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Research Article

SYNTHESIS AND BIOLOGICAL SCREENING OF SOME NEW 2, 5-DISUBSTITUTED 1, 3, 4-OXADIAZOLES

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ABSTRACT

Emergence of resistant bacterial and fungal strains towards existing antimicrobial agents is one of the major motives for research and development of new molecules to defend them. Substituted 1,3,4-oxadiazoles are of considerable pharmaceutical and material interest, which is documented by a steadily increasing number of publications and patents.1,3,4-oxadiazoles show various biological activities and have been synthesized from different compounds. Research on 1,3,4-oxadiazole and their synthetic analogs have revealed a variety of pharmacological activities including anti-microbial, anti-tubercular and insecticidal agents. Some of these compounds have also analgesic ,anti-inflammatory, anti-cancer, anti-HIV agent, anti-Parkinson and anti-proliferate agent, It was our interested to make novel derivatives of the titled compounds and evaluate the anti-bacterial, analgesic, anti-inflammatory and anti tubercular activities. A series of 2, 5-disubstituted 1,3,4-oxadiazoles were prepared which contain pyridine and piperidine ring .The structure of synthesized compounds has been characterized by spectroscopic data and elemental analysis. All the compounds have been screened for their antimicrobial activity.

Keywords: 1,3,4-oxadiazoles, Antimicrobial activity

INTRODUCTION

1,3,4-oxadiazoles belong to the group of heterocyclic compounds that have been attracting attention for last two decades due to their wide range of biological interactions. Many of them exhibit antibacterial, anticonvulsant, anticancer activities and are used to fight infections involving AIDS. 2,5-disubstituted-1,3,4-oxadiazoles have been reported as remarkable antidepressive,[1] anticonvulsive,[2,3] antiinflamatory,[4] antimitotic,[5] hypoglycemic,[6] antifungal,[7] antimicrobial,[8] anelgesic,[9] herbicidal,[10] muscle relaxant[1], ransquilising[11] agents as well as insecticides.[9] Due to the interesting activity of 2, 5-disubstituted 1,3,4-oxadiazole as biological agent's considerable attention has been focused on this class. The pharmaceutical importance of these compounds lies in the fact that they can be effectively utilizing as antibacterial, anti tubercular and insecticidal agents.[12,13,14,15] Some of these compounds have also analgesic, anti inflammatory, anticancer, anti-HIV agent, anti-Parkinson and anti-proliferate agent.[16,17,18,19,20] In addition, 1,3,4-oxadiazole have played a crucial part in the development of theory in heterocyclic chemistry and also used extensively in organic synthesis.[21,22] Among the methods employed in synthesis of 1,3,4-oxadiazole, condensation of hydrazide and its derivatives with variety of substituted acids and bases are commonly used.[23,24] 2,5-disubstituted 1,3,4-oxadiazole can be conveniently synthesized by the treatment of pyridine-4-carbohydrazide with different acids and bases and carbon disulfide in basic and acidic media.[25,26]

Therefore, as a part of our program focused on 1,3,4-oxadiazole with biological activity, and in connection with our interest in the chemistry of 2,5-disubstituted 1,3,4-oxadiazole. In this paper we report the synthesis of some novel 2,5-disubstituted 1,3,4-oxadiazoles and its anti-bacterial activity.

It was anticipated that the 1,3,4-oxadiazole ring 2,5-disubstituted with the potential drug molecules containing pyridine, piperidine and indole rings will enhance the activity of the molecule. This paper reports the synthesis and biological screening of some new 2,5-disubstituted 1,3,4-oxadiazoles using standard procedure with slight modification.

MATERIALS AND METHODS

Reagents

The course of reaction and purity were ascertained by performing TLC. Melting points were determined in open capillaries and is uncorrected.1H-NMR spectra were recorded at 300 MHz Bruker FT-NMR Spectrometer in $CDCl_3$ using tetra methyl silane (TMS) as internal standard. Elemental analysis (C, H, N and O) were carried out on an Elementar Vario EL III CHNO analyzer. The results are within 0.4% of the theoretical values.

Unless, otherwise stated all the starting materials and reagents were obtained from Aldrich (USA), Spectrochem Pvt. Ltd (India), Loba chemie (India) and Rankem Pvt. Ltd. (India) and were used without further purification.

General procedure for the preparation of acid hydrazide

A typical procedure for the preparation of piperidine-3-carbohydrazide:

Ethyl piperidine-3-carboxylate (5g, 0.0318mole) was added portion wise into 98% hydrazine hydrate (2ml, 0.04mole) in a RB flask. The reaction mixture was heated on a water bath for 15minutes followed by the addition of ethanol (50ml). It was then refluxed for another 2 hours. On cooling the white solid that separated was filtered and washed with cold ethanol and dried to give 93% (4.2g) yield, which was used as such with out any further purification in the next step.

General method for the preparation of acyl hydrazone:

A typical procedure for the preparation of (E)-N'-(pyridin-4-ylmethylene) piperidine-3-carbohydrazide:

Piperidine-3-carbohydrazide (4g, 0.027mole) and pyridine-4-aldehyde (3g, 0.028mole) were taken in ethanol (50ml) in a 100ml flask. Sodium acetate (2.3g, 0.027mole) was added to the reaction mixture and it was refluxed for five hours. After the reaction, the solvent was removed under reduced pressure to small volume. It was then extracted into ether layer. The solvent was evaporated off to get a yellow gummy mass. It was then re crystallized from alcohol to give cream color solid in 90% (5.8g) yield.

General procedure for the preparation of 2, 5-aryl-1, 3, 4-oxadiazoles:

A typical procedure for the preparation of 2-(piperidin-3-yl)-5-(pyridin-4-yl)-1, 3, 4-oxadiazole:

A mixture of (E)-N'-(pyridin-4-ylmethylene) piperidine-3-carbohydrazide(5g, 0.022mole) and $CAT.3H_2O$ (6g, 0.022mol) in ethanol (50ml) was heated to reflux with stirring for 3 hours. The sodium chloride formed in the reaction is filtered off and washed with ethanol. The combined filtrate and washings were evaporated in vacuum and the residue was extracted into ether and washed with 10% NaOH (3x20ml), water and finally with brine solution. The

ether layer after drying over anhydrous sodium sulphate was evaporated in vacuum. The resultant residue was dissolved in dichloromethane (10ml) and co-precipitated with hexane. Re-crystallization from ethanol gave oxadiazole as white crystalline solid in 82% (4.1g) yield. m.p: 235-237^{0}C.

2-(1H-indol-3-yl)-5-(piperidin-3-yl)-1,3,4-oxadiazole (5ad):

Obtained as cream color solid from 4ad (5g, 0.018 mole) and Chloramine-T trihydrate (5.2g, 0.018 mole)in ethanol with 91% yield (4.5g), m.p:152-154^{0}C; ^{1}HNMR ($CDCl_3$): δ 2.0 (s, NH), 1.4-3.2 (m, 9H, piperidine ring) 7.0-8.4 (m, 4H , indole ring) 8.7(s,1H,-CH) 12.4(s,-NH) ; Anal. Calcd for $C_{15}H_{16}N_4O$ (268.31): C, 67.15; H, 6.01; N, 20.88; O, 5.96 % Found: C, 67.01; H, 5.91; N, 20.78; O, 5.86%.

2-(piperidin-3-yl)-5-(pyridin-4-yl)-1,3,4-oxadiazole (5ae)

Obtained as off white color solid from 4ae (5g, 0.022 mole) and Chloramine-T trihydrate (6.0 g, 0.022 mole) in ethanol with 78% yield (4.1g), m.p: 235-237^{0}C; ^{1}HNMR ($CDCl_3$): δ 2.0 (s, NH), 1.4-3.2 (m, 9H, piperidine ring) 7.9-8.8 (m, 4H , pyridine ring); Anal. Calcd for $C_{12}H_{14}N_4O$ (230.27): C, 62.59; H, 6.13; N, 24.33; O, 6.95 % Found: C, 62.51; H, 5.99; N, 24.28; O, 6.86%.

2-methoxy-4-(5-(piperidin-3-yl)-1,3,4-oxadiazol-2-yl)phenol (5af)

Obtained as cream color solid from 4af (5g, 0.018 mole) and Chloramine-T trihydrate (5.07g, 0.018 mole) in ethanol with 78% yield (3.9g), m.p: 95-97^{0}C ; ^{1}HNMR ($CDCl_3$): δ 2.0 (s, NH), 1.4-3.1 (m, 9H, piperidine ring), 3.83(s, 3H, -OCH_3), 6.9-7.8 (dd, 2H, -CH), 7.4 (s, 1H, -CH), 9.8(s, 1H, -OH); Anal. Calcd for $C_{14}H_{17}N_3O_3$ (275.30): C, 61.08; H, 6.22; N, 15.26; O, 17.43% Found: C, 60.91; H, 5.99; N, 15.28; O, 17.31 %.

2-(3,4-dimethoxyphenyl)-5-(piperidin-3-yl)-1,3,4-oxadiazole(5ag)

Obtained as white color solid from 4ag (5g, 0.017mole) and Chloramine-T trihydrate (4.82g, 0.017mole) in ethanol with 81% yield (4.0g) , m.p: 88-92^{0}C; ^{1}HNMR(CDCl3): δ 2.0 (s, 1H, -NH), 1.4-3.1 (m, 9H, piperidine ring), 3.83(2s,6H, -OCH3), 6.9-7.9 (dd, 2H , -CH), 7.51 (s, 1H, -CH),; Anal. Calcd for $C_{15}H_{19}N_3O_3$ (289.33): C, 62.27; H, 6.62; N, 14.52; O, 16.59 % Found: C, 61.18; H, 6.43; N, 14.28; O, 16.45 %.

2-(1H-indol-3-yl)-5-(pyridin-4-yl)-1,3,4-oxadiazole(5bd)

Obtained as pinkish cream color solid from 4bd(5g, 0.018mole) and Chloramine-T trihydrate (5.32 g, 0.018 mole)in ethanol with 83% yield (4.1 g) , m.p: 135-138^{0}C; ^{1}HNMR ($CDCl_3$): δ 8.05-8.65 (m, 4H, pyridine ring), 7.2 -8.0 (m, 4H, -CH), 8.7(s,1H, -CH), 12.4(s, -NH),; Anal. Calcd for $C_{15}H_{10}N_4O$ (262.27): C, 68.69; H, 3.84; N, 21.36; O, 6.10 % Found: C, 68.18; H, 3.43; N, 21.28; O, 6.05 %.

2,5-di(pyridin-4-yl)-1,3,4-oxadiazole(5be)

Obtained as yellow color solid from 4be (5g, 0.022 mole) and Chloramine-T trihydrate (6.2g, 0.022mole) in ethanol with 87% yield (4.3 g), m.p: 225-229^{0}C; ^{1}HNMR ($CDCl_3$): δ 7.9-8.75(m, 8H, pyridine ring); Anal. Calcd for $C_{12}H_8N_4O$ (224.07): C, 64.28; H, 3.60; N, 24.99; O, 7.14% Found: C, 64.18; H, 3.43; N, 24.88; O, 7.15%.

2-methoxy-4-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)phenol(5bf)

Obtained as cream color solid from 4bf (5g, 0.018mole) and Chloramine-T trihydrate (5.19g, 0.018mole) in ethanol with 85% yield (4.2g), m.p: 155-158^{0}C; ^{1}HNMR ($CDCl_3$): δ 3.83 (s, -CH_3), 3.83(s, 3H, -OCH_3), 6.9-7.8 (dd, 2H, -CH), 7.56 (s, 1H, -CH), 7.9-8.75 (m, 4H, pyridine ring), 9.8(s,1H, -OH); Anal. Calcd for $C_{14}H_{11}N_3O_3$ (269.26): C, 62.45; H, 4.12; N, 15.61; O, 17.83% Found: C, 62.31; H, 4.09; N, 15.58; O, 17.71%.

2-(3,4-dimethoxyphenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole(5bg)

Obtained as off white color solid from 4bg (5g, 0.0175mole) and Chloramine-T trihydrate (4.94g, 0.0175 mole)in ethanol with 79% yield (4.0 g), m.p: 128-130^{0}C ; ^{1}HNMR ($CDCl_3$): δ 3.83 (s, -CH_3), 3.83(2s,6H, -OCH_3), 6.9-7.8 (dd, 2H, -CH), 7.56 (s, 1H, -CH), 7.9-8.75 (m, 4H, pyridine ring); Anal. Calcd for $C_{15}H_{13}N_3O_3$ (283.10): C, 63.60; H, 4.63; N, 14.83; O, 16.94% Found: C, 63.31; H, 4.59; N, 14.78; O, 16.81%.

2-(1-benzylpiperidin-4-yl)-5-(1H-indol-3-yl)-1,3,4-oxadiazole(5cd)

Obtained as light brown color solid from 4cd(5g, 0.013mole) and Chloramine-T trihydrate (3.91g, 0.013 mole)in ethanol with 80% yield (3.9g) , m.p: 135-138^{0}C; ^{1}HNMR ($CDCl_3$): δ 1.6-2.53 (m,9H,-CH_2, piperidine ring), 3.65(s,2H,-CH_2), 7.2-7.44(m,5H,-CH,benzene ring), 7.0-81.4(m, 4H, indole ring) 8.71(s,1H,-CH) 12.4(s, -NH); Anal. Calcd for $C_{22}H_{22}N_4O$ (358.18): C, 73.72; H, 6.19; N, 15.63; O, 4.46% Found: C, 73.61; H, 6.16; N, 15.59; O, 4.38%.

2-(1-benzylpiperidin-4-yl)-5-(pyridin-4-yl)-1,3,4-oxadiazole(5ce)

Obtained as light brown color solid from 4cd(5g, 0.015 mole) and Chloramine-T trihydrate (4.36g, 0.015 mole)in ethanol with 83% yield (4.1g) , m.p: 119-121^{0}C; ^{1}HNMR ($CDCl_3$): δ 1.6-1.95 (m,9H,-CH_2, piperidine ring), 3.65(s,2H,-CH_2), 7.2-7.44(m,5H,-CH,benzene ring), 7.95-8.74 (m, 4H , pyridine ring); Anal. Calcd for $C_{19}H_{20}N_4O$ (320.39): C, 71.23; H, 6.29; N, 17.49; O, 4.99% Found: C, 71.21; H, 6.23; N, 17.46; O, 4.86%.

4-(5-(1-benzylpiperidin-4-yl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenol (5cf)

Obtained as cream color solid from 4cd(5g, 0.013 mole) and Chloramine-T trihydrate (3.83 g, 0.013 mole)in ethanol with 85% yield (4.2g), m.p: 156-159^{0}C ; ^{1}HNMR ($CDCl_3$): δ 1.6-2.51 (m,9H,-CH_2,piperidine ring), 3.65(s,2H,-CH_2), 3.65 (s,3H,-CH_3), 7.2-7.44(m,5H,-CH,benzene ring), 7.95-8.74 (dd, 2H, -CH) 7.56(s,1H,-CH), 9.8(s,1H,-OH); Anal. Calcd for $C_{21}H_{23}N_3O_3$ (365.43): C, 69.02; H, 6.34; N, 11.50; O, 13.13% Found: C, 69.0; H, 6.33; N, 11.46; O, 13.04%.

2-(1-benzylpiperidin-4-yl)-5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazole (5cg)

Obtained as brown color solid from 4cd(5g, 0.013 mole) and Chloramine-T trihydrate (3.7g, 0.013mole) in ethanol with 88% yield (4.4g), m.p: 112-114^{0}C ; ^{1}HNMR ($CDCl_3$): δ 1.6-2.51 (m,9H,-CH_2, piperidine ring), 3.65(s,2H,-CH_2), 3.65(2s,6H,-CH_3), 7.2-7.44(m,5H,-CH, benzene ring), 7.95-8.74 (dd, 2H, -CH) 7.56(s,1H,-CH); Anal. Calcd for $C_{22}H_{25}N_3O_3$ (379.45): C, 69.64; H, 6.64; N, 11.07; O, 12.65% Found: C, 69.54; H, 6.53; N, 11.05; O, 12.61%.

ANTIMICROBIAL ASSAY

Antibacterial assay

All the synthesized compounds *viz* 2,5-aryl-1,3,4-oxadiazoles (**5**), were evaluated for their *in vitro* antibacterial activity against gram +ve and gram -ve bacterial strains *viz, Escherichia coli, Staphylococcus aereus, Klebeliessa pneumoniae, Pseudomonas areginosa* and *Bacillus substilis* by using the agar well diffusion method.[18]The bacterial strains were maintained on LB agar medium at 28°C. The bacteria were grown in LB broth, centrifuged at 10,000 rpm for 5 minutes; a pellet was dissolved in double distilled water and used to inoculate the plates. The autoclaved molten media (20 mL) was poured in each 90 mm sterilized petriplate and allowed to solidify. A circular well of diameter 6 mm was made exactly at the center of the plates by using cork borer and each well was filled with 0.1 ml of the test solution (10mg/ml). Streptomycin and DMSO were used as positive control and negative control respectively. All the compounds were tested in triplicate and inhibition zones were measured in mm after 24 hrs of incubation. The results were presented in **table-1.**

Antifungal activity

In vitro antifungal assays of all the synthesized compounds *viz* 2,5-aryl-1,3,4-oxadiazoles (**5**), were performed against fungal strains *Aspergillus niger, Aspergillus flavus* and *Fusarium monoliforme* using agar well diffusion method[19] . The fungal cultures were raised by growing on potato dextrose agar media at pH 7.4 for six days at

25°C. The spores were harvested in sterilized normal saline (0.9% NaCl in distilled water) and its concentration was adjusted to $1x10^6$/ml with a Haemometer. The autoclaved molten media (20 ml) was poured in each 90 mm sterilized Petri plate and allowed to solidify. To study the growth response of fungi species, 0.4 ml of the synthesized compound solution (5mg/ml) was poured into each plate and spread over the agar media. 10 µl spore suspension was poured in to small depression made at the center of the plate and kept for 6 days at 25°C. After six days of incubation, the fungal growth were measured and compared with the control. The control plates contained only DMSO for which fungal growth is taken as 100% (without inhibition). The fungal activity of all the synthesized compounds was assessed by comparing the zone of fungal growth in treated plates with that of control plates in mm. The results were presented in **table-2.**

REACTION SCHEME

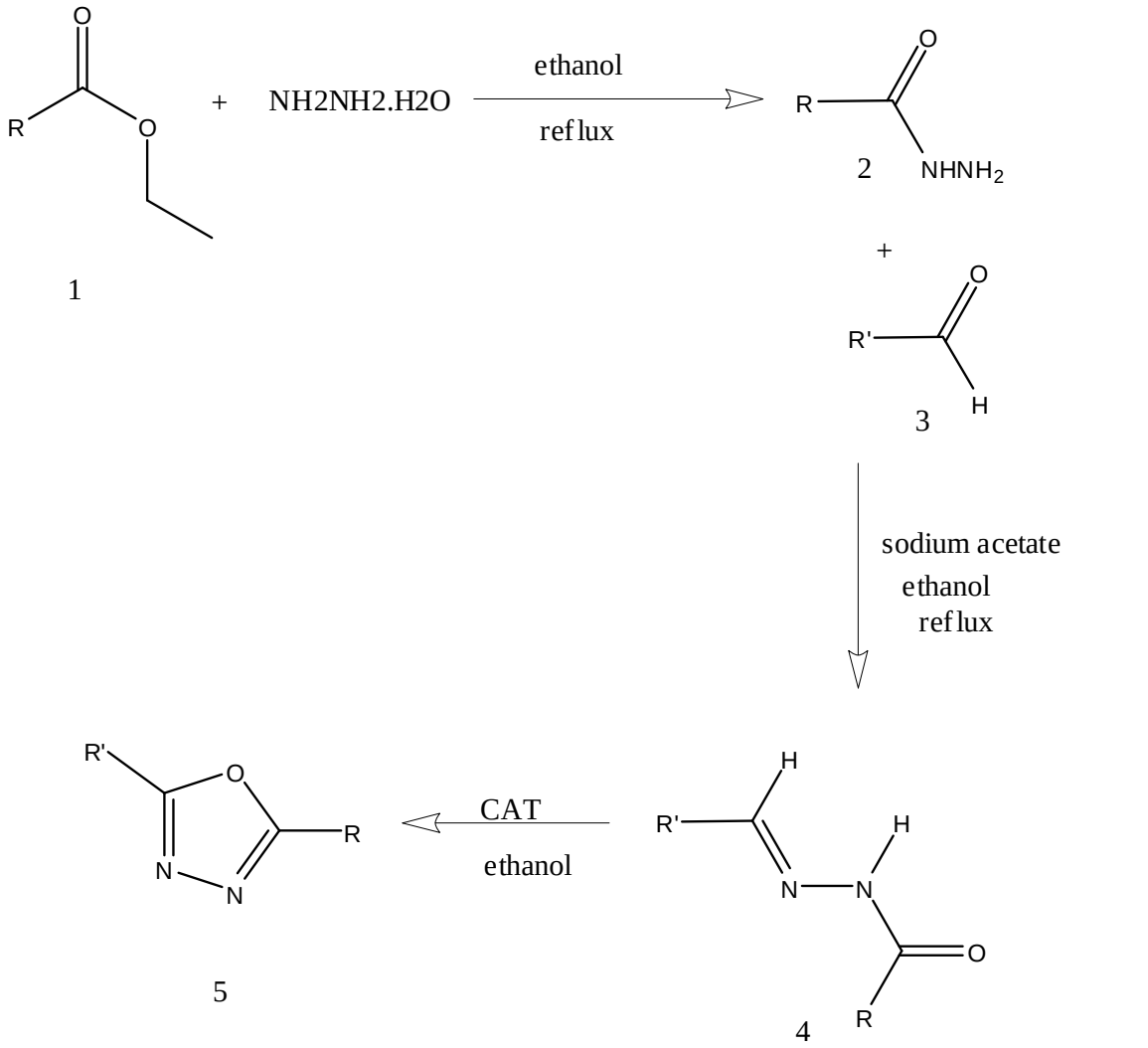

Table 1: Antibacterial activity of 2, 5-aryl-1, 3, 4-oxadiazoles (5)

Sl. No.	Inhibitory zone (diameter) mm [a]				
	Gram negative bacteria			Gram positive bacteria	
	E - coli	*Klebesiella pnemoniae*	*Pseudomonas auregenosa*	*Staphylococcus aureus*	*Bacillus Substilis*
5ad	06	05	05	04	05
5ae	05	04	06	04	05
5af	06	05	04	04	04
5ag	07	05	05	04	04
5bd	07	06	05	05	04
5be	08	08	06	05	04
5bf	05	05	06	04	04
5bg	05	04	04	04	04
5cd	06	05	04	05	04
5ce	07	06	06	05	05
5cf	07	06	06	05	05
5cg	08	06	06	05	08
Streptomycin	12	10	11	12	10

[a] Values are means of three determinations, the ranges of which are less than 5% of the mean in all cases.

Table 2: Antifungal activity of 2, 5-aryl-1, 3, 4-oxadiazoles (5)

Sl. No.	Inhibitory zone (diameter) mm[a]		
	Aspergillus niger	*Aspergillus flavus*	*Fusarium monoliforme*
5ad	04	04	03
5ae	05	04	04
5af	04	04	03
5ag	05	04	06
5bd	05	05	06
5be	06	06	05
5bf	05	05	06
5bg	04	05	03
5cd	06	06	07
5ce	06	06	08
5cf	04	03	03
5cg	05	06	06
Bavistin	09	10	09

[a] Values are means of three determinations, the ranges of which are less than 5% of the mean in all cases.

RESULTS AND DISCUSSION

We have synthesized 2,5-disubstituted 1,3,4-oxadiazoles by using standard procedure with slight modification. The course of reaction and purity were ascertained by performing TLC. Melting points were determined in open capillaries and are uncorrected. 1H-NMR spectra were recorded at 300 MHz Bruker FT-NMR Spectrometer in $CDCl_3$ using tetra methyl silane (TMS) as internal standard. Elemental analysis (C, H, N and O) were carried out on an Elementar Vario EL III CHN analyzer. The results are within 0.4% of the theoretical values.

In vitro antibacterial activities were performed against *Escherichia coli, Staphylococcus aereus*, *Klebsiella pneumoniae, Pseudomonas areginosa* and *Bacillus substilis* keeping Streptomycin as standard and In *vitro* antifungal activity was performed against *Aspergillus niger*, *Aspergillus flavus* and *Fusarium monoliform* keeping Bavistin as standard. All the compounds synthesized shown good and moderate activity against standard.

CONCLUSION

The structure proposed to the synthesized compound is well supported by spectroscopic data and elemental analysis. From the antimicrobial activity data (Table I & II), it may be concluded that all the synthesized compounds showed good and moderate activity against the antimicrobials.

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REFERENCES

1. Piala JJ,Yale H L; 2-amino-5-aryl-1,3,4-oxadiazoles US Patent 3141022,1964. Chem. Abstr: 1964, 61: 8317.
2. [a] Almasirad, A.; Tabatabai, S. A.; Faizi, M.; Kebriaeezadeh, A.; Mehrabi, N.; Dalvandi A.; Shafiee, A. Bioorg. Med. Chem. Lett. 2004, 14: 6057. [b] Zarghi, A.; Tabatabai, S. A.; Faizi, M.; Ahadian, A.; Navabi, P.; Zanganeh, V.; Shafiee, A. Bioorg. Med. Chem. Lett. 2005, 15: 1863. [c] Dogan, H. N.; Duran, A.;Rollas, S.; Sener, G.; Uysal, M. K.; Gulen, D. Bioorg. Med. Chem. 2002, 10: 2893.
3. Misra, U.; Hitkari, A.; Saxena, A. K.; Gurtu, S.; Shanker, K. Eur. J. Med. Chem. 1996, 31: 629.
4. [a] Palaska, E.; Sahin, G.; Kelicen, P.; Tuğba, N.; Altinok, G. Il Farmaco 2002, 57: 101. [b] Amir, M.; Shikha, K. Eur. J. Med. Chem. 2004, 39: 535.
5. Lokanatha Rai, K.M.; Linganna, N. IL Farmaco 2000, 55:389.
6. Girges, M. M. Arzneim.-Forsch. 1994, 44: 490.
7. Goswami, B. N.; Kataky, J. S.; Baruah, M. A. Eur. J. Med. Chem. 1996, 31: 819.
8. Papakonstantinou, G. S.; Marakos, P.; Tsantili, K. A.; Chytyroglon, L. A. Pharmazie 1998, 53: 300.
9. [a] Angilini I, Angilini L ,sparacc F; 2-phenyl-5-chloro alkyl-1,3,4-oxadiazoles, Britishpatent,,196,9 1:161,801: Chem. Abstract ,1969,71:112936.
10. [b] Najer H, Giudicelli R,Morale, Menin M; synthesis of some 5-aryl-2-dimethylamine 1,3,4-oxadiazoles with anti-inflammatory action. Bull SOC chem. France 1966, 153-159. Kennedy D.A, summers LA; chemical constitution and activity of Herbicides. J. Heterocyclic chem. 1981, 409-410.
11. Yale HL, Losee K: 2-amino 5-substituted 1, 3, 4-oxadiazoles and 5-imino substituted - 1,3,4-oxadiazolines. A group of novel muscle relaxants. J. med chem., 1966, 9: 478-483.

12. G.Grover, S.G.Kini. Indian Journal of Heterocyclic Chemistry, 2003, 12: 289.
13. J.Hazarika, J.C.S Kataky. Indian Journal of Heterocyclic Chemistry, 1997; 7: 47.
14. P.R.Kagthara, N.S.Shah, R.K.Doshi, H.H.Parekh,.Indian. Journal of Chemistry.1999; 38B: 572.
15. B.Shivarama Holla, C.S.Prasanna, Boja Poojary, K.S. Rao, K.Shridhara, U.Ganesha Bhat. Indian Journal of Chemistry. 2004; 43B: 864.
16. Zheng Li & Xicum Wang. Indian Journal of Chemistry.2003; 42B: 941.
17. M.Amir, S. Shahani. Indian Journal of Heterocyclic Chemistry.1998; 8:107.
18. H.P.Shah, B.R.Shah, J.J.Bhatt, N.C.Desai, P.B.Trivadi, N.K.Undavia. Indian Journal of Chemistry.1996; 37B: 180.
19. J.Hazarika, J.C.S.Kataky.Indian Journal of Heterocyclic Chemistry.1998; 7: 83.
20. Liszkiewicz, M.W.Kowalska, J.Wietrzyk A.Opolski. Indian Journal of Chemistry, 2003; 42B:2846.
21. K.Mogilaiah, N.Vasudeva Reddy,.Indian Journal of Chemistry. 2003; 42B:2124.
22. M.S.Chande, A.A.Godbole, E.Coutinho, P.Desai Indian Journal of Chemistry 2003; 42B: 397.
23. K.Subrahmanya Bhat, M.S.Karthikeyan, B.Shivarama Holla, N. Suchetha Shetty. Indian Journal of Chemistry 2004; 42B: 1765.
24. M.S.Y. Khan, M.Akhtar. Indian Journal of Chemistry.2003; 42B: 900.
25. P.Mishra, H.Rajak, A.Mehta. Journal of General Applied Microbial. 2005; 15: 133.
26. H.Rajak, M.Kharya, P.Mishra. International Journal of Chemical Science. 2007; 5:365.
27. Prez C, Paul M and Bazerque P. An antibiotic assay by the agar well diffusion method. Acta Biol Med Exp, 1990; 15: 113-115.
28. Singh I and Singh V. Antifungal properties of aqueous and organic solution extracts of seed plants against Aspergillus flavus and A.niger. Phytomorphology, 2000; 50(2): 151-157.