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Research Article

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Synthesis, characterization and biological activities of new bis-1,3,4-oxadiazoles

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ABSTRACT

In present work, a series of 1,n-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl]alkanes/benzene have been synthesized. Condensation of different dihydrazides with 5-nitrovanillin gave corresponding dihydrazones. Dihydrazones on oxidative cyclization using chloramine-T yielded the corresponding bis-1,3,4-oxadiazole derivatives. Structures of newly synthesized compounds were established using FTIR, ^1H NMR and elemental analysis. All compounds were screened for their anti-inflammatory activities. IC_{50} values reveal that newly synthesized compounds exhibit better anti-inflammatory activities. Representative samples were studied for cytotoxicity. Results of cytotoxicity reveal that compounds exhibit moderate cytotoxicity.

Keywords: 1,n-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl]alkanes, chloramine-T, anti-inflammatory activity.

INTRODUCTION

Oxadiazole derivatives belong to an important group of heterocyclic compounds. Among wide variety of heterocyclic compounds that have been explored for developing pharmaceutically important molecules -1,3,4-oxadiazole derivatives have played a vital role in medicinal chemistry. Large number of synthetic compounds with 1,3,4-oxadiazole nucleus have been screened for anti-bacterial [1-4], anti-tubercular [5], anticonvulsant [6], anticancer [7], anti-microbial [8,9], anti-inflammatory [10-12] and analgesic activities [10]. In recent years bis-heterocyclic compounds have been extensively studied [13]. In view of these observations, an attempt is made to develop a synthetic route for 1,n-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl]alkanes/benzene as potent anti- inflammatory agents.

Several methods are reported in literature for synthesis of 1,3,4-Oxadiazoles [14-17]. However, these methods suffer from disadvantages such as long reaction time, requirement of severe conditions [14, 16] and use of toxic oxidants [17]. Chloramine-T is a very versatile oxidizing agent and has been used in synthesis of various 1,3,4-oxadiazole derivatives [7].

In present study, we report synthesis of 1, n-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl] alkanes/benzene using chloramine-T as an oxidizing agent. Structures of newly synthesized compounds are established using FTIR, ^1H NMR and elemental analysis. Synthesized compounds were tested for their utility as possible anti-inflammatory agents and representative samples were studied for cytotoxicity.

Reaction scheme

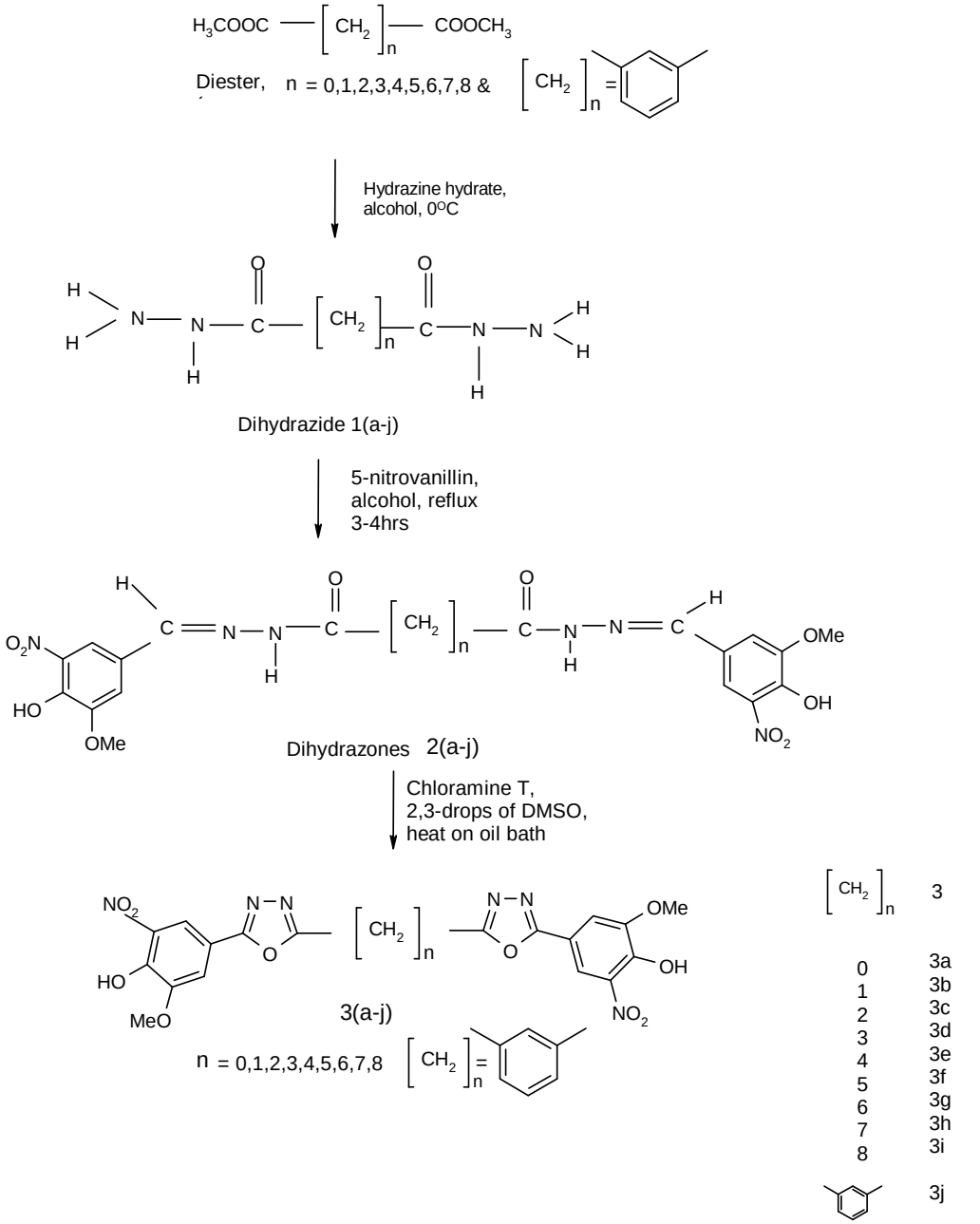


Figure 1. Reaction scheme leading to the formation 1, n-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl] alkanes/benzene

EXPERIMENTAL SECTION

All reagents were used as received from commercial suppliers. Uncorrected melting points (M.P.) of synthesized compounds were recorded in open capillary tubes**.** FTIR spectra were recorded using Bruker (Model 3000) spectrophotometer, 1H NMR spectra were recorded using Varian, Mercury Plus, 300 MHz NMR spectrophotometer. For recording NMR spectra DMSO was used as a solvent and TMS was used as an internal standard.

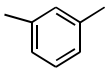
General procedure for synthesis of 1, n-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl] alkanes/benzene

To alcoholic solution of diester, hydrazine hydrate was added dropwise in molar ratio 1:2 to give corresponding dihydrazide. Dihydrazide obtained on condensation with 5-nitrovanillin gave dihydrazone which on oxidative cyclisation with chloramine-T yielded the corresponding bis-1,3,4-oxadiazole derivative.

Synthesis of dihydrazides 1(a-j)

To alcoholic solution of diester (dimethyl oxalate, dimethyl malonate, dimethyl succinate, dimethyl glutarate, dimethyl adipate, dimethyl pimelate, dimethyl suberate, dimethyl azelate, dimethyl sebacate and dimethyl isopthalate) (0.04 mol), hydrazine hydrate (0.08 mol) was added dropwise with constant stirring. The reaction was carried out at 0^oC. White products **1(a-j)** obtained were recrystallised in ethanol and are confirmed from their M.P. [18-21]. Physical parameters of the synthesized compounds **1(a-j)** are listed in Table 1.

Table 1. Physical parameters of the compounds 1(a-j)

Compound	Value of 'n' in $(CH_2)n$	Yield (%)	M. P. (^0C)
1a	$(CH_2)n$ =0	92	155
1b	1	90	152
1c	2	90	170
1d	3	87	162
1e	4	92	175
1f	5	85	182
1g	6	92	186
1h	7	86	187
1i	8	90	188
1j	$(CH_2)n$= 	92	Above 200

Synthesis of dihydrazones 2(a-j)

To alcoholic solution of dihydrazide (0.01 mol) **1(a-j)**, 5-nitrovanillin (3.30 g, 0.02 mol) was added and mixture was refluxed with continuous stirring for 3 to 4 h. Color product obtained was filtered and washed several times in warm alcohol to yield corresponding dihydrazone. As number of CH_2 groups between two rings increases the color of compound changes from yellow for n=0 to dark orange for n=8. Percentage yield and spectral data of dihydrazones were tabulated and is presented in Table 2.

Table 2. Physical and spectral data for compounds 2(a-j)

Compound	Value of 'n' in $(CH_2)_n$	Color	Yield (%)	FTIR data (cm^{-1})			
				C=N	C=O	N-H	NO_2
2a	0	Yellow	92	1617	1703	3284	1544
2b	1	Orange	87	1620	1681	3220	1530
2c	2	Orange	90	1612	1666	3193	1542
2d	3	Dark orange	90	1648	1684	3245	1543
2e	4	Dark orange	92	1618	1665	3273	1546
2f	5	Dark orange	90	1617	1668	3181	1537
2g	6	Dark orange	87	1617	1671	3171	1537
2h	7	Dark orange	83	1619	1651	3219	1541
2i	8	Dark orange	92	1620	1662	3191	1542
2j	$(CH_2)_n$= 	Dark orange	94	1615	1677	3208	1544

Synthesis of 1,n-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl]alkanes/ benzene 3(a-j)

To the mixture of dihydrazone (2 a-j, 0.006 mol) and Chloramine-T (3.380g, 0.012 mol), 2-3 drops of DMSO was added. Addition of DMSO brought both reactants in liquid phase and color changes to red. Red color liquid

obtained was heated on oil bath for ½ to 1 h at 120 ^{o}C followed by addition of alcohol that lead to the formation of color product, which was filtered and separated. Synthesized compounds were recrytallised using DMF: alcohol (25:75) mixture and washed with ether to obtain the pure compounds **3(a-j)**.

bis[5 -(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl] (3a)
Color of the compound obtained was: orange red yield: 2.605g (92%), mp above 260^{o}C; IR (KBr, ν cm^{-1}): 3382 (OH), 1616 (C=N), 1560 (NO_2), 1057 (N-N), 1281 and 1153(C-O-C);^{1}H NMR (DMSO-$d_{6,}$ δ ppm): 3.83 (s, 6H, 2 x OCH_3) , 7.0-8.4 (m, 4H, Ar-CH), 12.10(1s, 2H, 2OH). Analysis: for $C_{18}H_{12}N_6O_{10}$ found (calculated): C, 45.66(45.77); H, 2.56 (2.56); N, 17.83 (17.79)%.

1,1-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl]methane (3b)
Color of the compound obtained was dark red; yield: 2.507g (86%), mp charred at 252^{o}C; IR (KBr, ν cm^{-1}):3474 (OH), 1615 (C=N), 1531 (NO_2), 1063 (N-N), 1258 and 1159(C-O-C); ^{1}H NMR (DMSO-$d_{6,}$ δ ppm): 3.57(s , 2H, -CH_2), 3.81 (s, 6H, 2 x OCH_3) , 7.2-7.6 (m, 4H, Ar-CH), 11.32 (s,2H,2OH). Analysis: for $C_{19}H_{14}N_6O_{10}$ found (calculated): C, 46.82 (46.92); H, 2.90 (2.90); N, 17.25 (17.27)%.

1,2-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl]ethane (3c)
Color of the compound obtained was dark red; yield: 2.770g (93%), mp charred at 210^{o}C; IR (KBr, ν cm^{-1}): 3453 (OH), 1613 (C=N), 1546 (NO_2), 1060 (N-N), 1238 and 1157(C-O-C); ^{1}H NMR (DMSO-$d_{6,}$ δ ppm): 2.9 (s, 4H , 2 x ring-$\underline{CH_2}$), 3.75 (s, 6H, 2 x OCH_3) , 7.1-7.6 (m, 4H, Ar-CH), 11.0 and 11.20(2s, 2H, 2OH). Analysis: for $C_{20}H_{16}N_6O_{10}$ found (calculated): C, 47.91 (48.00); H, 3.21 (3.22); N, 16.79 (16.79)%.

1,3-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl]propane (3d)
Color of the compound obtained was dark red; yield: 2.819g (87%), mp above 260^{o}C; IR (KBr, ν cm^{-1}): 3488 (OH), 1614 (C=N), 1543 (NO_2), 1059 (N-N), 1240 and 1148(C-O-C); ^{1}H NMR (DMSO-$d_{6,}$ δ ppm): 1.88(p, 2H, ring-C-$\underline{CH_2}$, J=6.8 Hz), 2.24 and 2.62 (2t, 2X ring-$\underline{CH_2}$ – C, J=7.2 Hz), 3.71 (s, 2 x OCH_3, 6H) , 7.0-7.6 (m, Ar-CH, 4H), 11.06 and 11.090 (s, 2H, 2OH). Analysis: for $C_{21}H_{18}N_6O_{10}$ found (calculated): C, 49.13 (49.03); H, 3.51(3.52); N, 16.35(16.33)%.

1,4-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl]butane (3e)
Color of the compound obtained was dark red; yield: 2.819g (89%) , mp 242-243^{o}C; IR (KBr, ν cm^{-1}): 3393 (OH), 1612 (C=N), 1539 (NO_2), 1059 (N-N), 1250 and 1149(C-O-C); ^{1}H NMR (DMSO-$d_{6,}$ δ ppm): 1.62(p, 4H, 2 x ring-C-$\underline{CH_2}$, J=6.7 Hz), 2.24 and 2.62 (2t,4H,2 x ring- $\underline{CH_2}$–C, J=6.9 Hz), 3.79 (s, 6H, 2 x OCH_3) , 7.2-7.6 (m, 4H, Ar-CH), 11.06 and 11.09 (s, 2H, 2OH). Analysis:for $C_{22}H_{20}N_6O_{10}$ found (calculated):C, 50.10 (50.00); H, 3.82 (3.81); N, 15.89(15.90)%.

1,5-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl]pentane (3f)
Color of the compound obtained was dark red; yield: 2.912g (90%), mp charred at 230^{o}C; IR (KBr, ν cm^{-1}): 3314 (OH), 1632 (C=N), 1535 (NO_2), 1026 (N-N), 1263 and 1159(C-O-C); ^{1}H NMR (DMSO-$d_{6,}$ δ ppm): 1.34(p, 2H, ring –C-C-$\underline{CH_2}$, J=7.3 Hz), 1.60 (p, 4H, 2 x ring-C-$\underline{CH_2}$,) J=7.3 Hz, 2.15 and 2.54 (2t, 4H, 2 X ring-$\underline{CH_2}$, J=7.1 Hz), 7.0-7.6 (m, 4H, Ar-CH), 3.71 (s, 6H, 2 x OCH_3), 10.85 and 10.98 (s, 2H, 2OH). Analysis: for $C_{23}H_{22}N_6O_{10}$ found (calculated): C, 50.86 (50.92) ; H, 4.08 (4.08); N, 15.48 (15.49)%.

1,6-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl]hexane (3g)
Color of the compound obtained was dark red; yield: 2.620g (91%), mp 228-227^{o}C; IR (KBr, ν cm^{-1}): 3442 (OH), 1614 (C=N), 1539 (NO_2), 1058 (N-N), 1268 and 1148(C-O-C); ^{1}H NMR (DMSO-$d_{6,}$ δ ppm): 1.32(p, 4H, 2 x ring-C-C-$\underline{CH_2}$, J=7.1 Hz), 1.55 (p, 4H, 2 x ring-C- $\underline{CH_2}$-C, J=7.3 Hz), 2.18 &2.58(2t, 4H, 2 X ring- $\underline{CH_2}$, J=7.1 Hz), 3.80 (s, 6H, 2 x OCH_3) , 7.2-7.6 (m, , Ar-CH, 4H), 11.06 and 11.08 (s, 2H, 2OH). Analysis: for $C_{24}H_{24}N_6O_{10}$ found (calculated): C, 51.83 (51.80); H, 4.34 (4.34) ; N, 15.05 (15.10)%.

1,7-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl]heptane (3h)
Color of the compound obtained was dark red; yield: 3.009g (88%), mp 208-209^{o}C; IR (KBr, ν cm^{-1}): 3434 (OH), 1615 (C=N), 1540 (NO_2), 1060 (N-N), 1269 and 1146(C-O-C).^{1}H NMR (DMSO-$d_{6,}$ δ ppm): 1.30(s, 6H, ring-C-C-$\underline{CH_2}$-$\underline{CH_2}$-$\underline{CH_2}$-C-C-), 1.55 (bs, 4H, 2 x ring-C- $\underline{CH_2}$-C), 2.17 & 2.58(2t, 4H, 2 X ring- $\underline{CH_2}$, J=6.6Hz),3.80 (s,2 x OCH_3,6H), 7.2-7.9(m,Ar-CH,4H), 11.048 and11.052(s,2H,2OH). Analysis: for $C_{25}H_{26}N_6O_{10}$found (calculated): C, 52.64 (52.63); H, 4.60 (4.59); N,14.77 (14.73)%.

1,8-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl]octane (3i)
Color of the compound obtained was dark red; yield: 3.293g (94%), mp 214-215^{o}C; IR (KBr, ν cm^{-1}): 3481 (OH), 1616 (C=N), 1542 (NO_2), 1061 (N-N), 1272 and 1146(C-O-C);^{1}H NMR (DMSO-d_6, δ ppm): 1.27(s, 8H, ring-C-C-$\underline{CH_2}$-$\underline{CH_2}$-$\underline{CH_2}$-$\underline{CH_2}$-C-C-), 1.55 (p, 4H, 2 x ring-C- $\underline{CH_2}$-C, J=7.0 Hz), 2.17 & 2.59(2t, 4H, 2 X ring- $\underline{CH_2}$, J=7.0 Hz),3.85 (s, 2 x OCH_3, 6H), 7.3-7.7(m, Ar-CH, 4H), 11.36 and 11.38(s, 2H, 2OH). Analysis: for $C_{26}H_{28}N_6O_{10}$ found (calculated): C, 53.32 (53.42); H, 4.82 (4.82); N, 14.40 (14.37) %.

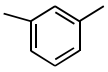
1,3-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl]benzene (3j)
Color of the compound obtained was dark red; yield: 2.926g (89%), mp charred at 190^{o}C; IR (KBr, ν cm^{-1}): 3454 (OH), 1616 (C=N), 1542 (NO_2), 1047 (N-N), 1271 and 1146 (C-O-C). ^{1}H NMR (DMSO-d_6, δ ppm): 3.88 (s, 2 x OCH_3, 6H), 7.42-7.70(m, Ar-CH, 4H), 8.35-8.43(m, Ar-CH, 5H, J=8.0, 0.267 Hz), 12.13(s,2H, 2OH). Analysis: for $C_{24}H_{16}N_6O_{10}$ found (calculated): C, 52.65 (52.56); H, 2.94 (2.94); N, 15.33 (15.32) %.

BIOLOGICAL ACTIVITIES

1) Anti-inflammatory activity

Anti-inflammatory potential of synthesized compounds were assessed using human RBC'S. Fresh human blood (5 mL) was collected and transferred to centrifugation tubes containing sodium citrate to prevent clotting. Tubes were centrifuged at 3000 rpm for 10 min and washed three times with equal volume of normal saline. Volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline. Reaction mixture consisting of 1.0 mL of test sample of various concentrations in normal saline and 0.5 mL of 10 % HRBC suspension, 1 mL 0.2 M phosphate buffer, 1mL hyposaline were incubated at 37 ^{0}C for 30 min and centrifuged at 3000 rpm for 30 min. Haemoglobin content of supernatant solution was estimated spectrophotometrically at 560 nm. Dichlorofenac was used as standard. Percentage of HRBC haemolysis and membrane stabilization was calculated and results obtained were tabulated and are presented in Table 3.

Table 3. Anti-inflammatory activities (IC_{50} values) of the synthesized compounds 3(a-j)

Sr. No.	Compound	Value of 'n' in $(CH_2)_n$	IC_{50}±S.D
1	3a	$(CH_2)_n$ =0	47.32 ± 1.15
2	3b	1	38.38 ± 1.063
3	3c	2	48.96 ± 1.063
4	3d	3	18.069 ± 1.063
5	3e	4	36.44 ± 1.063
6	3f	5	57.08 ± 1.063
7	3g	6	43.00 ± 2.36
8	3h	7	45.00 ± 1.32
9	3i	8	57.00 ± 2.79
10	3j	$(CH_2)_n$= 	79.32 ± 1.97
	Dichlorofenac		8.98 ± 0.136

2) In Vitro cytotoxicity study

Two representative samples were studied for short term in vitro cytotoxicity using Dalton's lymphoma ascites cells (DLA). Tumor cells aspirated from peritoneal cavity of tumor bearing mice were washed thrice with phosphate buffer saline (PBS). Cell viability was determined by typan blue exclusion method. Viable cell suspension (1 x10^6 cells in 0.1mL) was added to tubes containing various concentrations of test compounds and volume was made to1mL using PBS. Control tube contained only cell suspension. These assay mixture were incubated for 3 h at 37 ^{o}C. Further cell suspension was mixed with 0.1mL of 1% typan blue and kept for 3 min and loaded on a haemocytometer. Dead cells take up the blue color of typan blue while live cells do not take up the dye. Number of stained and unstained cells counted separately. Percentage cytotoxicity was calculated and data obtained is presented in Table 4.

Table 4. Cytotoxicity of compounds 3a and 3g.

Concentration (µg/mL)	Cytotoxicity (%)	
	Compound 3a	*Compound 3g*
200	40	38
100	23	19
50	11	10
20	05	02
10	00	00

RESULTS AND DISCUSSION

Hydrazones **2(a-j)** were prepared by condensation of acid hydrazides **1(a-j)** with 5-nitrovanillin. IR spectra of the dihydrazones showed peak in the range 1665 to 1703 cm^{-1} due to carbonyl of amide group and between 1612 to 1648 cm^{-1} due to C=N group. Reaction of dihydrazone with chloramine-T is difficult to take place in alcoholic medium. This problem was circumvented by adding two drops of DMSO in reaction mixture. Addition of two drops of DMSO to the mixture of dihydrazone and chloramine-T, brings both reactants in the same phase gives red color liquid which was heated on oil bath. Color solid 1,3,4-oxadiazole derivatives **3(a-j)** were then obtained by addition of alcohol. Formation of bis-1,3,4-oxadiazole derivative was confirmed by absence of peak due to amide carbonyl group and presence of peak in the range 1238 to 1281 cm^{-1} and 1148 to 1159 cm^{-1} gave the evidence for the ring closure, further peak in the range 1531 to 1560 cm^{-1} is due to NO_2 group. NMR spectra of synthesized compounds showed signals corresponding to different types of protons. Multiple signals between 7.0 and 8.4 ppm confirms aromatic protons, singlet signal in the range of 3.71 to 3.88 ppm corresponds to three protons of methoxy group, singlet between 11.0 and 12.1 ppm is due to protons of hydroxyl group. Further, multiplicities for the varying CH_2 groups are consistent with assigned structures. Results of anti-inflammatory activity reveal that synthesized compound **3d** exhibit excellent anti-inflammatory activity while other compounds exhibit moderate anti-inflammatory activity. Compound **3j** with phenyl group between the two oxadiazole rings shows poor anti-inflammatory activity. Representative compounds studied exhibits moderate cytotoxicity.

CONCLUSION

Bis-1,3,4-oxadiazole derivatives were synthesized by cyclization of acid hydrazone using Chloramine-T as an oxidizing agent. Method used for the synthesis is simple, easy and cost effective. Synthesized compounds possess better anti-inflammatory activity. It can be concluded that this class of compounds certainly hold great promise for discovering compounds with safer pharmacological properties.

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