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Synthesis, Microscopical Observation of Polymorphism and Antifungal, Antitubercular Activity of Novel Pyrrole Derivatives

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ABSTRACT

Pyrrole derivatives were synthesized with an approach to develop more potent and less side effects having antifungal and anti-tubercular activity. Benzoin with primary aromatic amines refluxing in ethanol resulted the formation of α-aminoketone intermediates. Which were condensed with malononitrile to yield the various 2-amino-4,5-diphenyl-1-substituted-1H-pyrrole-3-carbonitriles (Ia-e). Pyrrole (Ia-c) further reacted with acid such as Acetic acid, formic acid to yield different novel 2-methyl-7(phenyl aniine)-5,6 diphenyl-3,7dihydro-4H-pyrrolo[2,3-d]pyridine-4-one derivatives (IIa-c) by the Paal-knorr condensation. The synthesized compound IIAc were exist in different crystalline forms. The crystals were prepared from different polarity of solvents. The synthesized compounds were confirmed through spectral characterization using IR (JASCO 4100-FT/IR), Mass (QP 2013 Shimadzu) and 1H NMR (Bruker 300 MHz), XRD (Bruker AXS,Germany). Result indicated that these compounds showed promising antifungal, anti-tubercular activity in comparison to standard drugs.

1. INTRODUCTION

Pyrrole derivatives are display diverse biological activity. In the preparation of pyrrole derivative many disadvantages including harsh reaction condition and poor yields by applying Paal-Knorr reaction.

Pyrrole derivatives are considerable attention of synthetic importance and extensively used in drug discovery and pharmacological activity such as anti-microbial, anti-inflammatory and anti-tubercular. Nitrogen heterocycles are of special interest as they constitute an important class of natural and non-natural products, many of which exhibit useful biological activities[3].

1.2 Synthesis of pyrroles

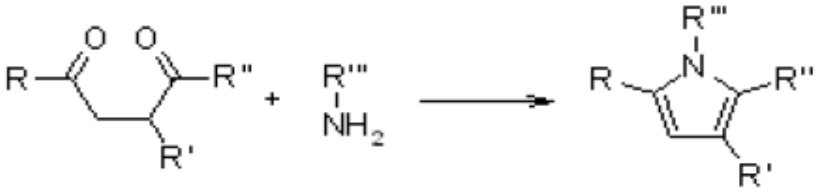
1.2.1 Ring synthesis:

1.2.1.1 From 1, 4-dicarbonyl compounds and ammonia or primary amines

1, 4-dicarbonyl compounds react with ammonia or primary amines to give pyrroles.

1.2.1.2 Paal-Knorr synthesis[3]

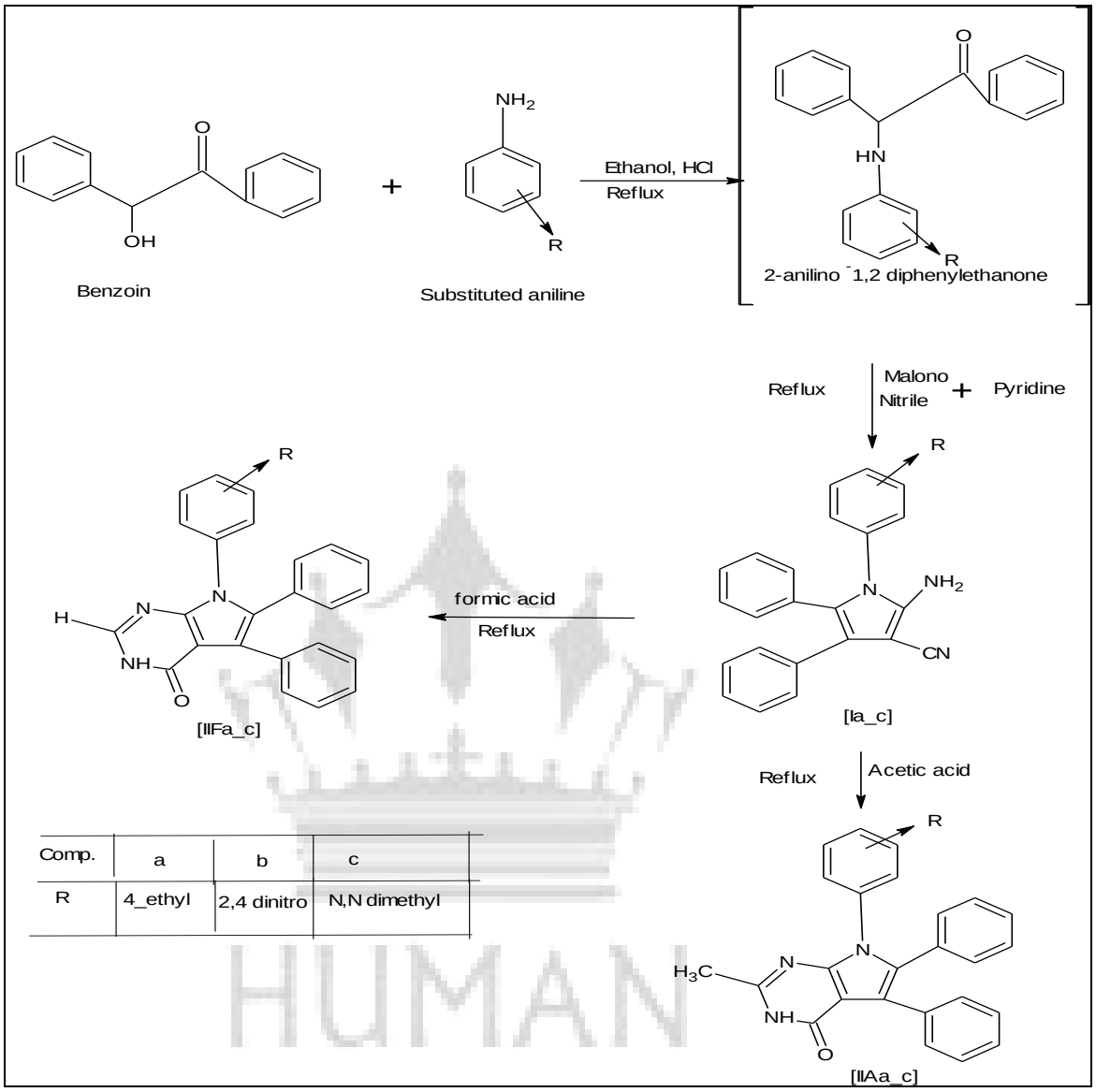
Pyrroles amines are formed by the reaction of ammonia or primary with a 1, 4-dicarbonyl compound. Successive nucleophilic addition of the amine nitrogen to the two carbonyl carbon atoms and the loss of two mol equivalent of water.



2. MATERIALS AND METHODS

Melting points were determined by open capillary tube method and are uncorrected. IR spectra were obtained using FTIR Jasco-4100 spectrophotometer. ^{1}H NMR spectra were recorded on Bruker 300MHz instrument using $CDCl_3$ as a solvent with tetramethylsilane as internal standard. Mass spectra were recorded on a QP 2010 Shimadzu, Shivaji University, Kolhapur. Reactions were monitored by TLC using 0.25mm silica gel G. The recrystallised polymorphic forms were screened under the morphological studies as optical microscopy for crystal morphological structures.

2.2 Reaction scheme[1]:



Scheme 1

2.3 Synthesis of substituted 5, 6-diphenyl pyrrole derivatives was carried out in two steps

1] Synthesis of 2-amino-4, 5-diphenyl-1-substituted-1H-pyrrole-3carbonitrile [Ia-c]

2] Synthesis of derivatives

i] Synthesis of 7-(phenyl aniline)-5, 6 diphenyl-3,7-dihydro-4H-pyrro [2,3-d] pyrimidin- 4-one [IIAa-c]

ii] Synthesis of 7-(phenyl aniline)-5, 6 diphenyl-3,7-dihydro-4H-pyrrolo[2,3-d] pyrimidin- 4-one [IIFa-c]

2.3.1 Synthesis of 2-amino-4,5-diphenyl-1-substituted-1H-pyrrole-3carbonitrile [Ia-c]

A mixture of benzoin, the substituted aniline [a,b,c] and conc. HCl in ethanol was refluxed and then cooled. Malononitrile was added, followed by a catalytic amount of pyridine portionwise and left to reflux until a solid formed. The solvent was evaporated under reduced pressure and the residue was recrystallised from methanol give the compounds 2-amino-4,5-diphenyl-1-substituted-1H-pyrrole-3carbonitrile [Ia-c].Yield: 63-69%, MP- 145-148 °C.

2.3.2 Synthesis of derivatives (IIAa-c and IIFa-c)

i] Synthesis of 2 methyl-7-(substituted aniline)-5, 6 diphenyl-3,7dihydro-4H-pyrrolo[2,3- d] pyrimidine-4-one . [IIAa-c]

The aminopyrrole, **Ia** (3.75 g, 0.01mol), **Ib** (4.25 g, 0.01mol) and **Ic** (3.15g, 0.01 mol) in acetic acid (40 ml) was refluxed for 4 hr, cooled, poured onto ice-water, neutralized with ammonia. Which were filtered off, dried and recrystallized from methanol gives the compounds 2-methyl-7-(substituted-aniline)-5,6diphenyl-3,7dihydro-4H-pyrrolo[2,3-d]pyrimidine-one[IIAa-c], which shown in **Scheme 1** and Melting point and % yields were recorded and reported **Table no.1.**

1 Synthesis of 2-methyl-7-(4-ethylphenyl)-5,6,diphenyl-3,7-dihydro-4H-pyrrolo [2,3-d]pyrimidin-4-one. **[IIAa]**

IR(cm^{-1}): 3410(NH str), 3095(Ali CH str), 3040(Aro CH str), 1710(C=O), 1620(C=N), 1440(C-N) ; NMR (δ, ppm): 7.3-7.9(m,Ar-13H of 4,5 diphenyl ring), 4.5(s,1H of CH of pyrimidin), 5.9(s,1Hof NH) ; MASS(m/z) 404(m+1), 106, 303(m-3), 255(m-2),178(m+2).

2 Synthesis of 2-methyl-7-(2,4 dinitrophenyl)-5,6 diphenyl-3,7-dihydro-4H-pyrrolo [2,3-d]pyrimidin-4-one. **[IIAb]**

IR(cm^{-1}): 3380(NH str), 3040(Aro CH str), 2390(Aryl NO2), 1705(C=O), 1610(C=N), 1460(C-N) ; NMR (δ, ppm): 7.3-7.9(m,Ar-13H of 4,5 diphenyl ring), 4.5(s,1H of CH of pyrimidin), 5.9(s,1Hof NH); MASS(m/z): 180(m+3), 110, 300(m-2), 168(m+2), 467.

3 Synthesis of 2-methyl-7-(2,2-dimethylphenyl)-5,6 diphenyl-3,7-dihydro-4H-pyrrolo [2,3-d]pyrimidin-4-one. **[IIAc]**

IR(cm^{-1}): 3420(NH str), 3060(Ali CH str), 3020(Aro CH str), 1710(C=O), 1610(C=N), 1460(C-N) ; NMR (δ, ppm): 7.3-7.9(m,Ar-13H of 4,5 diphenyl ring), 4.5(s,1H of CH of pyrimidin), 5.9(s,1Hof NH), 0.1(S,6H of phenyl ring CH3); MASS(m/z): 405(m+1), 107, 298(m+2), 112(m+2), 325(m+1).

ii] Synthesis of 7-(substituted aniline)-5,6 diphenyl-3,7dihydro-4H-pyrrolo[2,3-d]pyrimidine-4-one [IIFa-c].

The aminopyrrole, **Ia** (3.75 g, 0.01mol), **Ib** (4.25 g, 0.01mol) and **Ic** (3.15g, 0.01 mol) in formic acid (40 ml) was refluxed for 4 hr, cooled, poured onto ice-water, neutralized with ammonia. Which were filtered off, dried and recrystallized from methanol gives the compounds 7-(substituted-aniline)-5,6diphenyl-3,7dihydro-4H-pyrrolo[2,3-d]pyrimidine-one[IIAa-c], which shown in **Scheme 1,** and Melting point and % yields were recorded and reported in **Table no. 1**

1 Synthesis of 7-(4-ethylphenyl)-5,6,diphenyl-3,7-dihydro-4H-pyrrolo [2,3-d]pyrimidin-4-one. **[IIFa]**

IR(cm^{-1}): 3410(NH str), 3095(Ali CH str), 3040(Aro CH str), 1710(C=O), 1620(C=N), 1440(C-N) ; NMR (δ, ppm): 7.3-7.9(m,Ar-13H of 4,5 diphenyl ring), 4.5(s,1H of CH of pyrimidin), 5.9(s,1Hof NH) ; MASS(m/z): 178(m+2), 105(m+1), 284(m+2), 239, 392(m-1).

2 Synthesis of 7-(2,4 dinitrophenyl)-5,6 diphenyl-3,7-dihydro-4H-pyrrolo [2,3-d]pyrimidin-4-one. **[IIFb]**

IR(cm^{-1}): 3380(NH str), 3040(Aro CH str), 2390(Aryl NO2), 1705(C=O), 1610(C=N), 1460(C-N) ; NMR (δ, ppm): 7.3-7.9(m,Ar-13H of 4,5 diphenyl ring), 4.5(s,1H of CH of pyrimidin), 5.9(s,1Hof NH); MASS(m/z): 452(m+1), 387(m-2), 98(m-2), 288(m-2), 170(m-2),200(m-2).

3 Synthesis of 2-methyl-7-(2,2-dimethylphenyl)-5,6 diphenyl-3,7-dihydro-4H-pyrrolo 2,3-d]pyrimidin-4-one. **[IIFc]**

IR(cm^{-1}): 3420(NH str), 3060(Ali CH str), 3020(Aro CH str), 1710(C=O), 1610(C=N), 1460(C-N) ; NMR (δ, ppm): 7.3-7.9(m,Ar-13H of 4,5 diphenyl ring), 4.5(s,1H of CH of pyrimidin), 5.9(s,1Hof NH), 0.1(S,6H of phenyl ring CH3); MASS(m/z): 392, 105(m+2), 285(m+1), 325(m-1), 181(m-1).

Table No. 1: Physicochemical data of compounds (IIAa-c and IIFa-c)

Sr.No.	Name	Molecular formula	Melting Point [°c]	% yield	Rf value	Mobile phase
1.	IIAa	$C_{27}H_{23}N_2O$	108-112	70.83	0.79	T:EA
2.	IIAb	$C_{25}H_{17}N_5O_5$	116-120	80.87	0.69	T:EA:W
3.	IIAc	$C_{27}H_{24}N_3O$	112-114	69.31	0.70	T:EA
4.	IIFa	$C_{26}H_{21}N_3O$	70-72	63.88	0.79	T:EA
5.	IIFb	$C_{24}H_{15}N_5O_5$	78-80	65.60	0.73	T:EA:W
6.	IIFc	$C_{26}H_{22}N_3O$	68-70	79.42	0.76	T:EA

2.4 Microscopical observation of polymorphism[3]

2.4.1 Method for crystal preparation: IIAe derivative added in respective solvent system such as ethanol [P], Ethanol:Methanol[Q], Ethanol:Methanol:Acetone[R], Methanol:Acetone[S] stirred for 20 min with temp 50-60^{0}C then mixture is cooled at room temperature.

2.4.2 The visual comparison of the observed crystal morphology on the Optical microscopic images **Fig. 1** Indicate that **polymorphic form P,Q,R and S** shows **fine needle shape**, **needle crystal**, **Plate Shape, Rhombic shape** respectively.

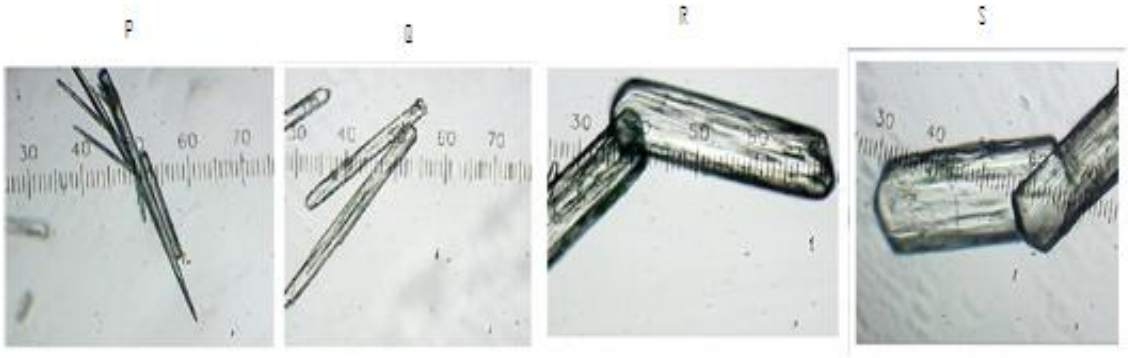


Fig. no.1: optical microscopical observation of polymorphic forms

2.4.3 Overlain IR Spectras of IIAe Derivative polymorphic forms[P,Q,R,S] were recrystallised from Ethanol, Methanol, Acetone or solvent mixtures.[3]

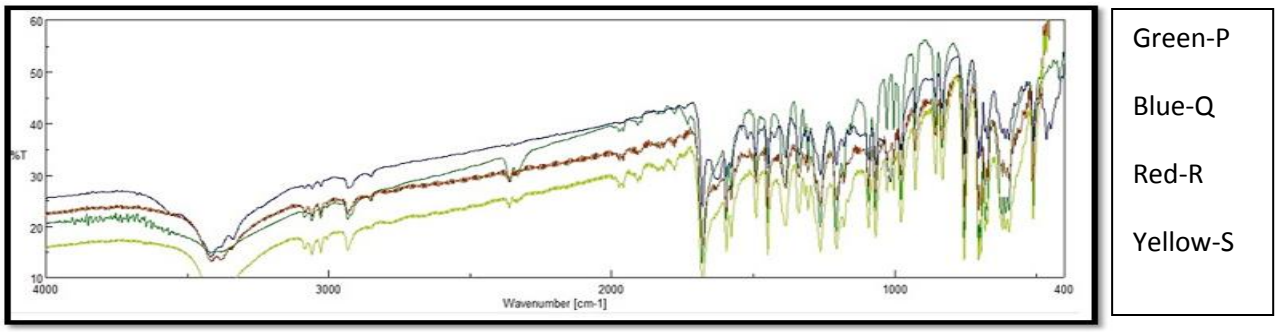


Fig. no.2: IR spectrum of compound IIAe derivative polymorphic forms

Table no.2: FTIR characterization of Polymorphic Forms

Polymorphic Forms	Wave number (cm^{-1}) of main interacting groups					
	NH str	Ali C-H str	Aro C-H str	C=O	C=N	C-N
P	3420	3060	3020	1710	1610	1460
Q	3422	3061	3023	1708	1609	1458
R	3421	3061	3021	1708	1609	1460
S	3420	3060	3023	1710	1609	1459

XRD diffraction of IIAc derivative [polymorphic form 'P']

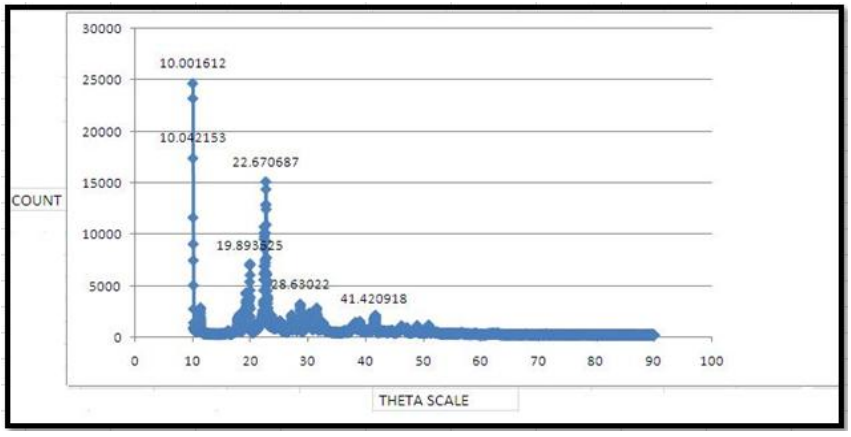


Fig. no.3: XRD spectrum of compound IIAc (polymorphic form P)

Table no. 3: XRD value of compound IIAc (polymorphic form P)

Sr. No.	Peak 2θ value	Intensity	Inference
1	10.0016	24700	Indicated Highly crystalline form
2	10.0218	23238	Indicated Highly crystalline form
3	19.8936	6986	Indicated Moderately crystalline form
4	22.6706	15143	Indicated Moderately crystalline form
5	28.6302	3242	Indicated Less crystalline form
6	41.4209	1785	Indicated Less crystalline form
7	51 to 90	841 to 228	No indication of crystalline form

3. Pharmacological activity:

3.1 Anti-fungal activity[7, 8]: The compounds (IIAa-c and IIFa-c) were evaluated for their antimicrobial activity against *E.coli, S.aureus, B.substilis* and *S.typhi* by disk diffusion method was performed using MacConkeys agar and Nutrient agar medium. Each compound was tested at a concentration at 100µg/ml in DMSO. The zone of inhibition was measured after 24h incubation at 37ºC[1, 4].

3.2 Anti-tubercular activity[9]: The compounds (IIAa-c and IIFa-c) were evaluated for their invitro antitubercular activity against Mycobacterium TB H37RV by nitrate reductase assay method was performed using LJ media. Each compound was tested at a concentration at 100µg/ ml, 150 µg /ml and 200µg/ ml in DMSO. If bottles didn't show any colour change and remain the same then it was confirmed that M.TB H37RV was sensitive to test samples[2].

4. RESULT AND DISCUSSION

4.1 IR, MASS, NMR Spectra studies :- The structural elucidation of the synthesized compounds was done by the interpretation of IR ,MASS and NMR spectra's. All the compounds show satisfactory IR, MASS and NMR.

4.2 Pharmacological Studies;

4.2.1 Antifungal activity[7]

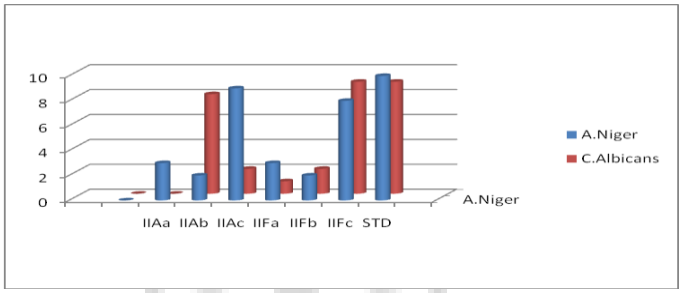


Fig. No.4: The Antifungal activities of synthesized compounds (IIAa-c and IIFa-c)

Candida albicans was used as representative of gram positive fungi. In case of gram positive species *C. albicans* compound IIAb and IIFc shown high activity.

Aspergillus niger was used as representative of gram negative fungi. In case of gram negative species *Aspergillus niger*, compound IIAc and IIFc shown high activity. As compared to standard drug Fluconazole.

4.2.2 Anti-tubercular activity[9]:

Table no.4: Anti-tubercular activity on synthesized compounds (IIAa-c- IIFa-c)

Comp. No.	Control	Std	IIAa	IIAb	IIAc	IIFa	IIFb	IIFc
100 µg/ml	C	NC	NC	C	NC	C	NC	NC

Where, C- Change , NC-not change.

From the observation of Nitrate Reductase Assay, it has been been showed that the compounds IIa and IIc are active against M.tuberculosis H37RV as compare to standard drugs Rifampicin and Isoniazid.

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