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Synthesis & Biological Evaluation of 1, 3, 4- Oxadiazoles as Anticancer Agents

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ABSTRACT: In the present study, novel derivatives of 1,3,4-oxadiazoles were synthesised using optimised chemical reaction methods. The compounds were also screened for their antioxidant and anticancer activity. Four compounds (**4**, **5b**, **5c** and **5i**) were found active against HepG2 cells and two compounds (**5d** and **5h**) against Hep 2 cells. Thus promising molecules were identified for their anticancer activity.

KEYWORDS: 1,3,4-Oxadiazoles; Anticancer Activity; Antioxidant Activity.

INTRODUCTION

The increase in diseases throughout the world especially economically backward countries is the major cause for deaths [1]. The cancer causing agents are increasing among under developed countries thus implying deaths due to cancer. Thus the need for the discovery of new drugs is increasing day by day. Many studies were carried out on various chemical families to discover new chemical entities. In the recent years, 1,3,4-oxadiazoles were reported to have a wide range of activities like antibacterial, antifungal, antitubercular and anticancer etc. In the present study we succeeded in synthesizing novel derivatives of 1,3,4-oxadiazoles and screened for their antimicrobial, anticancer and antioxidant activities.

MATERIALS & METHODS

Chemistry

Solvents and chemicals were purchased from Aldrich, Spectrochem Pvt. Ltd., and HI-MEDIA Pvt. Ltd. and used without further purification. The reactions were monitored by thin layer chromatography (TLC) carried out on 0.25mm Merck silica gel plates (silica gel 60 F254). Purification of synthesized compounds was performed by column chromatographic technique using silica gel 100-200 mesh (Merck). Melting points were determined using laboratory melting point apparatus (Toshniwal P. Ltd.) and are uncorrected. Chemical tests were performed to confirm the presence of functional groups and required elements.

UV absorption was detected in UV-2450 spectrophotometer (Shimadzu) to find the λ max of the compounds. IR spectra of the synthesized compounds were recorded on FT-IRAffinity-1 (Shimadzu) IR Spectrometer. Mass spectra were recorded on GC-MS-QP5050*A* (Shimadzu). NMR spectra were recorded on Bruker 400 MHz spectrometer using DMSO-*d*6 as the solvent.

General Procedure for Synthesis

Synthesis of aryl acid hydrazides (3): Aromatic acid hydrazides (**3**) were prepared by reacting (1eq.) aromatic acid ester (**2**) with (3 eq.) hydrazine hydrate in presence of methanol as solvent. The reaction mixture was refluxed for 6h. The reaction was monitored using TLC with hexane and ethyl acetate (8:2) as mobile phase. After reaction completed, the reaction mixture was concentrated using rotary evaporator. The concentrated product obtained was added to ice and the precipitated product was filtered and dried.

Preparation of 5-Biphenyl-4-yl-1,3,4 oxadiazole-2-thiol (4): A mixture of compound **3** (0.0047 mole), Potassium hydroxide (0.0047 mole) and CS_2 (0.0094 mole) were refluxed in 25mL of methanol for 12 hours. The reaction was monitored using TLC with hexane and ethyl acetate (8:2) as mobile phase. After reaction completed, the reaction mixture was concentrated using rotary evaporator. The reaction mixture was acidified with aq. HCl (10% v/v) to pH 7. The precipitated product was filtered, dried and recrystallized using ethanol.

5-Biphenyl-4-yl-[1,3,4]oxadiazole-2-thiol (**4**): Yield 83 %; mp 191 ^oC; FTIR (KBr, cm^{-1}): 3089 (C-H aromatic str.), 2675 (S-H str.), 1616 (C=N str.), 1176 (C-O str.). ^1H NMR (400 MHz, DMSO-d_6): δ 7.81 (d, *J*=8 Hz, 2H), 7.63 (d, *J*=8.4 Hz, 2H), 7.51 (d, *J*=7.2 Hz, 2H), 7.35 (t, *J*=7.6 Hz, 2H), 7.26 (t, *J*=7.4 Hz, 1H), 4.78 (s, 1H). GCMS (EI, *m/z*): 256 $[M+2]^+$, 254$[M]^+$, 222 $[M-SH]^+$.

Preparation of 2,5-diaryl- [1,3,4]oxadiazole (5a-i): To the aromatic hydrazide (1eq.) (**3**), aromatic acids (1eq.) (**1**) were added in presence of Phosphorous oxy chloride (5mL) and refluxed for 2-3h to form various 2,5-di-substituted oxadiazoles. The reaction was monitored using TLC with hexane and ethyl acetate (8:2) as mobile phase. After reaction completed, the reaction mixture was concentrated using rotary evaporator. Crude product was washed with ethanol and recrystallized using ethanol.

4-(5-Biphenyl-4-yl- [1,3,4] oxadiazol-2-yl)-pyridine (**5a**): Yield 58 %; mp 180 ^oC; FTIR (KBr, cm^{-1}): 3163 (C-H, str.), 3059 (C-H aromatic str.), 1681 (C=N str.), 1192 (C-O str.); ^1H NMR (400 MHz, DMSO-d_6): δ 7.76 (d, *J*=8 Hz, 2H), 7.55 (m, 4H), 7.51 (d, *J*=7.2 Hz, 2H), 7.49 (d, *J*=7.2 Hz, 2H), 7.26 (d, *J*=7.4 Hz, 2H), 7.24 (d, *J*=7.4 Hz, 2H); GCMS (EI, *m/z*): 299 $[M]^+$, 271 $[C_{19}H_{13}NO]^+$, 153 $[C_{12}H_{19}]^+$, 106 $[C_5H_2N_2O]^+$, 78 $[C_5H_4]^+$ **.**

2-Biphenyl-4-yl-5-(3,4-difluoro-phenyl)-[1,3,4]oxadiazole (**5b**): Yield 46 %; mp 150 ^oC; FTIR (KBr, cm^{-1}): 3232 (Aromatic str.), 3068 (C-H Aromatic str.), 1614 (C=N str.), 1193 (C-O str.) , 590 (C-F bending.); ^1H NMR (400 MHz, DMSO-d_6): δ 7.86 (m, 5H), 7.55 (m, 4H), 7.53 (d, *J*=7.2 Hz, 2H), 7.50 (d, *J*=7.2 Hz, 2H), 7.26 (d, *J*=7.4 Hz, 2H), 7.24 (s, 1H); GCMS (EI, *m/z*): 334 $[M]^+$, 315 $[M-F]^+$, 181$[C_8H_3F_2N_2O]^+$, 153 $[C_{12}H_9]$, 113 $[C_6H_3F_2]^+$, 76 $[C_6H_4]^+$.

2-Biphenyl-4-yl-5-(4-methoxy-phenyl)-[1,3,4]oxadiazole (**5c**): Yield 60 %; mp 120 ^oC; FTIR (KBr, cm^{-1}): 3064(Aromatic C-H str.), 2926 (Sp^3 C-H str.), 1600 (C=N str.), 1257(C-O str.), 1124 (C-O-C str.); ^1H NMR (400 MHz, DMSO-d_6): δ 7.54 (m, 4H), 7.48-7.22 (m, 5H), 7.37 (d, *J*=6.2Hz, 2H), 6.83 (d, *J*=6.8Hz, 2H), 3.71 (s, 3H, -OCH_3); GCMS (EI, *m/z*): 328 $[M]^+$, 299 $[M- OCH_3]^+$.

Acetic acid 4-(5-biphenyl-4-yl-[1,3,4]oxadiazol-2-yl)-phenyl ester (**5d**): Yield 59 %; mp 182 ^oC; FTIR (KBr, cm^{-1}): 3030(Aromatic C-H str.), 2841 (Sp^3 C-H str.), 1680(C=O str.), 1610 (C=N str.), 1263 (C-O str.). ^1H NMR (400 MHz, DMSO-d_6): δ 7.54 (m, 4H), 7.48-7.32 (m, 5H), 7.45 (d, *J*=7.2Hz, 2H), 7.13 (d, *J*=7.2Hz, 2H), 2.08 (s, 3H, -CH_3); GCMS (EI, *m/z*): 356 $[M]^+$, 300 $[M-55]^+$.

2-(3,4-Difluoro-phenyl)-5-phenyl-[1,3,4]oxadiazole (**5e**): Yield 54 %; mp 135 ^oC; FTIR (KBr, cm^{-1}): 3230 (Aromatic C-H str.), 3111 (C-H str.), 1595 (C=N str.), 1253(C-O str.), 574 (C-F str.); ^1H NMR (400 MHz, DMSO-d_6): δ 7.57-7.48 (m, 5H), 7.23 (d, *J*=7.2Hz, 1H), 7.17 (s, 1H), 7.01 (d, *J*=7Hz, 1H); GCMS (EI, *m/z*): 258 $[M]^+$.

2-(3,4-Difluoro-phenyl)-5-(4-methoxy)phenyl-[1,3,4]oxadiazole (**5f**): Yield 62 %; mp 125 ^oC; FTIR (KBr, cm^{-1}): 3076 (Aromatic C-H str.), 2953 (Sp^3 C-H str.), 1612 (C=N str.), 1259 (C-O str.), 516 (C-F str.); ^1H NMR (400 MHz, DMSO-d_6): δ 7.37 (d, *J*=6.8Hz, 1H), 7.23 (d, *J*=6Hz, 1H), 7.17 (s, 1H), 7.01 (d, *J*=6Hz, 1H), 6.83 (d, *J*=6.8Hz, 1H), 3.73 (s, 3H, -OCH_3); GCMS (EI, *m/z*): 288 $[M]^+$. 260 $[M- OCH_3]^+$.

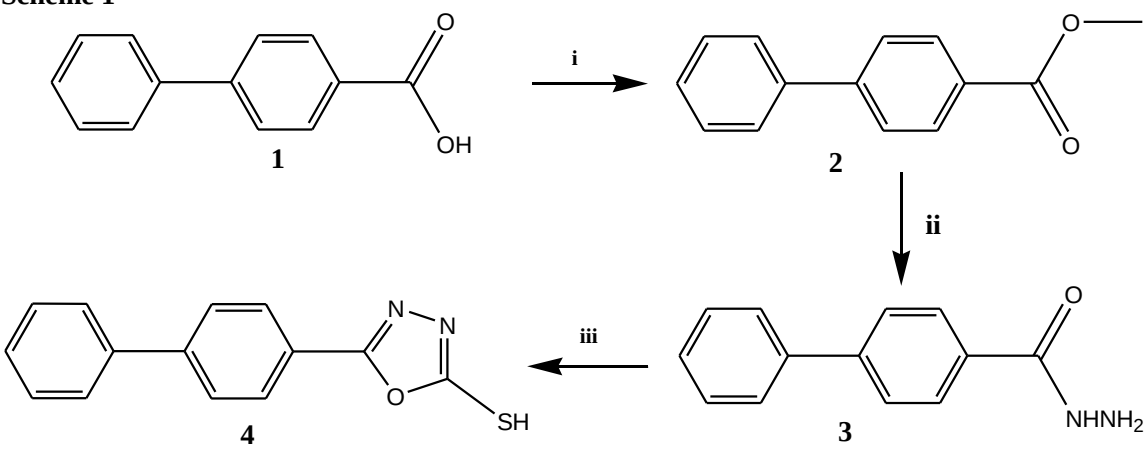
5-Chloro-2-[5-(2,4-di chloro-phenyl)-[1,3,4] oxadiazol-2-yl] phenylamine (**5g**): Yield 52 %; mp 143 ^oC; FTIR (KBr, cm^{-1}): 3256 (C-H str.), 2999 (Ar C-C str.), 1634 (C=N str.), 1200 (C-O str.), 700 (C-Cl str.); GCMS (EI, *m/z*): 338 $[M]^+$, 340 $[M+2]^+$.

5-Chloro-2-[5-(4-fluoro-phenyl)-[1,3,4]oxadiazol-2-yl]-phenylamine (**5h**): Yield 48 %; mp 130 ^oC; FTIR (KBr, cm^{-1}): 3331 (Ar. C-H str.), 2856 (Ar C-C str.), 1634 (C=N str.), 1212 (C-O str.), 656 (C-F str.); GCMS (EI, *m/z*): 289 $[M]^+$.

5-Chloro-2-[5-(4-chloro-phenyl)-[1,3,4]oxadiazol-2-yl]-phenylamine (**5i**): Yield 58 %; mp 136 ^oC; FTIR (KBr, cm^{-1}): 3352(N- Hstr.),3074 (Aromatic C-H str), 1602(C=N str.), 1236 (C-O str.); ^1H NMR (400 MHz, DMSO-d_6): δ 7.42 (d, *J*=6Hz, 2H), 7.33 (d, *J*=6.2Hz, 2H), 7.17 (d, *J*=7.2Hz, 1H), 6.69 (d, *J*=7.2Hz, 1H), 6.53 (s, 1H); GCMS (EI, *m/z*): 305 $[M]^+$.

Synthetic Schemes

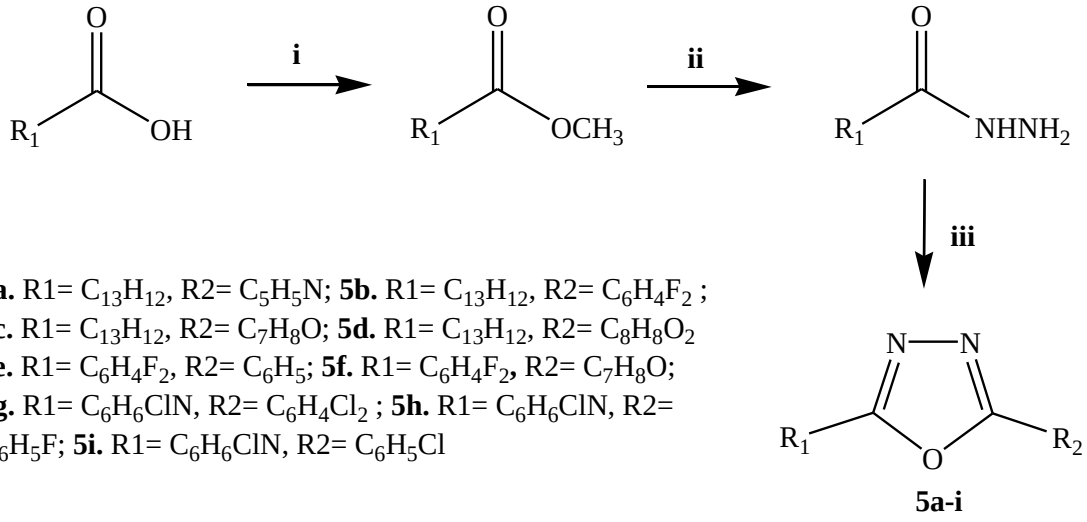
Scheme 1[a]



1 **2** **3** **4**

[a]**Reagents and Conditions**: **(i)** MeOH, H_2SO_4 (Cat.); **(ii)** $NH_2NH_2.H_2O$, MeOH; **(iii)** CS_2, Et_3N, MeOH.

Scheme 2[a]



5a. R1= $C_{13}H_{12}$, R2= C_5H_5N; **5b.** R1= $C_{13}H_{12}$, R2= $C_6H_4F_2$;
5c. R1= $C_{13}H_{12}$, R2= C_7H_8O; **5d.** R1= $C_{13}H_{12}$, R2= $C_8H_8O_2$
5e. R1= $C_6H_4F_2$, R2= C_6H_5; **5f.** R1= $C_6H_4F_2$, R2= C_7H_8O;
5g. R1= C_6H_6ClN, R2= $C_6H_4Cl_2$; **5h.** R1= C_6H_6ClN, R2= C_6H_5F; **5i.** R1= C_6H_6ClN, R2= C_6H_5Cl

5a-i

[a]**Reagents and Conditions: (i)** MeOH, H_2SO_4 (Cat.); **(ii)** NH_2NH_2. H_2O, MeOH; **(iii)** $POCl_3$, R_2COOH

Biological Evaluation

***In vitro* antioxidant study**

Diphenyl Picryl hydrazyl solution (DPPH, 200µM): 3.96 mg of DPPH was accurately weighed and dissolved in 50 mL of DMSO. A stock solution of 1000 µg/ml of synthesized compounds were prepared in DMSO. These solutions were serially diluted with DMSO to get required concentrations. The assay was carried out in a 96 well micro titre plate. To 100 µL of DPPH solution, 100 µL of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentrations of the test and standard solutions used were 1000 µg/ml to 7.8 µg/ml. The plates were incubated at 37 ^{0}C for 25 min and the absorbance of each solution was measured at 540 nm, using ELISA micro titre plate reader. The experiment was performed in triplicate and % scavenging activity was calculated using formula given below. IC_{50} (Inhibitory Concentration) is the concentration of the sample required to scavenge 50% of DPPH free radicals and it was calculated from the graph, % scavenging Vs concentration.

$$\% \text{ Scavenging} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

***In vitro* cytotoxicity by MTT assay**

A 96-well flat bottom tissue culture plate was seeded with $1x10^4$cells in 0.1 ml of MEM and DMEM medium supplemented with 10% FBS and allowed to attach for 24hrs. Test compounds were prepared just prior to the experiment in 0.5% DMSO and serially diluted with medium to get the working stock of 500 µg/ml, 250 µg/ml, 125 µg/ml solution, 62.5 µg/ml. After 24 h of incubation cells were treated with 20 µl of test solutions from respective top stocks and 80 µl of fresh medium was added and the cells were incubated for 48 hrs. The cells in the control group received only the medium containing 0.5% DMSO. Each treatment was performed in triplicates. After the treatment, drug containing media was removed and washed with 200µl of PBS. To each well of the 96 well plate, 100µl of MTT reagent (Stock: 1mg/ml in PBS) was added and incubated for 4h at 37ºC. After 4hrs of incubation the plate was inverted on tissue paper to remove the MTT reagent. To the wells, 100µl of 100% DMSO was added to each well. The optical density (O.D) was measured by an ELISA plate reader at 540 nm. Percentage cytotoxicity of each extract was calculated by using this formula:

$$\% \text{ Scavenging} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

Results were expressed as Mean ± SEM percentage O.D values (proportional to cell survival) were plotted against the tested drug concentrations.

RESULTS & DISCUSSION

Table 1: Free radical scavenging activity using DPPH.

S. No.	Compound	R*f* [a]	λ max[b]
1	4	0.76	237
2	5a	0.68	206
3	5b	0.53	302
4	5c	0.79	306
5	5d	0.89	278
6	5e	0.38	243
7	5f	0.64	297
8	5g	0.37	237
9	5h	0.73	227
10	5i	0.64	238

[a] Mobile phase: Hexane: Ethyl acetate (8:2), [b] Solvent: Methanol

Table 2: Free radical scavenging activity using DPPH.

S. No.	Compound	IC_{50} (µg/ml)
1	4	163.71
2	5a	>500
3	5b	>500
4	5c	>500
5	5d	469.32
6	5e	>500
7	5f	451.83
8	5g	>500
9	5h	497.14
10	5i	>500
11	Ascorbic acid	13.87

Chemistry

The acid hydrazides (**3**) were prepared from respective esters (**2**) using hydrazine hydrate without any impurities. The acid hydrazide on reacting with carbon disulphide in presence of triethylamine gave oxadiazole-3-thiol (**4**). The prepared acid hydrazides reacted with various aromatic acids in presence of $POCl_3$ giving respective oxadiazoles (**5a-i**). The synthesised compounds were purified by column chromatography. The structure of the synthesised compounds was characterised using UV, FTIR, GCMS and ^{1}HNMR spectroscopic techniques. Physical characterisation was determined using melting points and R*f* values.

Biological activity

Antioxidant Activity

Among the compounds screened for anti-oxidant activity, compound **4** (IC_{50} =163.71µg/mL) has shown moderate free radical scavenging activity. Most of the compounds screened were unable to scavenge the DPPH free radicals. Insignificant anti-oxidant activity of the synthesized compounds may be attributed to the absence of ideal electronic configuration in the scaffold to reduce the free radical by dissociation.

***In vitro* anticancer activity**

All the synthesized compounds were screened for cytotoxicity against HepG2 (hepatic human carcinoma cells) and Hep-2 (human epidermoid cancer cells or HeLa) cell lines using MTT. Cisplatin is used as standard. Compounds **4** (IC_{50} =20.26 µg/mL), **5b** (IC_{50} =41.72µg/mL), **5c** (IC_{50} =27.97µg/mL) and **5i** (IC_{50} =49.33µg/mL) showed promising activity against HepG2 cells. Compounds **5d** (IC_{50} =49.83µg/mL) and **5h** (IC_{50} =30.09 µg/mL) showed promising activity against Hep2 cells.

Table 3: *In Vitro* anticancer activity against Hep-2 cells using MTT.

S. No.	Compound	IC_{50} (µg/ml)
1	4	307.55
2	5a	228.43
3	5b	117.33
4	5c	187.83
5	5d	49.83
6	5e	262.08
7	5f	225.43
8	5g	87.93
9	5h	30.09
10	5i	69.06
11	Cisplatin	4.56

Table 4: *In Vitro* anticancer activity against HepG2 cells using MTT.

S. No.	Compound	IC_{50} (µg/ml)
1	4	20.26
2	5a	164.44
3	5b	41.72
4	5c	27.97
5	5d	197.91
6	5e	74.18
7	5f	182.85
8	5g	261.68
9	5h	126.27
10	5i	49.33
11	Cisplatin	3.69

CONCLUSION

In the present study, the novel derivatives of 1,3,4-oxadiazoles were found to be active against cancer cell lines indicating their promising anticancer property. Thus there is need to explore this nucleus further to improve their anticancer activity by structural designing. Although the compounds were found inactive as antimicrobial agents, it cannot be concluded with this information in exploring the activity further. This nucleus would be promising for future studies to explore.

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