IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
September 2017 Vol.:10, Issue:2

Synthesis and Biological Activity of Novel Bioactive Heterocyclic Compounds Containing Oxygen and Nitrogen

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Abhinandan A. Alman*[1], Pallavi S. Jadhav[2], Rajshekhar M. Chimkode[1]

1Sant Gajanan Maharaj College of Pharmacy, Mahagaon, Kolhapur - 416503, Maharashtra, India.

2Adarsh College of Pharmacy, Vita, Sangli - 415311, Maharashtra, India.

Submission: 25 August 2017
Accepted: 3 September 2017
Published: 30 September 2017

Keywords: 2, 5-disubstituted 1,3,4- oxadiazoles, Phosphorous oxychloride, Antimicrobial activity

ABSTRACT

A new series of substituted 2, 5-disubstituted 1,3,4-Oxadiazole were obtained by the reaction of substituted hydrazides and substituted aromatic carboxylic acids in presence of Phosphorous Oxychloride. The purity of the synthesized compounds was confirmed by their physical constant and TLC. The structure of the synthesized compounds was confirmed by IR, NMR and Mass spectral data. The inhibitory activity of synthesized compounds (IIIa-IIIj) was studied which is expressed as minimum inhibitory concentration (MIC) in µg/ml. MIC for antibacterial and antifungal activity was determined by broth microdilution method using BHI as media. All compounds were found moderately active.

INTRODUCTION

Antimicrobial resistance results in increased morbidity and mortality from treatment failures and increased health care costs. It is estimated that USD 30 billion is spent on the cumulative effects of antimicrobial resistance each year including multiple drug regimens, extra hospital days, additional medical care and lost productivity. And a recent study showed that multidrug-resistance was associated with increased incidence of invasive pneumococcal diseases in children younger than 5 years of age. It means that antibiotic resistance directly contributes to increased incidence of invasive diseases[1].

Heterocycles play a vital role in Pharmacological, Agricultural and Synthetic fields. Consequently, the development of methodologies useful for the assembly of molecules containing heterocyclic templates continues to attract the attention of both the academic and industrial communities[2]. Heterocyclic chemistry is the branch of chemistry dealing exclusively with synthesis, properties and applications of heterocycles especially vital to drug design. Incorporation of An Oxygen, Nitrogen, Sulfur, or an atom of a related element into an organic ring structure in place of a carbon atom gives rise to a heterocyclic compound. Since the heterocyclic atom must form more than one bond in order to be incorporated into a ring structure, halogens do not form heterocyclic compounds although they may be substituent's on a heterocyclic ring structure. Heterocyclic compounds, like polycyclic ring compounds, are usually known by non-systematic names[3]. Azoles are five membered heterocyclic compounds containing in their rings one or more heteroatoms, at least one of which is nitrogen. Standard drugs used in some of the medicinally important derivatives containing pyrazoles (azoles) are Novalgin, Aminopyrine etc. which possess NSAID properties. Apart from this, imidazoles, triazoles possess different biological activities like antimalarial, hypertensive and antifungal[4].

The presence of Oxygen and Nitrogen in heterocyclic system has attracted the attention of medicinal chemists because of the diverse biological activities and profound efficacy[5].

Oxadiazoles are important five-membered heterocyclic compounds. The synthesis of heterocyclic compounds has always drawn attention of organic chemists over the years mainly because of their important biological properties. The role of Nitrogen and Oxygen Containing hetero compounds which are endowed with unique structure and potent antibacterial activity need to be overemphasized. Moreover, Oxadiazoles have played a

crucial role in the development of theory in heterocyclic chemistry and also are extensively useful in organic chemistry. In the recent past, it has been observed and reported, that considerable antibacterial and antifungal activity has been exhibited by 1, 3, 4-oxadiazole derivatives, suitably substituted at 2nd and 5th positions[6].

1,3,4-Oxadiazole with different heterocyclic compounds known to have a wide range of biological activities such as anti-inflammatory[7], analgesic[8], anti- ulcerogenic[9], antimicobacterial[10], anthelmintic[11], muscle relaxant[12], antiviral[13], antitubercular[14], anticancer[15], anticonvulsant[16] and antifungal[17] activities.

The synthesis of compounds incorporating 1, 3, 4-oxadiazole ring has been attracting widespread attention due to their diverse pharmacological properties. Hydrazides and related compounds have been described as useful building blocks for the assembly of various heterocyclic rings[18]. We have synthesized a new series of substituted oxadiazol derivatives for the antimicrobial activities.

MATERIALS AND METHODS

Synthesis of 1, 3, 4-oxadiazole backbone:

Brief Chemical Synthesis:

The title compounds were synthesized as per scheme. Substituted benzoic acid is condensed with ethanol containing HCl gas and reflux to give ethyl-4- aminobenzoate **(I)**. this on condensation with hydrazine hydrate in presence of ethanol to give *p*-amino benzohydrazide **(II).** Thus formed hydrazide is condensed with different Substituted aromatic Carboxylic acids in the presence of Phosphorous oxychloride resulted in the synthesis of the derivatives of 2, 5-disubstituted-1,3,4- Oxadiazoles **(IIIa-IIIe).**

Isonicotinamide condensed with hydrazine hydrate to give pyridine-4-carbohydrazide **(II').** Pyridine-4-carbohydrazide condensed with different Substituted aromatic Carboxylic acids in the presence of phosphorous oxychloride resulted in the synthesis of the derivatives of 2-alkyl-5-aryl -1, 3, 4-oxadiazoles **(IIIf-IIIj).**

Synthesis of Ethyl-4-aminobenzoate[21] (I):

Place 80 ml of absolute ethanol in a 250 ml two necked flask equipped with a double surface reflux condenser and a gas inlet tube. Pass dry hydrogen chloride through the alcohol until saturated the increase in weight is about 20g remove the gas inlet tube, introduce 12g (0.088 mole) of p-aminobenzoic acid and heat the mixture under reflux for 2 hours. Upon cooling the reaction mixture sets to a solid mass of the hydrochloride of ethyl-4-aminobenzoate. It is better, however, to pour the hot solution into 300 ml of water (no hydrochloride separates) and add solid sodium carbonate carefully to the clear solution until it is neutral to litmus. Filter off the precipitated ester at the pump and dry in the air. Recrystallized from ethanol. mp 89-92^{0}C.

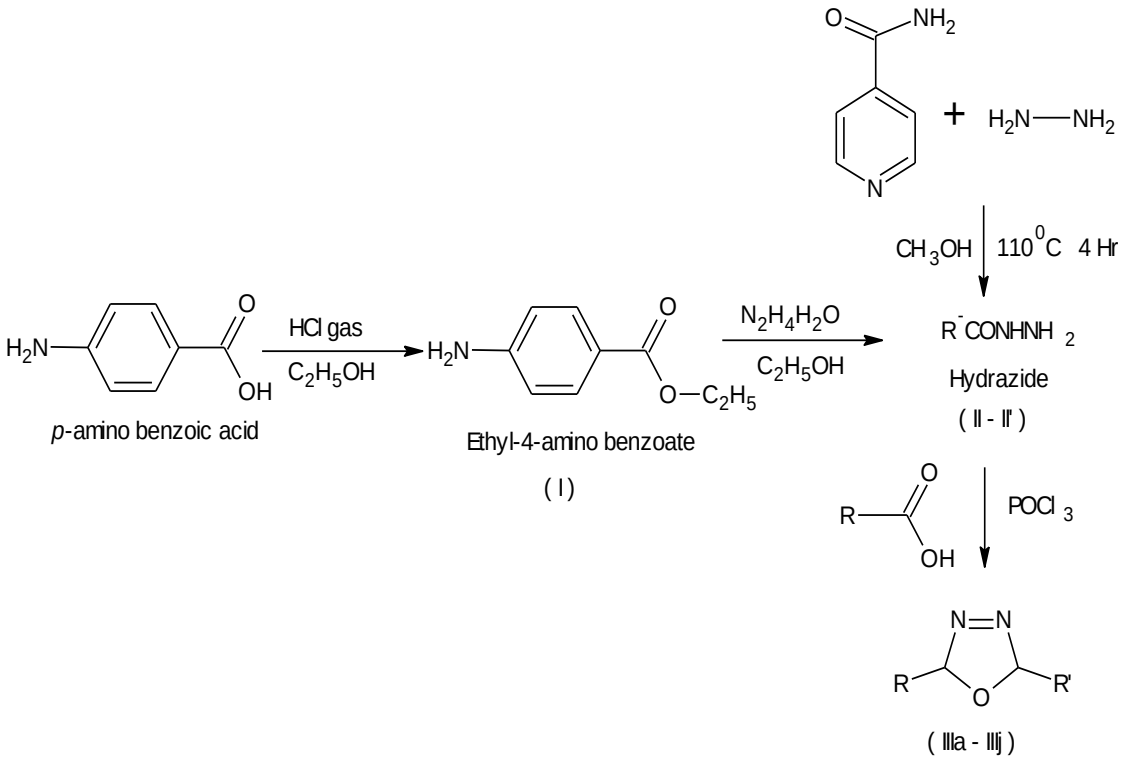
Synthesis of *p*-amino benzohydrazide[22] (II):

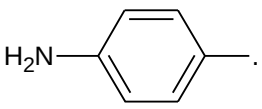
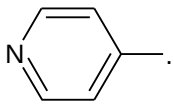
A mixture of ethyl-4-aminobenzoate (21.4g, 0.1mole) and 6.0ml of hydrazine hydrate in 90 ml of ethanol was refluxed over water bath for 6 hrs. The precipitate was filtered and recrystallised with ethanol. White precipitate was produced; mp 224°C and product yield is 85%.

Synthesis of Pyridine-4-carbohydrazide[19] (II'):

To a solution of Isonicotinamide (3.8g in 20 ml methanol), 3 ml of hydrazine hydrate was added and refluxed for 4 hr at 110°C. The reaction mixture was cooled, filtered, and the separated product was purified by recrystallization from ethanol. mp 170-174ºC

Scheme:



Compound	R	R'
IIIa		C_6H_5
IIIb		2-OH-C_6H_4
IIIc		2-NH_2-C_6H_4
IIId		4-NH_2-C_6H_4
IIIe		C_6H_5-CH=CH
IIIf		C_6H_5
IIIg		2-OH-C_6H_4
IIIh		2-NH_2-C_6H_4
IIIi		4-NH_2-C_6H_4
IIIj		C_6H_5-CH=CH

Synthesis of 2-alkyl-5-aryl-1,3,4-Oxadiazoles[20] (IIIa- IIIj):

A mixture of 0.01 mole (1.27g) of hydrazide **(II-II')** and 0.01 mole of different Substituted aromatic Carboxylic acids was dissolved in Phosphorous oxychloride and refluxed for 18-22 hrs. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid mass thus separated out was made to neutralize, then filtered, dried and purified by recrystallization from ethanol

Antimicrobial activity

For the antibacterial and antifungal activity, the compounds were dissolved in dimethylsulfoxide (DMSO). Further dilutions of the compounds and standard drugs in the test medium were prepared at the required quantities of 256, 128, 64, 32, 16, 8, 4, 2, 1 µg/ml concentrations with Mueller–Hinton broth and Sabouraud dextrose broth. The minimum inhibitory concentrations (MIC) were determined using the twofold serial dilution technique (National Committee for Clinical Laboratory Standards, 2000). A control test was also performed containing inoculated broth supplemented with only DMSO at the same dilutions used in our experiments and found inactive in the culture medium. All the compounds were tested for their in vitro growth inhibitory activity against different bacteria and fungi.

Antibacterial and antifungal activity

The cultures were obtained from Mueller–Hinton broth for all the bacterial strains after 24 h of incubation at 37 ± 1°C. Fungi were maintained in Sabouraud dextrose broth after incubation for 24 h at 25 ±1°C. Testing was carried out in Mueller–Hinton broth and Sabouraud dextrose broth at pH 7.4 and the two fold serial dilution technique was applied. The final inoculum size was 105 CFU/ml for the antibacterial assay and 104 CFU/ml for the antifungal assay. A set of tubes containing only inoculated broth was used as controls. For the antibacterial assay after incubation for 24 h at 37± 1°C and after incubation for 48 h at 25± 1°C for antifungal assay, the tube with no growth of microorganism was recorded to represent the MIC expressed in µg/ml. Every experiment in the antibacterial and antifungal assay was replicated twice.

Microorganisms used:

The antibacterial activity of synthetic compounds was determined against a panel of standard strains of the Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis*) and Gram-negative bacteria (*Klebsiella pneumoniae, Escherichia coli*) by broth microdilution method. The antifungal activity of synthetic compounds was determined against a panel of standard strains of *Candida albicans, Aspergillus niger* and *Aspergillus flavus.*

RESULTS AND DISCUSSION

Table 1: Physical Data of the Synthesized Compounds

Comp. No.	R'	Molecular Formula	Molecular Weight	M.P.(C)	Yield (%)	Rf value
I	----	$C_9H_{11}NO_2$	165	90-92	80	0.53
II	----	$C_7H_9N_3O$	151	222-224	65.12	0.55
II'	----	$C_6H_7N_3O$	137	174-178	80	0.36
III a	C_6H_5	$C_{14}H_{11}N_3O$	237	184-186	88.61	0.71
III b	2-OH-C_6H_4	$C_{14}H_{11}N_3O_2$	253	216-218	72.26	0.63
III c	2-NH_2-C_6H_4	$C_{14}H_{12}N_4O$	252	110-112	84.23	0.68
III d	4-NH_2- C_6H_4	$C_{14}H_{12}N_4O$	252	240-242	57.42	0.58
III e	C_6H_5-CH=CH	$C_{14}H_{13}N_4O$	264	210-212	53.41	0.72
III f	C_6H_5	$C_{13}H_9N_3O$	223	144-148	56.81	0.47
III g	2-OH- C_6H_4	$C_{13}H_9N_3O_2$	239	142	69.18	0.42
III h	2-NH_2- C_6H_4	$C_{13}H_{10}N_4O$	238	220-226	48.13	0.66
III i	4-NH_2- C_6H_4	$C_{13}H_{10}N_4O$	238	190-194	71.33	0.49
III j	C_6H_5-CH=CH	$C_{15}H_{13}N_4O$	251	126-128	57.63	0.77

Table 2: Spectral Data of the Synthesized Compounds

Compound	IR spectra (KBr cm^{-1}	$^{-1}$H-NMR spectra**(δ, ppm)**	Mass spectra (m/z value)
I	1684.11(C=O), 3424.21, 3341.25 (NH-NH_2), 2955.13 (C-H; aromatic)	----	----
II	1635.6(C=O), 3428.7, 3347.0 (NH NH_2), 3034.0(C-H; aromatic)	----	----
II'	1668.93(C=O), 3303.30, 3112.39 (NH-NH_2), 3013.44 (C-H; aromatic),	----	----
III a	1685.0, 1655.9 (C=N), 3005.9 (C-H; aromatic), 3326.1, 3260.1 (NH_2). 1073.9 (C-O-C)	-----	237
III b	1635.5 (C=N), 2922.2,2961.5 (C-H; aromatic), 3440.0 (OH), 3253.3(NH_2),1059.8 (C-O-C)	4.41-4.53(2H,d,NH_2), 10.17 (1H,S,OH) 7.56,7.64,7.82 (8H, m, aromatic protons)	253 (M-3)
III c	1639.62 (C=N), 3025.21 (C-H; aromatic), 3437.96, 3199.81 (NH_2), 1071,48 (C-O-C)	----	252 (M-2)
III d	1684.51 (C=N), 3010.44 (C-H; aromatic),3329.47, 258.08(NH_2), 973.52(C-O-C)	3.84, 4.00, 4.12, 4.19, 4.53 (4H,d,NH_2),7.62,7.67,7.83,7.89,7.96 (8H, m, aromatic protons),	252
III e	1611.56 (C=N), 2938.56.0 (C-H; aromatic), 3421.05,	4.03-4.11(2H,d,NH_2),6.29-6.34(2H,d,CH),7.28, 7.42, 7.54, 7.76, 7.85 (9H, m,aromatic protons)	266 (M-1)

	3187.37 (NH), 1009.08 (C-O-C)		
III f	1691.22 (C=N), 3014.98 (C-H; aromatic),1073.9 (C-O-C).	----	223 (M-1)
III g	1613.47, 1545.68 (C=N), 3048.86 (C-H; aromatic), 3443.30 (OH), 1068.38 (C-O-C)	----	239 (M+1)
III h	1600.83 (C=N), 2831.07 (C-H; aromatic), 3258.08(NH_2), 1084.98 (C-O-C)	----	238 (M-1)
III i	1678.19 (C=N), 3010.61 (C-H; aromatic), 3335.86,(NH_2), 940.98 (C-O-C)	----	238 (M-6)
III j	1691.06 (C=N), 2924.38 (C-H; aromatic), 1084.20 (C-O-C)	4.41-4.53 (2H,d,NH_2), 10.17 (1H,S,OH)7.56, 7.64,7.82 (8H, m, aromatic protons)	251 (M+2)

Table 3. MIC (µg /ml) values of the synthesized compounds (III a- III j) for antibacterial activity

Compound	Gram +ve		Gram –ve	
	Staphylococcus Aureus	*Bacillus Subtilis*	*Klebsiella Pneumoniae*	*Escherichia Coli*
IIIa	125	125	62.5	62.5
IIIb	250	125	62.5	125
IIIc	250	500	16.12	125
IIId	500	250	500	500
IIIe	250	250	62.5	125
IIIf	62.5	250	16.12	125
IIIg	250	250	62.5	250
IIIh	125	500	62.5	250
IIIi	500	250	125	250
IIIj	250	500	250	500
Ciprofloxacin	**2**	**2**	**1**	**2**
Norfloxacin	**2**	**2**	**1**	**12**

Table 4. MIC (µg /ml) values of the synthesized compounds (III a- III j) for antifungal activity

Compounds	***Candida Species***	***Aspergillus Species***	
	Candida Albicans	*Aspergillus Niger*	*Aspergillus Flavus*
IIIa	4.03	4.03	2
IIIb	1	8.06	4.03
IIIc	1	16.12	8.03
IIId	1	8.06	8.03
IIIe	1	8.06	4.03
IIIf	1	2	2
IIIg	1	4.03	4.03
IIIh	4.03	16.12	16.12
IIIi	2	4.03	16.12
IIIj	2	1	1
Fluconazole	**16**	**8**	**8**
Griseofulvin	**500**	**100**	**100**

The conventional method was followed which is used to synthesize substituted hydrazides from PABA and Isonicotinamide. These types of reactions are generally carried out in presence of hydrazine hydrate where alcohol is used as solvent. The final compounds 2, 5-disubstituted oxadiazoles were prepared by the cyclization of acetohydrazides at the 4th

position in presence of Phosphorous oxychloride. The formula, mp, yields, IR, NMR, Mass spectral values of the compound are listed earlier. All the spectral data were in accordance with the assumed structure.

The final compounds showed characteristic NMR signals. Pattern for aromatic hydrogen showed multiplets at range of δ 7.11 - 8.53 and the doublets in the range of δ 4.03- 4.53 showed NH_2 bonding for amino substituted acids for compound III d. Also, the singlet peak at the range of δ 10.17 was due to hydroxy proton in compound III b. Mass fragmentation pattern was also studied which gave an idea about the fragmentation of final compound with their corresponding mass. Compound III a showed molecular ion peak at 237 and compound III d showed molecular peak at 252.

The structural formula of organic compound, in principle, contains coded within it all of the information which predetermines the chemical, physical and biological properties of that compound. Biological activity may depend not only on how well the compound interacts with its receptor but also on whole arrange of physical features such as basicity, lipophilicity, electronic distribution and size. The idea of varying substituents is to attach a series of substituents such that these physical features are varied one by one. In reality, it is rarely possible to change one physical feature without affecting another. For aromatic compounds, a favorite approach is to vary the substitution pattern which may give increased activity.

Among the synthesized compounds, compound IIIc which is having NH_2 substitution at *ortho* position of aromatic ring is found active against Gram negative bacteria at concentration of (16.12µ g/ml) as compared to Ciprofloxacin, Norfloxacin as standard drugs and practical yield was 84.23%. Compound **IIIb** which is having hydroxyl substitution at *ortho* position of aromatic ring is found active against Gram negative bacteria at concentration of (62.05µg/ml).

Among the synthesized compounds, compound **III b** which is having hydroxyl substitution at *ortho* position of aromatic ring found active at concentration of (1µg/ml) as compared to Fluconazole and Griseofulvin as standard drugs and practical yield was 72.26%.

ACKNOWLEDGEMENT

We thank Dr. R. M. Chimkode, Principal and Adv. A. D. Chavan, President, SGM College of Pharmacy, Mahagaon, India, for providing necessary facilities. We also wish to thank Director, USIC, Karnataka University, Dharwad, India & Director, USIC, Shivaji University, Kolhapur, India for providing spectral data.

REFERENCES

1. Song JH Global Overview of Antimicrobial Resistance: Historical Perspectives Lectures Isaar 2003;14-16.
2. Rajanarendar E, Ramesh P, Kalyan Rao E, Mohan G, Srinivas M. *p*-TsOH catalysed KSF solid supported Michael addition with substituted isoxazoles and their reductive cyclisation to isoxazolo[4,5-*b*]azepines. ARKIVOC 2007;xiv:266-75.
3. http://www.3rd1000.com/chem301/chem302a.htm
4. http://prr.hec.gov.pk/Chapters/206-1.pdf
5. Alagwadi KR, Suresh S, Pattan SR, Pujar GV, Javali MC. Synthesis and antimicrobial evaluation of some 2-substituted oxadiazoles. Indian J heterocycl Chem 2007;17:93-94.
6. Zheng Li, Anguo Zhu, Xuerong Mao, Xiunan Sun, Xue Gong. Silica-supported dichlorophosphate: a recoverable cyclodehydrant for the eco-friendly synthesis of 2,5-disubstituted 1,3,4-oxadiazoles under solvent-free and microwave irradiation condition. J Braz Chem Soc 2008; 19:1622-26.
7. Husain A, Alam MM, Zaman MS, Ahuja P. Synthesis and biological evaluation of 2-[3-(4-methoxy phenyl)propan-3-one]-5-(substituted phenyl)-1,3,4-oxadiazoles. Indian J Hetrocycl Chem 2008;17:265-66.
8. Vagadevi HM, Vaidya VP. Studies in naphthofurans: Part III- Synthesis of 2- substituted naphtho [2,1-b] furans, 2-(2'-aryl-3'-acetyl-1',3',4'-oxadiazolyl) aminonaphtho [2,1-b] furans and their biological activities. Indian J Heterocycl Chem 2001;10:253-60.
9. Amir M, Shikha K. Synthesis and anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities of some new 2-[(2,6-dichloroanilino) phenyl]acetic acid derivatives. Eur J Med Chem 2004;39:535-45.
10. Rajak H, Gupta AK, Kharya MD, Mishra P. Synthesis and antimicrobial activity of new 2,5-disubstituted 1,3,4-oxadiazoles. Indian J Hetrocycl Chem 2009;19:25-28.
11. Manjunath SY, Biradar JS, Raga B. Synthesis and anthelmintic activity of triheterocycles:[5'-(5"-substituted-3"-phenylindol-2"-yl)-1',3',4',-oxadiazol-2'-yl-thiomethyl] benzimidazoles. Indian J Heterocycl Chem 2009;18:321-24.
12. Almasirad A, Vousooghi N, Tabatabai SA, Kebriaeezadeh A, Shafiee A. Synthesis anticonvulsant and muscle relaxant activities of substituted 1,3,4- oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole. Acta Chim slov 2007;54:317-24.
13. Kim RM, Rouse EA, Chapman KT, Schleif WA, Olsen DB, Stahlhut M. *et.al.*, P1' oxadiazole protease inhibitors with excellent activity against native and protease inhibitors–resistant HIV-1. Bioorg Med Chem Lett 2004;14:4651-54.
14. Chaudhari BR, Shinde DB, Shingare MS. Synthesis of some 1,4- benzothiazinyl thiosemicarbazides, triazoles, oxadiazoles, thiadiazoles and their antitubercular activity. Indian J Heterocycl Chem 1995;4:187-90.
15. Holla BS, Poorjary KN, Bhat KS, Mithun A, Poojary B. Synthesis and anticancer activity studies on some 2-chloro-1,4-bis-(5-substituted-1,3,4- oxadiazol-2-ylmethyleneoxy) phenylene derivatives. Indian J Chem. 2005;44B:1669-73.
16. Lankau HJ, Unverferth K, Grunwald C, Hartenhauer H, Heinecke K, Bernoster K *et.al.*, New GABA-modulating 1,2,4-oxadiazole derivatives and their anticonvulsant activity. Eur J Med Chem 2007;42:873-79.

17. Ahmed O Masalat, Mahmud Abussaud, Hasan Tashtoush, Mahmoud AL- Talib. Synthesis, antibacterial, antifungal and genotoxic activity of bis-1,3,4- oxadiazole derivatives. Pol J Pharmoclo 2002;54:55-59.
18. Khan MSY, Chawal G, MAsa Mueed. Synthesis and biological activity of some isoniazid based 1,3,4-oxadiazole derivatives. Ind J Chem 2004;43(B):1302-05.
19. Dewangan D, Pandey A, Sivakumar T, Rajavel R, Dubey RD. Synthesis of some Novel 2, 5-Disubstituted 1, 3, 4-Oxadiazole and its Analgesic, Anti- Inflammatory, Anti-Bacterial and Anti-Tubercular Activity. Int J of Chem Tech Research 2010;2:1397-1412.
20. Pattan SR, Rabara PA, Pattan JS, Bukitagar AA, Wakale VS, Musmade DS. Synthesis of some novel substituted 1,3,4-oxadiazole and pyrazole derivatives for antitubercular activity. Indian J Chem 2009;43B:1453-56.
21. Vogel's. Textbook of Practical Organic Chemistry, England, 1978.p. 664.
22. Dutkiewicz G, Chidan Kumar CS, Narayana B, Yathiranjan HS, Kubicki M et al. 2-(4-Chlorophenoxy)acetohydrazide. Acta Cryst. 2009: E65:3189.