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Synthesis of Some Aroylhydrazones and 2,5-Disubstituted-1,3,4-Oxadiazoles as DNA Photocleaving Agents

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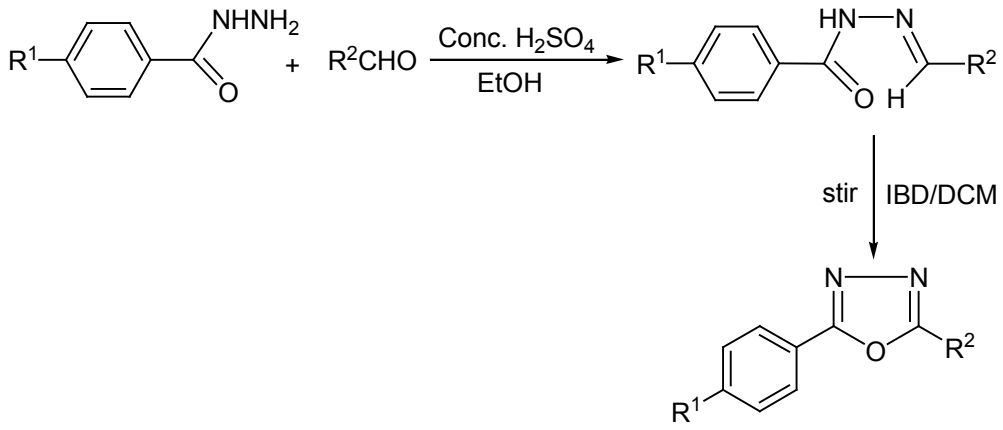
Abstract

Some 2,5-disubstituted-1,3,4-oxadiazole derivatives have been synthesized conveniently *via* oxidative cyclization of various synthesized aroylhydrazones by (diacetoxyiodo)benzene in dichloromethane under mild reaction conditions. In addition, the effect of electron-withdrawing/releasing groups on the formation of oxadiazole nucleus has also been studied. Compounds were obtained in good yields and their structures have been established on the basis of their FT-IR, ^{1}H, ^{13}C NMR and mass spectral data. Herein, a total of 42 compounds (hydrazones as well as oxadiazoles) were synthesized and investigated for their DNA photocleavage potential using plasmid DNA. It has been observed that aroylhydrazones showed good DNA photocleavage activity in comparison to their corresponding oxadiazoles.



Keywords: Oxadiazole; Pyrazole; Aroylhydrazone; (Diacetoxyiodo) benzene; DNA photocleavage

Introduction

Azoles are well known for their huge contribution in the pharmaceutical sector and among them pyrazole is still being considered as one of the leading pharmacophores in the development of bioactive compounds [1-10]. Pyrazole derivatives exhibited a broad spectrum of useful properties such as antitumor [11], antitubercular [12], antioxidant [13], anti-inflammatory [14,15], antibacterial [16], anti-obesity [17] and antidepressant [18] activities. Similarly, 1,3,4-oxadiazoles have been reported to exhibit various biological [19,20] and pharmacological activities [21-25]. These compounds, in particular, have been found to exhibit biological potential such as anticancer activity [26-28] specifically in the presence of some potent heterocyclic nuclei. In the past decade much attention has been paid towards the evaluation of the DNA photo cleavage potential of a variety of oxadiazole derivatives [29-31]. DNA is a primary site where most of the drugs interact and lead to inhibition or death of cancerous cells [32]. Therefore, compounds having binding or interacting ability with the DNA structure could be used as probes for DNA structure, as potential chemotherapeutic and diagnostic agents [33]. In view of the above facts, it was decided to synthesize some novel 2,5-disubstituted-1,3,4-oxadiazoles under mild reaction conditions and evaluate their DNA photo cleavage activity.

Experimental

Melting points of all the synthesized compounds were determined in an open capillary using digital melting point apparatus and are uncorrected. IR spectra were recorded as KBr discs on a Perkin-Elmer Spectrophotometer in the 4,000-450 cm^{-1} range. Both ^{1}H and ^{13}C NMR spectra of the compounds were recorded on a Bruker Advance NMR Spectrophotometer at 400 MHz and 100 MHz, respectively. Chemical shifts were measured relative to internal reference standard,

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tetramethylsilane (TMS) (δ=0) in $CDCl_3$ or DMSO-d_6, and were reported on δ scale (ppm). Coupling constants (J) were given in Hz. Mass spectra were recorded on Waters, Q-Tof micromass, ESI source, Mass Spectrometer. Carbon, nitrogen, hydrogen contents were analyzed using LECO 9320 analyzer. Aroylhydrazines **1** [34], 4-formylpyrazoles **2** [35] utilized in present investigation were synthesized according to the literature methods.

Synthesis of *Aroylhydrazones* (3a-u)

General procedure

A solution of 4-formylpyrazole or substituted benzaldehyde (0.01 mol) and sulfuric acid (one drop) in dichloromethane (DCM) was added to an ethanolic solution of aroylhydrazine (**1**, 0.01 mol) under stirring. Then reaction mass was refluxed for 40-45 min till completion of the reaction. The reaction was monitored by thin layer chromatography, excess of solvent was distilled out, and then the reaction mass was cooled to room temperature. The obtained product was filtered, washed with alcohol and recrystallised from ethanol. The melting points were noted before submitting the samples for analysis.

N-Benzoyl-N'-(1',3'-diphenyl-4'-pyrazolylmethylidene) hydrazine (3a)

Yield 89 %; m.p.: (Obs.) 180-181 °C, m.p.: (Lit.) 180-182 °C; TLC R_f = 0.37 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 11.76 (s, 1H, H-N, D_2O exchangeable), 8.96 (s, 1H, H-5'), 8.61 (s, 1H, H-6'), 7.92-8.00 (m, 4H, H-2, H-6 & H-2'', H-6''), 7.74 (d, 2H, J = 7.1 Hz, H-2''', H-6'''), 7.35-7.60 (m, 9H, H-3, H-4, H-5, H-Ph'' & H-Ph''') ppm; ^{13}C-NMR (100 MHz, $CDCl_3$+DMSO-d_6): δ = 162.9 (C, C-7), 151.8 (C, C-3'), 141.0 (CH, C-6'), 139.0 (C, C-1''), 133.3 (C, C-1), 132.0 (CH, C-4), 131.5 (C, C-1'''), 129.4 (CH, C-3''', C-5'''), 128.6 (CH, C-2, C-6), 128.4 (CH, C-3'', C-5''), 128.3 (CH, C-4'''), 128.2 (CH, C-2''', C-6'''), 127.6 (CH, C-3, C-5), 127.5 (CH, C-5'), 126.8 (CH, C-4''), 118.7 (CH, C-2'', C-6''), 116.9 (C, C-4') ppm; FT-IR (KBr): 3427 (N-H str.), 1668 (C=O str.) cm^{-1}; MS (ESI): m/z = 367.2 (M+1)$^+$; Anal. Calcd. for $C_{23}H_{18}N_4O$: C, 75.37; H, 4.91; N, 15.29. Found: C, 75.34; H, 4.89; N, 15.27.

N-Benzoyl-N'-(4'-nitrobenzylidene)hydrazine (3b)

Yield 86.8 %; m.p.: 238-239 °C; TLC R_f = 0.39 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 11.78 (s, 1H, H-N, D_2O exchangeable), 8.61 (s, 1H, H-7'), 8.34-8.48 (m, 4H, H-Ph'), 7.25-7.78 (m, 5H, H-Ph) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 163.0 (C, C-7), 148.0 (C, C-4'), 141.5 (C, C-1'), 140.8 (CH, C-7'), 132.8 (C, C-1), 132.4 (CH, C-4), 128.5 (CH, C-2, C-6), 127.7 (CH, C-2', C-6'), 127.4 (CH, C-3, C-5), 124.1 (CH, C-3', C-5') ppm; FT-IR (KBr): 3430 (N-H str.), 1668 (C=O str.), 1540 (NO_2 asymmetric str.), 1351 (NO_2 symmetric str.) cm^{-1}; MS (ESI): m/z = 270.3 (M+1)$^+$; Anal. Calcd. for $C_{14}H_{11}N_3O_3$: C, 62.38; H, 4.08; N, 15.59. Found: C, 62.36; H, 4.08; N, 15.58.

N-Benzoyl-N'-(4'-bromobenzylidene)hydrazine (3c)

Yield 87.5 %; m.p.: 201-202 °C; TLC R_f= 0.51 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 11.80 (s, 1H, H-N, D_2O exchangeable), 8.48 (s, 1H, H-7'), 7.89 (d, 2H, J = 7.4 Hz, H-2, H-6), 7.31-7.75 (m, 7H, H-Ph' & H-3, H-4, H-5) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 162.9 (C, C-7), 141.2 (CH, C-7'), 133.6 (CH, C-3', C-5'), 133.1 (C, C-1), 132.2 (CH, C-4), 130.1 (C, C-1'), 128.4 (CH, C-2, C-6), 127.8 (CH, C-3, C-5), 127.4 (CH, C-2', C-6'), 123.1 (C, C-4') ppm; FT-IR (KBr): 3433 (N-H str.), 1665 (C=O str.) cm^{-1}; MS (ESI): m/z = 303.4 (M+1)$^+$, 305.5 (M+2)$^+$ in the ratio showing typical bromine isotope profile (1:1); Anal. Calcd. for $C_{14}H_{11}BrN_2O$: C, 55.55; H, 3.64; N, 9.26. Found: C, 55.54; H, 3.62; N, 9.23.

N-Benzoyl-N'-(4'-methoxybenzylidene)hydrazine (3d)

Yield 87.9 %; m.p.: 189-190 °C; TLC R_f = 0.35 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 11.94 (s, 1H, H-N, D_2O exchangeable), 8.42 (s, 1H, H-7'), 7.32-7.92 (m, 7H, H-2', H-6' & H-Ph), 6.99 (d, 2H, J = 8.6 Hz, H-3', H-5'), 3.79 (s, 3H, 4'-OC**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ =161.4 (C, C-7), 160.0 (C, C-4'), 140.8 (CH, C-7'), 132.9 (C, C-1), 132.0 (CH, C-4), 129.1 (CH, C-2', C-6'), 128.1 (CH, C-2, C-6), 127.8 (CH, C-3, C-5), 122.4 (C, C-1'), 114.1 (CH, C-3', C-5'), 55.1 (OCH_3) ppm; FT-IR (KBr): 3421 (N-H str.), 1666 (C=O str.) cm^{-1}; MS (ESI): m/z = 255.2 (M+1)$^+$; Anal. Calcd. for $C_{15}H_{14}N_2O_2$: C, 70.81; H, 5.51; N, 11.01. Found: C, 70.78; H, 5.50; N, 11.00.

N-Benzoyl-N'-(4'-methylbenzylidene)hydrazine (3e)

Yield 88.7 %; m.p.: 195-196 °C; TLC R_f = 0.49 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 11.97 (s, 1H, H-N, D_2O exchangeable), 8.44 (s, 1H, H-7'), 7.40-7.93 (m, 7H, H-2', H-6' & H-Ph), 7.23 (d, 2H, J = 8.4 Hz, H-3', H-5'), 2.34 (s, 3H, 4'-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 161.0 (C, C-7), 140.7 (CH, C-7'), 140.1 (C, C-4'), 132.2 (C, C-1), 131.9 (CH, C-4), 129.4 (CH, C-2', C-6'), 129.2 (CH, C-3', C-5'), 128.6 (CH, C-2, C-6), 128.5 (C, C-1'), 127.5 (CH, C-3, C-5), 21.1 (CH_3) ppm; FT-IR (KBr): 3425 (N-H str.), 1665 (C=O str.) cm^{-1}; MS (ESI): m/z = 239.2 (M+1)$^+$; Anal. Calcd. for $C_{15}H_{14}N_2O$: C, 75.57; H, 5.88; N, 11.75. Found: C, 75.54; H, 5.87; N, 11.72.

N-Benzoyl-N'-(thien-2'-yl-methylidene)hydrazine (3f)

Yield 86.5 %; m.p.: 149-150 °C; TLC R_f = 0.36 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 11.82 (s, 1H, H-N, D_2O exchangeable), 8.69 (s, 1H, H-6'), 7.91 (d, 2H, J = 7.3 Hz, H-2, H-6), 7.11-7.61 (m, 6H, H-3, H-4, H-5 & H-3', H-4', H-5') ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 163.1 (C, C-7), 142.9 (CH, C-6'), 139.1 (CH, C-5'), 133.3 (C, C-1), 131.6 (CH, C-4), 130.6 (C, C-2'), 128.6 (CH, C-4'), 128.3 (CH, C-2, C-6), 127.6 (CH, C-3'), 127.5 (CH, C-3, C-5) ppm; FT-IR (KBr): 3421 (N-H str.), 1665 (C=O str.) cm^{-1}; MS (ESI): m/z = 231.1 (M+1)$^+$; Anal. Calcd. for $C_{12}H_{10}N_2OS$: C, 62.58; H, 4.34; N, 12.17. Found: C, 62.57; H, 4.33; N, 12.15.

N-Benzoyl-N'-(furl-2'-yl-methylidene)hydrazine (3g)

Yield 87 %; m.p.: 139-140 °C; TLC R_f= 0.32 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 11.85 (s, 1H, H-N, D_2O exchangeable), 8.72 (s, 1H, H-6'), 7.89 (d, 2H, J = 7.4 Hz, H-2, H-6), 7.09-7.58 (m, 6H, H-3, H-4, H-5 & H-3', H-4', H-5') ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 162.5 (C, C-7), 144.1 (CH, C-6'), 139.3 (C, C-2'), 138.8 (CH, C-5'), 132.6 (C, C-1), 131.8 (CH, C-4), 127.9 (CH, C-2, C-6), 126.8 (CH, C-3, C-5), 113.9 (CH, C-4'), 112.3 (CH, C-3') ppm; FT-IR (KBr): 3422 (N-H str.), 1663 (C=O str.) cm^{-1}; MS (ESI): m/z = 215.3 (M+1)$^+$; Anal. Calcd. for $C_{12}H_{10}N_2O_2$: C, 67.19; H, 4.67; N, 13.06. Found: C, 67.18; H, 4.65; N, 13.05.

N'-(1',3'-Diphenyl-4'-pyrazolylmethylidene)-N-(4-methylbenzoyl)hydrazine (3h)

Yield 88 %; m.p.: 192-193 °C; TLC R_f= 0.30 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 11.70 (s, 1H, H-N, D_2O exchangeable), 8.92 (s, 1H, H-5'), 8.62 (s, 1H, H-6'), 7.99 (d, 2H, J = 8.2 Hz, H-2'', H-6''), 7.85 (d, 2H, J = 8.0 Hz, H-2, H-6), 7.74 (d, 2H, J = 7.1 Hz, H-2''', H-6'''), 7.28-7.54 (m, 8H, H-3, H-5, H-Ph'' & H-Ph'''), 2.38 (s, 3H, 4-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 162.8 (C, C-7), 151.8 (C, C-3'), 141.4 (CH, C-6'), 140.7 (C, C-4), 139.0 (C, C-1''),

132.1 (C, C-1'''), 130.5 (C, C-1), 129.3 (CH, C-3''', C-5'''), 128.7 (CH, C-2, C-6), 128.4 (CH, C-3'', C-5''), 128.3 (CH, C-4'''), 128.2 (CH, C-2''', C-6'''), 127.4 (CH, C-3, C-5), 126.6 (CH, C-5'), 126.4 (CH, C-4''), 118.6 (CH, C-2'', C-6''), 116.9 (C, C-4'), 21.2 (CH_3) ppm; FT-IR (KBr): 3415 (N-H str.), 1664 (C=O str.) cm^{-1}; MS (ESI): m/z = 381.2 $(M+1)^+$; Anal. Calcd. for $C_{24}H_{20}N_4O$: C, 75.75; H, 5.26; N, 14.73. Found: C, 75.74; H, 5.23; N, 14.71.

4-Methyl-N'-(4'-nitrobenzylidene)-N-benzoylhydrazine (3i)

Yield 85.9 %; m.p.: 189-190 °C; TLC R_f = 0.33 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 12.05 (s, 1H, H-N, D_2O exchangeable), 8.56 (s, 1H, H-7'), 8.27 (d, 2H, J = 8.6 Hz, H-3', H-5'), 7.85-8.00 (m, 4H, H-2, H-6 & H-2', H-6'), 7.32 (d, 2H, J = 7.8 Hz, H-3, H-5), 2.41 (s, 3H, 4-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 163.3 (C, C-7), 147.7 (C, C-4'), 144.8 (C, C-4), 142.0 (C, C-1'), 140.7 (CH, C-7'), 130.1 (C, C-1), 128.8 (CH, C-2, C-6), 127.8 (CH, C-3, C-5), 127.7 (CH, C-2', C-6'), 123.8 (CH, C-3', C-5'), 21.1 (CH_3) ppm; FT-IR (KBr): 3433 (N-H str.), 1667 (C=O str.), 1543 (NO_2 asymmetric str.), 1349 (NO_2 symmetric str.) cm^{-1}; MS (ESI): m/z = 284.3 $(M+1)^+$; Anal. Calcd. for $C_{15}H_{13}N_3O_3$: C, 63.54; H, 4.59; N, 14.82. Found: C, 63.51; H, 4.58; N, 14.82.

N'-(4'-Bromobenzylidene)-N-(4-methylbenzoyl)hydrazine (3j)

Yield 89 %; m.p.: 231-232 °C; TLC R_f = 0.39 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 11.79 (s, 1H, H-N, D_2O exchangeable), 8.41 (s, 1H, H-7'), 7.55-7.83 (m, 6H, H-2, H-6 & H-Ph'), 7.27 (d, 2H, J = 7.8 Hz, H-3, H-5), 2.38 (s, 3H, 4-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 163.2 (C, C-7), 146.2 (C, C-4), 141.5 (CH, C-7'), 133.5 (CH, C-3', C-5'), 131.5 (CH, C-2, C-6), 130.3 (C, C-1'), 129.9 (C, C-1), 128.7 (CH, C-3, C-5), 127.6 (CH, C-2', C-6'), 123.2 (C, C-4'), 21.1 (CH_3) ppm; FT-IR (KBr): 3436 (N-H str.), 1665 (C=O str.) cm^{-1}; MS (ESI): m/z = 317.2 $(M+1)^+$, 319.2 $(M+2)^+$ in the ratio showing typical bromine isotope profile (1:1); Anal. Calcd. for $C_{15}H_{13}BrN_2O$: C, 56.92; H, 4.11; N, 8.85. Found: C, 56.90; H, 4.09; N, 8.84.

N'-(4'-Methoxybenzylidene)-N-(4-methylbenzoyl)hydrazine (3k)

Yield 89.1 %; m.p.: 199-200 °C; TLC R_f = 0.31 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 12.01 (s, 1H, H-N, D_2O exchangeable), 8.41 (s, 1H, H-7'), 7.66-7.72 (m, 4H, H-2', H-6' & H-2, H-6), 7.27 (d, 2H, J = 7.6 Hz, H-3, H-5), 6.99 (d, 2H, J = 8.6 Hz, H-3', H-5'), 3.82 (s, 3H, 4'-OC**H**$_3$), 2.36 (s, 3H, 4-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 161.3 (C, C-7), 159.8 (C, C-4'), 145.9 (C, C-4), 141.0 (CH, C-7'), 130.8 (C, C-1), 130.1 (CH, C-2, C-6), 128.9 (CH, C-2', C-6'), 128.6 (CH, C-3, 5), 122.4 (C, C-1'), 114.1 (CH, C-3', C-5'), 55.1 (OCH_3), 21.1 (CH_3) ppm; FT-IR (KBr): 3425 (N-H str.), 1664 (C=O str.) cm^{-1}; MS (ESI): m/z = 269.1 $(M+1)^+$; Anal. Calcd. for $C_{16}H_{16}N_2O_2$: C, 71.61; H, 5.97; N, 10.44. Found: C, 71.60; H, 5.94; N, 10.41.

N'-(4'-Methylbenzylidene)-N-(4-methylbenzoyl)hydrazine (3l)

Yield 86 %; m.p.: 209-210 °C; TLC R_f = 0.42 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 11.85 (s, 1H, H-N, D_2O exchangeable), 8.41 (s, 1H, H-7'), 7.64-7.70 (m, 4H, H-2', H-6' & H-2, H-6), 7.33 (d, 2H, J = 7.7 Hz, H-3, H-5), 7.26 (d, 2H, J = 8.2 Hz, H-3', H-5'), 2.30 (s, 6H, 4,4'-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 161.2 (C, C-7), 143.1 (C, C-4), 140.2 (CH, C-7'), 140.1 (C, C-4'), 131.0 (C, C-1), 129.3 (CH, C-2', C-6'), 129.1 (CH, C-3', C-5'), 128.8 (CH, C-2, C-6), 128.1 (C, C-1'), 127.6 (CH, C-3, C-5), 21.1 (CH_3) ppm; FT-IR (KBr): 3429 (N-H str.), 1667 (C=O str.) cm^{-1}; MS (ESI): m/z = 253.2 $(M+1)^+$; Anal. Calcd. for $C_{16}H_{16}N_2O$: C, 76.13; H, 6.34; N, 11.10. Found: C, 76.12; H, 6.33; N, 11.08.

N-Methylbenzoyl-N'-(thien-2'-yl-methylidene)hydrazine (3m)

Yield 85.9 %; m.p.: 165-166 °C; TLC R_f = 0.30 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 11.99 (s, 1H, H-N, D_2O exchangeable), 8.70 (s, 1H, H-6'), 7.68 (d, 2H, J = 7.6 Hz, H-2, H-6), 7.12-7.55 (m, 5H, H-3', H-4', H-5' & H-3, H-5), 2.36 (s, 3H, 4-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 162.9 (C, C-7), 143.0 (CH, C-6'), 140.5 (C, C-4), 138.2 (C, C-2'), 137.9 (CH, C-5'), 131.5 (C, C-1), 129.0 (CH, C-4'), 128.8 (CH, C-2, C-6), 127.8 (CH, C-3'), 127.4 (CH, C-3, C-5), 21.1 (CH_3) ppm; FT-IR (KBr): 3424 (N-H str.), 1666 (C=O str.) cm^{-1}; MS (ESI): m/z = 245.1 $(M+1)^+$; Anal. Calcd. for $C_{13}H_{12}N_2OS$: C, 63.91; H, 4.92; N, 11.47. Found: C, 63.89; H, 4.90; N, 11.46.

N'-(Fur-2'-yl-methylidene)-N-(4-methylbenzoyl)hydrazine (3n)

Yield 86.2 %; m.p.: 157-158 °C; TLC R_f = 0.29 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 12.01 (s, 1H, H-N, D_2O exchangeable), 8.68 (s, 1H, H-6'), 7.66 (d, 2H, J = 7.7 Hz, H-2, H-6), 7.02-7.45 (m, 5H, H-3', H-4', H-5' & H-3, H-5), 2.34 (s, 3H, 4-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 162.2 (C, C-7), 143.8 (CH, C-6'), 141.1 (C, C-4), 139.1 (C, C-2'), 138.6 (CH, C-5'), 130.7 (C, C-1), 128.3 (CH, C-2, C-6), 126.9 (CH, C-3, C-5), 114.0 (CH, C-4'), 111.6 (CH, C-3'), 21.1 (CH_3) ppm; FT-IR (KBr): 3420 (N-H str.), 1663 (C=O str.) cm^{-1}; MS (ESI): m/z = 229.1 $(M+1)^+$; Anal. Calcd. for $C_{13}H_{12}N_2O_2$: C, 68.39; H, 5.26; N, 12.27. Found: C, 68.38; H, 5.24; N, 12.25.

N'-(1',3'-Diphenyl-4'-pyrazolylmethylidene)-N-(4-nitrobenzoyl)hydrazine (3o)

Yield 87.6 %; m.p.: 232-233 °C; TLC R_f = 0.33 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 11.98 (s, 1H, H-N, D_2O exchangeable), 8.98 (s, 1H, H-5'), 8.61 (s, 1H, H-6'), 8.16-8.36 (m, 4H, H-2, H-6 & H-3, H-5), 8.02 (d, 2H, J = 8.0 Hz, H-2'', H-6''), 7.75 (d, 2H, J = 7.2 Hz, H-2''', H-6'''), 7.35-7.56 (m, 6H, H-Ph'' & H-Ph''') ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 160.9 (C, C-7), 152.0 (C, C-3'), 149.1 (C, C-4), 142.2 (CH, C-6'), 139.1 (C, C-1''), 139.0 (C, C-1), 132.0 (C, C-1'''), 129.4 (CH, C-3''', C-5'''), 129.0 (CH, C-3'', C-5''), 128.6 (CH, C-2, C-6), 128.5 (CH, C-4'''), 128.4 (CH, C-2''', C-6'''), 127.0 (CH, C-5'), 126.8 (CH, C-4''), 123.4 (CH, C-3, C-5), 118.7 (CH, C-2'', C-6''), 116.6 (C, C-4') ppm; FT-IR (KBr): 3434 (N-H str.), 1670 (C=O str.) cm^{-1}; MS (ESI): m/z = 412.1 $(M+1)^+$; Anal. Calcd. for $C_{23}H_{17}N_5O_3$: C, 67.14; H, 4.13; N, 17.03. Found: C, 67.10; H, 4.12; N, 17.01.

N-(4-Nitrobenzoyl)-N'-(4'-nitrobenzylidene)hydrazine (3p)

Yield 88.5 %; m.p.: 247-248 °C; TLC R_f = 0.36 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 12.10 (s, 1H, H-N, D_2O exchangeable), 8.67 (s, 1H, H-7'), 8.05-8.38 (m, 8H, H-Ph & H-Ph') ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 163.4 (C, C-7), 148.3 (C, C-4), 148.1 (C, C-4'), 141.9 (C, C-1'), 140.6 (CH, C-7'), 131.4 (C, C-1), 128.0 (CH, C-2, C-6), 127.9 (CH, C-2', C-6'), 123.7 (CH, C-3', C-5'), 123.6 (CH, C-3, C-5) ppm; FT-IR (KBr): 3436 (N-H str.), 1668 (C=O str.), 1546 (NO_2 asymmetric str.), 1354 (NO_2 symmetric str.) cm^{-1}; MS (ESI): m/z = 315.1 $(M+1)^+$; Anal. Calcd. for $C_{14}H_{10}N_4O_5$: C, 53.49; H, 3.18; N, 17.83. Found: C, 53.47; H, 3.16; N, 17.81.

N'-(4'-Bromobenzylidene)-N-4-nitrobenzoylhydrazine (3q)

Yield 86.5 %; m.p.: 227-228 °C; TLC R_f = 0.48 [ethylacetate: hexane

(3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 12.14 (s, 1H, H-N, D_2O exchangeable), 8.47 (s, 1H, H-7'), 8.17-8.36 (m, 4H, H-2, H-6 & H-3, H-5), 7.51-7.71 (m, 4H, H-Ph') ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 163.4 (C, C-7), 149.1 (C, C-4), 141.0 (CH, C-7'), 133.5 (CH, C-3', C-5'), 131.2 (C, C-1), 130.0 (C, C-1'), 127.7 (CH, C-2, C-6), 127.6 (CH, C-2', C-6'), 124.2 (CH, C-3, C-5), 123.4 (C, C-4') ppm; FT-IR (KBr): 3435 (N-H str.), 1669 (C=O str.) cm^{-1}; MS (ESI): m/z = 348.1 $(M+1)^+$, 350.2 $(M+2)^+$ in the ratio showing typical bromine isotope profile (1:1); Anal. Calcd. for $C_{14}H_{10}BrN_3O_3$: C, 48.40; H, 2.88; N, 12.10. Found: C, 48.38; H, 2.86; N, 12.09.

N'-(4'-Methoxybenzylidene)-N-(4-nitrobenzoyl)hydrazine (3r)

Yield 89.2 %; m.p.: 198-199 °C; TLC R_f = 0.33 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 11.96 (s, 1H, H-N, D_2O exchangeable), 8.40 (s, 1H, H-7'), 8.14-8.33 (m, 4H, H-2, H-6 & H-3, H-5), 7.68 (d, 2H, J = 8.6 Hz, H-2', H-6'), 6.97 (d, 2H, J = 8.7 Hz, H-3', H-5'), 3.80 (s, 3H, 4'-OC**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 161.5 (C, C-7), 160.3 (C, C-4'), 148.8 (C, C-4), 139.1 (CH, C-7'), 130.6 (C, C-1), 128.8 (CH, C-2', C-6'), 126.5 (CH, C-2, C-6), 123.3 (CH, C-3, C-5), 122.5 (C, C-1'), 114.1 (CH, C-3', C-5'), 55.1 (OCH_3) ppm; FT-IR (KBr): 3426 (N-H str.), 1670 (C=O str.) cm^{-1}; MS (ESI): m/z = 300.1 $(M+1)^+$; Anal. Calcd. for $C_{15}H_{13}N_3O_4$: C, 60.18; H, 4.35; N, 14.04. Found: C, 60.16; H, 4.33; N, 14.03.

N'-(4'-Methylbenzylidene)-N-(4-nitrobenzoyl)hydrazine (3s)

Yield 86.7 %; m.p.: 215-216 °C; TLC R_f = 0.45 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 12.05 (s, 1H, H-N, D_2O exchangeable), 8.44 (s, 1H, H-7'), 8.16-8.36 (m, 4H, H-2, H-6 & H-3, H-5), 7.65 (d, 2H, J = 8.6 Hz, H-2', H-6'), 7.25 (d, 2H, J = 8.4 Hz, H-3', H-5'), 2.32 (s, 3H, 4'-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 161.4 (C, C-7), 149.1 (C, C-4), 140.1 (CH, C-7'), 139.0 (C, C-4'), 130.6 (C, C-1), 129.3 (CH, C-2', C-6'), 129.0 (CH, C-3', C-5'), 128.2 (C, C-1'), 127.2 (CH, C-2, C-6), 123.3 (CH, C-3, C-5), 21.1 (CH_3) ppm; FT-IR (KBr): 3428 (N-H str.), 1667 (C=O str.) cm^{-1}; MS (ESI): m/z = 284.2 $(M+1)^+$; Anal. Calcd. for $C_{15}H_{13}N_3O_3$: C, 63.56; H, 4.59; N, 14.83. Found: C, 63.55; H, 4.57; N, 14.81.

N-4-Nitrobenzoyl-N'-(thien-2'-yl-methylidene)hydrazine (3t)

Yield 85.7 %; m.p.: 189-190 °C; TLC R_f = 0.30 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 12.04 (s, 1H, H-N, D_2O exchangeable), 8.67 (s, 1H, H-6'), 8.12-8.33 (m, 4H, H-2, H-6 & H-3, H-5), 7.09-7.58 (m, 3H, H-2', H-3', H-5') ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 161.2 (C, C-7), 149.1 (C, C-4), 143.9 (CH, C-6'), 138.9 (C, C-2'), 138.7 (CH, C-5'), 130.9 (C, C-1), 129.0 (CH, C-2, C-6), 128.9 (CH, C-4'), 127.5 (CH, C-3'), 123.3 (CH, C-3, C-5) ppm; FT-IR (KBr): 3425 (N-H str.), 1667 (C=O str.) cm^{-1}; MS (ESI): m/z = 276.2 $(M+1)^+$; Anal. Calcd. for $C_{12}H_9N_3O_3S$: C, 52.32; H, 3.27; N, 15.26. Found: C, 52.31; H, 3.24; N, 15.24.

N'-(Fur-2'-yl-methylidene)-N-(4-nitrobenzoyl)hydrazine (3u)

Yield 84.9 %; m.p.: 168-169 °C; TLC R_f = 0.27 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 12.06 (s, 1H, H-N, D_2O exchangeable), 8.38 (s, 1H, H-6'), 8.14-8.36 (m, 4H, H-2, H-6 & H-3, H-5), 6.58-7.74 (m, 3H, H-2', H-3', H-5') ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 161.3 (C, C-7), 149.2 (C, C-4), 144.9 (CH, C-6'), 138.8 (C, C-2'), 138.5 (CH, C-5'), 130.8 (C, C-1), 129.0 (CH, C-2, C-6), 123.3 (CH, C-3, C-5), 113.6 (CH, C-4'), 111.9 (CH, C-3') ppm; FT-IR (KBr): 3426 (N-H str.), 1666 (C=O str.) cm^{-1}; MS (ESI): m/z = 260.0 $(M+1)^+$; Anal. Calcd. for $C_{12}H_9N_3O_4$: C, 55.60; H, 3.47; N, 16.22. Found: C, 55.59; H, 3.45; N, 16.19.

Synthesis of 2,5-disubstituted-1,3,4-oxadiazoles (4)

General procedure

IBD (0.011 mol) was added in a portion-wise manner to the suspension or solution of an appropriate aroylhydrazone (**3**, 0.01 mol) in dichloromethane while stirring. The reaction mass was further stirred for 0.5-2.15 h and the reaction was monitored by TLC. After completion of the reaction, the solvent was evaporated and residue was triturated with petroleum ether twice to obtain the crude product **4** which was recrystallised from ethanol [36].

5-(1'',3''-Diphenyl-4''-pyrazolyl)-2-phenyl-1,3,4-oxadiazole (4a)

Yield 90 %; m.p.: (Obs.) 136-137 °C, m.p.: (Lit.) 136-138 °C; TLC R_f = 0.61 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.71 (s, 1H, H-5''), 7.92-7.97 (m, 4H, H-2'''', H-6'''' & H-2', H-6'), 7.84 (d, 2H, J = 7.6 Hz, H-2''', H-6'''), 7.26-7.55 (m, 9H, H-3', H-4', H-5', H-Ph''' & H-Ph'''') ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 164.0 (C, C-5), 159.9 (C, C-2), 152.0 (C, C-3''), 139.2 (C, C-1'''), 131.8 (C, C-1''''), 131.6 (CH, C-5''), 129.7 (CH, C-3'''', C-5''''), 129.4 (CH, C-4'), 129.1 (CH, C-3''', C-5'''), 129.0 (CH, C-4''''), 128.8 (CH, C-2'''', C-6''''), 128.3 (CH, C-2', C-6'), 127.6 (CH, C-4'''), 126.8 (CH, C-3', C-5'), 123.8 (C, C-1'), 119.5 (CH, C-2''', C-6'''), 106.5 (C, C-4'') ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1250 (C-O str.) cm^{-1}; MS (ESI): m/z = 365.2 $(M+1)^+$; Anal. Calcd. for $C_{23}H_{16}N_4O$: C, 75.78; H, 4.39; N, 15.38. Found: C, 75.75; H, 4.37; N, 15.34.

5-(4''-Nitrophenyl)-2-phenyl-1,3,4-oxadiazole (4b)

Yield 88.5 %; m.p.: (Obs.) 209-210 °C, m.p.: (Lit.) 209-210 °C; TLC R_f = 0.75 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.33-8.48 (m, 4H, H-Ph''), 7.35-7.75 (m, 5H, H-Ph') ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 162.1 (C, C-5), 160.7 (C, C-2), 148.9 (C, C-4''), 130.1 (CH, C-4'), 129.1 (C, C-1''), 128.7 (CH, C-2', C-6'), 127.0 (CH, C-3', C-5'), 126.5 (CH, C-2'', C-6''), 124.2 (CH, C-3'', C-5''), 123.9 (CH, C, C-1') ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1543 (NO_2 asymmetric str.), 1349 (NO_2 symmetric str.), 1252 (C-O str.) cm^{-1}; MS (ESI): m/z = 268.2 $(M+1)^+$; Anal. Calcd. for $C_{14}H_9N_3O_3$: C, 62.87; H, 3.37; N, 15.72. Found: C, 62.85; H, 3.34; N, 15.71.

5-(4''-Bromophenyl)-2-phenyl-1,3,4-oxadiazole (4c)

Yield 88.1 %; m.p.: (Obs.) 167-168 °C, m.p.: (Lit.) 167 °C; TLC R_f = 0.81 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.01 (d, 2H, J = 7.2 Hz, H-2', H-6'), 7.38-7.80 (m, 7H, H-3', H-4', H-5' & H-Ph'') ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 163.5 (C, C-5), 160.9 (C, C-2), 132.5 (CH, C-3'', C-5''), 129.8 (CH, C-4'), 128.9 (CH, C-2', C-6'), 128.1 (CH, C-2'', C-6''), 126.8 (CH, C-3', C-5'), 126.5 (C, C-1''), 124.0 (C, C-1'), 123.0 (C, C-4'') ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1245 (C-O str.) cm^{-1}; MS (ESI): m/z = 301.1 $(M+1)^+$, 303.1 $(M+2)^+$ in the ratio showing typical bromine isotope profile (1:1); Anal. Calcd. for $C_{14}H_9BrN_2O$: C, 55.98; H, 3.00; N, 9.33. Found: C, 55.96; H, 2.98; N, 9.32.

5-(4''-Methoxyphenyl)-2-phenyl-1,3,4-oxadiazole (4d)

Yield 91 %; m.p.: (Obs.) 150-151 °C, m.p.: (Lit.) 150 °C; TLC R_f = 0.73 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.35-8.10 (m, 7H, H-2'', H-6'' & H-Ph'), 7.12 (d, 2H, J = 8.5 Hz, H-3'', H-5''),

3.84 (s, 3H, 4''-OC**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 162.4 (C, C-5), 160.1 (C, C-2), 159.7 (C, C-4''), 130.0 (CH, C-4'), 128.7 (CH, C-2'', C-6''), 128.4 (CH, C-2', C-6'), 126.9 (CH, C-3', C-5'), 124.2 (C, C-1'), 120.8 (C, C-1''), 114.8 (CH, C-3'', C-5''), 55.3 (OCH$_3$) ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1249 (C-O str.) cm^{-1}; MS (ESI): m/z = 253.2 (M+1)$^+$; Anal. Calcd. for $C_{15}H_{12}N_2O_2$: C, 71.37; H, 4.76; N, 11.10. Found: C, 71.36; H, 4.75; N, 11.09.

5-(4''-Methylphenyl)-2-phenyl-1,3,4-oxadiazole (4e)

Yield 90.2 %; m.p.: (Obs.) 148-149 °C, m.p.: (Lit.) 148 °C; TLC R_f = 0.77 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.45-8.05 (m, 7H, H-2'', H-6'' & H-Ph'), 7.28 (d, 2H, J = 8.4 Hz, H-3'', H-5''), 2.40 (s, 3H, 4''-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 165.4 (C, C-5), 160.5 (C, C-2), 144.2 (C, C-4''), 130.2 (CH, C-2'', C-6''), 129.7 (CH, C-4'), 128.6 (CH, C-2', C-6'), 127.1 (CH, C-3', C-5'), 127.0 (CH, C-3'', C-5''), 123.9 (C, C-1'), 120.3 (C, C-1''), 21.7 (CH$_3$) ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1253 (C-O str.) cm^{-1}; MS (ESI): m/z = 237.3 (M+1)$^+$; Anal. Calcd. for $C_{15}H_{12}N_2O$: C, 76.17; H, 5.08; N, 11.85. Found: C, 76.16; H, 5.05; N, 11.84.

2-Phenyl-5-(thien-2''-yl)-1,3,4-oxadiazole (4f)

Yield 87.9 %; m.p.: 110-111 °C; TLC R_f = 0.75 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.12 (d, 2H, J = 6.9 Hz, H-2', H-6'), 7.18-7.84 (m, 6H, H-3', H-4', H-5' & H-3'', H-4'', H-5'') ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 164.0 (C, C-5), 160.9 (C, C-2), 131.8 (CH, C-5''), 130.2 (CH, C-3''), 129.8 (CH, C-4'), 129.1 (CH, C-2', C-6'), 128.2 (CH, C-4''), 127.0 (CH, C-3', C-5'), 125.2 (C, C-2''), 123.7 (C, C-1') ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1246 (C-O str.) cm^{-1}; MS (ESI): m/z = 229.0 (M+1)$^+$; Anal. Calcd. for $C_{12}H_8N_2OS$: C, 63.16; H, 3.51; N, 12.28. Found: C, 63.14; H, 3.48; N, 12.26.

5-(Fur-2''-yl)-2-phenyl-1,3,4-oxadiazole (4g)

Yield 85.9 %; m.p.: (Obs.) 103-104 °C, m.p.: (Lit.) 103 °C; TLC R_f = 0.64 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.10 (d, 2H, J = 7.0 Hz, H-2', H-6'), 7.12-7.88 (m, 6H, H-3', H-4', H-5' & H-3'', H-4'', H-5'') ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 164.3 (C, C-5), 159.8 (C, C-2), 133.4 (CH, C-5''), 130.1 (CH, C-4'), 128.8 (CH, C-2', C-6'), 128.5 (C, C-2''), 127.1 (CH, C-3', C-5'), 123.9 (C, C-1'), 114.8 (CH, C-3''), 114.5 (CH, C-4'') ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1244 (C-O str.) cm^{-1}; MS (ESI): m/z = 213.1 (M+1)$^+$; Anal. Calcd. for $C_{12}H_8N_2O_2$: C, 67.89; H, 3.77; N, 13.20. Found: C, 67.86; H, 3.75; N, 13.18.

5-(1'',3''-Diphenyl-4''-pyrazolyl)-2-(4'-methylphenyl)-1,3,4-oxadiazole (4h)

Yield 88.6 %; m.p.: 145-146 °C; TLC R_f = 0.57 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.61 (s, 1H, H-5''), 7.75-7.87 (m, 6H, H-2''', H-6''', H-2'''', H-6'''' & H-2', H-6'), 7.18-7.47 (m, 8H, H-3', H-5', H-Ph''' & H-Ph''''), 2.34 (s, 3H, 4'-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 163.9 (C, C-5), 162.3 (C, C-2), 151.9 (C, C-3''), 142.3 (C, C-4'), 139.3 (C, C-1'''), 132.0 (C, C-1''''), 131.7 (CH, C-5''), 129.6 (CH, C-2', C-6'), 129.5 (CH, C-3''', C-5'''), 129.2 (CH, C-3'''', C-5''''), 128.9 (CH, C-4'''), 128.8 (CH, C-2''', C-6'''), 127.6 (CH, C-3', C-5'), 127.3 (CH, C-4''''), 120.5 (C, C-1'), 119.4 (CH, C-2'''', C-6''''), 106.5 (C, C-4''), 21.3 (CH$_3$) ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1252 (C-O str.) cm^{-1}; MS (ESI): m/z = 379.1 (M+1)$^+$; Anal. Calcd. for $C_{24}H_{18}N_4O$: C, 76.17; H, 4.76; N, 14.81. Found: C, 76.16; H, 4.74; N, 14.79.

2-(4'-Methylphenyl)-5-(4''-Nitrophenyl)-1,3,4-oxadiazole (4i)

Yield 90.2 %; m.p.: 168-169 °C; TLC R_f = 0.69 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.36-8.44 (m, 4H, H-Ph''), 8.03 (d, 2H, J = 8.2 Hz, H-2', H-6'), 7.41 (d, 2H, J = 8.1 Hz, H-3', H-5'), 2.34 (s, 3H, 4'-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 164.9 (C, C-2), 162.3 (C, C-5), 149.0 (C, C-4''), 142.5 (C, C-4'), 129.8 (CH, C-2', C-6'), 129.0 (C, C-1''), 127.8 (CH, C-3', C-5'), 126.7 (CH, C-2'', C-6''), 124.4 (CH, C-3'', C-5''), 120.2 (C, C-1'), 21.2 (CH$_3$) ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1545 (NO_2 asymmetric str.), 1347 (NO_2 symmetric str.), 1255 (C-O str.) cm^{-1}; MS (ESI): m/z = 282.1 (M+1)$^+$; Anal. Calcd. for $C_{15}H_{11}N_3O_3$: C, 64.03; H, 3.91; N, 14.94. Found: C, 64.02; H, 3.90; N, 14.92.

5-(4''-Bromophenyl)-2-(4'-methylphenyl)-1,3,4-oxadiazole (4j)

Yield 87 %; m.p.: 205-206 °C; TLC R_f = 0.75 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.82-8.02 (m, 4H, H-2'', H-6'' & H-2', H-6'), 7.69 (d, 2H, J = 8.4 Hz, H-3'', H-5''), 7.35 (d, 2H, J = 7.9 Hz, H-3', H-5'), 2.46 (s, 3H, 4'-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 164.9 (C, C-2), 163.6 (C, C-5), 142.5 (C, C-4'), 132.4 (CH, C-3'', C-5''), 129.8 (CH, C-2', C-6'), 128.3 (CH, C-2'', C-6''), 126.9 (CH, C-3', C-5'), 126.3 (C, C-1''), 122.9 (C, C-4''), 120.9 (C, C-1'), 21.7 (CH$_3$) ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1248 (C-O str.) cm^{-1}; MS (ESI): m/z = 315.2 (M+1)$^+$, 317.1 (M+2)$^+$ in the ratio showing typical bromine isotope profile (1:1); Anal. Calcd. for $C_{15}H_{11}BrN_2O$: C, 57.29; H, 3.50; N, 8.91. Found: C, 57.28; H, 3.48; N, 8.89.

5-(4''-Methoxyphenyl)-2-(4'-methylphenyl)-1,3,4-oxadiazole (4k)

Yield 88.7 %; m.p.: (Obs.) 148-149 °C, m.p.: (Lit.) 148-150 °C; TLC R_f = 0.67 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.68-8.00 (m, 4H, H-2'', H-6'' & H-2', H-6'), 7.40 (d, 2H, J = 8.0 Hz, H-3', H-5'), 7.11 (d, 2H, J = 8.3 Hz, H-3'', H-5''), 3.84 (s, 3H, 4''-OC**H**$_3$), 2.44 (s, 3H, 4'-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 164.2 (C, C-2), 162.8 (C, C-5), 159.7 (C, C-4''), 142.7 (C, C-4'), 129.7 (CH, C-2', C-6'), 128.5 (CH, C-2'', C-6''), 127.3 (CH, C-3', C-5'), 121.2 (C, C-1'), 120.6 (C, C-1''), 114.5 (CH, C-3'', C-5''), 55.3 (OCH$_3$), 21.2 (CH$_3$) ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1256 (C-O str.) cm^{-1}; MS (ESI): m/z = 267.1 (M+1)$^+$; Anal. Calcd. for $C_{16}H_{14}N_2O_2$: C, 72.15; H, 5.26; N, 10.52. Found: C, 72.14; H, 5.24; N, 10.49.

5-(4''-Methylphenyl)-2-(4'-methylphenyl)-1,3,4-oxadiazole (4l)

Yield 88 %; m.p.: (Obs.) 179-180 °C, m.p.: (Lit.) 179-180 °C; TLC R_f = 0.73 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 7.74-7.95 (m, 4H, H-2'', H-6'' & H-2', H-6'), 7.38 (d, 2H, J = 8.4 Hz, H-3', H-5'), 7.28 (d, 2H, J = 8.6 Hz, H-3'', H-5''), 2.45 (s, 6H, 4', 4''-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 166.8 (C, C-2), 165.9 (C, C-5), 144.7 (C, C-4''), 142.2 (C, C-4'), 130.1 (CH, C-2'', C-6''), 129.8 (CH, C-2', C-6'), 127.2 (CH, C-3'', C-5''), 127.0 (CH, C-3', C-5'), 120.3 (C, C-1''), 120.1 (C, C-1'), 21.7 (CH$_3$), 21.3 (CH$_3$) ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1255 (C-O str.) cm^{-1}; MS (ESI): m/z = 251.1 (M+1)$^+$; Anal. Calcd. for $C_{16}H_{14}N_2O$: C, 76.77; H, 5.60; N, 11.19. Found: C, 76.76; H, 5.59; N, 11.18.

2-(4'-Methylphenyl)-5-(thien-2''-yl)-1,3,4-oxadiazole (4m)

Yield 85 %; m.p.: 126-127 °C; TLC R_f = 0.72 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.06 (d, 2H, J = 8.1 Hz, H-2',

H-6'), 7.28-7.98 (m, 5H, H-3'', H-4'', H-5'' & H-3', H-5'), 2.42 (s, 3H, 4'-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 165.9 (C, C-2), 164.4 (C, C-5), 142.3 (C, C-4'), 132.0 (CH, C-5''), 130.0 (CH, C-2', C-6'), 129.5 (CH, C-3''), 128.6 (CH, C-4''), 126.9 (C, C-2''), 126.8 (CH, C-3', C-5'), 120.6 (C, C-1'), 21.3 (CH$_3$) ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1249 (C-O str.) cm^{-1}; MS (ESI): m/z = 243.3 (M+1)+; Anal. Calcd. for $C_{13}H_{10}N_2OS$: C, 64.38; H, 4.13; N, 11.55. Found: C, 64.36; H, 4.10; N, 11.54.

5-(Fur-2''-yl)-2-(4'-methylphenyl)-1,3,4-oxadiazole (4n)

Yield 84.9 %; m.p.: (Obs.) 131-132 °C, m.p.: (Lit.) 131-132 °C; TLC R_f = 0.60 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 7.95 (d, 2H, J = 8.0 Hz, H-2', H-6'), 7.21-7.85 (m, 5H, H-3'', H-4'', H-5'' & H-3', H-5'), 2.44 (s, 3H, 4'-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 165.4 (C, C-2), 164.3 (C, C-5), 142.0 (C, C-4'), 133.5 (CH, C-5''), 129.9 (CH, C-2', C-6'), 128.6 (C, C-2''), 127.0 (CH, C-3', C-5'), 120.9 (C, C-1'), 115.2 (CH, C-3''), 113.8 (CH, C-4''), 21.3 (CH$_3$) ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1251 (C-O str.) cm^{-1}; MS (ESI): m/z = 227.1 (M+1)$^+$; Anal. Calcd. for $C_{13}H_{10}N_2O_2$: C, 69.00; H, 4.42; N, 12.38. Found: C, 69.00; H, 4.40; N, 12.37.

5-(1'',3''-Diphenyl-4''-pyrazolyl)-2-(4'-nitrophenyl)-1,3,4-oxadiazole (4o)

Yield 86.4 %; m.p.: 210-211 °C; TLC R_f= 0.60 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 9.38 (s, 1H, H-5''), 8.26-8.44 (m, 4H, H-Ph'), 7.42-8.03 (m, 10H, H-Ph''' & H-Ph'''') ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 164.1 (C, C-5), 161.6 (C, C-2), 150.8 (C, C-3''), 149.0 (C, C-4'), 138.6 (C, C-1'''), 131.4 (C, C-1''''), 131.1 (CH, C-5''), 129.4 (CH, C-2', C-6'), 128.9 (CH, C-3'''', C-5''''), 128.8 (CH, C-3''', C-5'''), 128.7 (CH, C-4''''), 128.4 (CH, C-2'''', C-6''''), 127.9 (CH, C-4'''), 127.2 (C, C-1'), 124.3 (CH, C-3', C-5'), 118.9 (CH, C-2''', C-6'''), 105.5 (C, C-4'') ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1256 (C-O str.) cm^{-1}; MS (ESI): m/z = 410.1 (M+1)$^+$; Anal. Calcd. for $C_{23}H_{15}N_5O_3$: C, 67.46; H, 3.67; N, 17.11. Found: C, 67.44; H, 3.65; N, 17.10.

2-(4'-Nitrophenyl)-5-(4''-nitrophenyl)-1,3,4-oxadiazole (4p)

Yield 89 %; m.p.: (Obs.) 127-128 °C, m.p.: (Lit.) 127-129 °C; TLC R_f = 0.68 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.32-8.54 (m, 8H, H-Ph' & H-Ph'') ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 163.1 (C, C-2), 162.6 (C, C-5), 149.3 (C, C-4'), 149.1 (C, C-4''), 130.1 (C, C-1''), 129.3 (C, C-1'), 127.2 (CH, C-2', C-6'), 126.9 (CH, C-2'', C-6''), 124.6 (CH, C-3', C-5'), 124.4 (CH, C-3'', C-5'') ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1546 (NO_2 asymmetric str.), 1351 (NO_2 symmetric str.), 1257 (C-O str.) cm^{-1}; MS (ESI): m/z = 313.0 (M+1)$^+$; Anal. Calcd. for $C_{14}H_8N_4O_5$: C, 53.85; H, 2.56; N, 17.95. Found: C, 53.83; H, 2.54; N, 17.93.

5-(4''-Bromophenyl)-2-(4'-nitrophenyl)-1,3,4-oxadiazole (4q)

Yield 85 %; m.p.: 197-198 °C; TLC R_f = 0.74 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.39-8.46 (m, 4H, H-Ph'), 7.63-7.81 (m, 4H, H-Ph'') ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 163.6 (C, C-5), 163.2 (C, C-2), 148.8 (C, C-4'), 132.3 (CH, C-3'', C-5''), 129.8 (C, C-1''), 128.4 (CH, C-2'', C-6''), 127.3 (CH, C-2', C-6'), 126.3 (C, C-1''), 124.3 (CH, C-3', C-5'), 122.9 (C, C-4'') ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1250 (C-O str.) cm^{-1}; MS (ESI): m/z = 346.1 (M+1)$^+$, 348.1 (M+2)$^+$ in the ratio showing typical bromine isotope profile (1:1); Anal. Calcd. for $C_{14}H_8BrN_3O_3$: C, 48.68; H, 2.32; N, 12.17. Found: C, 48.66; H, 2.30; N, 12.14.

5-(4''-Methoxyphenyl)-2-(4'-nitrophenyl)-1,3,4-oxadiazole (4r)

Yield 90 %; m.p.: 176-177 °C; TLC R_f = 0.66 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.37-8.46 (m, 4H, H-Ph'), 8.10 (d, 2H, J = 8.4 Hz, H-2'', H-6''), 7.15 (d, 2H, J = 8.2 Hz, H-3'', H-5''), 3.90 (s, 3H, 4''-OC**H**$_3$) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 162.3 (C, C-5), 162.1 (C, C-2), 159.6 (C, C-4''), 149.1 (C, C-4'), 129.1 (C, C-1'), 128.6 (CH, C-2'', C-6''), 127.6 (CH, C-2', C-6'), 124.3 (CH, C-3', C-5'), 120.9 (C, C-1''), 114.6 (CH, C-3'', C-5''), 55.3 (OCH$_3$) ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1248 (C-O str.) cm^{-1}; MS (ESI): m/z = 298.2 (M+1)$^+$; Anal. Calcd. for $C_{15}H_{11}N_3O_4$: C, 60.56; H, 3.70; N, 14.13. Found: C, 60.54; H, 3.69; N, 14.11.

5-(4''-Methylphenyl)-2-(4'-nitrophenyl)-1,3,4-oxadiazole (4s)

Yield 87.9 %; m.p.: 188-189 °C; TLC R_f = 0.70 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.31-8.42 (m, 4H, H-Ph'), 8.05 (d, 2H, J = 8.4 Hz, H-2'', H-6''), 7.37 (d, 2H, J = 8.2 Hz, H-3'', H-5''), 2.46 (s, 3H, 4''-C**H**$_3$) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 165.7 (C, C-5), 162.6 (C, C-2), 149.5 (C, C-4'), 143.1 (C, C-4''), 130.0 (CH, C-2'', C-6''), 129.0 (C, C-1'), 127.7 (CH, C-2', C-6'), 127.1 (CH, C-3'', C-5''), 124.4 (CH, C-3', C-5'), 120.5 (C, C-1''), 21.8 (CH$_3$) ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1254 (C-O str.) cm^{-1}; MS (ESI): m/z = 282.3 (M+1)$^+$; Anal. Calcd. for $C_{15}H_{11}N_3O_3$: C, 63.99; H, 3.91; N, 14.93. Found: C, 63.97; H, 3.89; N, 14.91.

2-(4'-Nitrophenyl)-5-(thien-2''-yl)-1,3,4-oxadiazole (4t)

Yield 84.9 %; m.p.: 165-166 °C; TLC R_f = 0.57 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.35-8.46 (m, 4H, H-Ph'), 7.29-7.96 (m, 3H, H-3'', H-4'', H-5'') ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 164.3 (C, C-5), 162.4 (C, C-2), 148.9 (C, C-4'), 133.5 (CH, C-5''), 131.6 (CH, C-3''), 130.6 (CH, C-4''), 128.7 (C, C-1'), 128.4 (C, C-2''), 127.7 (CH, C-2', C-6'), 124.3 (CH, C-3', C-5') ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1248 (C-O str.) cm^{-1}; MS (ESI): m/z = 274.0 (M+1)$^+$; Anal. Calcd. for $C_{12}H_7N_3O_3S$: C, 52.75; H, 2.56; N, 15.38. Found: C, 52.73; H, 2.54; N, 15.36.

5-(Fur-2''-yl)-2-(4'-nitrophenyl)-1,3,4-oxadiazole (4u)

Yield 85.5 %; m.p.: 145-146 °C; TLC R_f = 0.55 [ethylacetate: hexane (3:7)]; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.31-8.49 (m, 4H, H-Ph'), 7.01-7.85 (m, 3H, H-3'', H-4'', H-5'') ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 164.5 (C, C-5), 163.0 (C, C-2), 149.4 (C, C-4'), 133.8 (CH, C-5''), 129.7 (C, C-2''), 129.0 (C, C-1'), 127.8 (CH, C-2', C-6'), 124.4 (CH, C-3', C-5'), 114.6 (CH, C-3''), 114.0 (CH, C-4'') ppm; FT-IR (KBr): transparent in the region of (N-H str.) and (C=O str.), 1249 (C-O str.) cm^{-1}; MS (ESI): m/z = 258.1 (M+1)$^+$; Anal. Calcd. for $C_{12}H_7N_3O_4$: C, 56.01; H, 2.72; N, 16.34. Found: C, 56.00; H, 2.71; N, 16.32.

Biological Activity

DNA photocleavage study

DNA photocleavage experiment was performed by taking 10 µL solution containing plasmid DNA in TE (*Tris* 10mM, EDTA 0.01mM, pH 8.0) buffer in the presence of 40 µg of synthesized compounds. The sample solution held in caps of polyethylene microcentrifuge tubes were placed directly on the surface of a trans-illuminator (8000 mW/cm) at 360 nm and were irradiated for 30 min at room temperature.

After irradiation, samples were further incubated at 37°C for 1h. Irradiated samples were mixed with 6X loading dye containing 0.25 % bromophenol blue and 30 % glycerol. The samples were then analyzed by electrophoresis on a 0.8 % agarose horizontal slab gel in *Tris*-acetate EDTA buffer (40 mM *Tris*, 20 mM acetic acid, 1 mM EDTA, pH: 8.0). Untreated plasmid DNA was maintained as a control in each run of gel electrophoresis which was carried out at 5V/cm for 2.0 h. Gel was stained with ethidium bromide (1 µg/mL) and photographed under UV light [37]. To account the effect of synthesized compounds on DNA, the band intensities were analyzed using the MYImage Analysis software provided by Thermo Fisher Scientific Inc.

Results and Discussion

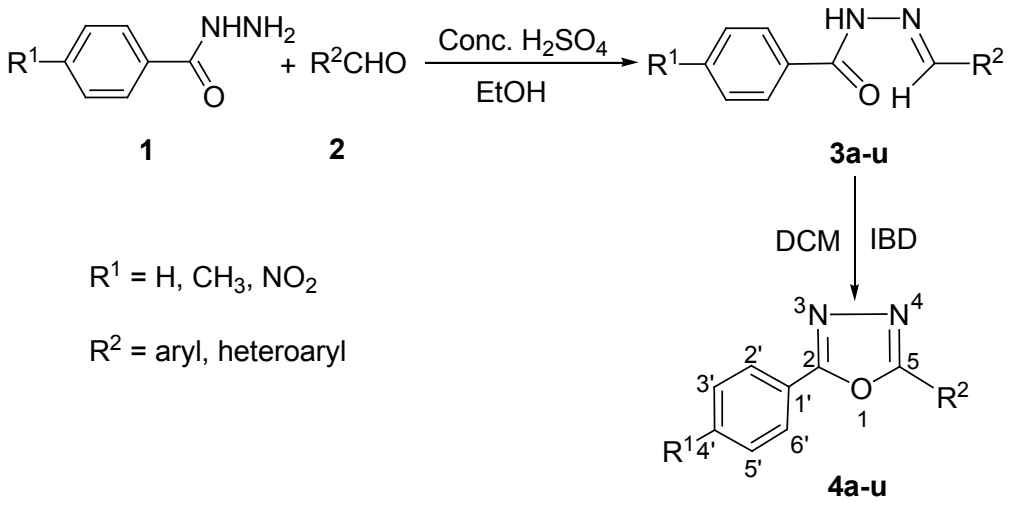
Chemistry

A variety of disubstituted-1,3,4-oxadiazoles had already been synthesized using different toxic reagents like phosphorus oxychloride [38], phosphorus pentaoxide [39] and acetic anhydride [40]. In the past few years, organic synthesis acquired various advantages such as shorter reaction time, higher regio-selectivity [41] and use of greener solvents or reagents with low toxicity profile. In this concern, organoiodine(III) reagents are well known for their non-toxic and eco-friendly behavior in organic synthesis [42,43]. Due to low toxicity and selective nature [44], they have also been extensively used for the synthesis of various heterocycles. In continuation of our interest to synthesize biological active azole derivatives, herein, we report the synthesis of some new 2,5-disubstituted-1,3,4-oxadiazoles by oxidative transformation of various newly synthesized hydrazones by iodobenzene diacetate (IBD), a hypervalent iodine (III) reagent in dichloromethane under mild reaction conditions.

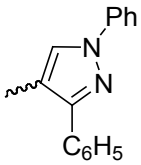
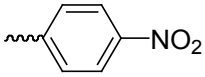
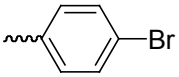
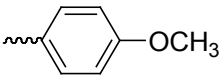
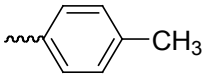
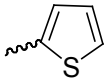
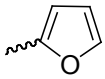
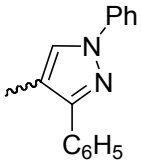
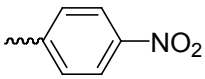
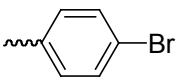
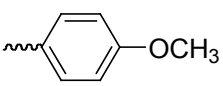
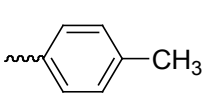
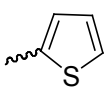
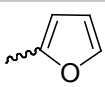
The substituted aroylhydrazones **3** were obtained by the condensation of aroylhydrazines **1** with 3-aryl-1-phenyl-1*H*-pyrazole-4-carbaldehydes/benzaldehyde derivatives **2** in a mixture of ethanol and dichloromethane in presence of catalytic amount of concentrated sulfuric acid under reflux according to a literature method which was used to synthesize some different derivatives [45, 46]. The final products **4** were obtained in 84-91 % yields with high purity *via* oxidative cyclization of **3** by treating with 1.1 equivalent of IBD under mild conditions (Scheme 1). Although some 1,3,4-oxadiazole derivatives had been prepared *via* oxidation of a few substituted hydrazones such as *N*-acyl hydrazones [47] with 1.1 equivalent of iodobenzene diacetate in dichloromethane (DCM) at room temperature, in the present investigation total 42 compounds were prepared. The compounds were characterized on the basis of their FT-IR, ^{1}H, ^{13}C NMR and mass spectral data. The FT-IR spectra of compounds **3a**-**u** showed absorption bands for –*NH* and –C=O stretching vibrations at 3427 and 1668 cm^{-1}, respectively. In ^{1}H NMR spectra, the compounds **3a**, **3h** and **3o** displayed two characteristic signals due to 5'-H of pyrazole ring and N=C**H** at δ 8.96 and 8.61, respectively. A characteristic downfield signal at δ 11.76 was appeared due to the -*NH* proton and rest of the protons exhibited multiplet in the aromatic region. The chemical shifts in ^{13}C NMR spectra of **3a**, **3h** and **3o** (considering as representative cases) at δ 140.1-144.1, 126.6-127.5 and 160.9-163.4 correspond to N=**C**H, pyrazole-5' and carbonyl carbon atoms, respectively.

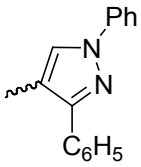
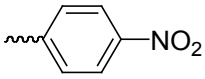
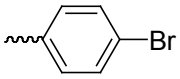
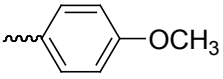
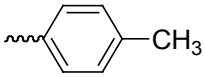
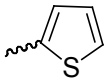
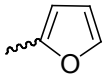
The structures of final products **4a**-**u** were established by comparing FT-IR, ^{1}H and ^{13}C NMR spectral data with those of the compounds **3a**-**u**. The FT-IR spectra of **4** were found transparent in region of -*NH* and –C=O stretching and thus confirmed the successful oxidation of **3** into **4**. Disappearance of chemical shifts at δ 8.38-8.72 (N=CH) and 11.70-12.14 (-*NH*) in ^{1}H NMR spectra of the products (**4a**-**u**) confirmed the successful conversion of aroylhydrazones into 2,5-disubstituted-1,3,4-oxadiazoles. The ^{13}C NMR spectra displayed signals at around δ 159.9, 164.0 for two oxadiazole carbons, however, signals at δ 152.0, 106.5 and 131.6 were appeared due to pyrazole ring carbons 3'', 4'' and 5'', respectively. In ^{13}C NMR spectrum, disappearance of a characteristic signal in range of δ 140.1-144.1 due to N=**C**H functionality further confirmed the formation of oxadiazoles. The physical data of all the synthesized compounds **4** is given in the Table 1.

The probable mechanism involved in synthesis of oxadiazole is given in Figure 1. The intermediate **I** is formed by the attack of hydrazone nitrogen on iodine of iodobenzene diacetate followed by elimination of acetate ion. The rate of reaction may depend upon the electron density on the carbon-a and carbon-b. Higher electro-positive character on carbon-b accelerates the attacking tendency of oxygen and the rate of reaction. Whereas, increased in positive charge on carbon-a reduces the attacking tendency of oxygen and hence retards the rate of reaction. On this basis, it is assumed that the presence of electron-withdrawing group at *para* position of phenyl ring (R^2) facilitates the rate of reaction. On the other hand, electron-releasing group at *para*



Scheme 1: Synthesis of substituted aroylhydrazones (3a-u) and oxadiazoles (4a-u).

No.	R^1	R^2	Exact Mass	m.p. (°C)		Reaction Time (min)
				a	b	
4a	H		364.13	136-138	136-137	30-35
4b			267.06	209-210	209-210	95
4c			299.99	167	167-168	30
4d			252.09	150	150-151	155
4e			236.09	148	148-149	120
4f			228.04	-----	110-111	30
4g			212.06	103	103-104	45
4h	*p*-CH_3		378.15	-----	145-146	90
4i			281.08	-----	168-169	100
4j			314.01	-----	205-206	70
4k			266.11	148-150	148-149	215
4l			250.11	179-180	179-180	155
4m			242.05	-----	126-127	150
4n			226.07	131-132	131-132	165

4o			409.12	-----	210-211	70
4p			312.05	127-129	127-128	90
4q	*p*-NO_2		344.97	-----	197-198	30
4r			297.07	-----	176-177	190
4s			281.08	-----	188-189	130
4t			273.02	-----	165-166	70
4u			257.04	-----	145-146	40

[a]Literature m.p.; [b]Observed m.p.

Table 1: Physical data of oxadiazoles (4).

position of phenyl ring (R^2) decreases rate of reaction by increasing the electron density at carbon-b.

On the other side, it is assumed that electron-withdrawing group attached to *para* position of phenyl ring (R^1) decreases the electron density on carbon-a and thus facilitates the elimination of proton attached to nitrogen but retains the attacking tendency of oxygen to carbon-b. While electron-releasing group attached to *para* position at phenyl ring decreases the electro-positive character of carbon-a and rate of elimination of proton as a result the rate of reaction gets decreased. Thus, phenyl ring having electron-releasing group attached to either carbon-a or b decreases the rate of reaction (Figure 2).

Biological Evaluation

Plasmid DNA photocleavage study

The DNA photocleavage study was performed using agarose gel electrophoresis method and results are presented in Figures 3 and 4. There was a significant decrease in intensity of DNA band in case of aroylhydrazones and oxadiazoles as compared to the control DNA.

In case of substituted aroylhydrazones **3a-c**, **3h**, **3j**, **3o-s** (lane 2-4, 9, 11 and 17-21), decreased intensity of plasmid DNA as compared to control (lane 1) indicated the cleavage of DNA forms. In lane 11, 17-21 the compounds **3j** and **3o-s** were responsible for a complete fragmentation of supercoiled (Form I) into open circular (Form II) and linear (Form III) DNA. The intensity of Form III (linear form) was found to be increased in case of compounds **3a-c**, **3h**, **3j** (lane 2-4, 9 and 11) while Form I (supercoiled DNA) was decreased. Moreover, an appearance of the Form III in between Form I and II was observed due to nicking of super coiled DNA. Aroylhydrazones were found to be more active cleaving agent causing fragmentation of plasmid DNA into linear (Form III) DNA.

Unsymmetrical oxadiazoles derivatives (**4**) also exhibited efficient DNA cleavage property as presented in Figure 4. On irradiation with UV light, the compounds **4a-b**, **4d-h**, **4s** (lane 2-3, 5-9 and 21) were found to show cleavage of supercoiled (Form I) into open circular (Form II) DNA. In lane 12, the compound **4k** containing methoxy group at *para*-position of phenyl ring (R^2) was found to be the most effective cleaving agent which was particularly responsible for the complete fragmentation of plasmid DNA. It was found that compounds bearing electron-releasing substituent at *para*-position of phenyl ring (R^2) increase the cleavage potential of oxadiazole compounds. Moreover, higher intensity of the Form II (open circular DNA) was observed in case of aroylhydrazones containing pyrazole moiety. Furthermore, *para* substitution on phenyl ring also increases the cleavage action of the aroylhydrazone compounds on the plasmid DNA.

Conclusion

In the present investigation, we reported the synthesis of total 21 unsymmetrical 1,3,4-oxadiazole derivatives *via* oxidative cyclization of their corresponding 21 aroylhydrazones using IBD under solvent conditions and thus explored potential utility of organoiodine(III) reagents on a variety of hydrazone derivatives bearing different electron-withdrawing as well as electron-donating group substituents. It has been observed that phenyl ring (R^1) having electron-releasing group at *para*-position attached to carbon-a or b decreases the rate of reaction. Structures of the synthesized compounds were established on the basis of results obtained from their NMR spectral data. In DNA photocleavage study, it has been observed that compounds **3a-u** and **4a-u** have shown moderate DNA photocleavage activity. However, **3a-u**, in particular, exhibited more photocleavage potential as compared to **4a-u**.

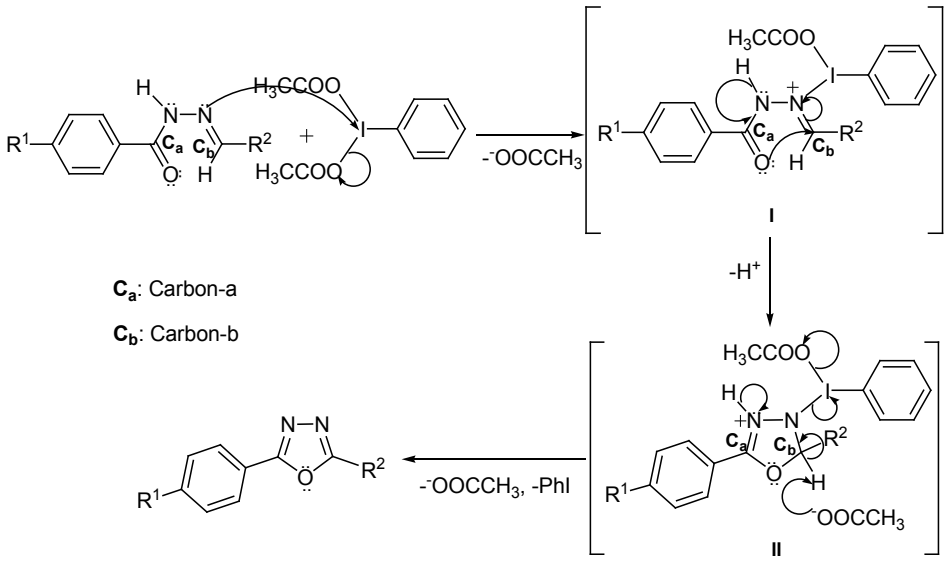


Figure 1: Proposed mechanism for the synthesis of oxadiazoles 4.

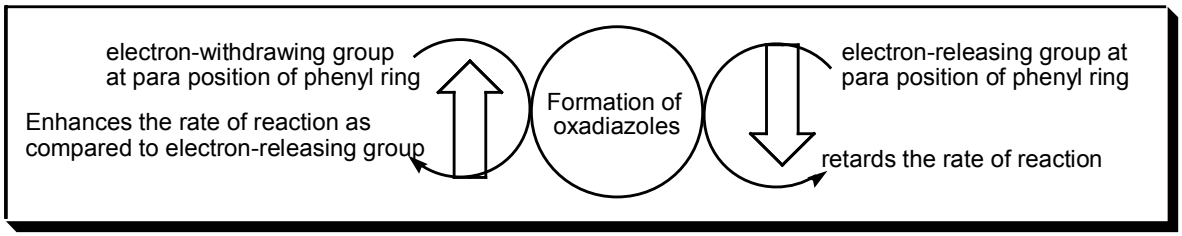
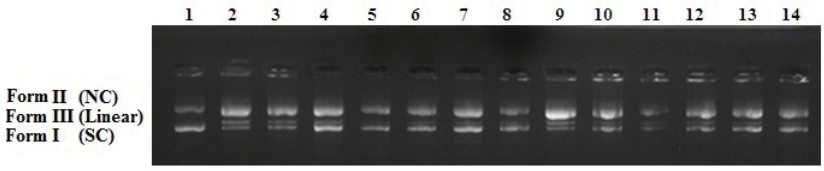
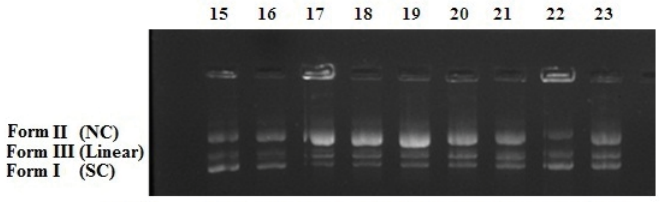


Figure 2: Effect of substitution on the formation of oxadiazoles.



%SC	67.0	21.8	25.5	41.0	35.4	43.6	40.0	42.4	16.8	30.7	29.0	29.8	29.9	38.7
%NC	32.3	57.3	53.5	41.2	47.6	42.6	46.3	38.9	60.6	56.7	42.8	53.5	54.0	49.0
%Linear	0.0	20.8	20.8	17.6	12.9	13.6	13.6	18.6	22.4	12.5	28.1	16.5	16.0	12.2



%SC	50.8	43.9	18.6	19.2	16.7	19.2	19.1	52.0	38.0
%NC	35.6	34.5	53.1	56.1	64.1	56.7	51.0	20.8	36.1
%Linear	13.5	21.9	28.2	24.5	19.0	23.9	29.7	27.1	25.7

Figure 3: Plasmid DNA photocleavage of hydrazones **3a-u**. Lane 1: Control plasmid DNA+UV+DMSO, Lane 2: DNA+40 µg **3a**, Lane 3: DNA+40 µg **3b**, Lane 4: DNA+40 µg **3c**, Lane 5: DNA+40 µg **3d**, Lane 6: DNA+40 µg **3e**, Lane 7: DNA+40 µg **3f**, Lane 8: DNA+40 µg **3g**, Lane 9: DNA+40 µg **3h**, Lane 10: DNA+40 µg **3i**, Lane 11: DNA+40 µg **3j**, Lane 12: DNA+40 µg **3k**, Lane 13: DNA+40 µg **3l**, Lane 14: DNA+40 µg **3m**, Lane 15: Control plasmid DNA+UV+DMSO, Lane 16: DNA+40 µg **3n**, Lane 17: DNA+40 µg **3o**, Lane 18: DNA+40 µg **3p**, Lane 19: DNA+40 µg **3q**, Lane 20: DNA+40 µg **3r**, Lane 21: DNA+40 µg **3s,** Lane 22: DNA+40 µg **3t,** Lane 23: DNA+40 µg **3u**, respectively. Values under each lane represent percentage contribution of Form I and II.

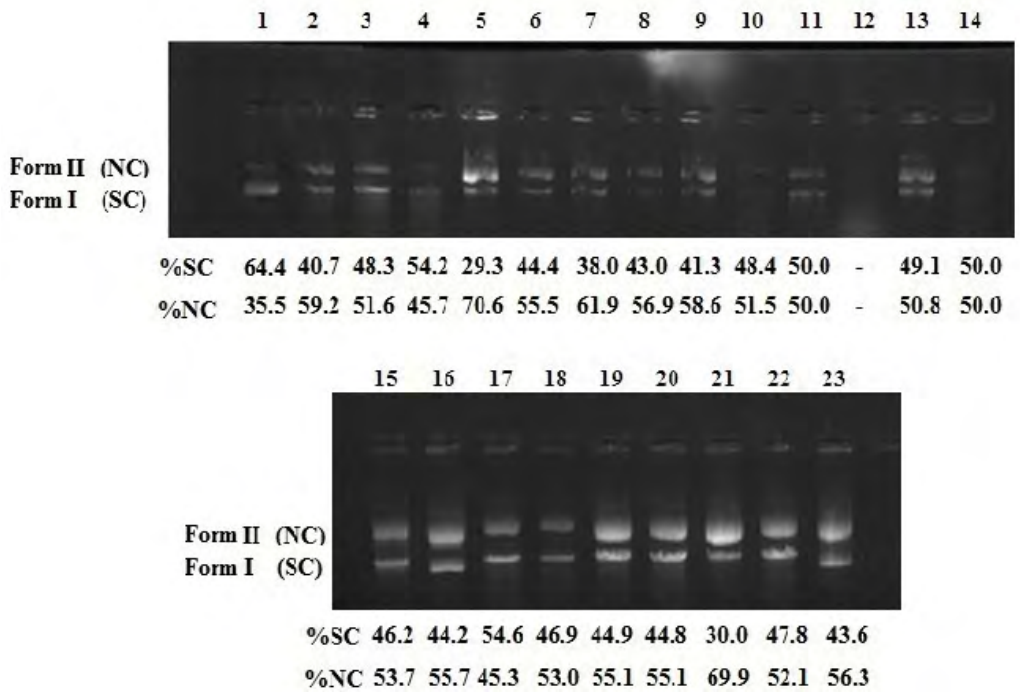


Figure 4: Plasmid DNA photocleavage of oxadiazoles **4a-u**. Lane 1: Control plasmid DNA+UV+DMSO, Lane 2: DNA+40 µg **4a**, Lane 3: DNA+40 µg **4b**, Lane 4: DNA+40 µg **4c**, Lane 5: DNA+40 µg **4d**, Lane 6: DNA+40 µg **4e**, Lane 7: DNA+40 µg **4f**, Lane 8: DNA+40 µg **4g**, Lane 9: DNA+40 µg **4h**, Lane 10: DNA+40 µg **4i**, Lane 11: DNA+40 µg **4j**, Lane 12: DNA+40 µg **4k**, Lane 13: DNA+40 µg **4l**, Lane 14: DNA+40 µg **4m**, Lane 15: Control plasmid DNA+UV+DMSO, Lane 16: DNA+40 µg **4n**, Lane 17: DNA+40 µg **4o**, Lane 18: DNA+40 µg **4p**, Lane 19: DNA+40 µg **4q**, Lane 20: DNA+40 µg **4r**, Lane 21: DNA+40 µg **4s,** Lane 22: DNA+40 µg **4t,** Lane 23: DNA+40 µg **4u**, respectively. Values under each lane represent percentage contribution of Form I and II.

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