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Research Article

Synthesis of 5-p-chlorophenyl-2-substituted-1,3,4-oxdiazole and evaluation of its biological activities

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Abstract

A variety of new compounds 2-(1-pyrodinyl)-5-p-chlorophenyl-1,3,4-oxdiazole (III), 2-(benz-2',5'-methoxy)-amino-5-p-chlorophenyl-1,3,4-oxdiazole (IV) and 2-(benz-2,`5`methyl-1-H-pyrazol-4-yl)azo-5-p-chlorophenyl-1,3,4-oxdiazole (V) was synthesized by the action of ester (p-chloromethylbenzoate) with hydrazine hydrate. All proposed structures were supported by IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis. These newly synthesized compounds (III,IV,V) have been screened for their antibacterial activity on *E. coli, P. aeruginosa, S. aureus and S. pyrogenes* and antifungal activity on *C. albicans, A. niger* and *A. clavatus*.

Key words: 1,3,4-oxdiazole,2-substituted, antimicrobial activity .

1. Introduction

In last few decades, the research in the fields of new materials arise more interest in organic substances, which are effective against various microbes. These materials are contain heterocyclic ring and used as medicine in treatment of infectious diseases. Heterocyclic ring has great interest academically as well as biologically. These compounds occur widely in nature and in variety of non-naturally occurring compounds which are also essential to life. These are constituent of various compounds such as alkaloids, antibiotics, vitamins, hemoglobin and are also used in the formation of drugs. Heterocyclic compounds containing five-membered oxdiazole nucleus possess a variety of useful biological effects. The compounds bearing 1,3,4-oxdiazole nucleus are known to be effective as analgesic, anti-inflammatory [1-3], hyperglycaemic and anticancerous substances[4]. Many substituted of oxdiazole also have biological activities such as antimicrobial[5], antitubercular[6], anticonvulsant[7] and antihepatitis-B-virus[8]. 1,3,4-oxdiazoles also play important role in agricultural fields as herbicidal[9] and insecticidal[10].Oxdiazole derivatives are also functioning as enzyme inhibitor, diuretic, CNS dispersant and antihypertensive[11] and are effective on central nervous system[12]. In the present work we have synthesized some novel 5-p-chlorophenyl-2-substituted 1,3,4-oxdiazole and evaluated their antimicrobial activities.

2. Material and Methods

2.1 Experimental section

The proposed compounds were synthesized in many steps, melting points were determined in open capillary method, purity of the compounds was checked on silica gel T.L.C. plate and compounds were analyzed with the help of IR spectra, ^{1}H-NMR & ^{13}C-NMR.

Following methods are used for the synthesis of 5-p-chlorophenyl-2-substituted 1,3,4-oxdiazole-

2.1.1 Synthesis of p-Chlorobenzoyl hydazide(I) :

This compound was prepared by refluxing ester and hydrazine hydrate in 1:1 volume in ethanolic medium.

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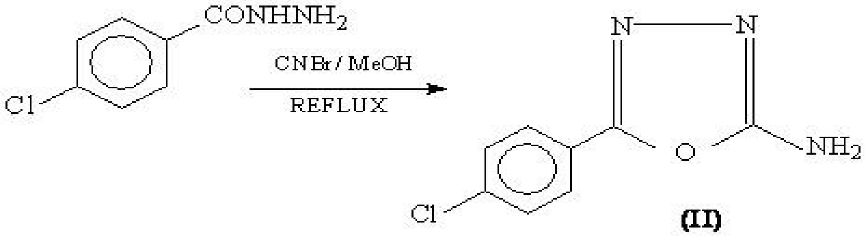
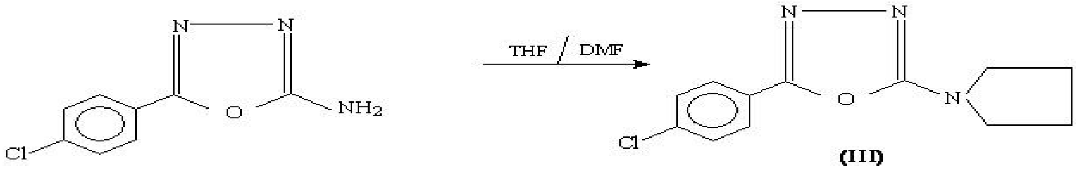
In1.0ml of hydrazine hydrate was taken in conical flask with a short reflex condenser and then added 1.0ml p-Chlorophenyl methyl ester drop wise and heated the mixture gently under reflux for 15minutes. Now start adding ethanol through the condenser. Again reflux the content for further 2-3hours. The solution was kept to settle where the crystal of p-Chlorobenzoyl hydazide was formed, which was filtrated, washed and dried in air. Analysis of compound (I) found C %=32.33, H%=8.20, N%=37.75, calculated for $C_7H_6N_2O$, C%=32.43, N%=37.81, H%=8.16.

2.1.2 Synthesis of 2-Amino-5-(p-Chlorophenyl)-1,3,4-oxdiazole (II): This compound was prepared by refluxing p-Chlorobenzoyl hydazide (I) and cynogen bromide in the presence of methanol at 40^0C.



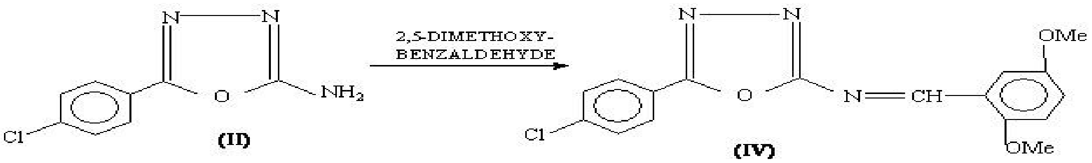
In 1.0ml p-Chlorobenzoyl hydazide taken in a conical flask fitted with a short reflex condenser added 0.0015mol of cynogen bromide and methanol through condenser. Now mixture was stirred for 1-2 hours. After complete stirred, 10 ml of saturated solution of sodium bicarbonate was added. The solution was kept to settle for some time. The solid washed with water, filtrated and dried in air. Analysis of compound (II) found C%=54.23, H%=3.38, N%=23.72, Cl%=9.60 and calculated for $C_8H_6N_3OCl$, C%=54.14, H%=3.29, N%=23.65, and Cl%=9.51.

2.1.3 Synthesis of 2-(1-pyrodinyl)-5-p-(chlorophenyl)-1,3,4-oxdiazole (III): This compound was prepared by refluxing compound (II) and tetahydrofuronin glacial acetic acid.



In 0.015mol of compound (II) taken in a conical flask, 0.015mol tetrahydrofuran and 15 ml glacial acetic acid was added drop wise through the hallow condenser in conical flask. The reaction mixture was refluxed for 8 hours. After the refluxtion of reaction, the solvent was reduced to one third of its volume under pressure. The solution was kept to settle for some time. The solid product separated, washed and dried in air. Analysis of compound (III) found C%=62.33, H%=5.19, N %=18.18, Cl%=7.35 and calculated for $C_{12}H_{12}N_3OCl$, C%=62.24, H%=5.09, N%=18.11, and Cl%=7.25.

2.1.4 Synthesis of 2-(benz-2',5'-methoxy)-amino-5-p-chlorophenyl-1,3,4-oxdiazole (IV): This compound was prepared by refluxing of compound (II) with 2, 5-dimethoxybenzaldehyde in the presence of methanol.



In 0.01mol of compound (II) taken in a conical flask fitted with a short reflex condenser added 0.01mol of 2, 5-dimethoxybenzaldehyde and methanol through condenser. The reaction mixture was stirred for 8 hours. After the refluxing, the solution was kept to settle for some time. The solid product separated, washed and dried in air. Analysis of compound

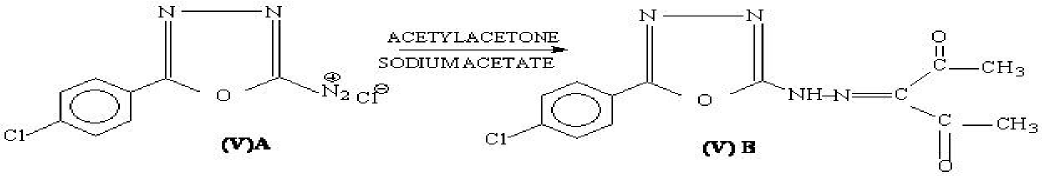
(IV) found C%=62.76, H%=4.30, N%=12.09, Cl%=5.23 and calculated for $C_{17}H_{14}N_3OCl$, C%=62.65, H%=4.26, N%=12.0 and Cl%=5.11.

2.1.5 Synthesis of 2-(benz-2,`5-`methyl-1-H-pyrazol-4-yl)azo-5-p-(chlorophenyl)-1, 3, 4-oxdiazole (V): This compound was prepared by refluxing of compound (II) with acetalacetone and hydrazine hydrate. This compound was formed in following step-

2.1.5.1 Synthesis of 2-Diazoniumchloride-5-p-chlorophenyl-1, 3, 4-oxdiazole (V)A: This compound was prepared by diazotization reaction of compound (2) at 0-6^{o}C.

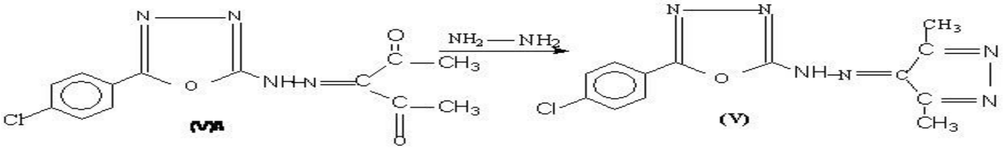
In 0.01molof compound (II) dissolved in 0.03 mol HCl taken in conical flask and cooled on ice bath. An aqueous solution of sodium nitrite also cooled on ice bath, was added to it drop wise. The temperature was maintained at about 5-10^{o}C. The addition of sodium nitrite was stopped as soon as the reaction mixture just gave blue color with starch potassium iodide paper. The excess of sodium nitrite must be avoid as it interference with subsequent reaction of the diazonium salt. This compound was very reactive and unstable.

2.1.5.2 Synthesis of 2-(3-hydrazinopentan 2, 4-dione)-5-p-chlorophenyl-1,3,4-oxdiazole(V) B: This compound was prepared by refluxing of compound (V)A with acetalacetone in ethanol.



In freshly prepared compound (V)A taken in a conical flask, added ice cooled mixture of acetalacetone 0.01mol and 0.01mol of sodium acetate in 25ml ethanol drop wise with content stirring. The stirring continued 30 minute. After the reflution, mixture was left for 2-hours at room temperature for settle. Then the solid product was collected, washed, dried in air.

2.1.6 Synthesis of 2-(benz-2,`5`methyl-1-H-pyrazol-4-yl)azo-5-p-(chlorophenyl)-1,3,4-oxdiazole (V): This compound was prepared by refluxing of compound (V)B with hydrazine hydrate.



In 0.01mol of compound (V)B taken in a conical flask fitted with a short reflex condenser added 0.02mol hydrazine hydrate drop wise and heated under reflux in ethanol for 10-12 hours. The solvent was concentrated and the reaction product was allowed to cool. The separated product was filtered washed and dried in air. Re-crystal in chloroform's solvent. Analysis of compound (V) found C%=54.54, H%=3.84, N%=30.06, Cl%=5.94 and calculated for $C_{13}H_{11}N_6OCl$, C %=54.49, H%=3.79, N%=30.02, and Cl%=5.86. ^{1}H NMR 6.68-7.30 (6H, Ar),1.98 (6H, 2CH_3), 4.01(NH) and ^{13}C NMR 167 (2C), 147 (2C), 130-135 (2C+4H), 14.8 (2x CH_3).

3. Result and Discussion

The synthesis of proposed compounds involved in many steps. The ester (p-chloromethylbenzoate) was taken in a conical flask as initial reactant and in which hydrazine hydrate was added to form p-chlorobenzoyl hydrazide. The Compound (I) was treated with cynogen bromide which gave compound (II). The Compound (III) was prepared by the reaction of compound (II) and tetrahydrofuron in acidic medium. The Compound (II) also gives three different products (compounds III, IV, and V) with the action of tetrahydrofuron, 2,5-dimethoxybenzaldehy and hydrazine respectively. The reaction of compound (II) and 2,5,-methoxy benzaldehyde in ehanolic medium produced compound (IV). The synthesis compound (V),the compound (II) was formed to unstable diazonium salt by diazotization reaction which was allowed to react with acetylacetone to form intermediate compound. This intermediate compound was treated with hydrazine hydrate finally produced compound (V). All these synthesized compounds were analyzed for C, N, and H as well as spectroscopic studies like ^{1}H NMR and ^{13}C NMR. The ^{1}H NMR spectrum of the compound (V) clearly indicated the presence of 4-aromatic proton at δ 6.88-7.30. The signals of methyl proton appear at around δ 2.00and that of NH proton at around δ 4.00. In the ^{13}C NMR spectrum down field signals are seen at 167 clearly assignable to tertiary carbon of oxdiazole signals. Another signals at around 150 are done to tertiary carbon of pyrazole ring while there of phenyl ring signals appeared at the range of 130-135. These results are compactable with the proposed structures of the compounds. These compounds were also evaluated for their antimicrobial activities.

3.1 Evaluation of antimicrobial activity

3.1.1 Antibacterial activity:

All the above synthesized compounds (III to V) were evaluated for *in vitro* antibacterial activity against gram positive and gram negative bacterial strains such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Strophylococcus aureus* and *Streptococcus pyrogenes* at concentration 100µgm/ml by disc diffusion method by using DMSO (Dimethylsulphooxide) as solvent control and nutrient agar was employed as culture medium. After 24 hours of incubation at 37^{0}C, the zone of inhibition was measured in mm. The activity was compared with known antibiotic Ciprofloxacin and result was represented in table-1.

Table-1: Antibacterial activity of compounds III to V in terms of diameter of inhibition zone in mm.:

Compound code	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pyrogenes*
III	17	16	18	14
IV	16	18	15	17
V	19	17	18	16
Ciprofloxacin	25	24	26	23

3.1.2 Antifungal activity:

The synthesized compounds III to V were tested for antifungal activity against *Candida albicanes*, *Aspergillus niger* and *Aspergillus clavatus* by disc diffusion method at 100µgm/ml concentration. The results were expressed in terms of diameter of zone of inhibition in mm. Nystatin is used as standard antibiotic to compare antifungal activitivities with synthesized compounds and the results are represented in table-2

Table-2: Antifungal activity of compounds 5a to 7b in terms of diameter of inhibition zone in mm.:

Compound code	*C. albicanes*	*A. niger*	*A. clavatus*
III	18	19	15
IV	16	15	18
V	19	17	16
Nystatin	23	25	21

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References

1. F. A. Omar, N. M. Mahfouz and M. A. Rahman, Design, synthesis and anti-inflammatory activity of some 1,3,4-oxadiazole derivatives, *Eur. J. Med. Chem*. 1996, 31, 819–825.
2. B. Narayana, K. K. Vijayaraj, B. V. Ashlatha and N. S. Kumari, Synthesis of some new 2-(6-methoxy-2-naphthyl)-5-

aryl-1,3,4-oxadiazoles as possible non-steroidal anti-inflammatory and analgesicagents, *Arch. Pharm*. (Weinheim) , 2005, 338, 373–377.

3. M. Amir and S. Kumar, Synthesis and evaluation of anti-inflammatory, analgesic and lipid peroxidation properties of ibuprofen derivatives, *Acta Pharm*., 2007, 57, 31–45.
4. Rekha Rani and Makrandi J.K, A focile synthesis of symmetrical and unsymmetrical 2,5,-disubstituted 1,3,4-oxdiazoles. *I. J. of heterocyclic chemistry*,2008,vol 18,81-82.
5. S. L. Gaonkar, K. M. Rai and B. Prabhuswamy, Synthesis and antimicrobial studies of a new series of 2-_4-[2-(5-ethylpyridin-2-yl)ethoxy] phenyl_-5-substituted-1,3,4-oxadiazoles, *Eur. J. Med.Chem*., 2006, 41, 841–846.
6. M. A. Ali and M. S. Yar, Oxadiazole Mannich bases: Synthesis and antimycobacterial activity, Bioorg. *Med. Chem. Lett*., 2007, 17 , 3314–3316.
7. A. Zarghi, S. A. Tabatabai, M. Faizi, A. Ahadian, P. Navabi, V. Zanganeh and A. Shafiee, Synthesis and anticonvulsant activity of new 2-substituted-5-(2-benzyloxyphenyl)-1,3,4-oxadiazole, Bioorg. *Med. Chem. Lett*., 2005, 15, 1863–1865.
8. T. M. Tan, Y. Chen, K. H. Kong, J. Bai, Y. Li, S. G. Lim, T. H. Ang and Y. Lam, Synthesis and the biological evaluation of 2-benzenesulfonylalkyl-5-substituted-sulfanyl-(1,3,4)-oxadiazoles as potential anti-hepatitis B virus agents, *Antiviral Res*., 2006, 71, 7–14.
9. Mulwod V.V, and Atul C.C, Synthesis and biological activity of new oxdiazolo[1,3,5]-trizine, 1,2,4-triazolo and thiadiazolo1,3,4-oxdiazole derivatives, *I.J.of chemistry*, 2006, 45B,1710-1715.
10. Shivarama Holla B, Parsanna C.S,Poojary Boja,Rao K.S, Shridharana K, and Ganesha Bhat, U, Synthesis and characterization of 1,3,4-thiodiazole and 1,3,4-oxdiazole derivatives containing 2-chloropyridin-5-methyl moiety, I. J. of chemistry, 2004, 43B,2170-2174.
11. Deshmukh R, Jha A.K, Thakur A.S, and Dewangan D, Synthesis and biological activity of some 1,3,4-oxdiazole derivative and their thione analogous, *I.J. of Res. in pharmaceutical and Bio. Med. Science*, 2011, 215-219.
12. Vosoya S.L, Patel M.R, Dobari S.V, and Joshi H.S, Facile synthesis of some new azetidinones and acetyl oxadiazole bearing benzo[b] thiophene nucleus as a potent biological active agent, I. *J. of chemistry*, 2005, 44B,405-409.

Flow chart