SYNTHESIS AND BIOLOGICAL EVALUATION OF 1,3,4 OXADIAZOLE/ OXADIAZOLINE DERIVATIVES

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ABSTRACT: ***The Main Aim of the study is to synthesise and Biological Evaluation Of 1,3,4-Oxadiazole/Oxadiazoline Derivatives. The present study concluded the beneficial effect of synthesized novel 1, 3, 4-Oxadiazole derivatives for its antibacterial and anticonvulsant action. This study confirms the rational basis for its use in synthesized 1, 3, 4-Oxadiazole and its derivatives for the treatment of bacterial infection and anticonvulsant activity in patients. Further pharmacological investigations are under way to characterize active novel 1, 3, 4-Oxadiazole and to establish exact mechanism of anticonvulsant action, which may have fewer side effects. This work, we believe, will be useful for further inflammation research works. Additional pharmacological studies should be done to clarify the exact mechanism of anticonvulsant efficacy and to define active novel 1, 3, 4-Oxadiazole, which could have fewer side effects. We hope that our work will help move the field of inflammation research forward.***
Key Words: 1,3,4- Oxadiazole/Oxadiazoline Derivatives, anticonvulsant efficacy

I. INTRODUCTION

Heterocyclic organic compounds are major class of cyclic compounds having one element at least other than carbon, such as oxygen, sulfur or nitrogen. Heterocyclic compounds involve almost all aspects of medicinal chemistry, modern organic chemistry and biochemistry because of more than half of the known compounds as heterocycles.

Heterocyclic compounds are widely spread as natural products like alkaloids, and terpenes and playing an important role in biological process and human life. Many antibiotics including penicillin, ciprofloxacin, and streptomycin and natural pigments such as hemoglobin, indigo, and anthocyanin have heterocyclic ring systems. Heterocyclic compounds possess a stable ring structure, which does not involve readily hydrolysis or de-polymerization. These are simple forms of either nonaromatic or aromatic rings. Aliphatic compounds are the cyclic ring of amines, ethers, and thioethers and their properties influence by ring system. The three and four-member heterocyclic rings are more reactive and strained than five or six-member rings. Synthesized heterocycles are designed by organic researcher used for pharmaceutical industries. A large number of marketed products are pharmaceutical drugs possess nitrogen-containing heterocyclic ring system such as Raltegravir, Zibotentan. Heterocyclic compounds give promising lead structures for design novel drugs.

Heterocyclic chemistry includes a large class of compounds, **azoles** being one among them. Azoles are five-membered heterocyclic compounds containing nitrogen atom and at least one other non-carbon atom of either nitrogen, sulfur, or oxygen [1]. It includes the following heterocyclic rings.

II. MATERIALS AND METHODS

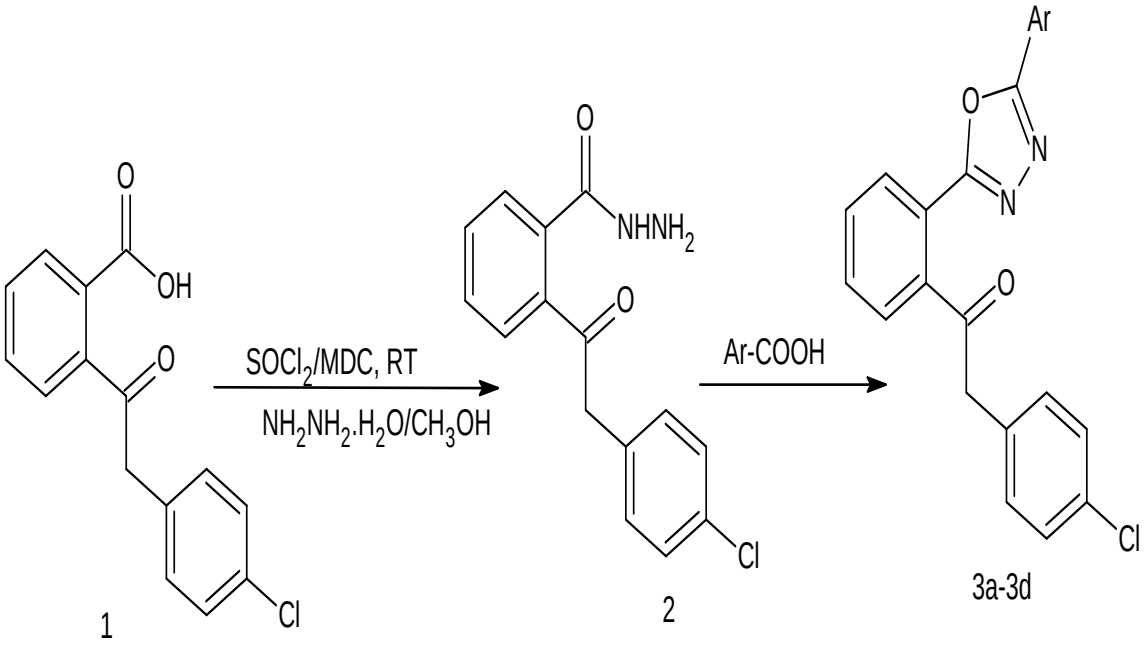
All melting points were determined using open capillaries in a liquid paraffin bath and were uncorrected. The completion of the reaction was monitored frequently by thin layer chromatography (TLC) using silica gel-G as absorbent and Toluene: Ethyl acetate (75:25) was employed as mobile phase. The visualization of TLC was accomplished by UV light and Iodine. IR spectra (KBr pallet) were recorded on FT-IR, Perkin Elmer RX1 spectrophotometer, and NMR spectra on BRUKER AVANCE II (400 MHz) using TMS as internal standard (chemical shifts in δ ppm). The mass spectrums of compounds were recorded on Waters, Q-TOF, Micro mass LC-MS spectrophotometer.

Experimental

Synthesis of 2-[2-(4'-Chloro-phenyl)-acetyl]-benzoic acid hydrazide (2):

Compound **1** (2.37 g, 0.01 mol) and SOCl2 (2.17g 0.03 mol), MDC in 100 mL round bottom flask were stirred for 30 mins at room temperature to obtained acid chloride of compound **1**. Excess of reagent and solvents were

distilled off. Acid chloride and excess of hydrazine hydrate in dry methanol were transferred in 100 mL round bottom flask and subjected for Microwave irradiation for 4-5 mins. After completion of the reaction (monitored by TLC), the reaction mixture was cooled, poured on crushed ice, on neutralization of the contents with sodium bicarbonate solution (20%) a solid mass separated out, which was filtered, washed with water, dried and recrystallized from methanol to get **2.** Yield 75 %, m.p. 224-226°C



2-[(4-chlorophenyl)acetyl]benzoic acid 2-[(4-chlorophenyl)acetyl]benzohydrazide

Ar 3a: Phenyl,3b: 4-methylphenyl,3c: 4-nitrophenyl,3d: 4-hydroxyphenyl

Synthesis of 2-(4'-Chloro-phenyl)-1-{2-[5"-(substituted-phenyl)-[1, 3, 4] oxadiazol-2-yl]-phenyl} - ethanone (3 a-d):

Compound **2** (14.425g, 0.01 mol) was dissolved in phosphorous oxychloride (50mL) and to it was added substituted aromatic acid (0.05 mol). The reaction mixture, after refluxing for 6 hrs, was cooled to room temperature and poured onto crushed ice. The product was isolated in a similar manner as described above to obtain the desired product.

Under similar conditions **3a-d** were prepared.

III. BIOLOGICAL EVALUATION

Evaluation of Acute Toxicity Studies [2-4]

The study was performed based on Miller and Tainer method. The method is used to determine LD50 value of the synthesized compounds. Male Albino mice (25-30 g) were used for this study. The animals were divided into 15 groups of 6 mice each. The synthesized compounds were administered orally. The animals were observed for 2h for death because of acute toxicity. The LD50 value of the synthesized compounds found to be 200mg/kg. The doses of test compounds were fixed based on their acute toxicity.

Anticonvulsant Activity [5]

Anticonvulsant activity was carried out by using maximal electro shock (MES) induced convulsion method. Albino mice of either sex (25-30g) were used for the study and divide to 14 groups of 6 mices each. They were given electrical shock through corneal electrodes of 150mA for 0.2 sec by using electro convulsiometer. Group I were treated with 0.5% tween 80 suspension and served as a control. Group II were treated with phenytoin (25mg/kg) serve as standard. Group III-VII were treated with synthesized 3a,3b,3c and 3d compounds (20 mg/kg) respectively. After 30 min, seizure induction onset time of tonic flexion, extension and clonic, phase was noted. The protective index was observed as reduction time of tonic extensor phase and all the data was observed as reduction time of tonic extensor phase and all the data (Mean±SEM) were analyzed statistically by students "t" test and tabulated in table No.1.

Antibacterial Activity:

Preparation of compound concentrations

One mg/ml of synthesized compounds powder (1:10 ratios) was dissolved in dimethyl sulfoxide (99%). The

resulting solution was kept at -18℃ in sterile test tubes.

Antibacterial activity

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)/ minimum fungicidal concentration (MFC) of the synthesized compounds were evaluated using agar well diffusion method according to the Clinical & Laboratory Standards Institute standards**[6].**

Preparation of bacterial and fungal suspensions

The lipophilic ampoules containing S. aureus, S. epidermidis, A. baumannii and K. pneumoniae were transferred to nutrient broth and incubated for 24 hours at 37 °C. A. flavus and A. fumigatus were transferred to sabouraud dextrose broth and incubated for 24 hours at 37 °C. Using a sampler, 1 ml from 24-hour culture of microbial suspension was transferred to a tube containing sterile nutrient broth to reach turbidity of the microbial suspension equal to half McFarland standard (1.5 × 109 CFU/ml).

Agar well diffusion method

To perform this experiment, wells of 5 mm in diameter were created by a sterile pipette in agar media containing bacterial or fungal suspension. The wells were then filled with the synthesized compounds (4a-4d). Ciprofloxacin and fluconazole were used as the positive controls. The plates were incubated at 37 °C for 24 hours. The experiment was performed in triplicate.

Broth dilution method

The antibacterial and antifungal activity of the compounds was evaluated by broth dilution method. First, 10 µl of inoculums containing 1.5×10-9C.F.U/ml of tested microorganism was added to sterile test tubes. Different concentrations (1.95-1000 µg/ml) of the synthesized compounds were added to the test tubes. Lowest concentration of the compounds that inhibited growth of bacteria was recorded as the MIC. Lowest concentration that reduced the viability of bacteria and fungi by ≥99.9% was recorded as the MBC and MFC, respectively.

IV. RESULT AND DISCUSSION

Physicochemical properties

Table 1: Physical data of 2-(4'-Chloro-phenyl)-1-{2-[5"-(substituted phenyl)-[1, 3, 4] oxadiazol-2-yl]-phenyl}- ethanone

Compound	Ar	Mf	MW	MP	% yield
3a	Phenyl	C22H15O2N2Cl	374.5	190	58
3b	4-methylphenyl	C23H17O2N2C	388.5	138	63
3c	4-nitrophenyl	C22H15O3N3Cl	419.5	180	62
3d	4-hydroxyphenyl	C22H15O3N2Cl	390.5	300	63

Analysis of compounds

Compound 3a 2-(4'-Chloro-phenyl)-1-[2-(5"-phenyl-[1, 3, 4] oxadiazol-2-yl) phenyl]-ethanone

White compound obtained was found to have the molecular composition $C_{22}H_{15}O_2N_2Cl$ showed the following results on elemental analysis.

Elemental analysis of the compound (3a)

Molecular formula	Elemental analysis					
	Calculate			Found		
	C	H	N	C	H	N
$C_{22}H_{15}O_2N_2Cl$	70.49	4.01	7.48	70.65	4.23	7.96

Spectral Characteristic data

IR (cm^{-1}) (Fig.5.1): 3040(CH), 2984, 1675 (C=O), 1238 (N-N=C), 1095 (C-O-C)

^{1}H NMR interpretation **(Fig. 5.2) ((δ ppm)):** 4.70 (s, 2H, CH_2); 7.24- 8.3 (m, 13H, Ar-H)

^{13}C NMR **(δ ppm) (Fig. 5.3):** 50.2 (CH_2); 122-139 (ArC); 156 C=N), 158 (C=N), 195.4(C=O)

Mass interpretation **(Fig. 5.4): m/e:** M^+ 374.9

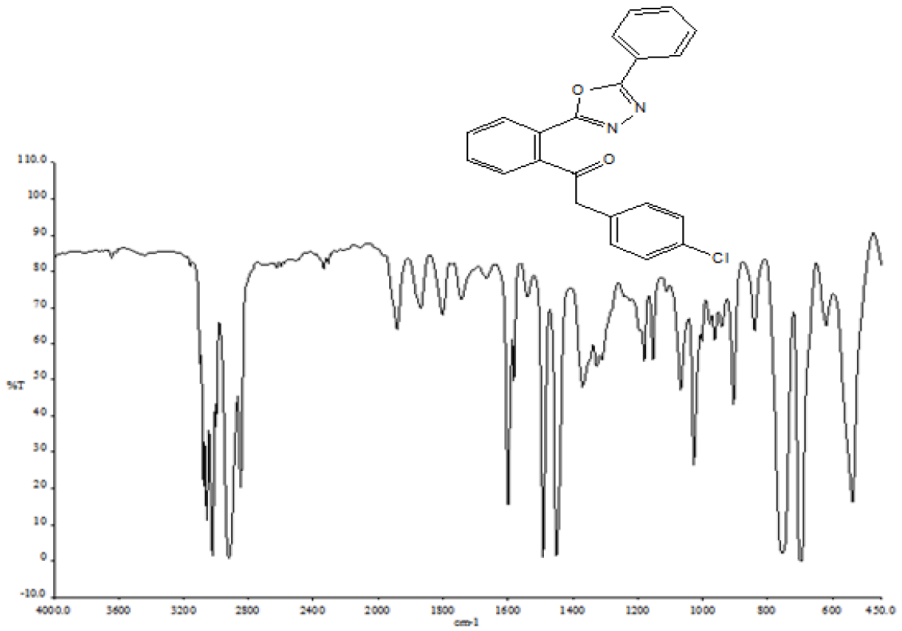


Fig.1. IR spectra of compound 3a

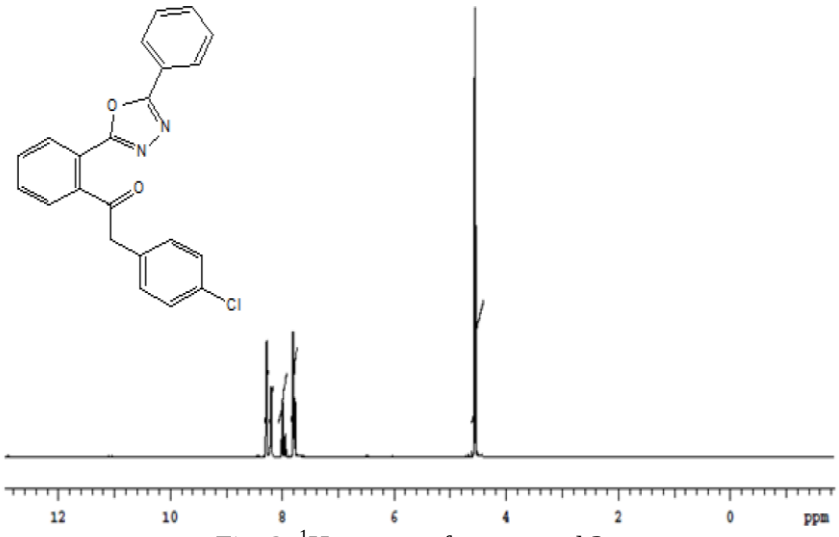


Fig. 2. ^{1}H spectra of compound 3a

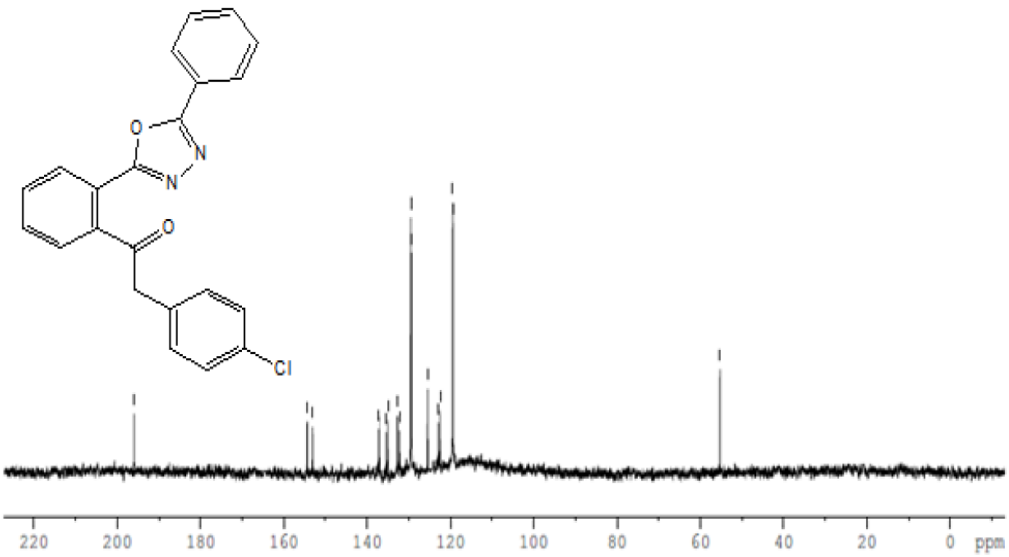


Fig.3. ^{13}C spectra of compound 3a

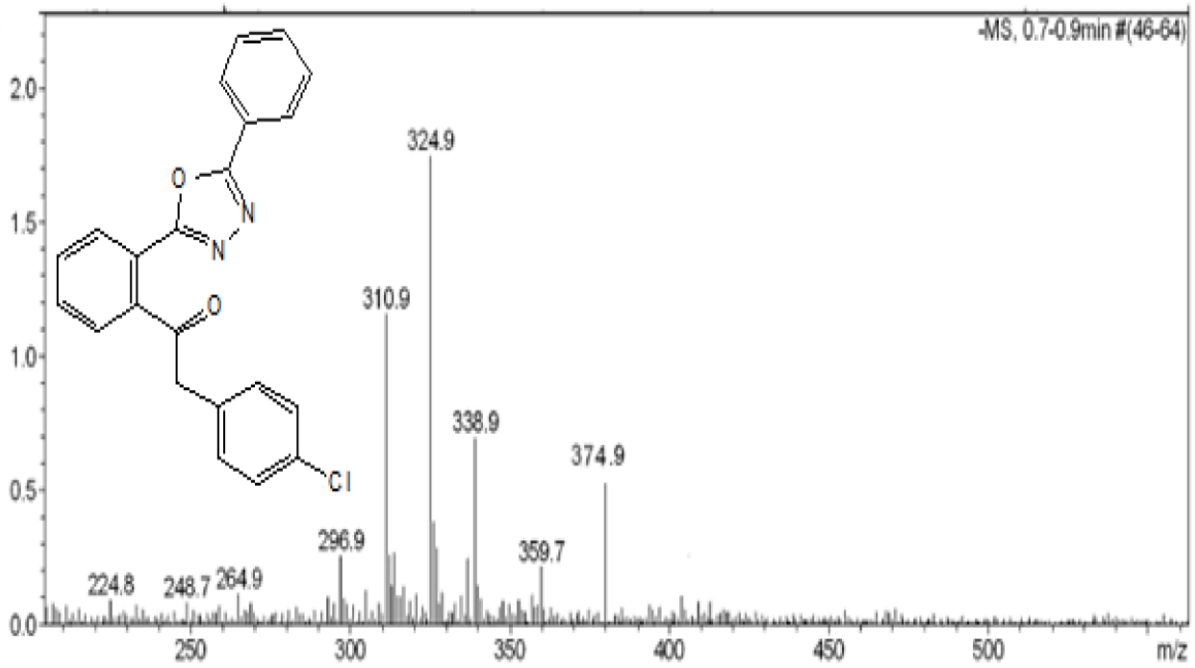


Fig. 4. Mass spectra of compound 3a

Compound 3b (2-(4'-Chloro-phenyl)-1-[2-(5"-p-tolyl-[1, 3, 4] oxadiazol-2-yl)-phenyl]-ethanone)

White compound obtained was found to have the molecular composition $C_{23}H_{17}O_2N_2Cl$ showed the following results on elemental analysis.

Elemental analysis of the compound (3b)

Molecular formula	**Elemental analysis**					
	Calculate			**Found**		
	C	H	N	C	H	N
$C_{23}H_{17}O_2N_2Cl$	71.04	4.38	7.21	71.46	4.56	7.96

Spectral Characteristic data

IR (cm^{-1}) (Fig. 5.5): *1690* (C=O), *1665* (C=O), 1245 (N-N=C), *1090* (C-O).

^{1}H NMR (δ ppm) (Fig. 5.6): 2.35 (s, 3H, CH_3), 4.78 (s, 2H, CH_2), 7.34- 8.40 (m, 12H, ArH)

^{13}C NMR (δ ppm) (Fig. 5.7): 20.9 (CH3), 54.2 (CH2), 120.6-140.5 (ArC), 155.3(C=N), 158.5(C=N), 196.6 (C=O)

Mass (Fig. 6.8): m/e: M+ 388.5

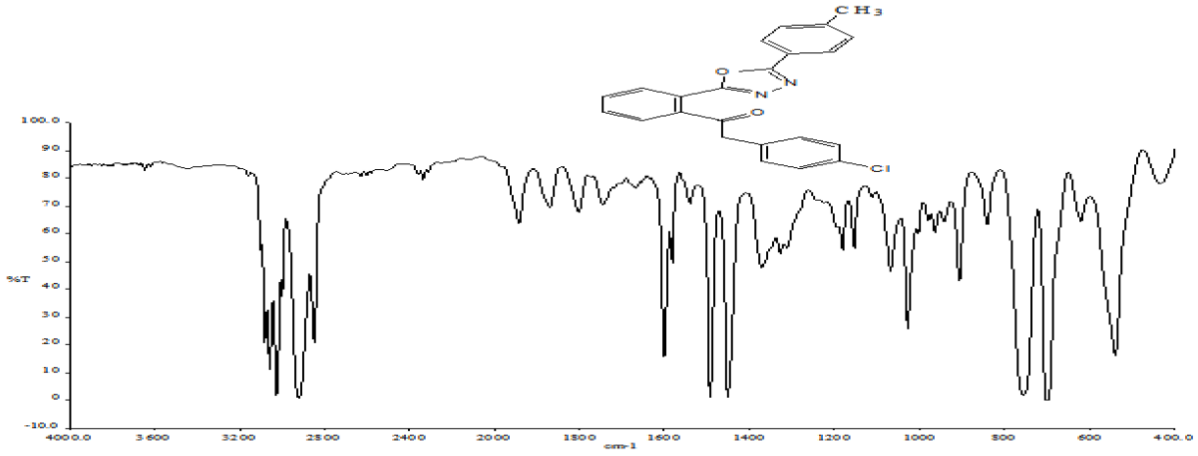


Fig.5. IR spectra of compound 3b

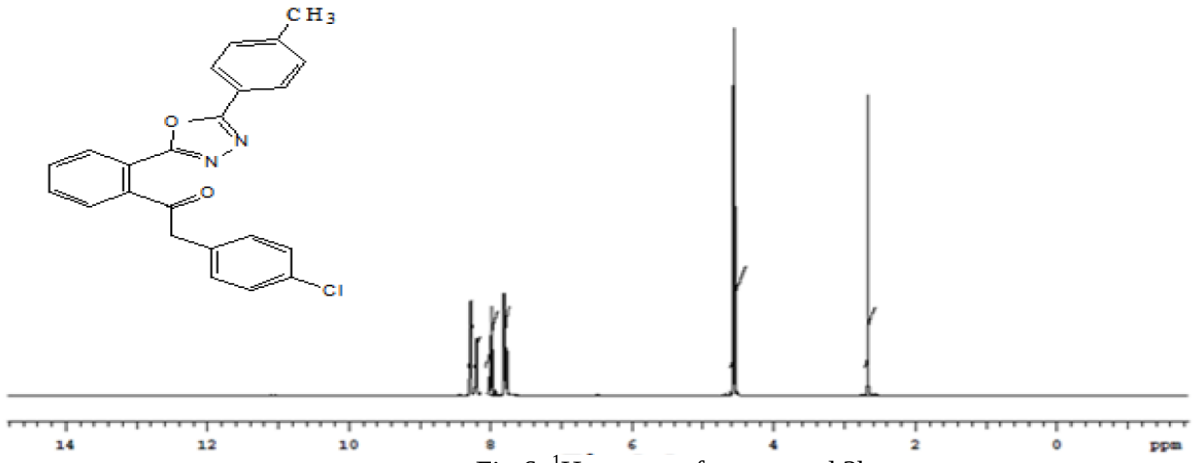


Fig.6. ^{1}H spectra of compound 3b

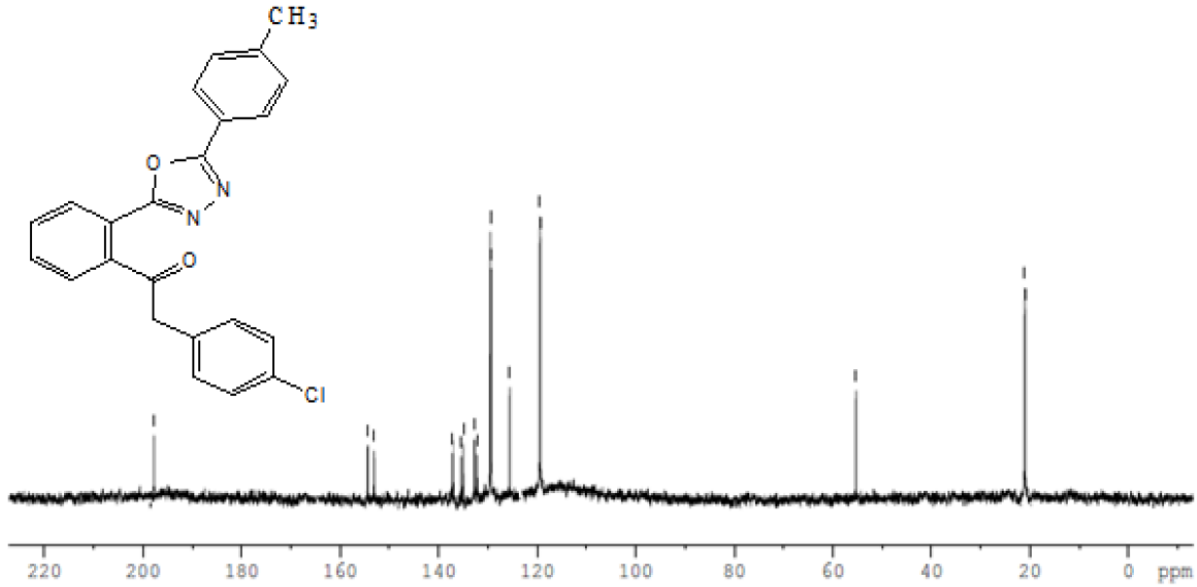


Fig.7. ^{13}C spectra of compound 3b

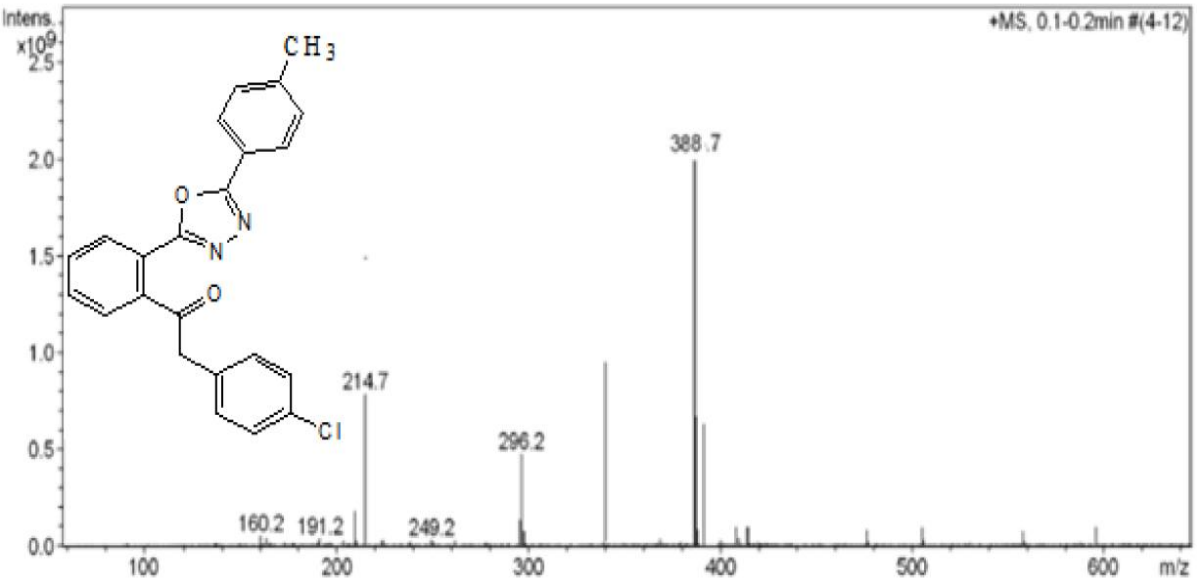


Fig. 8. Mass spectra of compound 3b

Compound 3c (2-(4'-Chloro-phenyl)-1-{2-[5"-(4-nitrophenyl)-[1, 3, 4] oxadiazol-2-yl]-phenyl}-ethanone)
Yellow compound obtained was found to have the molecular composition $C_{22}H_{14}O_4N_3Cl$ showed the following results on elemental analysis.

Elemental analysis of the compound (3c)

Molecular formula	Elemental analysis					
	Calculate			Found		
	C	H	N	C	H	N
$C_{22}H_{14}O_4N_3Cl$	63.08	3.58	13.38	63.56	4.95	13.99

Spectral Characteristic data
IR (cm^{-1}) (Fig. 5.9):*1680* (C=O), *1560-1340* (NO2), 1230 (N-N=C), *1085* (C-O).
^{1}H NMR (δ ppm) (Fig. 5.10): 4.79 (s, 2H, CH2), 7.54- 8.65 (m, 12H, ArH)
^{13}C NMR (δ ppm) (Fig. 5.11): 50.2 (CH2), 127.3-145.5 (ArC), 156.2 (C=N), 159.2 (C=N), 195.4 (C=O)
Mass (Fig. 6.8): m/e: M+ 419.5

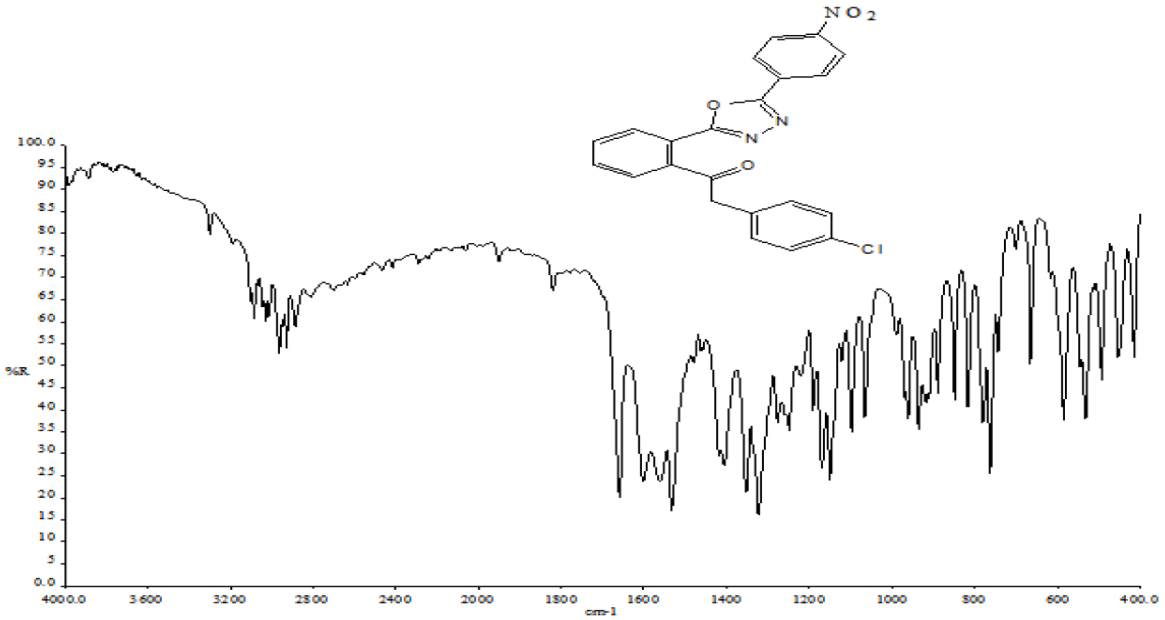


Fig.9. IR spectra of compound 3c

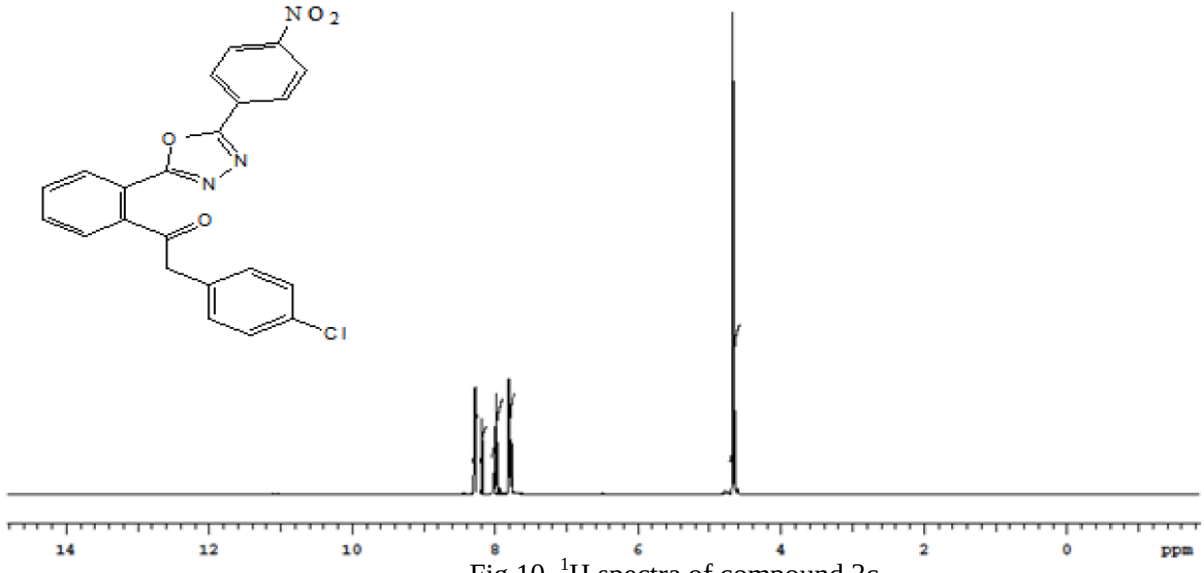


Fig.10. ^{1}H spectra of compound 3c

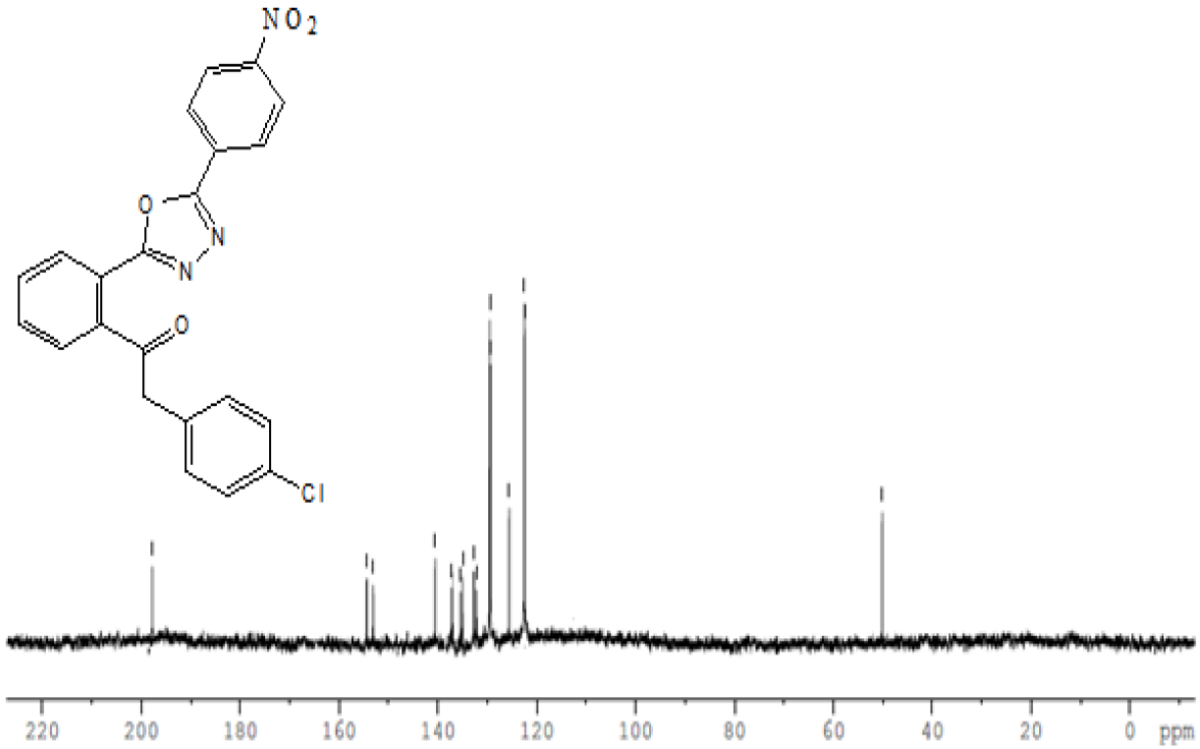


Fig.11. ^{13}C spectra of compound 3c

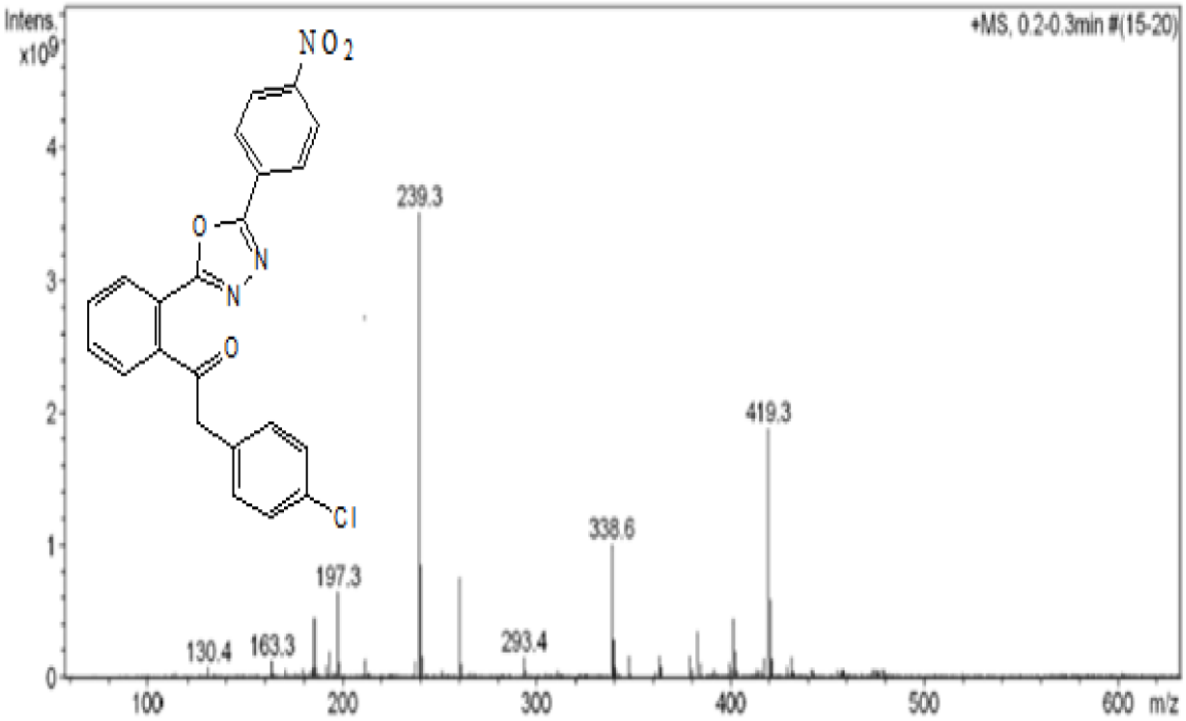


Fig.12. Mass spectra of compound 3c

Compound 3d (1-{2-[5"-(4'-Hydroxy-phenyl)-[1, 3, 4] oxadiazol-2-yl]-phenyl}-2-phenyl-ethanone

White compound obtained was found to have the molecular composition C22H15O3N2Cl showed the following results on elemental analysis.

Elemental analysis of the compound (3c)

Molecular formula	**Elemental analysis**					
	Calculate			**Found**		
	C	H	N	C	H	N
C22H15O3N2Cl	67.61	3.84	7.17	67.98	3.94	7.65

Spectral Characteristic data
IR (cm^{-1}) (Fig. 5.13): 3310 (OH), 3010 (CH); *1670* (C=O), 1242 (N-N=C), 1090(C-O)
^{1}H NMR (δ ppm) (Fig. 5.14): 4.81 (s, 2H, CH2), 5.45 (s, 1H, OH), 7.50- 8.56 (m, 12H, ArH);
^{13}C NMR (δ ppm) (Fig. 5.15): 50.2 (CH2); 128.2-149.6 (ArC), 156.3(C=N), 159.5 (C=N), 195.4(C=O)
Mass (Fig. 6.16): m/e: m/e: M+ 390.5

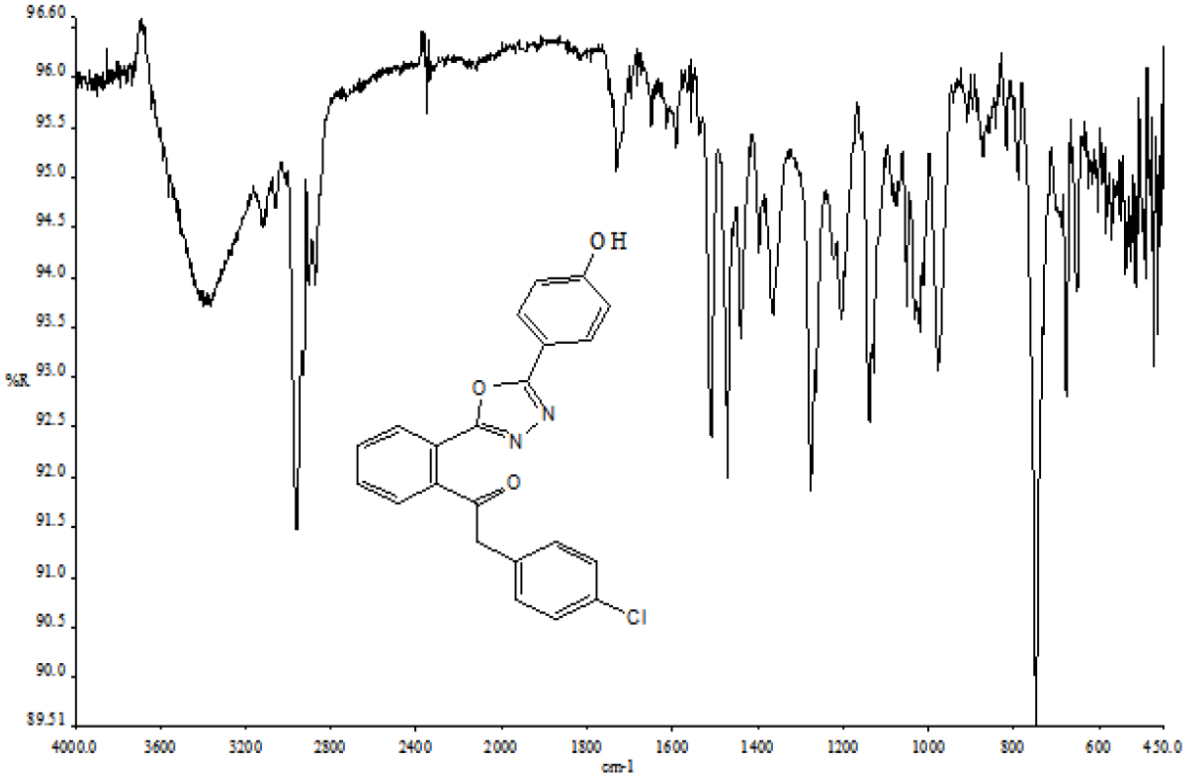


Fig. 13: IR spectra of compound 3d

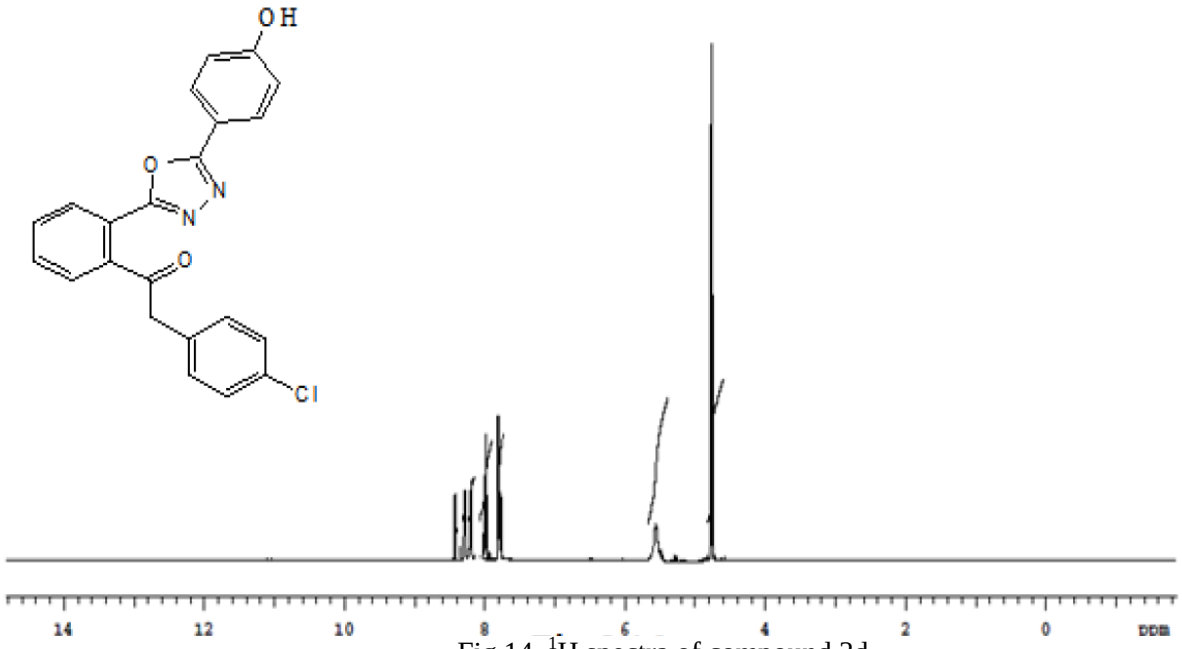


Fig.14. ^{1}H spectra of compound 3d

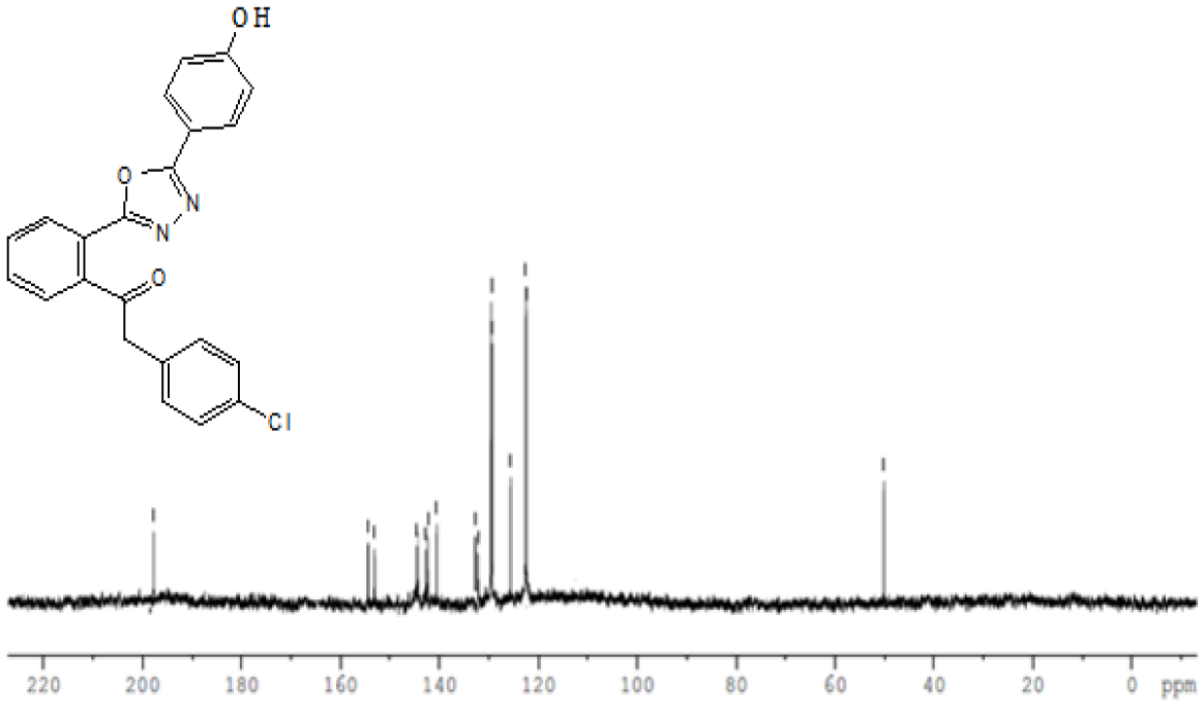


Fig.15. ^{13}C spectra of compound 3d

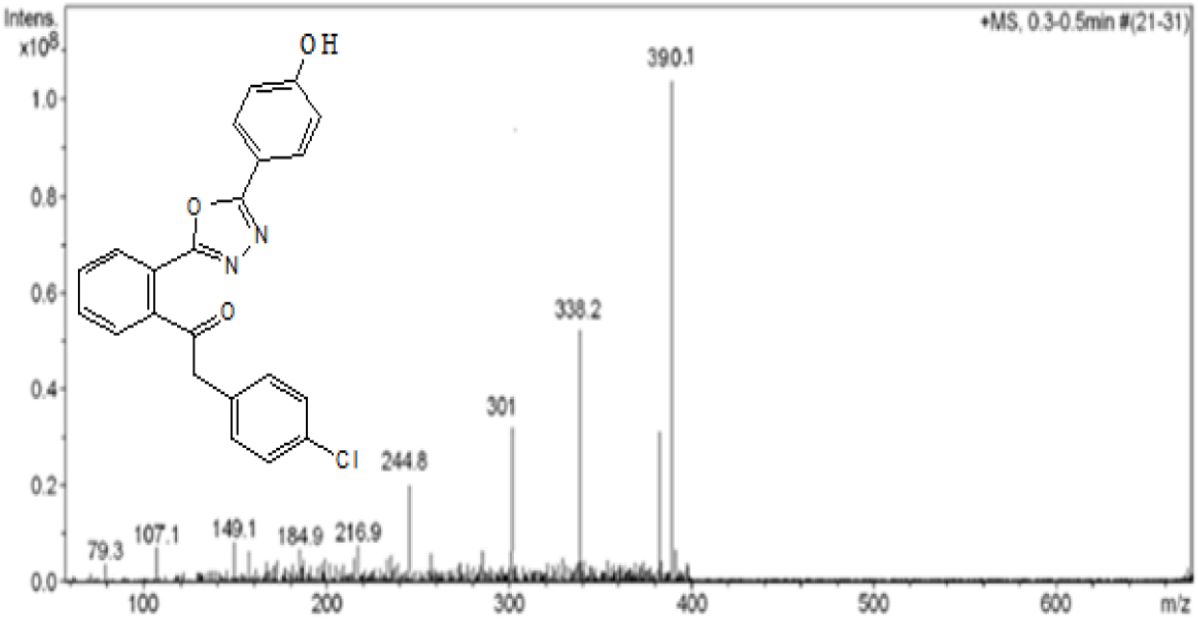


Fig.16. Mass spectra of compound 3d

Anticonvulsant Activity

Table No: 2: Anticonvulsant activity

S.No.	Treatment	Extensor(sec) (Mean±SEM)	Clonus(sec) (Mean±SEM)	Stupor(sec) (Mean±SEM)	Mortality
1	Control	27.81±0.9097	21.65±0.8431	50.66±0.8818	Recovery
2	STD	09.01±0.5773**	10.49±0.8850	27.16±0.6539	Recovery
3	3a	21.12±0.0013*	26.51±0.7653	38.53±0.3425	Recovery
4	3b	16.15±0.7031**	20.77±0.7864	31.13±0.5431	Recovery
5	3c	14.16±0.7031**	18.02±0.5642	28.66±0.8767	Recovery
6	3d	19.32±0.8027**	24.13±0.5122	33.44±0.7611	Recovery

** P < 0 .001 indicates the highly significant difference compared with control.

* P < 0 .05 indicates the significant difference compared with control.

Table 3: BBB predictor results

S.NO	COMPOUND NAME	SCORE	BBB+/BBB
1	Valproic acid	0.088	BBB+
2	Phenytoin	0.080	BBB+
3	**3a**	0.015	BBB^-
4	**3b**	0.052	BBB+
5	**3c**	0.058	BBB+
6	**3d**	0.021	BBB+

The new synthesized compounds 3a, 3b, 3c and 3d were screened for anti convulsant activity by using maximal electroshock induced convulsions method at the dose of 20mg/kg.

The compounds which shown highly significant reduction in the extensor phase are 3c. The compounds 3a, 3b and 3d shown significant activity.

In comparison to Phenytoin, Valproic acid, all the test compounds showed comparable anti convulsant activity. The results shown in the anti convulsant activity report which coincides with the results obtained in the BBB predictor.

Antimicrobial activity

Determination of the in vitro antibacterial and antifungal activity Antibacterial and antifungal activities of the prepared 1, 3, 4-oxadiazole derivatives (4a-4d) moieties were evaluated. The diameters of inhibition zone (IZ) for each compound are reported in table 1. Compound (4d) showed powerful antibacterial activity against S. aureus, S. epidermidis and A. baumannii (Figure 1). Other compounds also showed acceptable antibacterial effects.

Table 4-Antibacterial and antifungal activities of 1, 3, 4-oxadiazol derivatives by agar well diffusion method

Compound	K. pneumonia PTCC1290	A. baumannii PTCC1855	A. baumannii PTCC1855	S. aureus PTCC 1189
Minimum Bactericidal Concentration				
3a	NA	1000	1000	1000
3b	NA	NA	1000	500
3c	NA	NA	1000	500
3d	NA	**125**	125	62.50
Ciprofloxacin	1000	62.50	62.50	31.25
Minimum inhibitory concentration				
3a	NA	100	500	500
3b	NA	NA	500	125
3c	NA	NA	500	125
3d	NA	62.50	62.50	31.25
Ciprofloxacin	**500**	31.25	31.25	15.62
Inhibition of zone				
3a	11.33 ± 0.5	31.66±0.5	38.66±0.5	36.66±0.5
3b	14.66 ± 0.5	16.66±0.5	39.66±0.5	42.33±0.5
3c	13.66 ± 0.5	20.66±0.5	36.66±0.5	41.33±0.5
3d	17.33 ± 1.15	48.66±0.5	47.66±0.5 6	53.66±0.5
Ciprofloxacin	30.5± 0.3	51.66±0.5	56.66±0.5	64.66±0.5

Results are related to 1 mg/ml of each compound.

NA: no activity

Table 5- Antifungal activities of 1, 3, 4-oxadiazol derivatives by agar well diffusion method

Compound	A. flavus PTCC5006	A. fumigates PTCC5009
Minimum fungicidal concentration		
3a	NA	NA
3b	1000	1000
3c	NA	NA
3d	NA	NA
Fluconazole	NA	NA
Minimum inhibitory concentration		

3a	NA	NA
3b	1000	1000
3c	NA	NA
3d	NA	NA
Fluconazole	NA	NA
Inhibition of zone		
3a	11.33± 0.5	NA
3b	11.33± 0.5	12.33± 0.5
3c	11.66± 0.5	NA
3d	11.66± 0.5	11.66± 0.5
Fluconazole	11.33± 0.5	11.66± 0.5

Results are related to 1 mg/ml of each compound.
NA: no activity
Antibacterial and antifungal activities of the prepared 1, 3, 4-oxadiazole derivatives (4a-4d) moieties were evaluated. The diameters of the inhibition zone (IZ) for each compound are reported in table 1 and 2. Compound (4d) showed powerful antibacterial activity against S. aureus, S. epidermidis and A. baumannii. Other compounds also showed acceptable antibacterial effects. However, the compounds showed no notable antifungal activity.

V. DISCUSSION

Four novel 1, 3, 4-oxadiazole derivatives were synthesized by taking 2[4-chloriophenyl)acetyl] benzoic acid. All synthesized compound were analyzed for their phycochemical characteristics and spectral analysis like IR, H NMR, C NMR and Mass spectroscopy.
The objective of this study was to evaluate the antibacterial and antifungal activities of 1, 3, 4-oxadiazole derivatives against some pathogenic bacteria and fungi. In recent years, a number of new 1, 3, 4-oxadiazole analogues has been introduced as potential antimicrobial agents (17). The latest study about 1, 3, 4- oxadiazole with methoxyphenyl group reported that methoxyphenyl group in 1, 3, 4- oxadiazole structure have favorable antibacterial effect against gram-positive and gram-negative bacteria, which is in line with our findings.
This finding is like results of some previous studies[7,8]. In our study, we synthesized new 1, 3, 4- oxadiazole derivatives with inhibitory properties against gram-positive and gramnegative bacteria. However, the compound exhibited no antifungal activity.
Therefore, it can be inferred that in similar structures, the presence of these groups, especially methoxy phenyl group, enhances the antimicrobial activity. It is suggested to use other functional groups of carboxylic acids in the synthesis of new derivatives. It seems that the ability of oxadiazole structures is influenced by addition of different functional groups.
Synthesized compounds 3c compounds posses highly significant anti-convulsant activity.

VI. CONCLUSION

The present study concluded the beneficial effect of synthesized novel 1, 3, 4-Oxadiazole derivatives for its antibacterial and anticonvulsant action. This study confirms the rational basis for its use in synthesized 1, 3, 4-Oxadiazole and its derivatives for the treatment of bacterial infection and anticonvulsant activity in patients. Further pharmacological investigations are under way to characterize active novel 1, 3, 4-Oxadiazole and to establish exact mechanism of anticonvulsant action, which may have fewer side effects. This work, we believe, will be useful for further inflammation research works.

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