methanol) λ_{max} (log ε) 252 (4.43); MS (m/z) 298 (M^+, 2), 242 (5), 227 (30), 211 (100), 91 (70), 83 (27), 77 (3). Anal. calcd for $C_{18}H_{22}N_2O_2$: C, 72.46; H, 7.43; N, 9.39. Found: C, 72.37; H, 7.52; N, 9.30.

***N*′-Benzoyl-4-benzyloxybenzohydrazide (3e).** Yield 16 g (62%); mp 184 °C; $R_f=0.41$ (ethyl acetate); ^1H NMR (300 MHz, C_3D_6O) δ 5.21 (s, 2H, $PhCH_2O$), 6.94 (d, 2H, $J=8.3$ Hz, H-3/H-5), 7.95–7.09 (m, 10H, Ar-H), 7.99 (d, 2H, $J=8.3$ Hz, H-2/H-6), 9.63 (br, s, 1H, NH), 9.69 (s, 1H, NHCO), 9.75 (s, 1H, NHCO); ^{13}C NMR (100 MHz, DMSO-d_6) δ 69.4 ($PhCH_2O$), 114.5 (C-3/C-5), 124.9 (C-3″/C-5″), 127.4 (C-4″), 127.7 (C-3′/C-5′), 127.9 (C-4′), 128.4 (C-2′/C-6′), 129.3 (C-2/C-6), 131.7 (C-2/C-6″), 132.6 (C-1), 136.7 (C-1′), 138.7 (C-1″), 165.3 (C-4),165.8 (CONH). IR (KBr) ν_{max} 3258, 3053, 1684, 1645, 1612, 1581, 1533, 1504, 1254, 1287 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 251 (4.39). MS (m/z) 347 (M^+, 1), 324 (2), 240 (10), 211 (6), 105 (100). Anal. calcd for $C_{21}H_{18}N_2O_3$: C, 72.82; H, 5.24; N, 8.09. Found: C, 72.75; H, 5.31; N, 8.16.

Preparation of hydrazide (3h). To a solution of 4-hydroxybenzoyl hydrazine (7 mmol) in acetone (30 mL), was added K_2CO_3 (20 mmol) and methyl chloroformate (20 mmol). After refluxing for 4 h, **3h** was afforded which was crystallized from chloroform, methanol and hexane. Yield 2 g (86%); mp 218 °C; $R_f=0.48$ (ethyl acetate); ^1H NMR (300 MHz, DMSO-d_6) δ 3.61 (s, 3H, OCH_3), 6.81 (d, 2H, $J=8.7$ Hz, H-3/H-5), 7.72 (d, 2H, $J=8.7$ Hz, H-2/H-6), 9.08 (s, 1H, NH), 10.03 (br, s, NH), 10.07, (br, s, 1H, OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 52.8 (OCH_3), 115.2 (C-3/C-5), 123.2 (C-1), 129.6 (C-2/C-6), 157.3 (C-4), 160.9 (CONH), 166.2 (PhCO); IR (KBr) ν_{max} 3383, 2954, 1725, 1648, 1614, 1581, 1523, 1456, 1268, 1245, 1179 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 255 (4.51); MS (FD) (m/z) 210 (M^+). Anal. calcd for $C_{11}H_{10}N_2O$: C, 70.95; H, 5.41; N, 15.04. Found: C, 70.99; H, 5.37; N, 15.11.

General procedure for the preparation of (2g), (2l), (2m), (2q), (2s) and (2t). Hydrazine hydrate (263 mmol) was added to methyl 4-(hydroxy) benzoate (66 mmol)/ methyl 3-methoxy benzoate/ methyl benzoate (66 mmol)/ methyl 4-methylbenzoate (66 mmol)/ methyl nicotinate (66 mmol) and methyl 3-methylbenzoate (66 mmol). After refluxing for 5 h the solid obtained was washed with hexane to afford **2g**, **2l**, **2m**, **2q**, **2s** and **2t** respectively.

4-Hydroxybenzohydrazide (2g). Yield 11 g (91%); mp 261 °C; $R_f=0.48$ (methanol/ethyl acetate, 1:9); ^1H NMR (400 MHz, DMSO-d_6) δ 4.39 (bs, 2H, NH_2), 6.77 (d, 2H, $J=8.7$ Hz, H-3/H-5), 7.68 (d, 2H, $J=8.7$ Hz, H-2/H-6), 9.48 (bs, 1H, CONH), 9.91 (br, s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) 114.7 (C-3/C-5), 123.8 (C-1), 128.7 (C-2/C-6), 159.5 (C-4), 165.9 (CO); IR (KBr) ν_{max} 3306, 3211, 1618, 1585, 1543, 1508, 1283 cm^{-1}; UV (methanol) λ_{max} (log ε) 253 (4.23); MS (m/z) 152 (M^+, 16), 121 (100), 93 (52), 65 (55). Anal. calcd for

$C_7H_8N_2O_2$: C, 55.26; H, 5.30; N, 18.41. Found: C, 55.19; H, 5.37; N, 18.34.

2-Methoxybenzohydrazide (2l). Yield 12 g (90%); R_f 0.50 (hexane/ethyl acetate, 5:5); ^{1}H NMR (500 MHz, $CDCl_3$) λ 3.86 (s, 3H, OCH_3), 4.29 (br, 2H, NH_2), 6.89 (dd, 1H, J = 8.3, 1.9 Hz, H-5), 7.00 (dt, 1H, J = 8.3, 1.9 Hz, H-4), 7.36 (dt, 1H, J = 8.3, 1.9 Hz, H-3), 8.11 (dd, 1H, J = 8.3, J = 1.9 Hz, H-2), 9.43 (br, s, 1H, CONH); ^{13}C NMR (125 MHz, $CDCl_3$) 56.2 (CH_3) 113.1 (C-5), 119.8 (C-4), 122.3 (C-3), 125.2 (C-2), 135.4 (C-1), 156.9 (C-6), 168.9 (CO); IR (KBr) ν_{max} 3331, 3008, 1633, 1578, 1499, 1321, 1277, 765, 608 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 207 (2.8); MS (m/z) 166 (M^+, 26), 152 (19), 135 (100), 121 (59), 105 (5), 77 (55), 65 (18), 51 (10). Anal. calcd for $C_8H_{10}N_2O_2$: C, 57.82; H, 6.07; N, 16.86. Found: C, 57.73; H, 6.16; N, 16.77.

Benzohydrazide (2m). Yield 11 g (79%); R_f = 0.45 (hexane/ethyl acetate, 3:7); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.52 (br. s, 2H, NH_2), 7.56–7.01 (m, 5H, Ar-H), 10.01 (br. s, 1H, CONH); ^{13}C NMR (125 MHz, $CDCl_3$) 127.3 (C-3/C-5), 128.6 (C-2/C-6), 131.2 (C-4), 134.2 (C-1), 169.8 (CO); IR (KBr) ν_{max} 3325, 1614, 1571, 1491, 1329, 833, 666, 516 cm^{-1}; UV (methanol) λ_{max} (log ε) 201 (3.9; MS (m/z) 136 (M^+, 8), 105 (78), 77 (100), 51 (62). Anal. calcd for $C_7H_8N_2O$: C, 61.75; H, 5.92; N, 20.58. Found: C, 61.66; H, 6.01; N, 20.49.

4-Methylbenzohydrazide (2q). Yield 13 g (74%); R_f = 0.48 (ethyl acetate); ^{1}H NMR (300 MHz, CD_3OD) δ 2.36 (s, 3H, CH_3), 4.48 (br, s, 2H, NH_2), 7.19 (d, 2H, J = 8.3 Hz, H-3/H-5), 7.63 (d, 2H, J = 8.3 Hz, H-2/H-6), 9.54 (bs, 1H, CONH). ^{13}C NMR (75 MHz, CD_3OD) δ 21.7 (CH_3), 126.8 (C-2/C-6), 129.6 (C-3/C-5), 136.4 (C-4), 170.1 (CO). IR (KBr) ν_{max} 3319, 3189, 1627, 1563, 1489, 1276, 855, 611 cm^{-1}; UV (methanol) λ_{max} (log ε) 197.4 (4.73); MS (m/z) 150 (M^+, 67), 135 (30), 119 (99), 91 (100), 77 (10), 63 (36), 49 (1). Anal. calcd for $C_8H_{10}N_2O$: C, 63.98; H, 6.71; N, 18.65. Found: C, 63.91; H, 6.78; N, 18.58.

Nicotinohydrazide (2s). Yield 9 g (99%); R_f = 0.50 (ethyl acetate/methanol, 9:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.47 (br, s, 2H, NH_2), 7.54 (dt, 1H, J = 7.9, 2.1 Hz, H-5), 8.24 (t, 1H, J = 7.9 Hz, H-4), 8.71 (dt, 1H, J = 7.9, 2.1 Hz, J = 2.1 Hz, H-6), 8.98 (t, 1H, J = 2.1 Hz, H-2), 9.54 (br. s, 1H, CONH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 125.4 (C-3), 132.4 (C-5), 135.2 (C-4), 149.1 (C-6), 151.1 (C-2), 169.3 (CO); IR (KBr) ν_{max} 3323, 3178, 1639, 1565, 1483, 1285, 833, 629 cm^{-1}; UV (methanol) λ_{max} (log ε) 198 (4.7) MS (m/z) 137 (M^+, 86), 122 (25), 106 (100), 78 (100), 51 (54). Anal. calcd for $C_6H_7N_3O$: C, 52.55; H, 5.14; N, 30.64. Found: C, 52.46; H, 5.23; N, 30.55.

2-Methylbenzohydrazide (2t). Yield 14 g (71%); R_f 0.54 (ethyl acetate/methanol, 9:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.28 (s, 3H, CH_3), 4.21 (br, s, 2H, NH_2), 7.03 (dd, 1H, J = 8.1, 1.7 Hz, H-5), 7.10 (dt, 1H, J = 8.1, 1.7 Hz, H-3), 7.25 (dt, 1H, J = 8.1, 1.7 Hz, H-4), 7.71 (dd, 1H, J = 8.1, 1.7 Hz, H-2), 10.01 (bs, 1H, CONH); ^{13}C NMR (75 MHz, DMSO-d_6) 19.5 (CH_3), 125.6 (C-3), 127.7 (C-2), 128.5 (C-4), 136.6 (C-6), 171.3 (CO); IR (KBr) ν_{max} 3323, 1629, 1566, 1491, 1273, 865, 619 cm^{-1}; UV (methanol) λ_{max} (log ε) 274 (4.87); MS (m/z) 150 (M^+, 54), 119 (100), 91 (70), 77 (52), 63 (44). Anal. calcd for $C_8H_{10}N_2O$: C, 63.98; H, 6.71; N, 18.65. Found: C, 63.89; H, 6.80; N, 18.56.

General procedure for the preparation of (2i) and (2j). Hydrazine hydrate (236 mmol) was added to methyl 2-naphthoate (59 mmol)/methyl 2-phenylacetate (59 mmol). After refluxing for 5 h the solid obtained was washed with hexane to afford **2i** and **2j** respectively.

2-Naphthoylhydrazine (2i). Yield 15 g (71%); mp 152 °C; R_f 0.54 (methanol/ethyl acetate, 1:9); ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.54 (br, s, 2H, NH_2), 8.41–7.61 (m, 7H, Ar-H), 9.89 (bs, 1H, CONH); ^{13}C NMR (75 MHz, DMSO-d_6) 123.9 (C-5), 126.6 (C-8), 127.2 (C-4), 127.4 (C-3), 127.5 (C-1), 127.8 (C-7), 128.7 (C-6), 130.7 (C-2), 132.2 (C-10), 134.1 (C-9), 166.0 (CO); IR (KBr) ν_{max} 3308, 3210, 3048, 1622, 1558, 1505 cm^{-1}; UV (methanol) λ_{max} (log ε) 280 (4.97); MS (m/z) 186 (M^+, 40), 155 (100), 127 (46), 77 (5). Anal. calcd for $C_{11}H_{10}N_2O$: C, 70.95; H, 5.41; N, 15.04. Found: C, 70.87; H, 5.49; N, 14.96.

Phenyl acetyl hydrazine (2j). Yield 18 g (49%); R_f = 0.45 (methanol/ethyl acetate, 1:9); ^{1}H NMR (300 MHz, CD_3OD) δ 3.45 (s, 2H, $PhCH_2CO$), 4.26 (br, s, 2H, NH_2), 7.19–7.29 (m, 5H, Ar-H), 9.51 (br, s, 1H, CONH); ^{13}C NMR (75 MHz, CD_3OD) 41.8 ($PhCH_2$), 127.4 (C-3/C-5), 128.9 (C-4), 129.3 (C-2/C-6), 130.3 (C-1), 171.6 (CO); IR (KBr) ν_{max} 3305, 3023, 1643, 1534, 1358 cm^{-1}; UV (methanol) λ_{max} (log ε) 205 (3.98); MS (m/z) 149.8 (M^+, 56), 117 (66), 91 (100), 65 (22). Anal. calcd for $C_8H_{10}N_2O$: C, 63.98; H, 6.71; N, 18.65. Found: C, 63.91; H, 6.78; N, 18.58.

General procedure for the preparation of (2k), (2n–2p), (2r) and (2u). Hydrazine hydrate (133 mmol) was added to ethyl acetate (102 mmol)/methyl butyrate (102 mmol)/ methyl propionate (102 mmol)/ methyl 2-methylpropanoate (102 mmol)/ methyl hexanoate (102 mmol) or methyl nonanoate (102 mmol). After refluxing for 5 h the solid obtained was washed with hexane to afford (**2k**), (**2n–2p**), (**2r**) and **2u**) respectively.

Acetohydrazide (2k). Yield 8 g (97%); R_f = 0.45 (hexane/ethyl acetate, 1:9); ^{1}H NMR (400 MHz, CD_3OD) δ 1.90 (s, 3H, CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 18.51 (CH_3), 172.53 (CO); IR (KBr) ν_{max} 3479, 2953, 1653, 1283, 944, 699, 614 cm^{-1}; UV (methanol) λ_{max} (log ε) 202 (3.3); MS (m/z) 74 (M^+, 100), 56 (86). Anal. calcd for $C_2H_6N_2O$: C, 32.43; H, 8.16, N, 37.81. Found: C, 32.37, H, 8.23, N, 37.88.

Butanohydrazide (2n). Yield 12 g (89%); R_f = 0.55 (hexane/ethyl acetate, 2:8); ^{1}H NMR (300 MHz, D_2O) δ 2.08 (t, 2H, J = 7.5 Hz, H-2), 1.50 (m, 2H, H-3), 0.81 (t, 3H, J = 6.5 Hz, CH_3); ^{13}C NMR (75 MHz, D_2O) δ 177.1 (CO), 36.1 (C-2), 16.9 (C-3), 13.8 (CH_3); UV (methanol) λ_{max} (log ε) 195 (2.97); IR (KBr) ν_{max} 3254, 2961, 1681, 1266, 965, 799, 633 cm^{-1}; MS (m/z) 102 (M^+, 38),

71(41), 55 (3). Anal. calcd for $C_4H_{10}N_2O$: C, 47.04; H, 9.87, N, 27.43. Found: C, 46.94, H, 9.96, N, 27.38.

Propanohydrazide (2o). Yield 14 g (65%); $R_f = 0.45$ (ethyl acetate/methanol, 9:1); ^{1}H NMR (300 MHz, D_2O) δ 0.93 (m, 3H, H-3), 2.03 (m, 2H, H-2); ^{13}C NMR (75 MHz, D_2O) δ 12.84 (CH_3), 26.04 (C-2), 177.18 (CO); IR (KBr) ν_{max} 3256, 2979, 1645, 1279, 949, 801, 666 cm^{-1}; UV (methanol) λ_{max} (log ε) 189 (3.01); MS (*m*/*z*) 88 (M^+, 24), 71 (80), 57 (100). Anal. calcd for $C_3H_8N_2O$: C, 40.90; H, 9.15, N, 31.79. Found: C, 40.83, H, 9.22, N, 31.73.

2-Methylpropanohyrdrazide (2p)

Yield 12 g (84%); $R_f = 0.55$ (ethyl acetate); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (d, 6H, $J = 7.02$ Hz, H-3/4), 2.13 (m, 1H, H-2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.9 (C-3/4), 29.8 (C-2), 176.4 (CO); IR (KBr) ν_{max} 3259, 2955, 1639, 1278, 941, 811, 671 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 192 (3.18); MS (*m*/*z*) 129 (M^+, 22), 102 (88), 71 (100), 56 (12). Anal. calcd for $C_4H_{10}N_2O$: C, 47.04; H, 9.87, N, 27.43. Found: C, 46.96, H, 9.94, N, 27.36.

Hexanohydrazide (2r). Yield 20 g (68%); $R_f = 0.56$ (ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 (t, 3H, $J = 7.5$ Hz, H-6), 1.24 (m, 2H, H-5), 1.56 (m, 2H, H-4), 2.1 (m, 2H, H-3), 2.19 (t, 2H, $J = 7.8$ Hz, H-2); ^{13}C NMR (75 MHz, $CDCl_3$) 13.9 (CH_3), 21.5 (C-5), 27.6 (C-4), 29.7 (C-3), 32.8 (C-2), 174.3 (C-1); IR (KBr) ν_{max} 3299, 2975, 1683, 1278, 795, 639 cm^{-1}; UV (methanol) λ_{max} (log ε) 195 (4.36); MS (*m*/*z*) 130 (M^+, 67), 99 (100), 71 (99), 55 (58). Anal. calcd for $C_6H_{14}N_2O$: C, 55.35; H, 10.84; N, 21.52. Found: C, 55.26, H, 10.93, N, 21.43.

Nonanohydrazide (2u). Yield 26 g (67%); $R_f = 0.44$ (hexane/ethyl acetate, 4:6); ^{1}H NMR (300 MHz, CD_3OD) δ 0.89 (t, 3H, $J = 6.5$ Hz, H-9), 1.31 (m, 10H, H_2-4/H_2-5/H_2-6/H_2-7/H_2-8), 1.59 (m, 2H, H-3), 2.31 (t, 2H, $J = 7.5$ Hz, H-2); ^{13}C NMR (75 MHz, CD_3OD) δ 12.5 (C-9), 16.6 (C-8), 17.8 (C-7), 18.8 (C-6), 22.4 (C-5), 26.4 (C-4), 29.5 (C-3), 31.7 (C-2), 175.9 (C-1); IR (KBr) ν_{max} 3294, 2965, 1679, 1273, 959, 788, 639 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 186 (3.23); MS (*m*/*z*) 172 (M^+, 48), 141 (100), 71 (89), 57 (97). Anal. calcd for $C_9H_{20}N_2O$: C, 62.75; H, 11.70; N, 16.26. Found: C, 62.69, H, 11.76, N, 16.20.

Leishmanicidal activity (in vitro). Leishmania major (MHOM/PK/88/DESTO) promastigotes, cultivated in bulk were aseptically be sedimented down at 300 rpm, counted with the help of improved Neubaver chamber under the microscope and diluted with the fresh medium to a final concentration of 2×10^6 parasites/mL. The compounds to be checked were dissolved to a final concentration of 1.0 mg in 0.1 mL of PBS (Phosphate Buffered Saline, pH 7.4 containing 0.5% MeOH, 0.5% DMSO).

In a 96-well microtiter plate, 90 μL of the parasite culture (2.0×10^6 parasites/mL) was added in different wells. 10 μL of the experimental compound was added in culture and serially diluted so that minimum concentration of the compound is 0.1 μg/mL. 10 μL of PBS (Phosphate buffered saline, pH 7.4 (containing 0.5% MeOH, 0.5% DMSO) was added as negative control while glucantime, amphotericin B, pentamidine and ampicilline to a final concentration of 1.0 mg/mL was added separately as positive control. The plate was incubated between 21–22 °C in dark for 5 days during which control organisms multiply 6 times. The culture was examined microscopically on an improved neubaver chamber and IC_{50} values of compounds possessing antileishmanial activity were calculated.[24] All assays were run in duplicate.

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