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"SYNTHESIS AND CHARACTERIZATION OF NEW PYRROLYL OXADIAZOLE AS ANTIMICROBIAL AGENTS"

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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 benzocaine 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 ethanol. Then this 4-pyrrol-1-yl-benzoic acid hydrazide (3) treated with appropriate benzaldehyde in presence of TCCA and ethanol to yield compound substituted 2-(4-(1H-pyrrol-1-yl)phenyl)-5-phenyl-1,3,4-oxadiazole 04(a-f).Structures of newly synthesized compounds were confirmed on the basis of physico-chemical and spectral data (IR, ^{1}H &^{13}C-NMR, Mass spectra). All the synthesized 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.

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INTRODUCTION

Discovery of antimicrobial agents was the most brilliant achievement of modern medicine in the 20th century. In the "golden age of antibiotics" from 1940s to 1970s, antimicrobial agents have significantly increased human life expectancy by curing previously fatal infectious diseases. At the end of 1960s, some authorities impetuously declared that infectious diseases would have been the history of human life. Currently, however, half a century after the introduction of "miracle drugs", scientific community and public fear the re-emergence of infectious diseases caused by antibiotic-resistant bacteria. Most cardinal infectious diseases which account for more than 85% of the mortality from infection worldwide such as acute respiratory infections, diarrheal diseases, AIDS, malaria, and tuberculosis have serious problems in the treatment due to widespread emergence of antimicrobial resistance. For example, treatment of acute respiratory infections is complicated by the emergence of pneumococcal resistance to penicillin, macrolides, and fluoroquinolones. Antimicrobial resistance among major human pathogens including bacteria, virus, fungi, and mycobacteria is obviously one of the most serious threats to public health globally in the 21st century.

The main mycobacterial infections in humans are *tuberculosis* and *leprosy* both typically chronic infections; caused respectively by *Mycobacterium tuberculosis* and *Mycobacterium leprae*. 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.

Pyrrole is one of the most appearing heterocycles in the plant and animal kingdom because of its presents as a subunit of chlorophyll in plant cells and hemin and vitamin B_{12} in animal cells. Several macromolecular antibiotics having pyrrole structure were isolated from biological sources and their activities were defined. Pyrrole and its derivatives have shown to possess biological activities such as antibacterial [1], antitumor[2], analgesic[3], antitubercular[4,5], anti-inflammatory and anti-allergic.[6]Several macromolecular antibiotics were isolated from biological sources and their activities were defined.[7,8].

1,3,4-Oxadiazole ring is associated with many types of biological properties such as anti-inflammatory [9-11], hypoglycemic [12], antifungal and antibacterial [13-17] activities. On the other hand, some 5-[isoxazolo[5,4- d]pyrimidinyloxymethyl] - 2 - substitutedphenylamino1,3,4-oxadiazole and -1,3,4-oxadiazole-2(3H)-thione derivatives[17] 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

Chemicals:

Chemicals used in the synthesis of the titled compounds were purchased from, Sigma-Aldrich Pvt Ltd, HiMedia laboratories, S.D. Fine Chem. Pvt Ltd and Spectrochem Pvt. Ltd. They were Benzocaine, Hydrazine hydrate, Isoniazid, 2,5-dimethoxytetrahydrofuran, acetonyl acetone etc. All the solvents and chemicals were purified before use by distillation/recrystallization.

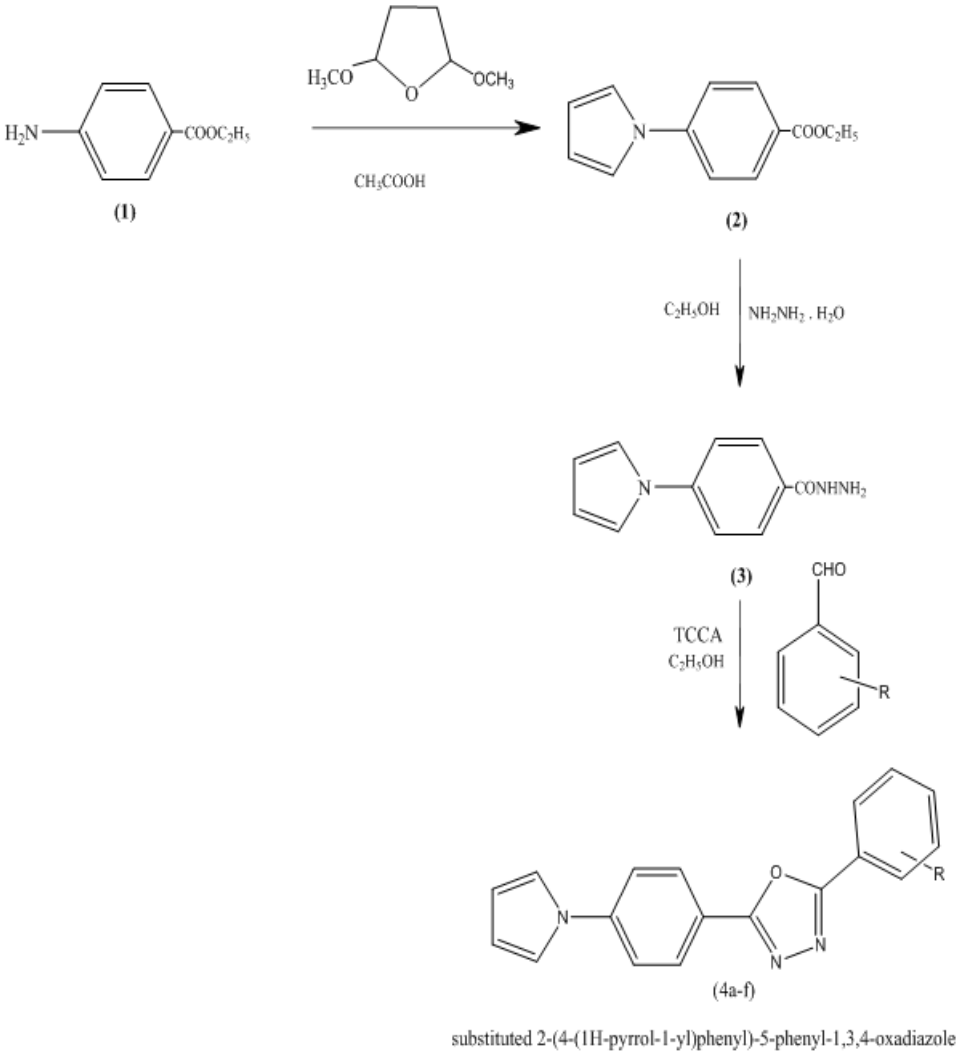
Instruments:

All the melting points and boiling points of synthesized compounds were determined by capillary method in a paraffin bath/digital melting point apparatus and are uncorrected. TLC was prepared with silica gel GF (HiMedia/Spectrochem) on plates (3×8 cm), which were activated at 115 ^{0}C for 30 min. The colorless spots were detected by exposure to iodine vapors; FT-IR spectra were recorded on spectrophotometer by using KBr pellets and values are expressed in cm^{-1}. The ^{1}H NMR were recorded on Bruker 400/500 MHz instruments using deutoriated trichloro methane ($CDCl_3$) and dimethyl sulphoxided_6 (DMSO-d_6) as solvent and tetramethylsilane (TMS) as internal standard, chemical shifts are expressed as δ values (ppm). EI Mass spectra were recorded on JEOL GCmate Mass spectrometer.

Chemical synthesis:

Pyrrole ring was constructed by reacting benzocaine 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 ethanol. Then this 4-pyrrol-1-yl-benzoic acid hydrazide (3) treated with appropriate benzaldehyde in presence of TCCA and ethanol to yield compounds substituted 2-(4-(1H-pyrrol-1-yl)phenyl)-5-phenyl-1,3,4-oxadiazole **04(a-f).**

SCHEME:



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

Synthesis

Procedure for Synthesis of ethyl 4-(1*H*-pyrrol-1-yl) benzoate (02):

Compound **(02)** was synthesized by refluxing a mixture of ethyl 4-aminobenzoate **(02)** (2 g, 0.1 mol) in dried acetic acid (15 ml) and 2,5-dimethyltetrahydrofuran (1.9 ml, 0.12 mol) for 45 min. at 150-160 ^{o}C. Then the reaction mixture was poured onto crushed ice then it was neutralized by the addition of saturated sodium bicarbonate, the solid separated was filtered and recrystallized from ethanol.

Procedure for Synthesis of 4-(1*H*-pyrrol-1-yl) benzo hydrazide (03):

4-(1*H*-pyrrol-1-yl) benzo hydrazide (03)was synthesized by refluxing a mixture of ethyl 4-(1*H*-pyrrol-1-yl)benzoate **(02)** (2 g, 0.015 mol) with hydrazine hydrate (10 ml) in absolute ethanol (10 ml) for 3 hr the reaction mixture was cooled and crystalline mass obtained was recrystallized from ethanol to secure yellow crystals.

General procedure for synthesis of substituted **2-(4-(1*H*-pyrrol-1-yl) phenyl)-5-phenyl-1,3,4-oxadiazole (04a-f)**

To a well stirred solution of TCCA (1mmol) in ethanol (5ml), appropriate aldehyde (2mmol) was added. After stirring reaction mixture for 10 min, a solution of 4-(1*H*-pyrrol-1-yl) benzo hydrazide (03) in ethanol (5 ml) was added. To the reaction mixture, the remaining part of TCCA (1mmol) was added, stir continuously until completion of reaction which is monitored by TLC. The reaction mixture extracted with ether (30ml) and dried over anhydrous calcium carbonate; the ether was evaporated. The residue was recrystallized from ethyl acetate.

BIOLOGICAL SCREENING

Evaluation of antibacterial activity:

The Minimum Inhibitory Concentration (MIC) determination of the tested compounds was investigated in side-by-side comparison with ciprofloxacin, norfloxacin against Gram-positive [*Staphylococcus aureus (ATCC-12598), Bacillus subtilis (ATCC-6633)*] and Gram-negative bacteria [*Klebsiella pneumonia (ATCC-29665), Escherichia coli (ATCC-25922)*] by broth microdilution method. [18, 19]

MATERIALS AND METHODS

1. Mueller–Hinton agar
2. McFarland turbidity standards
3. Scrupulously clean, acid-washed borosilicate glass tubes
4. Micropipette
5. Nutrient agar

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 15 lb pressure for about 20 min. The test tubes and pipettes were sterilized in hot air oven at 160 ^{0}C for 1 hr.

Preparation of solution of test compounds:

Serial dilutions of the test compounds and reference drugs were prepared in Mueller-Hinton agar. Drugs (1 mg) were dissolved in dimethylsulfoxide/$CDCl_3$ (1 ml). Further progressive dilutions with melted Mueller-Hinton agar were performed to obtain the required concentrations of 0.2, 0.4, 0.8, 1.6, 3.125, 6.25, 12.5, 25, 50 and 100 µg/ml.

Preparation of the Inoculums:

Test organisms were sub-cultured on to nutrient agar and incubated overnight at 35 ^{0}C. The tubes that contain 2 ml of Muller-Hinton agar were inoculated with five or more colonies from the agar plate and turbidity was adjusted to match a 1 McFarland standard (10^5 cfu/ml) and incubated at 37 °C for 18 hr. 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 DMSO/$CDCl_3$ at the same dilutions as used in the experiments and DMSO/$CDCl_3$ had no effect on the microorganisms in the concentrations studied.

Antitubercular Activity:[20]

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 radiometric method.
3) Briefly, 200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized 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 0.2 µg/ml.
6) Plates were covered and sealed with para film 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 as growth.
9) The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink.

RESULTS OF SPECTRAL ANALYSIS

2-(4-(1H-pyrrol-1-yl)phenyl)-5-phenyl-1,3,4-oxadiazole. (4a)

Yield (%)75,M.P 228-230,FTIR(KBr cm^{-1})3134.19 (CH Arom.), 1612.82 (C = N), 1329.10 (C – N) 1244.23 (C – O of oxadiazole). ^{1}HNMR(δppm) 6.33 (dd, 2H, pyrrole C_3 and C_4-H), 7.02 (t, 1H, phenyl C_4-H), 7.28 (dd, 2H, pyrrole C_2 and C_5-H), 7.30-7.98 (m, 8H, bridging phenyl C_2, C_6, C_3, C_5-H and phenyl C_2, C_3, C_5, C_6-H), 10.43.^{13}C NMR111.01 (pyrrole C_3 and C_4), 117.02 (phenyl C_2 and C_6), 118.49 (bridging phenyl C_2 and C_6), 119.46 (pyrrole C_2 and C_5), 120.57 (bridging phenyl C_4), 121.64 (phenyl C_4), 126.88 (phenyl C_3 and C_5), 128.65 (bridging phenyl C_3 and C_5), 138.43 (phenyl C_1), 141.45 (bridging phenyl C_1), 157.24 (oxadiazole C_5), 159.79 (oxadiazole C_2). Mol.wt 287.32g.

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

Yeild(%)43, M.P 198-200, FTIR(KBr cm^{-1}) 3154.35 (CH Arom.), 1619.05 (C = N), 1327.93 (C – N) 1278.85 (C – O of oxadiazole), (C-Cl) 835.39.

2-(4-(1H-pyrrol-1-yl)phenyl)-5-(4-flurophenyl)-1,3,4-oxadiazole. (4c)

Yeild(%)41, M.P 218-220, FTIR (KBr cm^{-1}) 3139.72 (CH Arom.), 1610.37 (C = N), 1316.52 (C – N) 1270.83 (C – O of oxadiazole), (C-F) 1418.60.

4-(5-(4-(1H-pyrrol-1-yl)phenyl)-1,3,4-oxadiazol-2-yl)phenol. (4d)

Yeild(%)80, M.P 202-204, FTIR (KBr cm^{-1}) 3529.01 (-OH) 3094.85 (CH Arom.), 1611.94 (C = N), 1334.46 (C – N) 1281.19 (C – O of oxadiazole).

2-(4-(1H-pyrrol-1-yl)phenyl)-5-(4-bromophenyl)-1,3,4-oxadiazole. (4e)

Yeild(%)47, M.P 282-284, FTIR (KBr cm^{-1}) 3141.24 (CH Arom.), 1609.44 (C = N), 1315.41 (C – N) 1239.29 (C – O of oxadiazole), (C-Br) 660.39.

2-(4-(1H-pyrrol-1-yl)phenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole. (4f)

Yeild(%)65, M.P 188-190, FTIR (KBr cm^{-1}) 3144.95 (CH Arom.), 1690.16 ($-NO_2$), 1610.65 (C = N), 1332.78 (C – N) 1203.37 (C – O of oxadiazole), (C-Br) 660.39.

Antibacterial Activity

All the newly synthesized compounds were screened for the antibacterial activities of substituted 2-(4-(1H-pyrrol-1-yl) phenyl)-5-phenyl-1,3,4-oxadiazole 04(a-f) by broth microdilution assay method (MIC µg/ml).

Table No. 1: MIC (µg/ml) of synthesized compounds 4(a-f).

Compounds	R	Gram Positive Bacteria		Gram Negetive Bacteria	
		S. aureus	***B. subtilis***	***K. pneumoniae***	***E. Coli***
04 a	-H	25	6.25	1.6	1.6
04 b	*p*-Cl	12.5	3.12	3.12	1.6
04 c	*p*-F	12.5	3.12	6.25	12.5
04 d	*p*-OH	3.125	1.6	1.6	0.8
04 e	*p*-Br	6.25	1.6	12.5	12.5
04 f	*p*-NO_2	6.25	6.25	1.6	0.8
Ciprofloxacin	-	2	2	1	2
Norfloxacin	-	3	1	1	12

Antitubercular Activity

All the newly synthesized compounds were screened for the antitubercular scrennning of substituted 2-(4-(1H-pyrrol-1-yl) phenyl)-5-phenyl-1,3,4-oxadiazole 04(a-f) against *M. tuberculosis* $H_{37}Rv$ by Microplate Almar Blue Assay (MABA) method.

Table No. 2: MIC (µg/ml) of synthesized compounds 4(a-f).

Compound	MIC values (µg ml^{-1}) *M. tuberculosis* $H_{37}Rv$
4a	50
4b	6.25
4c	3.12
4d	12.5
4e	6.25
4f	12.5
Pyrazinamide	3.12
Streptomycin	6.25

DISCUSSION

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. Biheterocyclic compounds of pyrrole clubbed with oxadiazole were synthesized as described in scheme.

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 synthesized 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 synthesized 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 methodand antitubercular activity against *Mycobacterium tuberculosis* H_{37}Rv by using Microplate Almar Blue Assay (MABA) method.

CONCLUSION

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).**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*. 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.* 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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Authors' agreements

Authors hereby declare that there is no conflict of interest for the publication.

List of Abbreviations Used

C	-	Degree centigrade
$CDCl_3$	-	Deuterated chloroform
Conc.	-	Concentrated
DMSO	-	Dimethyl sulphoxide
FT-IR	-	Fourier Transform Infrared
gm	-	Gram
hr	-	Hour
M.P.	-	Melting Point
Min.	-	Minutes
Mol	-	Mole
NMR	-	Nuclear Magnetic Resonance
pH	-	Hydrogen ion concentration
ppm	-	Parts per million
R_f	-	Retardation factor
TLC	-	Thin Layer Chromatography
TMS	-	Tetra methyl silane
MIC	-	Minimum inhibitory concentration
TCCA	-	Trichloroisocyanuric acid

REFERENCES

1. Demirayak S, Burun K, Kiraz N. Some pyrrole substituted aryl pyridazinone and pyhalazinone derivatives and their antihypertensive activities. Eur J Med Chem. 1989;39:95.
2. Halazy S, Magnus P. Studies on antitumour agents CC-1065:1-Phenylsulphonyl-1,3-butadiene for the synthesis of 3,3[1]-bipyrroles. Tetrahedron Lett. 1984;25:1421.
3. Li B, Zhang, Goadeng X, Xuebao. 1985;6:917.
4. Bijev A. Pyrrole based hydrazones synthesized and evaluated invitro as potential tuberculostastics. 2006;56:96-103.
5. Sbardella G. Synthesis and in vitro antimycobacterial activity of novel 3-(1*H*-pyrrol-1-yl)-2-oxazolidinone analogues of PNU-100480. Bioorg Med Chem Lett. 2004;14:1537-1541.
6. Brikner C, Leyck S, Christ B, Kesselring K.German.Patent. 1986:3:506
7. Jones A R, Bean P G. The chemistry of pyrroles. Academic Press London. 1997.
8. Jones A R. pyrroles part 2. The chemistry of heterocyclic compounds. Wiley New York. 1992.
9. Mullican MD, Wilson MW, Conner DT, Kostlan CR, Schrier DJ, Dyer RD. Design of 5-(3, 5-di-tert-butyl-4-hydroxyphenyl)-1, 3, 4-thiadiazoles,-1, 3, 4-oxadiazoles, and-1, 2, 4-triazoles as orally active, nonulcerogenicantiinflammatory agents. Journal of medicinal chemistry. 1993 Apr;36(8):1090-9.
10. Boschelli DH, Connor DT, Bornemeier DA, Dyer RD, Kennedy JA, Kuipers PJ, Okonkwo GC, Schrier DJ, Wright CD. 1, 3, 4-Oxadiazole, 1, 3, 4-thiadiazole, and 1, 2, 4-triazole analogs of the fenamates: in vitro inhibition of cyclooxygenase and 5-lipoxygenase activities. Journal of medicinal chemistry. 1993 Jun;36(13):1802-10.
11. Raman K, Singh HK, Salzman SK, Parmar SS. Substituted thiosemicarbazides and corresponding cyclized 1, 3, 4- oxadiazoles and their anti- inflammatory activity. Journal of pharmaceutical sciences. 1993 Feb;82(2):167-9.
12. Sahin G, Palaska E, Kelicen P, Demirdamar R, Altinok G. Synthesis of Some New 1-Acylthiosemicarbazides, 1, 3, 4-Oxadiazoles, 1, 3, 4-Thiadiazoles and l, 2, 4-Triazole-3-thiones and their Anti-inflammatory Activities. Arzneimittelforschung. 2001 Jun;51(06):478-84.
13. Husain MI, KumaraA, Srivastava RC.Synthesis of N-(2-Naphthyloxyacetyl) thiosemicarbazide and2-arylamino-5-(2-naphthyloxymethyl)-1,3,4-thiadiazoles/oxadiazoles as oral hypoglycemic agents. Current Sci. 1986 Jul 20:644-6.
14. Singh H, Yadav LD. Synthesis of Some 5-Aryl-2-heteroaryl/heteroarylamino-l, 3, 4-oxadiazoles as Potential Fungicides. Agri and Biolog Chem. 1976 Apr 1;40(4):759-64.
15. Misra HK. Synthesis of Some New Substituted 1, 3, 4 Oxadiazoles as Potential Insecticidal, Antibacterial and Anti acetylcholine Esterase Agents. Archiv der Pharmazie. 1983;316(6):487-93.
16. Giri S, Singh H, Yadav LD. Studies in Oxadiazoles Synthesis of Some 2-Mercapto-l, 3, 4-oxadiazoles and Related Compounds as Potential Fungicides. Agricultural and Biological Chemistry. 1976 Jan 1;40(1):17-21.
17. Adhikari VA, Badiger VV. Synthesis and Biological Activities of Isoxazolo (5, 4, d) pyrimidinyloxymethylthiadiazoles, oxadiazoles and triazoles. Chem Inform. 1988 Nov 22;19(47)
18. Dundappa SD, Raghu AV, Gadaginamath GS. Synthesis and antimicrobial activity of some new 1-substituted-3-pyrrolyl aminocarbonyl/oxadiazolyl/triazolyl/5-methoxy-2-methyl indoles and benz[*g*]indoles. Ind J Chem 2006;45(B):689-96.
19. Conley NR, Hung RJ, Willson CG. A New Synthetic Route to Authentic *N*-Substituted Aminomaleimides. J Org Chem 2005;70:4553-5.
20. Narule MN, Gaidhane MK, Gaidhane KP. Synthesis, characterization, biologically and antioxidant active of some 2-substituted 3,5-dimethyl-4-ethoxy carbonyl pyrrole derivatives. J Pharm Research. 2013; 6-626-632.

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