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DEVELOPMENT OF NOVEL 1, 3, 4-OXADIAZOLE DERIVATIVES AS NEW ANTI-MICROBIAL AGENTS

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ABSTRACT

2-(arylideneamino)-4-(5-Aryl-1,3,4-Oxadiazol-2-yl) phenol (VII) derivatives were evaluated for antimicrobial activity by using cup and plate method. The synthesized compounds were characterized and evaluated for antibacterial activity against Bacillus subtilis Staphylococcus aureus, Escherichia coli and Proteus vulgaris and antifungal activity against Aspergillus niger, Curvularia lunata and Candida albicans by using Ampicillin sodium and Clotrimoxazole as a standard respectively. Among all the compounds, Compound VIId and Compound VIIl were more effective against bacteria. Among the series Compound VIIp and Compound VIIi were exhibited more inhibition against fungal.

KEY WORDS

1,3,4-Oxadiazole, IR Spectrum, NMR Spectrum, Mass Spectrum, Antibacterial, Antifungal

INTRODUCTION:

The major drawback of current treatment of infectious diseases are challenging due to resistance to antimicrobial agents and their side effects. In order to overcome this situation, it is necessary to continue the search for new antibacterial agents. In recent scenario heterocycles plays a major role in drug synthesis. In that respect oxadiazole plays a significant role among other heterocycles. From the literature survey oxadiazole was found to be having diverse activity like antimicrobial (Banday MR, et al, 2010), anti-tumor (El-Hamouly et al, 2011), anti-convulsant (Tabatabai SA et al, 2013), anti-tuberculosis (Rajesh A et al, 2013), anti-oxidant (Nevena Mihailovic et al, 2017), anti-inflammatory (Ega Durga shivaprasad et al, 2013) etc. So, it was planned to synthesize a novel series of 1,3,4 oxadiazole derivatives and to check their activity as antimicrobial and antifungal agent.

MATERIALS AND METHODS

All melting points were taken in open capillaries on a veego VMP-1 apparatus and are uncorrected IR spectra were recorded as KBr pellets on a Perkin-Elmer FT IR 240-c spectrometer. The ^{1}H NMR spectra were recorded on Varian-Gemini 200 MHz spectrometer in DMSO-d6 using TMS as an internal standard and mass spectras were recorded on Schimadzu QP 5050A spectrometer.

Synthesis of methyl-4-hydroxy-3-nitrobenzoate (II)

To a solution of aluminium nitrate (40grms) in acetic acid- acetic anhydride (1:1) mixture (160ml), was added an appropriate phenol (I, 40grms) in small portions, while cooling and shaking occasionally. The reaction mixture was left at room temperature for 1.5 hours while shaking the contents intermittently to complete the nitration. The resulting brown solution was diluted to complete the nitration. The resulting brown solution was diluted with ice-cold water and acidified with concentrated Nitric acid to get a bulky, yellow precipitate. It was filtered washed with small quantity of

methanol and purified by recrystallization from alcohol to get a yellow crystalline solid (K. Rajmohan et al, 1973).

Synthesis of methyl 3-amino-4-hydroxybenzoate (III)

Methyl-4-hydroxy-3-nitrobenzoate (II, 10 grams) was dissolved in boiling alcohol (50%, 100ml) and sodium dithionite was added to this boiling alcohol solution until it becomes almost colourless. Then the alcohol was reduced to one-third of its volume by distillation and the residual liquid was triturated with crushed ice. The resulting colourless, shiny product was filtered, washed with cold water and dried in the air. Its purification was affected by recrystallization from benzene to get colourless, shiny scales. (Finhorn et al, 1900)

Synthesis of methyl-3-(Arylideneamino)-4-hydroxybenzoates (IV)

Methyl-3-amino-4-hydroxybenzoate (III, 0.01mol) was condensed with Four different aromatic aldehydes i.e, Anisaldehyde, 4-chlorobenzaldehyde, 4-dimethylaminobenzaldehyde and Vaniline (0.015mol) by refluxing in 20ml of absolute alcohol containing few drops of acetic acid for 7-8hrs. The product thus separated was filtered, dried and purified by recrystallization from suitable solvents. These compounds were characterized by their spectral data.

Synthesis of 3-(Arylideneamino)-4-hydroxybenzohydrazides (V)

A mixture of methyl-3-(Arylideneamino)-4-hydroxybenzoates (IV, 0.01mol) and hydrazine hydrate (99%) (0.01mol) were taken in 50ml of alcohol, heated under reflux on a water bath for 5hrs. The alcohol was reduced to half of its volume and cooled. The product separated was filtered and washed with small portions of cold alcohol first and then with cold water repeatedly and dried. The product was purified by recrystallization from suitable solvents.

Synthesis of 3-(Arylideneamino)-N'-(Arylidene)-4-hydroxybenzohydrazides(VI)

3-(Arylideneamino)-4-hydroxybenzohydrazides (V, 0.01mol) was condensed with Four different aromatic aldehydes i.e, 4-chlorobenzaldehyde, 4-dimethylaminobenzaldehyde 3,4,5-trihydroxybenzaldehyde and Vaniline (0.015mol) by refluxing in 20ml of absolute alcohol containing few drops of acetic acid for 6-7hrs. The product thus separated was filtered, dried and purified by recrystallization from suitable solvents.

Adopting this procedure, sixteen 3-(Arylideneamino)-N'-(Arylidene)-4-hydroxybenzohydrazides (VI) have been prepared and characterized.

Synthesis of 2-(Arylideneamino)-4-(5-(Aryl)-1,3,4-oxadiazol-2-yl) phenols (VII)

3-(Arylideneamino)-N'-(Arylidene)-4-hydroxybenzohydrazides (VI, 0.01 M) and sodium acetate (0.02 M) were dissolved in 30–40 ml of glacial acetic acid taken in a (100 ml) round-bottomed flask equipped with a separating funnel for the addition of bromine. Bromine (0.7 ml in 5 ml glacial acetic acid) was added drop by drop, while stirring magnetically. After half an hour stirring, the solution was poured on crushed ice. The resulting solid was separated, dried and recrystallized from aldehyde free ethanol. **(**Khan MTH et al, 2005)

Adopting this procedure, sixteen 2-(Arylideneamino)-4-(5-(Aryl)-1,3,4-oxadiazol-2-yl) phenols (VII) have been prepared and characterized. They are presented in Table 1.

SCREENING FOR ANTI-MICROBIAL PROPERTIES

1. **Antibacterial activity by cup plate method (**Indian Pharmacopoeia, 1996)

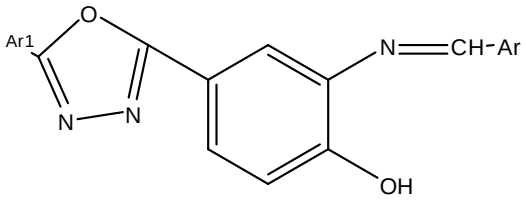
The antibacterial activity of synthesized compounds was conducted against two gram-positive bacteia viz., *Bacillus subtilis* and *Staphylococcus aureus* and two gram-negative bacteia viz., Escherichia *coli* and *Salmonella typhi* by using cup plate method. Ampicillin sodium was employed as standard to compare the results.

The test organisms were sub cultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37°C ± 1°C for 24 hours, they were stored in refrigerator. The stock cultures were maintained. Bacteria inoculum was prepared by transferring a loopful of stock culture to nutrient broth (100 ml) in conical flasks (250 ml). The flasks were incubated at 37°C ± 1°C for 48 hours before the experimentation. Solution of the test compounds were prepared by dissolving 10 mg each in dimethylformamide (10 ml, AnalaR grade). A reference standard for both gram-positive and gram-negative bacteria was made by dissolving accurately weighed quantity of ampicillin sodium in sterile distilled water, separately.

The nutrient agar medium was sterilized by autoclaving at 121°C (15 lb/sq. inch) for 15 min. The petriplates, tube and flasks plugged with cotton were sterilized in

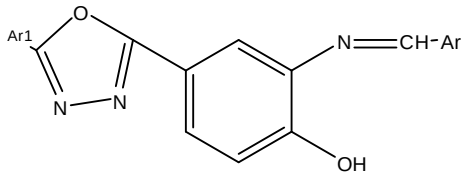
hot-air oven at 160°, for an hour. Into each sterilized petriplate (10 cm diameter), about 27 ml of molten nutrient agar medium was poured and inoculated with the respective strain of bacteria (6 ml of inoculum to 300 ml of nutrient agar medium) was transferred asceptically. The plates were left at room temperature to allow the solidification. In each plate, three cups of 6 mm diameter were made with sterile borer. Then 0.1 ml of the test solution was added to the respective cups asceptically and labeled, accordingly. The plates were kept undisturbed for atleast 2 hours in refrigerator to allow diffusion of the solution properly into nutrient agar medium. After incubation of the plates at 37° ± 1°C for 24 hours, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader. All the experiments were carried out in triplicate. Simultaneously, controls were maintained employing 0.1 ml of dimethyl formamide to observe the solvent effects. The results are presented in Table 2.

Table 1: Physical Data of 2-(Arylideneamino)-4-(5-(Aryl)-1,3,4-oxadiazol-2-yl) phenols (VII)



S.N	Compd	Ar	Ar^1	Chemical Formula (M)	Melting Point (°C)	Yield (%)
1	VIIa	4-chlorophenyl	Phenyl	$C_{21}H_{14}ClN_3O_2$	154	67
2	VIIb	4-dimethylamino phenyl	Phenyl	$C_{23}H_{20}N_4O_2$	156	60
3	VIIc	4-hydroxy-3-methoxyphenyl	Phenyl	$C_{22}H_{17}N_3O_4$	184	61
4	VIId	3,4,5-trihydroxyphenyl	Phenyl	$C_{21}H_{15}N_3O_5$	147	62
5	VIIe	4-chlorophenyl	4-methoxyphenyl	$C_{22}H_{16}ClN_3O_3$	125	60
6	VIIf	4-dimethylamino phenyl	4-methoxyphenyl	$C_{24}H_{22}N_4O_3$	194	60
7	VIIg	4-hydroxy-3-methoxyphenyl	4-methoxyphenyl	$C_{23}H_{19}N_3O_5$	148	61
8	VIIh	3,4,5-trihydroxyphenyl	4-methoxyphenyl	$C_{22}H_{17}N_3O_6$	144	60
9	VIIi	4-chlorophenyl	4-chlorophenyl	$C_{21}H_{13}Cl_2N_3O_2$	159	65
10	VIIj	4-dimethylamino phenyl	4-chlorophenyl	$C_{23}H_{19}ClN_4O_2$	184	61
11	VIIk	4-hydroxy-3-methoxyphenyl	4-chlorophenyl	$C_{22}H_{16}ClN_3O_4$	149	61
12	VIIl	3,4,5-trihydroxyphenyl	4-chlorophenyl	$C_{21}H_{14}ClN_3O_5$	184	60
13	VIIm	4-chlorophenyl	3,4,5-trihydroxyphenyl	$C_{21}H_{14}ClN_3O_5$	197	58
14	VIIn	4-dimethylamino phenyl	3,4,5-trihydroxyphenyl	$C_{23}H_{20}N_4O_5$	165	59
15	VIIo	4-hydroxy-3-methoxyphenyl	3,4,5-trihydroxyphenyl	$C_{22}H_{17}N_3O_7$	184	65
16	VIIp	3,4,5-trihydroxyphenyl	3,4,5-trihydroxyphenyl	$C_{21}H_{15}N_3O_8$	148	66

TABLE-2: Antibacterial activity of 2-(Arylideneamino)-4-(5-(Aryl)-1,3,4-oxadiazol-2-yl) phenols VII(a-p)



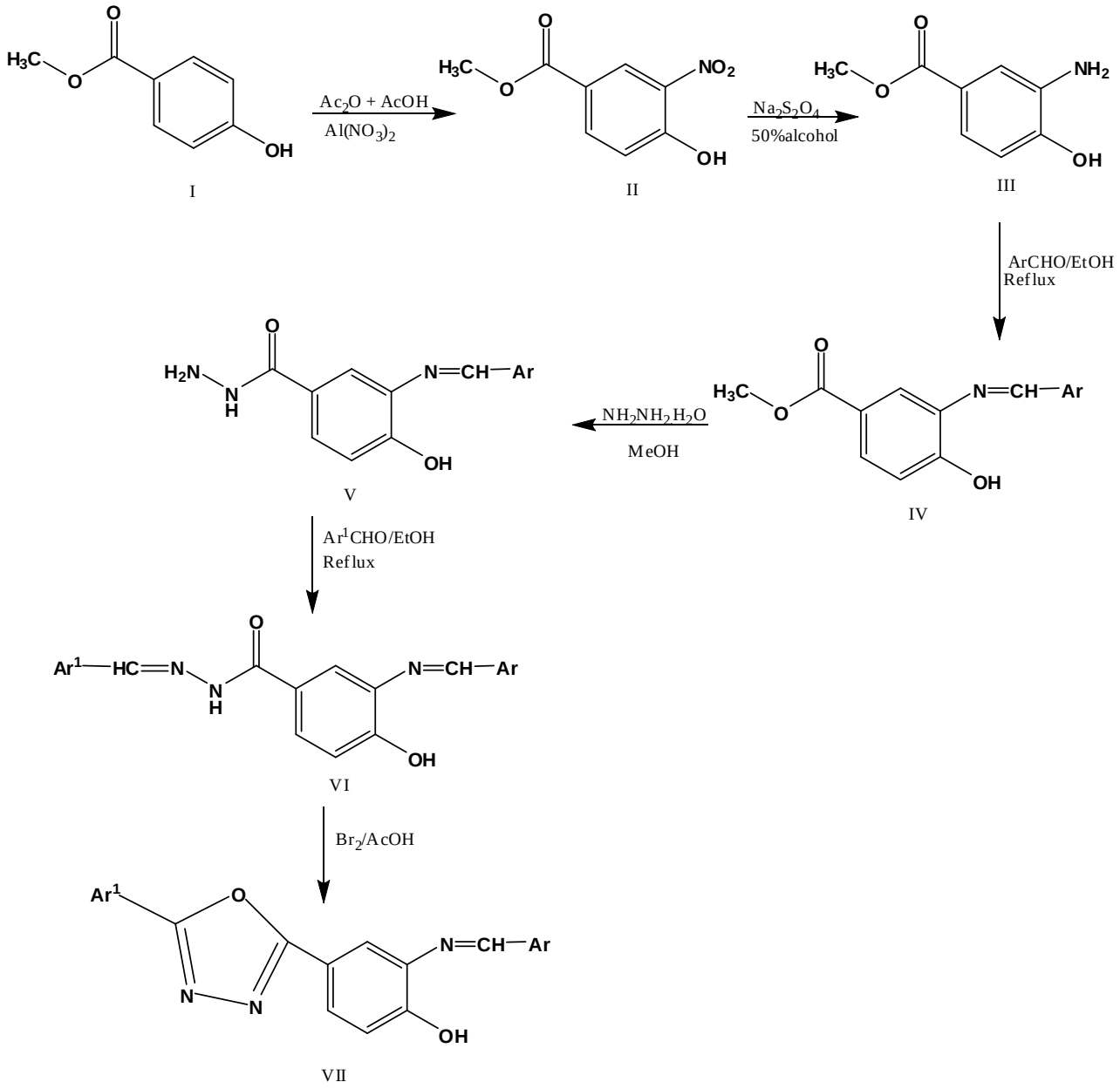
S.No	Compound.	Zone of inhibition (mm)			
		Gram positive bacteria		Gram negative bacteria	
		Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Proteus vulgaris*
1	**VIIa**	18	20	22	27
2	**VIIb**	16	21	23	27
3	**VIIc**	19	18	21	28
4	**VIId**	24	28	28	34
5	**VIIe**	14	17	21	22
6	**VIIf**	20	21	24	25
7	**VIIg**	18	16	20	24
8	**VIIh**	12	14	18	22
9	**VIIi**	19	21	18	24
10	**VIIj**	15	19	22	24
11	**VIIk**	19	24	28	28
12	**VIIl**	22	26	28	34
13	**VIIm**	21	27	24	26
14	**VIIn**	20	24	28	27
15	**VIIo**	12	14	17	18
16	**VIIp**	20	26	28	32
Standard	**Ampicillin(10µg/ml)**	**22**	**26**	**26**	**32**

Solvent: Dimethylformamide; **Concentration**: 0.1 mg/ml

TABLE-6: Antifungal activity of 2-(Arylideneamino)-4-(5-(Aryl)-1,3,4-oxadiazol-2-yl) phenols (GEMSa-p)

S.No	Compound.	Zone of inhibition (mm)		
		Aspergillus niger	*Candida Albicans*	*Curvularia lunata*
1	**VIIa**	10	10	08
2	**VIIb**	08	---	05
3	**VIIc**	19	19	19
4	**VIId**	---	12	10
5	**VIIe**	11	06	---
6	**VIIf**	12	05	07
7	**VIIg**	09	---	11
8	**VIIh**	---	11	06
9	**VIIi**	19	22	14
10	**VIIj**	08	08	---
11	**VIIk**	---	14	10
12	**VIIl**	03	---	09
13	**VIIm**	06	11	---
14	**VIIn**	09	09	08
15	**VIIo**	10	07	11
16	**VIIp**	18	24	15
Standard	**Clotrimazole (10µg/ml)**	**18**	**21**	**14**

Solvent: Dimethylformamide; **Concentration**: 0.1 mg/ml



2) Antifungal activity [British Pharmacopoeia,1953**]:**
All those compounds screened for antibacterial activity were also tested for their antifungal activity. The fungi employed for screening were: *Candida albicans* and *Aspergillus niger.*

The test organisms were sub-cultured using potato-dextrose-agar medium. The tubes containing sterilized medium were inoculated with test fungi and after incubation at 25°C for 48 hours, they were stored at 4°C in refrigerator. The inoculum was prepared by taking a loopful of stock culture to about 100 ml of nutrient broth, in 250 ml conical flasks. The flasks were incubated at 25°C for 24 hours before use. The solutions of test compounds were prepared by a similar procedure described under the antibacterial activity. A reference standard (1 mg/ml conc.) was prepared by dissolving 10 mg of Clotrimazole in 10 ml of dimethylformamide (AnalaR grade). Further, the dilution was made with dimethylformamide itself to obtain a solution of 100 µg/ml concentration.

The potato-dextrose-agar medium was sterilized by autoclaving at 121°C (15 lb/sq. inch) for 15 minutes. The petriplates, tubes and flasks with cotton plugs were sterilized in hot-air oven at 150°, for an hour. In each sterilized petriplate, about 27 ml of molten potato-dextrose-agar medium inoculated with respective fungus (6 ml of inoculum in 300 ml of potato-dextrose medium) was added, aseptically. After

solidification of the medium at room temperature three discs of 6 mm diameter were made in each plate with a sterile borer. Accurately 0.1 ml (100 µg/disc) of test solution was transferred to the discs aseptically and labelled, accordingly. The reference standard 0.1 ml (10 mg/cup) was also added to the discs in each plate. The plates were kept undisturbed at room temperature for 2 hours, at least to allow the solution to diffuse properly into the potato-dextrose-agar medium. Then the plates were incubated at 25°C for 48 hours. The diameter of the zone of inhibition was read with the help of an antibiotic zone reader. The experiments were performed in triplicate in order to minimize the errors. The results are presented in Table 3.

RESULTS AND DISCUSSION

Methyl-4-hydroxy-3-nitrobenzoate was reduced with sodium dithionite in 50% boiling alcohol and it was condensed with various aromatic aldehydes to give the methyl-3-(Arylideneamino)-4-hydroxybenzoates (IV). The compound IV was condensed hydrazine hydrate (99%) in alcohol again the resultant compound was refluxed with various aromatic aldehydes and finally the targeted compound was synthesized by cyclization with Bromine in Acetic acid lead to 2-(Arylideneamino)-4-(5-Aryl)-1,3,4-oxadiazol-2-yl) phenols.

All the synthesized compounds were characterized and evaluated for antibacterial activity against *Bacillus subtilis Staphylococcus aureus*, *Escherichia coli and Proteus vulgaris* and antifungal activity against *Aspergillus niger*, *Curvularia lunata* and *Candida albicans* by using Ampicillin sodium and Clotrimoxazole as a standard.

The antibacterial activity results of novel 2-(Arylideneamino)-4-(5-(Aryl)-1,3,4-oxadiazol-2-yl)phenols (VII), Compound VIId (Ar=3,4,5-trihydroxyphenyl, Ar^1=phenyl) was comparatively more active against Gram positive and Gram negative with zone of inhibition of 24mm, 28mm, 28mm and 34mm against *B.subtilis, S.aureau, E.coli* and *P.vulgaris* respectively and Compound VIII (Ar=3,4,5-trihydroxyphenyl, Ar^1=4-chlorophenyl)was next in the order of exhibiting antibacterial activity against both Grame positive and Gram negative bacteria with zone of inhibition of 22mm, 26mm, 28mm and 34mm respectively against the test organism. Rest of the compounds showed mild to moderate activity against both Gram positive and Gram-negative bacteria.

The results revealed that compounds 2-(Arylideneamino)-4-(5-(Aryl)-1,3,4-oxadiazol-2-yl) phenols (VII), Compounds VIIp and VIIiare more active among all the compounds. Compound VIIp(Ar=3,4,5 trihydroxy phenyl, Ar^1=3,4,5-trihydroxyphenyl) is more active against *A.niger, C.albicans* and *C.lunata,* with zone of inhibition of 18mm, 24mm, and 15mm respectively and compound VIIi(Ar=4-chlorophenyl, Ar^1=4-chlorophenyl) was more active against *A.niger, C.albicans* and *C.lunata* with zone of inhibition of 19mm, 22mm, and 14mm respectively. Remaining compounds showed mild to moderate activity against test organisms. Surprisingly some of the compounds were inactive against test organism.

CONCLUSION

This study reports the successful synthesis of the title compounds in good yields and moderate to potent anti-microbial activity of these derivatives containing 1,3,4-oxadiazole moiety which is comparable with standard drug.

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