INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

SYNTHESIS AND ANTITUBERCULAR EVALUATION OF CERTAIN PYRROLE DERIVATIVES

Sandhya Chinnamulagund, Ashwini S. Joshi, Chetana, Sravanthi Avunoori, V. H. Kulkarni, Shrinivas D. Joshi*

Novel Drug Design and Discovery Laboratory, Department of Pharmaceutical Chemistry, SET's College of Pharmacy, Sangolli Rayanna Nagar, Dharwad 580 002, India.

ARTICLE INFO

Article history
Received 06/03/2023
Available online
21/03/2023

Keywords
Pyrrole,
Antitubercular activity,
Antibacterial activity.

ABSTRACT

A series of 2-aryl-5-[4-(1*H*-pyrrol-1-yl)phenyl]-1,3,4-oxadiazole derivatives (**VIa-g**) have been synthesized in good yields. These compounds were synthesized with an approach to reduce the growing anti-tubercular resistance and to develop more potent and less side effects having antitubercular activity. The reaction of 2,5-dimethoxytetrahydro furan with 4-aminobenzoate (**II**) in presence of ethanol, which yields ethyl 4-pyrrol-1-yl benzoate (**III**). This ethyl-4-pyrrol-1-yl benzoate (**III**) on reaction with hydrazine hydrate produced 4-pyrrol-1-yl benzoic acid hydrazide (**IV**). This carbohydrazide on treatment with different substituted benzoyl chlorides gave intermediates (**Va-g**), which on cyclisation with P_2O_5 in the presence of DMF yielded of 2-aryl-5-[4-(1*H*pyrrol-1-yl)phenyl]-1,3,4-oxadiazoles **VI(a-g)**. Structure of newly synthesized pyrrole derivatives were confirmed on the basis of physicochemical and spectral data (IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectra). All the synthesized compounds were screened for their antitubercular activity using Microplate Alamar Blue Assay (MABA) method. Compounds showed anti-tubercular activity at MIC values between 1.6 to 12.5 µg/ml, compounds **VIb**,**VIc** and **VId** showed highest activity of 1.6 µg/ml. Selected compounds were evaluated for anti-bacterial activity against gram positive (*S. aureus*) and gram negative (*E. coli*). Compounds showed moderate activity. Physicochemical properties of the selected compounds satisfieds with the Lipinski's rule of 5.

Correspondingauthor
Dr. Shrinivas D. Joshi
Professor and Head,
Department of Pharmaceutical Chemistry
SET's College of Pharmacy,
S. R. Nagar, Dharwad – 580002, Karnataka.
+91 9986151953
shrinivasdj@rediffmail.com

*Please cite this article in press as **Shrinivas D. Joshi** et al. Synthesis and Antitubercular Evaluation of Certain Pyrrole Derivatives.Indo American Journal of Pharmaceutical Research.2023:13(03).*

INTRODUCTION

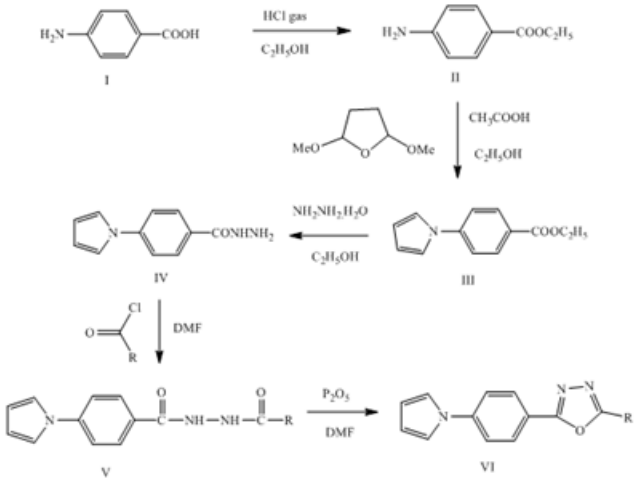
Tuberculosis (TB), a disorder, caused by *Mycobacterium tuberculosis (M. tuberculosis),* is the second leading disease causing the death along with HIV [1]. In the first update on the tuberculosis since the UN General Assembly High Level meeting on Sep 26, 2018, in which All UN Member States committed to end the global TB epidemic by 2030, WHO published the Global Tuberculosis Report on Oct 17, 2019. The report details data from 202 countries and territories that account for more than 99% of the world's population and estimated the number of TB cases. Ahead of first 2020 milestone, TB still accounts for the highest mortality from any infectious diseases worldwide, even surpassing HIV/AIDS, causing 1.5 million deaths in 2018. According to WHO's report, the current pace of change is not enough: the global cumulative rate of reduction for TB incidence was only 6.3% between 2015 and 2018, which is much less than that of the 2020 milestone of 20% [2]. TB is a major threat; killing about 2 million people each year. The World Health Organisation 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 [3].

Pyrrole is an important heterocycle of the plant and animal kingdom as a subunit of chlorophyll in plant cells, hemin and vitamin B_{12} in animal cells. First isolated in 1857 from the product of bone pyrolysis, it showed biological activities that are characteristic of haemoglobin [4]. Earlier, pyrrole derivatives have shown *in vitro* antitubercular activity [5,6] and recently, much of the research was carried out on anti-TB drug design using pyrrole as templets for the synthesis [7,8] including molecular modelling along with laboratory investigations [8,9]

Experimental

All the chemicals used in the synthetic experiment were purchased from Sigma-Aldrich, S. D. Fine-Chem Limited and spectrochem Pvt. Ltd. Solvent used were of reagent grade and they were purified and dried by standard methods. Melting points were determined using the Shital-digital programmable melting point apparatus and are uncorrected. FTIR spectra in KBr pellets were recorded on a Bruker FTIR spectrophotometer. The ^{1}H spectra were recorded on a Bruker AVANCE II at 400 MHz, chemical shifts are expressed in parts per million (*ppm*) relative to TMS. The abbreviations used to describe the peak patterns are: (s) singlet, (d) doublet, (t) triplet and (m) multiplet. Mass spectra (MS) were recorded in a JEOL GCMATE II GC – Mass spectrometer and Schimadzu QP 20105 GC-Mass spectrometer. Analytical thin layer chromatography (TLC) sheets of silica gel 60F254 (Merck, Darmstadt, Germany) visualized by long- and short- wavelength ultraviolet (UV) lamps.

SCHEME



R = 3-Br C_6H_4 , 4- F C_6H_4, 3-F C_6H_4 ,2-F C_6H_4, 2-Cl C6H4, 3-Cl C6H4,4-Cl C_6H_4

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

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

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

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

General procedure for the synthesis of N-(aryl carbonyl)-4-(1H-pyrrol-1-yl)benzhydrazide derivatives (Va-g)

A mixture of 4-pyrrol-1-yl benzoic acid hydrazide 1mmol and substituted acyl chlorides 1.5 mmol in 10 ml of dry DMF was refluxed for 7-8 hr (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.

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

To a suspension of **V(a-g)** (0.001 mol) in dry DMF (50 ml), phosphorous pentoxide (2.0 g) was added portion wise with stirring at room temperature and the reaction mixture was refluxed for 4 hr. 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.

2-(4-(1H-pyrrol-1-yl)phenyl)-5-(3-bromophenylphenyl)-1,3,4-oxadiazole (VIa)

IR (KBr) $\upsilon_{max,}$ cm^{-1}:1644.99 (C=N), 1068.44 (C-O-C), 2918.57 (Ar CH=CH Stre); ^{1}H NMR (400MHz, δ ppm, DMSO-d_6): 7.50-8.17 (m, 12H's of pyrrole-C_2, C_3, C_4, C_5-H, bridging phenyl- C_2, C_3, C_5, C_6-H and phenyl-C_2, C_4, C_5, C_6-H); ^{13}C NMR (500MHz, δ ppm, DMSO-d_6): 118.45, 119.50, 120.44, 121.70, 126.46 , 126.89, 127.41, 130.02, 136.69, 142.04, 165.23, 164.34.

2-(4-(1H-pyrrol-1-yl)phenyl)-5-(4-fluorophenyl)-1,3,4-oxadiazole (VIb)

IR (KBr) $\upsilon_{max,}$ cm^{-1}: 1604.81 (C=N), 1101.05 (C-O-C), 2924.34 (Ar CH=CH Stre); ^{1}H NMR (400MHz, δ ppm, DMSO-d_6): 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 and phenyl-C_2, C_3, C_5, C_6-H); MS (EI, DMSO-d_6): Found = 306.22 [M^{+1}], $_{Calcd}$ = 305.31.

2-(4-(1H-pyrrol-1-yl)phenyl)-5-(3-fluorophenyl)-1,3,4-oxadiazole (VIc)

IR (KBr) $\upsilon_{max,}$ cm^{-1}: 1647.04 (C=N), 1071.28 (C-O-C), 2923 (Ar CH=CH Stre); ^{1}H NMR (400MHz, δ ppm, DMSO-d_6): 6.33 (t, 2H pyrrole-C_3, C_4-H), 7.53 (t, 2H pyrrole-C_2, C_5-H) and 7.60-8.03 (m, 8H, bridging phenyl-C_2, C_3, C_5, C_6-H and phenyl- C_2, C_4, C_5, C_6-H).

2-(4-(1H-pyrrol-1-yl)phenyl)-5-(2-fluorophenyl)-1,3,4-oxadiazole (VId)

IR (KBr) $\upsilon_{max,}$ cm^{-1}: 1637.46 (C=N), 2922.5 (Ar CH=CH Stre); ^{1}H NMR (400MHz, δ ppm, DMSO-d_6): 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 and phenyl-C_3, C_4, C_5, C_6-H).

2-(4-(1H-pyrrol-1-yl)phenyl)-5-(2-chlorophenyl)-1,3,4-oxadiazole (VIe)

R (KBr) $\upsilon_{max,}$ cm^{-1}: 1642.52 (C=N), 2922.55 (Ar CH=CH Stre), 1182089 (C-O-C).

2-(4-(1H-pyrrol-1-yl)phenyl)-5-(3-chlorophenyl)-1,3,4-oxadiazole (VIf)

IR (KBr) $\upsilon_{max,}$ cm^{-1}: 2846.91 (Ar CH=CH Stre), 1686 (C=O), 1094 (C-O-C).

2-(4-(1H-pyrrol-1-yl)phenyl)-5-(4-chlorophenyl)-1,3,4-oxadiazole (VIf)

IR (KBr) $\upsilon_{max,}$ cm^{-1}: 2922.41 (Ar CH=CH Stre), 1645.20 (C=O).

Biological ativity:

Antitubercular activity

MIC values were determined for pyrrole derivatives against *M. tuberculosis* H37Rv strain by Microplate Alamar Blue assay (MABA) [10] using INH as the standard drug. The 96 wells plate received 100 µL of Middlebrook 7H9 broth and serial dilution of compounds were made directly on the plate with drug concentrations of 0.2, 0.4, 0.8, 1.6, 3.125, 6.25, 12.5, 25, 50 and 100 µg/mL. Plates were covered, sealed with parafilm and incubated at 37^0C for 5 days. Then, 25 µL of freshly prepared 1:1 mixture of alamar blue reagent and 10% Tween 80 were added to the plate and incubated for 24 hr. A blue color in the well was interpreted as no bacterial growth and pink color was scored as the growth. The MIC was defined as the lowest drug concentration, which prevented the color change from blue to pink. Table 2 reveals antitubercular activity (MIC) data.

Antibacterial activity

MIC determination of the tested compounds was investigated by aside-by-side comparison with norfloxacin and ciprofloxacin against Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. Coli*) by the broth microdilution method [11,12]. Serial dilution of the test compounds and reference drugs were prepared in Muller-Hinton agar. Drugs (10mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with the molten Muller-Hinton agar were performed to obtain the required concentrations of 0.2, 0.4, 0.8, 1.6, 3.125, 6.25, 12.5, 25, 50 and 100 mg/mL. The tubes were inoculated with 105 cfu mL^{-1} (colony forming unit/mL) and incubated at 37 C for 18h. MIC was the lowest concentration of the tested compound that yielded no visible growth on the plate. To ensure that solvent had no effect on bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and DMSO had no effect on the microorganisms in the concentration studied. Table 3 reveals antibacterial activity (MIC values) data.

RESULT AND DISCUSSION

Synthesis

Synthetic root adapted to obtain the target compounds are depicted in scheme. FTIR, ^{1}H NMR, ^{13}C NMR and mass spectral data are in agreement with the proposed structures of all the synthesized compounds. The physicochemical properties of the newly synthesized compounds are depicted in Table 1.

Table 1. Physical data of the synthesized compounds:

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

All the synthesized compounds of pyrrolyl oxadiazole derivatives (**VIa-g**) were obtained in a good yield and their structures were established by NMR spectral analysis, FTIR spectra of a compound **VIa** showed the presence of C=N peak at 1644.99 cm^{-1} and Ar CH=CH stretch at 2918.57 cm^{-1}, further the structure of the compound **VIa** was confirmed by ^{1}H NMR spectra which showed the presence of 12 hydrogens of pyrrole-C_2, C_3, C_4, C_5-H, bridging phenyl- C_2, C_3, C_5, C_6-H and phenyl-C_2, C_4, C_5, C_6-H, as multiplet at 7.50-8.17 ppm. ^{13}C NMR spectra showed a peak at 118.45 ppm for pyrrole C_3, C_4, 119.50 ppm for bridging phenyl C_2, C_6, 120.44 pyrrole C_2, C_5, 121.70 for bridging phenyl C_4, 126.46 for bridging phenyl C_3, C_5, 126.89 for phenyl C_5, 127.41 for phenyl C_2, 130.02 for phenyl C_1, 136.69 for phenyl C_3,C_6, 142.04 for bridging phenyl C_1, for 165.23 oxadiazole-C_5 and 164.34 oxadiazole-C_2.

Biological activity

Antitubercular Activity

All the synthesized compounds (**VIa-g**) were screened against *M. tuberculosis* H37Rv strain which showed MIC value ranging from 1.6-12.5µg/ml. Among all the screened compounds, compounds **VIb**, **Vlc**, **Vld** showed highest activity at MIC value of 1.6 µg/ml. The compounds **Vle**, **Vlf**, **Vlg** showed antitubercular activity at MIC value of 12.5 µg/ml. The compounds Vlb, Vlc, Vld showed antitubercular activity at MIC value of 3.25 µg/ml respectively (Table **2**).

Antibacterial Activity

The results of antimicrobial activities (expressed in MIC) of the compounds against Gram-positive and Gram-negative bacteria are illustrated in Table **2**. The activity of ciprofloxacin is used for comparison. Pyrrolyl oxadiazole derivatives have shown antibacterial activity between MIC of 25-50 µg/ml. Compound **Vlc** showed highest activity against Gram-positive bacteria with MIC value of 25 µg/ml. Compound **Vlb** showed highest activity against Gram-negative bacteria with MIC value of 25 µg/ml. The compound screened for antibacterial activity against gram-positive (*S. aureus*) and gram negative bacteria (*E. coli*) using broth microdilution method.

Table 2: *In vitro* antitubercular and antibacterial activity results of newly synthesized compounds.

Comp.	*In vitro antitubercular activity* MIC value (µg/ml)	*In vitro antibacterial activity* MIC value (µg/ml)	
		Gram +ve (*S. aureus*)	Gram –ve (*E. coli*)
VIa	3.25	--	--
VIb	1.6	50 µg/mL	25 µg/mL
VIc	1.6	25 µg/mL	50 µg/mL
VId	1.6	--	--
VIe	12.5	--	--
VIf	12.5	--	--
VIg	12.5	--	--
Pyrazinamide	3.12 µ/ml	--	--
Streptomycin	6.25 µ/ml	--	--
Ciprofloxacin	--	2 µg/mL	2 µg/mL

CONCLUSION

Novel series of 2-aryl-5-[4-(1H-pyrrol-1-yl) phenyl]-1,3,4-oxadiazole derivatives (**VIa-g**) were synthesized and identified as potent antitubercular and antibacterial agents. Among all the compounds **Vlb**, **Vlc** and **Vlc** have displayed significant activity against *M. tuberculosis* with MIC value 1.6 µ/ml. These compounds will be useful as the lead compounds for developing antitubercular and antibacterial agents.

LIST OF ABBREVATIONS

^{0}C -Degree centigrade
DMSO -Dimethyl sulfoxide
FTIR -Fourier Transfer Infrared Spectroscopy
NMR -Nuclear Magnetic Resonance
ppm -Parts per million
MP -Melting point
Rf -Retention factor
Min -Minutes
H -Hours
Mol -Mole
TLC -Thin Layer Chromatography
MIC -Minimum Inhibitory Concentration
DMF -NN-Dimethylformamide

Conflict of Interest

All the authors have no conflict of interests.

ACKNOWLEDGEMENTS

Authors immensely thank research support from the Vision Group on Science and Technology, VGST/GRD/NO-567/2016-17/2020-21/47, Dated: 03.08.21. We also thank Dr. V. G. Jamakandi, Dean, Dr. T. M. Aminabhavi, Research Director and Dr. H. V. Dambal, President, S.E.T's College of Pharmacy, Dharwad, Karnataka, India for providing the facilities. We thank Dr. K. G. Bhat of Maratha Mandal's Dental College, Hospital and Research Centre, Belgaum, Karnataka, India, for providing antibacterial and antitubercular activities. SAIF, Panjab, University, Chandigarh, Panjab, India provided some of the NMR and mass spectral data.

REFERENCES

1. Raviglione M, M arias B, Floyd K, Lonnroth K, Getahun H, Migliori GB, et al. Scaling up interventions to achieve global tuberculosis control progress and new developments. Lancet. 2012;379: 1902-1913.
2. WHO report, Global Tuberculosis Report, oct 17- 2019.
3. Mamoru koketsu, Kohsuke Tanaka, Yuichi Takenaka, Cecil D kwong and Hideharu Ishihara. Synthesis of 1,3-thiazine derivatives and their evaluation as potential antimicrobial agents. Eur J of pharm sci 2002;15: 307-10.
4. Yang XD, Wan WC, Deng XY, Li Y, Yang LJ, Zhung HB, et al. Design, synthesis and cytotoxic activity of novel hybrid compounds between 2-phenylbenzofuron and imidazole. Bioorg Med Chem Lett. 2012;22: 2726-2729.
5. Miyachi H, Kiyota H, Segawa M. Novel imidazole derivatives with subtype-selective antimuscuranic activity. Bioorg Med Chem Lett. 1998;8(14): 1807-1812.
6. Estevez V, Villacampa M, Menenbez JC. Multicomponent reaction for the synthesis of pyrroles. Chem Soc Rev. 2010;39: 4402-4421.
7. Yale H, Losse K, Martins J, Holsing M, Perry F, Bernstein J. Chemotherapy of experimental tuberculosis. VIII. The synthesis of acid hydrazides, their derivatives and related compounds. Am J Chem Soc. 1953;75: 1933-1942.
8. Joshi SD, Kulkarni VH, More UA. Antitubercular activity of pyrrole Schiff base and computational study of M tuberculosis InhA. Int J Drug Design and Discovery. 2013;4(4): 1163-1173.
9. Joshi SD. Vagdevi HM, Vaidya VP, Gadaginmath GS. Synthesis of some new pyrrole derivatives as potential antimicrobial and antitubercular agents. Indian J Hetro Chem. 2007;17(7): 165-168.
10. Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, et al. Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue assay, J Clin Microbiol. 1998; 36: 362-66.
11. Banerjee A, Dubnau E, Quemard A, Balasubramaniyan V, Um KS, Wilson T, et al. InhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. Science. 1994; 263: 227-30.
12. Estevez V, Villacampa M, Menendez JC. Multicomponent reactions for the synthesis of pyrroles. Chem Soc Rev. 2010; 39: 4402-21.

5487847845**1230304**