



SYNTHESIS OF SOME NOVEL 1-H PYRAZOLE DERIVATIVES AND THEIR ANTIBACTERIAL ACTIVITY STUDIES

**P. Bharath Rathna Kumar^{1*}, S.Subramaniyan¹, K.Yamini²
and R.Suthakaran³**

^{1*}Department of Pharmaceutical Chemistry, Bharat Institute of Technology (Pharmacy),
Manganapaly, Ibrahimpatnam, Hyderabad-501510

²Department of Pharmacognosy, Teegala Ram Reddy College of Pharmacy,
Meerpet, Hyderabad- 500097

³Department of Pharmaceutical Chemistry, Teegala Ram Reddy College of Pharmacy,
Meerpet, Hyderabad- 500097

⁴Email: sudha_sudhar@rediffmail.com

ABSTRACT

When one biologically active molecule is linked to another, the resultant molecule generally has increased potency. Hence two pharmacophores, i.e. pyrazole ring and indole moiety are fused to obtain highly potent, more specific and less toxic agent. In the present study, synthesis of title compounds 1-[(2-methyl-1*H*-indol-3-yl)carbonyl] -3-substituted phenyl-1*H*-pyrazole-4-carbaldehyde derivatives (VI-e) by using Vilsmeier-Haack reagent (DMF/POCl₃). The facial synthesis of 1-[(2-methyl-1*H*-indol-3-yl) carbonyl] -3-substituted phenyl-1*H*-pyrazole-4-carbaldehyde derivatives (VI-e) has been achieved by the reaction of phenyl hydrazine(1) with a mixture of ethyl acetoacetate (II) and glacial acetic acid to synthesize ethyl-2-indole-3-carboxylate (III) which on condensation with different acetophenones in methanol in the presence of glacial acetic acid affords hydrazones (IVa-e). The hydrazones (IVa-e) on treatment with Vilsmeier-Haack reagent furnished 1-[(2-methyl-1*H*-indol-3-yl) carbonyl] -3-substituted phenyl-1*H*-pyrazole-4-carbaldehyde derivatives (VI-e).

Key words: Indole, pyrazole, antibacterial activity.

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INTRODUCTION

Pyrazoles¹ represents one of the most active classes of compounds possessing wide spectrum of biological activities. Many of the therapeutically useful compounds such as phenylbutazone, oxyphenbutazone and Antipyrine, belong to pyrazoles², the literature survey reveals that pyrazole derivatives exhibit anti diabetic, herbicidal activity, anti-inflammatory, antipyretics, analgesics and muscle relaxants². Indole and its derivatives are also biologically very important heterocyclic systems. It was thought that a pyrazole ring coupled to an indole moiety, the resulting compound might possess enhanced biological activity. In view of these facts, it was planned to synthesize title compounds³.

The required starting compound is ethyl-2-indole-3-carboxylate (III) was prepared by the Fischer-Indole synthesis⁴. These compounds under went condensation with hydrazine hydrate in ethanol to furnish the Indole-3-carboxy hydrazide (IV). The reaction of hydrazides with different Acetophenones in methanol in the presence of Acetic acid to form (V). These compounds treated with Vilsmeier Haack reagent (DMF/POCl₃) to form Title compounds (VIa-e)⁵.

EXPERIMENTAL

Material & Methods

All chemicals used were of synthetic grade. The purity of compounds was ascertained by TLC on precoated silica F254 plates (Merck, Mumbai, India) using iodine vapors and UV light as detecting agents. The melting points of the synthesized compounds were determined by open capillary method and

are uncorrected⁶. Melting points were determined in open capillaries in electrical apparatus and are Uncorrected. IR spectra were recorded on a FT-IR spectrometer using KBR pellet. The NMR spectra were recorded in JMR spectrometer using TMS as internal standard⁷. The Mass spectra were recorded in NCMS spectrometer The physical data of the compounds is given in table-1.

Synthesis

Synthesis of Ethyl-2-methyl-1H-indole-3-carboxylate (III).

In a flat bottom flask (250 ml) fitted with a dropping funnel, a sealed stirrer unit and reflux condenser, a mixture of ethylacetoacetate (II) (6.3 ml; 0.05 mol) and glacial acetic acid (3ml, 0.05mol) was placed in the flat bottom flask and heated under reflux with stirring⁸. Add phenyl hydrazine (5 ml; 0.05 mol) slowly during first 1hr. Continue the stirring for further 1hr. Pour the reaction mixture into a 50ml beaker and stir vigorously while it solidifies. Then add sufficient water and filter. Dry the crude product, the crude product thus obtained was recrystallized from ethanol.

Synthesis of 2-methyl-1H-indole-3-carbohydrazide (IV).

Ethanol solution of Ethyl-2-methyl-1H-indole-3-carboxylate (III) (10gm 0.05mol) was refluxed with hydrazine hydrate (2.5 gm; 0.05 mol) for 3 hr at 70°C. The reaction mixture was allowed to cool and poured over crushed ice⁹. The solid thus obtained was filtered and dried. The crude product thus obtained was recrystallized from ethanol.

General procedure of synthesis of 2-methyl-N'-[(1E)-1-phenylethylidene]-1 H- indole-3-carbohydrazide (Va-e).

A mixture of 2-methyl-1H-indole-3-carbohydrazide (IV) (9.45 gm; 0.05mol) and acetophenone (5.85 ml, 0.05 mol) in 25ml of methanol containing 3-4 drops of glacial acetic acid was refluxed for 2 hr at 70°C. The reaction mixture was allowed to cool to room temperature¹⁰. Pour the reaction mixture into cool water and stir well and keep it aside for over night in refrigerator. The crude product was recrystallized by mixture of solvents acetone and water.

General procedure of synthesis of Title compounds

Synthesis of 1-[(2-methyl-1H-indol-3-yl) carbonyl]-3-phenyl-1H-pyrazole-4-carbaldehyde (VI-e)

The compound 2-methyl-N'-[(1E)-1-phenylethylidene]-1H-indole-3-carbohydrazide (Va) (14.5 gm; 0.05 mol) was added to the mixture of Vilsmeier-Haack reagent, prepared by drop wise addition of phosphorus oxychloride (1.5 ml; 0.05 mol) to an ice cold solution of N,N dimethyl formamide (20ml). The reaction mixture was refluxed for 4hrs at room temperature by using magnetic stirrer. Then the reaction mixture was poured into ice cold water and neutralized with sodium bicarbonate solution. The product obtained was filtered, washed with water and recrystallized from ethanol.

Antibacterial activity

The newly prepared compounds were screened for their antibacterial activity two gram-positive bacteria *B.Subtilis*, *S.aureus* and two gram-negative bacteria [*E.coli*, *P.aeruginosa*] using Nutrient agar medium [Muller Hinton Agar medium]¹¹.

Antibacterial activity was carried out by Disc-diffusion method [Zone of inhibition] and serial dilution method [MIC]¹². Antibacterial activities were studied by subjecting the compounds to pharmacological screening by standard procedures.

All the compounds synthesized in the present investigation were tested for their antibacterial activity. The antibacterial activities were tested on nutrient medium against *Bacillus pumilus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Preparation of nutrient as a medium

The nutrient agar media was prepared by using the following ingredients-

1. Peptone 20g.
2. Beef extract 5g.
3. Sodium chloride 5g.
4. Agar Agar 20 g.
5. Distilled water up to 1000 ml

Weighed quantities of peptone, beef extract were dissolved in distilled water and pH was adjusted to 7.2-

7.4 using pH paper. Then the specified amount of agar was added kept the beaker on hot water bath and allowed the agar to melt, it was dispensed in suitable containers and plugged them with non-adsorbent cotton they were sterilized by autoclave at 121^oC for 20 minutes.

Preparation of solutions of test compounds: Now 1mg/ml concentration of the test compounds was prepared using DMF as solvent it is considered, as stock solution from this 100µg/ml concentration is prepared using DMF as solvent and this was used for antimicrobial activity studies.

Preparation of standard antibiotic solution

Ampicillin 20µg/ml was used as a standard antibiotic for comparison and it was prepared by using sterile water.

Procedure

Sterile nutrient agar medium was cooled to 45^oC this media was inoculated with 18-24 hours old bacterial culture under aseptic conditions mixed well by gentle shaking then it was poured in to sterile petri dishes and allowed the medium to set. After setting all the seeded petri dishes were transferred to laminar flow unit and 5 cups were made by using sterile cork borer. Out of 5cups, 2cups were added with 50µl of the standard antibiotic (Ampicillin) and solvent control one in each bore, test compounds were added to the remaining 3 bores one in each bore. Then they were allowed for diffusion for 2 hours and incubated at 37^oC for 24hrs.

Determination of MIC

Organism: (*Escherichia coli*)

Minimum inhibitory concentrations of the synthesized compounds were determined by taking different concentrations of the compound in DMF. Increasing order of concentrations were added by using sterilized pipettes to different test tubes which contains sterilized broth medium inoculated with sensitive organism with respect to the particular compound. Then all the test tubes were incubated at 37^oC for 24 hours. Then after incubation period presence of growth (turbidity) was observed. Turbidity was not observed from concentration 100µg/ml this is the MIC of the compound.

RESULTS AND DISCUSSION

In the present study, synthesis of title compounds 1-[(2-methyl-1*H*-indol-3-yl) carbonyl] -3-substituted phenyl-1*H*-pyrazole-4-carbaldehyde derivatives (VI-e) by using Vilsmeier-Haack reagent (DMF/POCl₃): As a starting compound ethyl-2-indole-3-carboxylate (III) used to produce Schiff bases. The structure of compounds were characterized by IR, ¹H, NMR, Mass spectral data and Elemental analysis. The IR spectrum of the all the compounds C=N, C=O bands were observed at about 1460-1600 cm⁻¹ region.

In the ¹H NMR spectra of compounds (Va-e) taken in NH proton of the indole was seen as singlet at about 11.0 to 11.6 ppm. Indole methyl protons observed as single at 2.5 ppm Pyrazole proton observed as singlet at

IR, NMR spectra and Mass spectra of the title compounds are given following-

Compound Via

Yield 66%, m.p.55-56. IR(KBR): 3413 (indoleNH), 3154 (C-H), 1621(C=O),1499(C=N),1116 (C-O).¹H NMR (PPM): 11.5 (1H,s,indoleNH),10.2 (1H,s,CHO),8.3(1H,s,PyrazoleCH),6.5-8.0(9H.m,aromaticprotons),2.2 (3H,s, CH₃)M.S(m/z): 329(M+1)

Compound Vib

Yield 91%, m.p.59-60; IR(KBR): 3402 (indoleNH), 3044(C-H), 1667(C=O),1580(C=N),1254 (C-O),576(C-Br) ¹H NMR (PPM): 11.6 (1H,s,indoleNH),10.2 (1H,s,CHO),8.4 (1H,s,Pyrazole CH),6.5-8.1 (8H.m,aromaticprotons),2.5 (3H,s, CH₃)M.S(m/z): 408(M+1)

Compound Vic

Yield 91%, m.p.59-60 IR (KBR): 3425 (indoleNH), 3043(C-H), 1675(C=O),1600(C=N),1517 (No₂),1252(C-O) ¹H NMR (PPM): 11 (1H,s,indoleNH),10.(1H,s,CHO),8.3 (1H,s,Pyrazole CH),6.5-7.9 (8H.m,aromaticprotons),2.5 (3H,s, CH₃). M.S(m/z): 408(M+1)

Compound Vid

Yield 80%, m.p.64-65. IR (KBR): 3405 (indoleNH), 3120(NH₂), 2922(C-H), 1700 (C=O),1593(C=N),1252 (C-O).¹H NMR (PPM): 11.3 (1H,s,indoleNH),10.2 (1H,s,CHO),8.3 (1H,s,Pyrazole CH),6.5-8.8 (10H.m,aromaticprotons),2.3 (3H,s, CH₃)M.S(m/z): 344(M+1)

Compound Vie

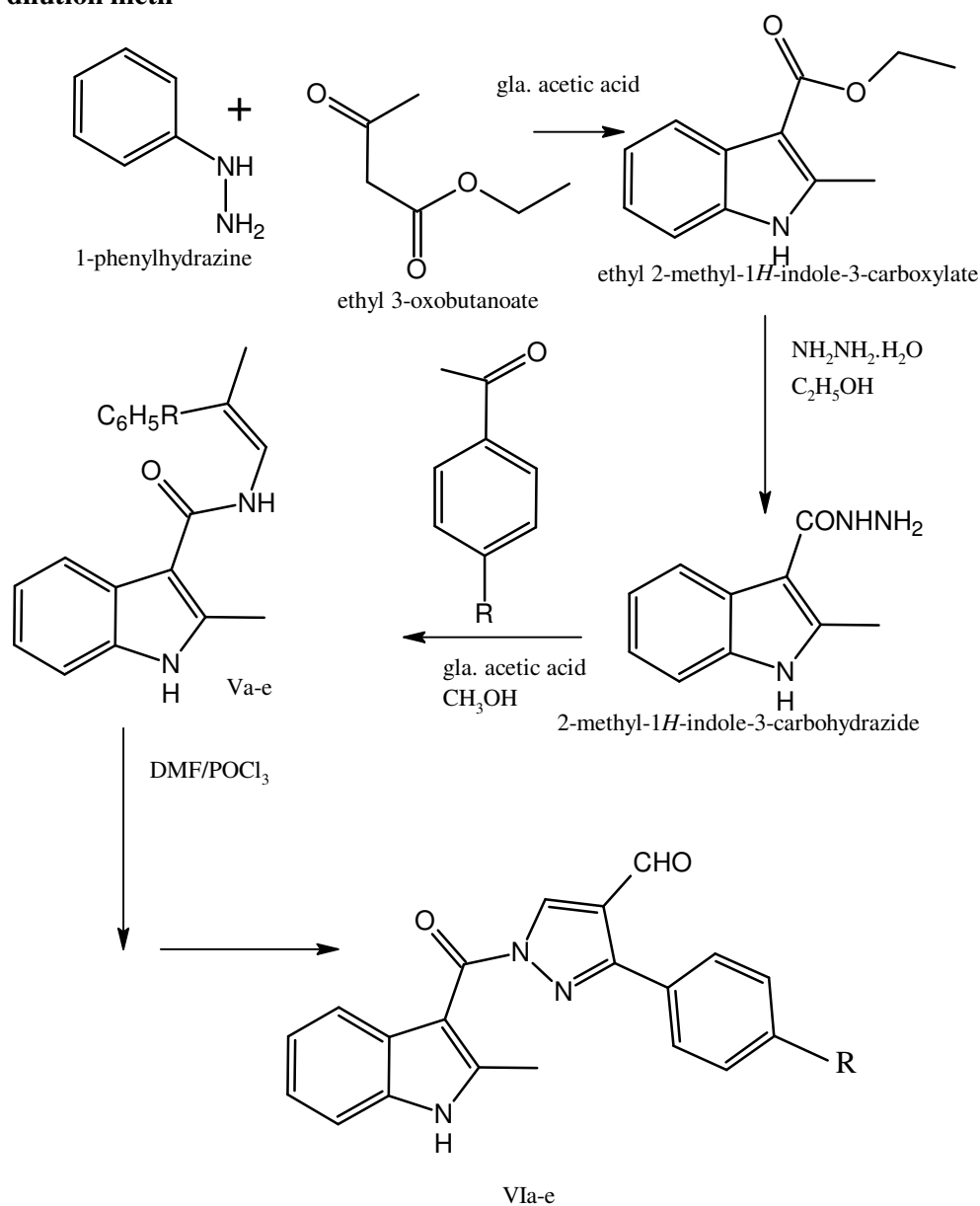
Yield 57%, m.p.60-61. IR(KBR): 3410 (indoleNH), 3065(C-H), 2924(phenolic OH) 1660 (C=N), 1273 (C-O). ^1H NMR (PPM): 11.2 (1H,s,indoleNH), 9.8 (1H,s,CHO), 8.7 (1H,s,Pyrazole CH), 6.7-8.0 (9H,m,aromaticprotons), 2.5 (3H,s, CH₃). M.S(m/z): 408(M+1)

Antibacterial Activity

(a) Disc diffusion method

All the compounds synthesized have shown potent to weak antibacterial activity. Compounds VIc and VIId shows good activity against *Bacillus Subtilis* and *Pseudomonas Aeruginosa* when compared with standard. Compounds VIb and VIa showed moderate antibacterial activity. Compound VIe show weak antibacterial activity when compared to standard.

(b) Serial dilution meth



R: VIa=H, VIb=Br, VIc=NO₂, VIId=NH₂, VIe=OH

Scheme-1

The results show that the compounds VIc and VIId have an MIC of 125µg/ml respectively against B.Subtilis, which was considered as a good activity when compared to standard. Compounds VIb and VIa showed moderate activity with an MIC of 250µg/ml. Compound Vie show MIC 500µg/ml considered as weak antibacterial activity in comparison against the standard.

Compound VIId is considered to have good antibacterial activity with an MIC of 62.5 µg/ml against S.Aureus. Compounds VIc showed moderate activity with an MIC of 250µg/ml. Compound VIa, VIb and Vie show weak activity at MIC 500µg/ml in comparison with the standard.

The MIC of VIc and VIId was 62.5µg/ml, it is considered as the compounds having good activity against E.Coli. Compounds VIa and VIb are said to be moderately active MIC 250µg/ml. Compound Vie is considered as weak antibacterial as they show MIC of 1000µg/ml.

Compound VIId have good activity with an MIC 62.5 µg/ml against *Pseudomonas Aeruginosa*. Compounds VIa, VIb and VIc exhibited moderate activity showing an MIC of 250µg/ml. Compound Vie show 500µg/ml MIC indicating weak antibacterial activity in comparison with standard.

The observed data on the antimicrobial activity of the compounds and control drugs are given in table-2 and table-3.

CONCLUSION

The two moieties i.e. 3-methoxybenzofuran and thiazolidin-4-one substituted 1, 2, 4-triazole moieties independently are antibacterial agents. Here when the two moieties are fused and screened for possible antimicrobial studies, they showed a broad spectrum of antibacterial activity against G (+ve) and G (-ve) bacteria. Benzofuran and triazole molecule is responsible for antibacterial activity, but it is interesting to note that thiazolidin-4-one substituted 1, 2, 4-triazole moiety showed a broad-spectrum antibacterial activity. The above results establish the fact that thiazolidin-4-one substituted 1, 2, 4-triazole benzofuran can be a potential source for exploitation in search of new generation of antibiotics. It may be worthwhile to explore the possibility in this area by fusing other heterocyclic moieties and increase the potency of the synthesized compounds.

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