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Synthesis And Anti-Tubercular Evaluation of Certain Pyrrole Oxadiazole Derivatives

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Abstract

The various novel nitrogen containing heterocyclic compounds were synthesized and are screened for antibacterial and antitubercular activities, purity of newly synthesized compounds confirmed by using TLC and structures for the same compounds were confirmed by using IR, NMR, ^{13}C and Mass spectrum. Pyrrole ring was constructed by reacting it with 2,5-dimethoxytetrahydrofuran in presence of glacial acetic acid to obtain ethyl 4-pyrrol-1-ylbenzoate **(2)** in good yield. Conversion of ethyl 4-pyrrol-1-ylbenzoate **(2)** into 4-pyrrol-1-yl-benzoic acid hydrazide **(3),** which was achieved by refluxing ethyl 4-pyrrol-1-ylbenzoate (**2)** with hydrazine hydrate in refluxing ethanol. Then this 4-pyrrol-1-yl-benzoic acid hydrazide **(3)** treated with appropriate benzaldehyde to yield compound substituted 2-(4-(1H-pyrrol-1-yl) phenyl)-5-phenyl-1,3,4-oxadiazole **04(a-f).** Structures of newly synthesised compounds were confirmed on the basis of physico-chemical and spectral data (IR, ^{1}H &^{13}C-NMR, Mass spectra). All the synthesised compounds were screened for anti-tubercular activity using Microplate Almar Blue Assay (MABA) method. Compounds showed anti-tubercular activity at MIC values between 50 to 3.12 µg/ml when compared with standard drugs Pyrazinamide (3.125 µg/ml) and Streptomycin (6.25 µg/ml). Newly synthesised compounds were also screened for antibacterial activity using broth micro dilution assay method. Compounds showed antibacterial activity at MIC values between 25 to 0.8 µg/ml when compared with standard drugs Ciprofloxacin and Norfloxacin.

Keywords

- pyrrole oxadiazole, anti-tubercular, anti-bacterial, dimethyl sulfoxide.

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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 increasing incidence of invasive pneumococcal diseases in children younger than 5 years of age. It means that antibiotic resistance directly contributes to increasing incidence of invasive diseases.[1]

The main mycobacterial infections in humans are *tuberculosis* and *leprosy* both typically chronic infections; caused respectively by *Mycobacterium tuberculosis* and *Mycobacterium leprae*. Tuberculosis was for centuries a major killer disease, less than 40 years ago, new drugs were developed and put to use and tuberculosis came to be seen as an easily curable infection. This is so, no longer the mycobacterium which causes it has come back to haunt us, the multidrug-resistant strains are now common and

strains with increased virulence have emerged.[2-3] Tuberculosis is a major threat; killing about 2 million people each year. The World Health Organization estimates that 1 billion people will be newly infected in the period 2000-2020, resulting in 35 million more deaths. TB is currently the leading killer of youths, women, and AIDS patients in the world.[4]

HIV infection is the highest risk factor so far identified which increases the chance of latent infection with tubercle bacilli progressing to active tuberculosis by reducing the protection provided by cell mediated immunity. HIV infected persons have a higher frequency of extra-pulmonary tuberculosis, which is more difficult to diagnose than pulmonary tuberculosis. As the association between tuberculosis and HIV infection becomes more widely known, the diagnosis of tuberculosis will begin to carry an additional social stigma. Tuberculosis is out of control in many parts of the world and it is now the world's leading cause of death from a single agent.[5-8]

The situation has become more critical because of the presence of some complicating factors like, emergence of multi-drug resistant tuberculosis[9], HIV co-infection[6], lack of patient compliance with chemotherapy, and variable efficacy of Bacilli-Calmette Guerin (BCG) vaccine. Multi drug resistant tuberculosis is defined as disease due to *M. tuberculosis* that is resistant to Isoniazid and Rifampicin with or without resistance to other drugs. In the years to come tuberculosis is bound to be an important health problem, particularly in immuno-compromised host.

Tuberculosis (TB) has infected man since the birth of civilization and despite the introduction of TB chemotherapy in the 1950s it has made a dramatic resurgence in the past decades and it still remains a leading infectious disease worldwide.[10] the major factors explaining the explosion in numbers of infections with *Mycobacterium Tuberculosis* (MTB) are: (i) the deadly synergy with HIV,[11-12] (ii) the development and spread of multidrug-resistant strains of MTB (MDR-TB),[13–15] resistant to all the first-line drugs, and, (iii) more recently, the emergence of extensively drug resistant MTB strains (XDR-TB),[16-17] also resistant to three or more of the six classes of second-line drugs and untreatable using currently available anti-TB drugs. With this background, there have been no new drugs to treat TB, except fluoroquinolone antibiotics recently introduced,[18] in the last 40 years. This reflects the inherent difficulties in developing and clinical testing new agents as well as the lack of pharmaceutical industry research in the area.[19] The priority is the development of a new agent that will shorten the duration of chemotherapy from the current 6-8 months to 2 months or less, although new drugs with activity against MDR- and XDR-TB and latent TB are also needed.

The recent rise in tuberculosis cases and especially the increase of drug resistant Mycobacteria indicate an urgent need to develop new anti-tuberculosis drugs.

Although many active anti-TB agents have since been developed, a disturbing co-occurrence with the use of these drugs as single agents has been the development of drug resistance. While the development of this resistance can be forestalled through the use of combination regimens.[20]

Pyrrole and its derivatives have shown to possess biological activities such as antibacterial[21], antitumor[22], analgesic[23], antitubercular[24,25], anti-inflammatory and anti-allergic.[26] Several macromolecular antibiotics were isolated from biological sources and their activities were defined.[27,28.]

1,3,4-Oxadiazole ring is associated with many types of biological properties such as anti-inflammatory[29-31], hypoglycemic[32], antifungal and antibacterial[33-37] activities. On the other hand, some 5- [isoxazolo [5,4-d] pyrimidinyloxy methyl] - 2 - substitutedphenylamino1,3,4-oxadiazole and -1,3,4-oxadiazole-2(3H)-thione derivatives[37] have been reported as significantly active antimicrobials against Staphylococcus aureus and Candida albicans.

In continued attempts to identify new potent antimycobacterial compounds, we now want to report the investigations on the use of 4-pyrrol-1-yl benzoic acid hydrazide for the synthesis of new pyrrolyl oxadiazole derivatives and evaluate the newly synthesized compounds for antitubercular activity.

MATERIALS AND METHODS USED:

Chemicals are used in the synthesis of the compounds described were purchased from Sigma Aldrich, Spectrochem Pvt Ltd, and S. D. Fine Chem. Ltd, Avra All the chemicals and solvents are purified either by recrystallization or distillation method.

Instruments:

Melting points of synthesized compounds were determined in SHITAL-Digital programmable melting point apparatus [SSI -22(B)] and sometimes using Thiles Tube are uncorrected; IR spectra were recorded on Bruker- α T spectrophotometer by using KBr pellets. The ^{1}H NMR and ^{13}C NMR was recorded on Bruker Avance**III** NMR400/100 MHz instruments using dimethyl sulfoxide (**DMSO-d6)** as solvents and TMS as internal standard, chemical shifts are expressed as δ values (ppm) and the splitting of the

NMR spectra are termed as singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quartret (q), multiplet (m). Mass spectra on Water-Q-Tof Premier-HAB213, all the compounds' spectra showed the data in consistent with the projected structure and analytical thin layer chromatography (TLC) was performed by using Silica Gel GF and the movement of the solvent is identified by using ultravioletlamp.

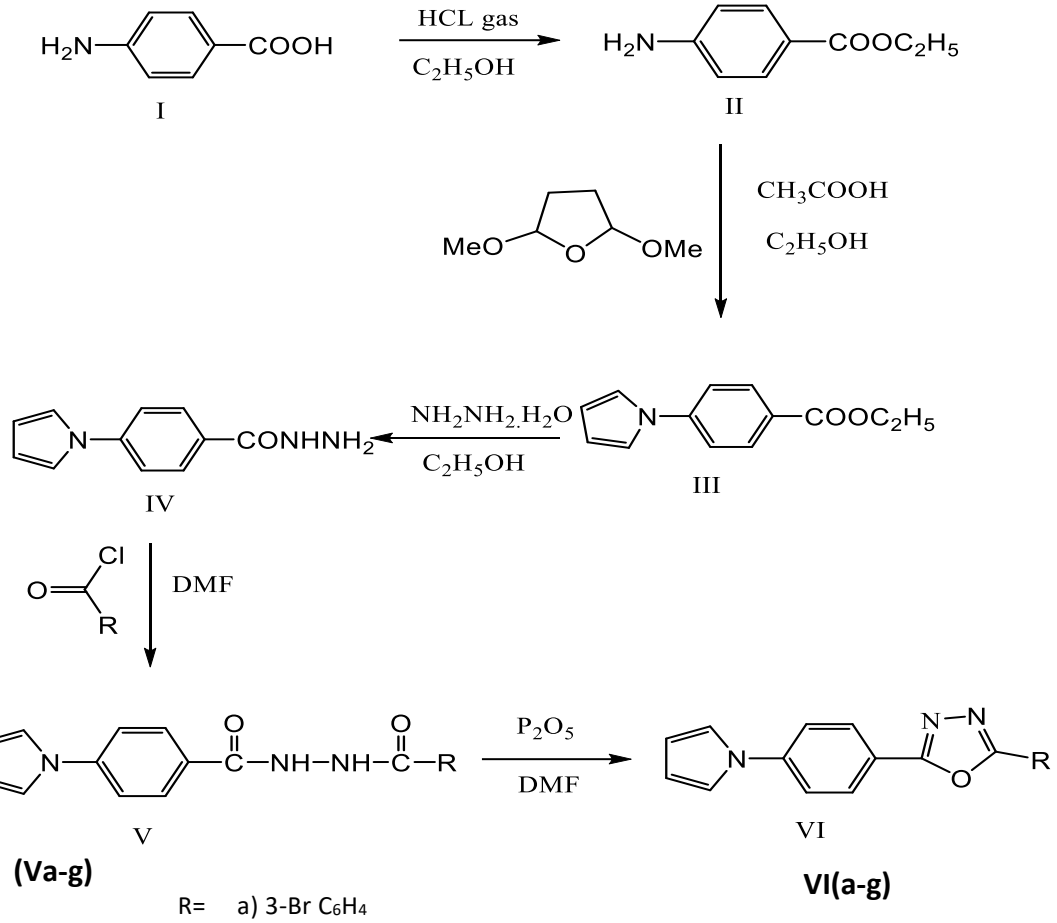
Chemical synthesis:

aminobenzoate II by passing HCl gas in dry ethanolic solution of compound **I.** By utilizing amino functionality in **II,** pyrrole ring was constructed by reacting it with 2,5-dimethoxytetrahydrofuran in presence of glacial acetic acid to obtain ethyl 4-pyrrol 1-yl- benzoate **III** in good yield.

Conversion of ester **III** into 4-pyrrol 1-yl-benzoic acid hydrazide **IV** was straightforward, which was achieved by refluxing ester **III** with hydrazine hydrate in refluxing ethanol.

Then this hydrazide **IV** treated with appropriate acyl chlorides produced the intermediates **V(a-g)** which on cyclisation with P_2O_5 in dry DMF gave 5-substituted oxadiazole **VI(a-g).**

SCHEME:

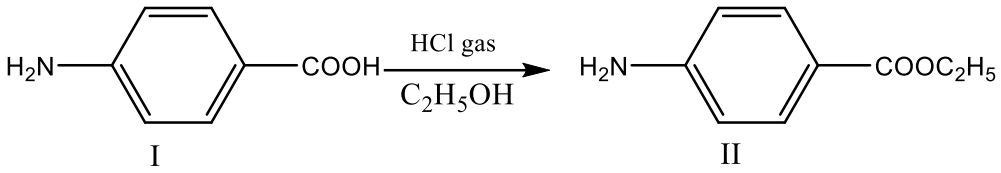


(Va-g) **VI(a-g)**

R= a) 3-Br C_6H_4
b) 4- F C_6H_4
c) 3-F C_6H_4
d) 2-F C_6H_4
e) 2-Cl C_6H_4
f) 3-Cl C_6H_4
g) 4-Cl C_6H_4

Synthesis of ethyl 4-aminobenzoate (II)

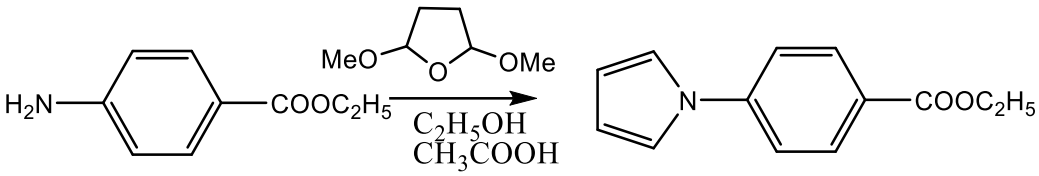
Dry absolute ethanol (80) ml was placed in two necked flask equipped with a double surface reflux condenser and a gas inlet tube. Dry hydrogen chloride gas was passed through the dry ethanol until saturated. 4-Aminobenzoic acid (12g, 0.088 mol) was introduced and the mixture was heated under reflux for 2 hr. upon cooling, the reaction mixture sets to a solid mass of the hydrochloride of ethyl 4-aminobenzoate. The hot solution was poured into water (300 ml) and sodium carbonate was added carefully to the clear solution until it was neutral to litmus. The product thus separated was filtered and recrystallized from ethanol.



I II

Synthesis of ethyl 4-pyrrol-1-yl Benzoate (III):

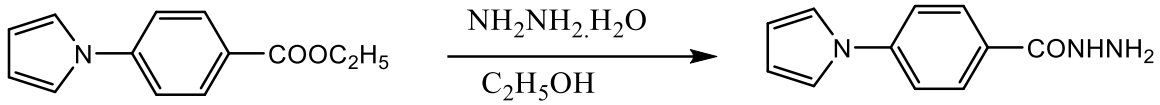
2,5-Dimethoxytetrahydrofuran (16g, 0.12 mol) was added to ethyl 4-aminobenzoate (16.5 0.1 mol) in ethanol (100ml) and this mixture was heated at reflux in presence of catalytic amount of acetic acid (0.05ml) for 3hour. After removal of solvent, product is filtered and recrystallized from ethanol.



III

Synthesis of 4-Pyrrol-1-yl benzoic acid hydrazide (IV):

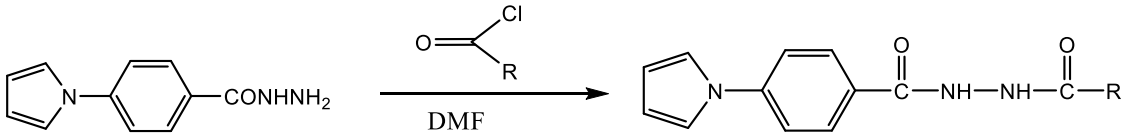
Compound IV was synthesized by refluxing a mixture of ethyl 4-pyrrol-1-yl benzoate (3.22 g, 0.015 mol) reflux for 3 hours with hydrazine hydrate (10 ml) in absolute ethanol (10 ml). The reaction mixture was cooled, and crystalline mass obtained was recrystallized from ethanol to secure yellow crystals.



IV

General procedure for the synthesis of N-(aryl carbonyl)-4-(1H-pyrrol-1-yl) benzo hydrazide derivatives:

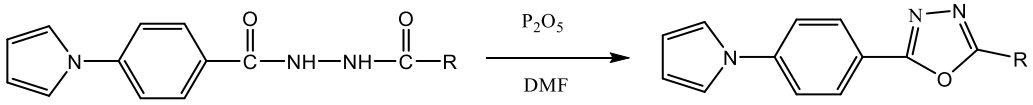
A mixture of 10 (1 mmol) and substituted acyl chlorides (1.5 mmol) in 10 mL of dry DMF was refluxed for 7–8 h (monitored by TLC). The reaction mixture was cooled and slowly quenched onto crushed ice with stirring and neutralized with saturated sodium bicarbonate solution. The solid which separated was filtered, washed with cold ethanol, dried and recrystallized from aqueous DMF to give the products.



V

General procedure for the synthesis of 2-aryl-5-[4-(1Hpyrrol-1-yl) phenyl]-1,3,4-oxadiazole derivatives (VIa-g)

To a suspension of V(a-g) (0.6g. 0.001) in dry DMF (50 mL), phosphorus pentoxide (2.0 g) was added portion wise with stirring at room temperature and the reaction mixture was refluxed for 4 h. The reaction mixture was cooled and then filtered. The resulting solid that separated was washed with water, dried and recrystallized from aqueous DMF to give the products.



Va-g V VI **VIa-g**

BIOLOGICALSCREENING

Evaluation of antitubercular Activity: Micro Plate Almar Blue Assay method

Procedure: -

1. The anti-mycobacterial activity of compounds was assessed against *M.tuberculosis* using micro plate Alamar Blue assay (MABA) method.
2. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometricmethod.
3. Briefly, 200 µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation.
4. The 96 wells plate received 100µl of the Middle brook 7H9 broth and serial dilution of compounds were made directly on plate.
5. The final drug concentrations tested were 100 to 0.2µg/ml.
6. Plates were covered and sealed with parafilm and incubated at 37 ºC for five days.
7. After this time, 25 µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hr.
8. A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored asgrowth.
9. The MIC was defined as the lowest drug concentration which prevented the colour change from blue topink.

Evaluation of antibacterial activity:

The Minimum Inhibitory Concentration (MIC) determination of the tested compounds was investigated in side-by-side comparison with ciprofloxacin against Gram-positive (Staphylococcus aureus, Bacillus subtilis) and Gram-negative bacteria (Klebsiella pneumoniae, Escherichia coli) by broth micro dilution method.

Materials and methods:

1. Mueller-Hintonagar
2. McFarland turbiditystandards
3. Scrupulously clean, acid-washed borosilicate glass tubes
4. Micropipette
5. Nutrientagar

Preparation of media:

Sterilization of media and glassware:

The media used in the present study, Mueller-Hinton agar and nutrient agar were sterilized in conical flasks of suitable capacity by autoclaving at 15lb pressure for about 20 minutes. The test tubes and pipettes were sterilized in a hot air oven at 160^0C for 1 h.

Preparation of solution of test compounds:

Serial dilutions of the test compounds and reference drugs were prepared in Mueller-Hinton agar. Drugs (1mg) were dissolved in chloroform (CHCl3, 1ml). Further progressive dilutions with melted Mueller-Hinton agar were performed to obtain the required concentrations of 0.2, 0.4, 0.8, 1.6, 3.12, 6.25, 12.5, 25, 50 and 100 µg/ml.

Preparation of the Inoculums:

The organisms were sub-cultured on to nutrient agar and incubated overnight at 35^0C. The tubes that contain 2ml of Muller-Hinton agar inoculated with five or more colonies from the agar plate and turbidity was adjusted to match a 1 McFarland standard (10^5cfu/ml) and incubated at 37^0 C for 18hrs. The MIC was the lowest concentration of the tested compound that yields no visible growth on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with CHCl3 at the same dilutions as used in the experiments and CHCl3 had no effect on the microorganisms in the concentrations studied.

Enzyme inhibition studies:

***InhA* expression and purification:**

The production and purification of InHA-6xHis protein from a protease-deficient strain of *E. coli* (BL21) transformed with pHAT5/*InhA* plasmid were performed as follows. A

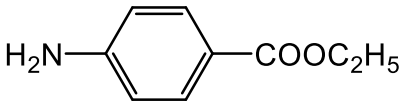
1 mL of bacteria was grown in 100 mL of Lysogeny broth (LB) medium containing ampicillin (100 µg/mL) and 2% glucose at 37°C. After 4 h, the solution was re-diluted in 1 L of the same mediumandre-grown at 37°C.After attaining the proper concentration (OD595=0.6 - 0.8), the culture was centrifuged at 3300 g factor for 10 min at 4°C and bacteria were suspended in LB medium containing ampicillin (100 µg/mL). Protein expression was induced for overnight incubation in 1 mM isopropyl-β-D-galactopyranoside (IPTG) at 20°C. Cells were harvested by centrifugation at 6000 g for 30 min at 4°C. Dry pellet was kept at -80°C for several months and purification was done with Ni-NTA Agarose from QIAGEN following the manufacturer's protocol. The purified recombinant protein was applied to PD- 10 desalting columns (GE Healthcare, Piscataway, NJ) equilibrated with PIPES 30 mM pH 6.8 and 150 mM NaCl to remove the imidazole. Samples were analyzed using SDS-PAGE and Coomassie blue staining and then stored at 4°C for a short-term storage at -80°C with 20% glycerin for long-term storage.

***InhA* activity inhibition**

Triclosan and NADH were obtained from Sigma-Aldrich. Stock solutions of the selected compounds were prepared in DMSO such that the final concentration of this co- solvent was constant at 5% (v/v) in the final volume of 1 mL for all the kinetic reactions. Kinetic assays were performed using trans-2-dodecenoyl-coenzyme A (DDCoA) and wild type *InhA*as previously described. Briefly, the reactions were performed at 25°C in an aqueous buffer (30 mM PIPES and 150 mM NaCl pH 6.8) containing 250 µM cofactor (NADH), 50 µM substrate (DDCoA) and the test compound (at 50 µM). Reactions were initiated by adding *InhA* (100 nM final) and NADH oxidation was monitored at the fixed wavelength of 340 nm. Inhibitory activity of each derivative was expressed as % inhibition of *InhA*activity (initial velocity of the reaction) with respect to control reaction without the inhibitor.

RESULTS AND DISCUSSION

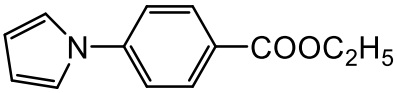
Table 1. Physico-chemical data of ethyl 4-aminobenzoate (II)



II

Compound	R	Yield (%)	Recrystallization solvent	M.P. (°C)	Molecular Formula
II	-	65	Ethanol	89-90	$C_9H_{11}NO_2$

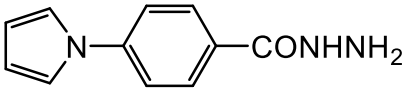
Table 2. Physico-chemical data of ethyl 4-pyrrol 1-yl-benzoate (III):



III

Compound	R	Yield (%)	Recrystallization solvent	M.P. (°C)	Molecular Formula
III	-	70	Ethanol	74-75	$C_{13}H_{13}NO_2$

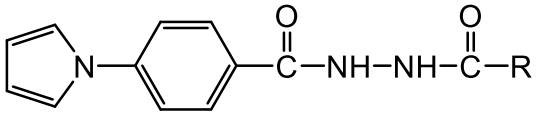
Table 3: Physicochemical data of 4-pyrrol-1-yl benzoic acid hydrazide (IV)



IV

Compound	R	Yield (%)	Recrystallization solvent	M.P. (°C)	Molecular Formula
IV	-	75	Ethanol	180-182	$C_{11J}H_{11}N_3O$

Table 3: Physico-chemical data of *N*'-(arylcarbonyl)-4-(1H-pyrrol-1-yl) benzohydrazide derivatives (Va-g)

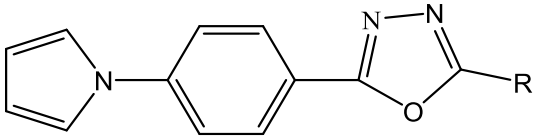


Va-g

Comp.	R	Yield (%)	Recrystallization solvent	M.P.(^{0}c)	Rf *	Molecular Formula	Mol. weight
Va	3-Br C_6H_4	66	DMF	244-246	0.735	$C_{18}H_{14}BrN_3O_2$	283.03
Vb	4-F C_6H_4	76	DMF	260-262	0.754	$C_{17}H_{14}FN_3O_2$	323.23
Vc	3-F C_6H_4	65	DMF	288-290	0.777	$C_{17}H_{14}FN_3O_2$	323.23
Vd	2-F C_6H_4	63	DMF	264-266	0.715	$C_{18}H_{14}FN_3O_2$	323.23
Ve	2-Cl C_6H_4	68	DMF	278-280	0.759	$C_{18}H_{14}ClN_3O_2$	339.78
Vf	3-Cl C_6H_4	79	DMF	277-279	0.712	$C_{18}H_{14}ClN_3O_2$	339.78
Vg	4-Cl C_6H_4	74	DMF	251-253	0.723	$C_{18}H_{14}ClN_3O_2$	339.78

Silica Gel was used as stationary phase, Chloroform: methanol (9:1) as mobile phase and iodine vapour as visualizing agent

Table 4. Physico-chemical data of 2-aryl-5-[4-(1H-pyrrol-1-yl) phenyl-1,3,4-oxadiazole derivatives (VIa-g)

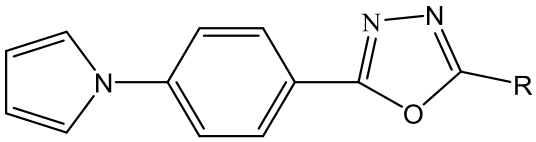


VIa-g

Comp.	R	Yield (%)	Recrystalisation solvent	M.P.(^{0}c)	Rf *	Molecular Formula	Mol. weight
VIa	3-Br C_6H_4	64	DMF	246-249	0.881	$C_{18}H_{12}BrN_3O$	287.33
VIb	4-F C_6H_4	76	DMF	252-254	0.843	$C_{17}H_{12}FN_3O$	305.31
VIc	3-F C_6H_4	66	DMF	263-265	0.804	$C_{17}H_{12}FN_3O$	305.31
VId	2-F C_6H_4	56	DMF	250-252	0.854	$C_{18}H_{12}FN_3O$	305.31
VIe	2-Cl C_6H_4	55	DMF	248-250	0.818	$C_{18}H_{12}ClN_3O$	321.76
VIf	3-Cl C_6H_4	69	DMF	233-235	0.846	$C_{18}H_{12}ClN_3O$	321.76
VIg	4-Cl C_6H_4	70	DMF	251-255	0.803	$C_{18}H_{12}ClN_3O$	321.76

Silica Gel was used as stationary phase, Chloroform: methanol (9:1) as mobile phase and iodine vapour as visualizing agent.

Table 5. Physico-chemical data of 2-aryl-5-[4-(1H-pyrrol-1-yl) phenyl-1,3,4-oxadiazole derivatives (VIa-g)



VIa-g

Comp.	R	Yield (%)	Recrystallization solvent	M.P.(^{0}c)	Rf *	Molecular Formula	Mol. weight
VIa	3-Br C_6H_4	64	DMF	246-249	0.881	$C_{18}H_{12}BrN_3O$	287.33
VIb	4-F C_6H_4	76	DMF	252-254	0.843	$C_{17}H_{12}FN_3O$	305.31
VIc	3-F C_6H_4	66	DMF	263-265	0.804	$C_{17}H_{12}FN_3O$	305.31
VId	2-F C_6H_4	56	DMF	250-252	0.854	$C_{18}H_{12}FN_3O$	305.31
VIe	2-Cl C_6H_4	55	DMF	248-250	0.818	$C_{18}H_{12}ClN_3O$	321.76
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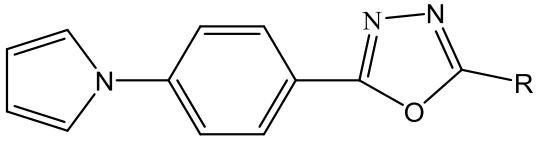
VIg	4-Cl C_6H_4	70	DMF	251-255	0.803	$C_{18}H_{12}ClN_3O$	321.76

Silica Gel was used as stationary phase, Chloroform:methanol (9:1) as mobile phase and iodine vapour as visualizing agent.

Table 6. Spectral data of2-aryl-5-[4-(1H-pyrrol-1-yl) phenyl-1,3,4-oxadiazole derivatives (VIa-g)



Via-g

Compound	R	IR spectra KBr cm^{-1}	^{1}H NMR spectra(δ-ppm)	^{13}C NMR Spectra (δ-ppm)	MASS Spectra
VIa	3-BrC_6H_4	**KBr:** 1644.99 (C=N), 1068.44 (C-O-C), 2918.57 (Ar CH=CH Stre). **Spectrum no :01**	**(DMSO-*d_6*): 400 MHz:** 7.50-8.17 (m, 12H pyrrole, $C_{3,C4}$, C_5-H, bridging phenyl C_2, C_3, C_5, C_6-H & Phenyl C_2, C_4, C_5, C_6-H). **Spectrum no :02**	**(DMSO-*d_6*): 500 MHz:** 118.45 (pyrrole C_3, C_4), 119.50 (bridging phenyl C_2, C_6), 120.44 (pyrrole C_2, C_5), 121.70(bridging phenyl C_4), 126.46 (bridging phenyl C_3, C_5), 126.89 (phenyl C_5), 127.41(phenyl C_2), 130.02 (phenyl C_1), 136.69 (phenyl C_3, C_6), 142.04 (bridging phenyl C_1),	------

				165.23 (Oxadiazole C_5), 164.34 (Oxadiazole C_2). **Spectrum no :03**	
VIb	4-F C_6H_4	**KBr:** 1604.81 (C=N), 1101.05 (C-O-C), 2924.34 (Ar CH=CH Stre). **Spectrum no :04**	**(DMSO-*d*$_6$): 400 MHz** 7.36-8.08 (m, 12H pyrrole C_2, C_3, C_4, C_5-H, bridging phenyl C_2, C_3, C_5, C_6-H & Phenyl C_2, C_3, C_5, C_6-H). **Spectrum no :05**	-------	**(DMSO-*d*$_6$):** Found= 306.22 [M^{+1}] Calcd= 305.31 SPECTRU NO: 13
VIc	3- F C_6H_4	**KBr:** 1647.04 (C=N), 1071.28 (C-O-C), 2923 (Ar CH=CH Stre). **Spectrum no :07**	**(DMSO-*d*$_6$): 400 MHz** 6.33 (t, 2H pyrrole C_3, C_4-H), 7.53(t, 2H Pyrrole C_2, C_5-H) & 7.60-8.03(m, 8H, bridging phenyl C_2, C_3, C_5, C_6-H & Phenyl C_2, C_4, C_5, C_6-H). **Spectrum no :08**	------	------
VId	2- F C_6H_4	**KBr:** 1637.46 (C=N), 2922.5 (Ar CH=CH Stre). **Spectrum no :09**	**(DMSO-*d*$_6$): 400 MHz** 6.33-8.12 (m, 12H pyrrole C_2, C_3, C_4, C_5-H, bridging phenyl C_2, C_3, C_5, C_6-H & Phenyl C_3, C_4, C_5, C_6-H). **Spectrum no :10**	------	------
VIe	2- ClC_6H_4	**KBr:** 1642.52 (C=N), 2922.55 (ArCH=CHStre), 1182.89 (C-O-C). **Spectrum no :11**	-----	-------	-------
VIf	3- ClC_6H_4	**KBr:** 2846.91 (Ar CH=CH Stre), 1686 (C=O), 1094 (C-O-C). **Spectrum no :12**	------	-------	------
VIg	4- ClC_6H_4	**KBr:** 2922.41 (Ar CH=CHstre), 1645.20 (C=O). **Spectrum no :13**	--------	-------	-------

Table no: 7 LIPINSKI RULE OF 5

Compound	Mol. wt	Hydrogen bond donor	Hydrogen bond acceptor	Log p	Lipinskis violations
VIa	365	0	3	4.95	0
VIb	305	0	3	4.33	0
VIc	305	0	3	4.33	0
VId	305	0	3	4.33	0
VIe	321	0	3	4.84	0
VIf	321	0	3	4.84	0
VIg	321	0	3	4.84	0

Physicochemical properties of the all the compounds shows the 0 (Zero) violations of Lipinski's Rule of Five.

Biological Evaluation

Antitubercular activity:

All the newly synthesized compounds were screened for the antitubercular activity by Alamar Blue Dye using Microplate Alamar Blue Assay (MABA) method. Pyrazinamide and Streptomycin were used as standard drugs and activity of all the newly synthesized compounds was measured against them. The MIC was measured.

Table 8: Reveals the antitubercular activity (MIC) of2-aryl-5-[4-(1H-pyrrol-1-yl) phenyl-1,3,4-oxadiazole deruvatives(VIa-g)

Table 8: Antitubercular screening-Alamar Blue Dye using Microplate Alamar Blue Assay (MABA) method (MIC µg/mL) and enzyme inhibition assay:

Compounds	R	*M. tuberculosis* $H_{37}Rv$ MIC values (µg/mL)	% Inhibition of InhA at 50 µM
VIa	3-Br C_6H_4	3.25	Compounds will be sent to InhA inhibition assay
VIb	4-F C_6H_4	1.6	
VIc	3- F C_6H_4	1.6	
sVId	2- F C_6H_4	1.6	
VIe	2- Cl C_6H_4	12.5	
VIf	3- Cl C_6H_4	12.5	
VIg	4- Cl C_6H_4	12.5	
Pyrazinamide	----------	3.12 µ/ml	
Streptomycin	----------	6.25 µ/ml	
Triclosan	----------	---------	>99

NT – NOT TESTED, **NI** – NO INHIBITION

Table 9: *In vitro* evaluation of antibacterial activity (MIC values in µg/mL)

Compounds	Gram Negative	Gram Positive
	***E. coli* (µg/mL)**	***S. aureus* (µg/mL)**
VIb	25 µg/mL	50 µg/mL
VIc	50 µg/mL	25 µg/mL
Ciprofloxacin	2 µg/mL	2 µg/mL

SUMMARY AND CONCLUSION

The present work was intended to synthesize and evaluation of certain pyrrolyl oxadiazole derivatives for their antibacterial and antitubercular activities. The detailed review of literature was carried out for the synthesis. Heterocyclic compounds of pyrrole clubbed with oxadiazole were synthesised as described in scheme **I.**

Pyrrole ring was constructed by reacting it with 2,5-dimethoxytetrahydrofuran in presence of glacial acetic acid to obtain ethyl 4-pyrrol-1-ylbenzoate **(2)** in good yield. Conversion of ethyl 4-pyrrol-1-ylbenzoate **(2)** into 4-pyrrol-1-yl-benzoic acid hydrazide (3), which was achieved by refluxing ethyl 4-pyrrol-1-ylbenzoate (2) with hydrazine hydrate in refluxing ethanol. Then this 4-pyrrol-1-yl-benzoic acid hydrazide (3) treated with appropriate benzaldehyde to yield compound substituted 2-(4-(1H-pyrrol-1-yl) phenyl)-5-phenyl-1,3,4-oxadiazole **04(a-f).**

Structures of these newly synthesised compounds were confirmed on the basis of their physico-

chemical and spectral data such as IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectra.

The compounds synthesised in the present work were screened for antibacterial activity against Gram +ve *(Staphylococcus aureus, Bacillus subtilis)* and Gram –ve (*Klebsiella pneumoniae, Escherichia coli)* by using microdilution broth method and antitubercular activity against *Mycobacterium tuberculosis* H_{37}Rv by using Microplate Almar Blue Assay (MABA) method.

The investigation of anti-tubercular screening data revealed that all the tested compounds showed significant activity against *M. Tuberculosis* at range of 3.12 to 50 µg ml. Compound **4c** were showed highly significant activity at 3.12 µg/ml and Compounds **4b and 4e** showed significant activity at 6.25µg/ml against *M. Tuberculosis*. Results were depicted in **Table No-06**. From the investigation of antibacterial screening data, it is revealed that all tested compounds showed moderate to good microbial inhibition. Compounds showed antibacterial activity at MIC values of 25-0.8 µg/ml. The compounds 4d and 4e shows highly significant activity at MIC value of 1.6 µg/ml against gram positive bacteria such as *B. subtilis.* Compounds 4d and 4f shows highly significant activity at MIC value of 0.8 µg/ml against gram negative bacteria such as *E. Coli.* Compounds 4a, 4d and 4f shows significant activity at MIC value of 1.6 µg/ml against gram negative bacteria such as *K. pneumonia and E. Coli.* Results were depicted in **Table No-07.**

Further studies in this area will not only help to explore the structural features for better efficacy of this class of compounds but also will be instrumental in development of newer classes of antitubercular and antibacterial compounds.

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