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Synthesis, Characterization and Antimicrobial Screening of 1-Substituted -3-Methyl-4-Arylhydrazino- 2-Pyrazolin-5-Ones and Isoxazolinones

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ABSTRACT

The objective of the present work was to prepare and evaluate the derivatives for antimicrobial activity confirming with spectral data. The facile synthesis of 1-substituted-3-methyl-4-aryl hydrazine2-pyrazolin-5-ones and isoxazolinones has been achieved by the diazotization of chlorinated aniline with ethylacetoacetate in the presence of sodium acetate and ethanol furnished the compound (II), while the diazotization of chlorinated aniline with ethyl cyano acetate in the presence of sodium acetate and ethanol yielded (v). Reaction of the intermediates (II) and (V) with aryl hydrazines yielded the compounds (IIIa-c) and (VIa-c) while the reaction of the intermediates with hydroxylamines furnished the compounds (IV) and (VII). Structure of these compounds confirmed on the basis of spectral data. The antibacterial and antifungal activities have been evaluated for the synthesized derivatives. The antimicrobial studies for the derivatives was studied by disc diffusion and serial dilution techniques and compared with standard microbes active for gram positive and gram negative organisms against a standard therapeutic drug. Some of the compounds were found to exhibit promising good anti- bacterial and anti-fungal activities.

Keywords: Pyrazole, Isoxazole, Anti-bacterial, Anti-fungal.

INTRODUCTION

The main objective of the present study is to synthesize different derivatives with pyrazole and isoxazole as basic heterocyclic nucleus and screening for their antimicrobial activities. Pyrazolinone is a five membered lactam ring compound containing two nitrogens and ketone in the same molecule. Lactam structure is an active nucleus in pharmacological activity. In some specific cases of 1-aryl-substituted pyrazolin-5-ones (Ali *et al.*, 2008). Pyrazolinone is a nonsteroidal anti-inflammatory agent used in the treatment of arthritis and other musculoskeletal and joint disorders. 5-pyrazolinones are biologically important group of compounds having activities like antibacterial, antifungal , anti-inflammatory , antidiabetic , analgesic , antipyretic , antiviral , antineoplastic activity and other biological Isoxazolinones showed a broad spectrum of biological activities like antitumor, CNS activity, antimicrobial, antitubercular and analgesic activities (Suresh *et al.*, 2009).

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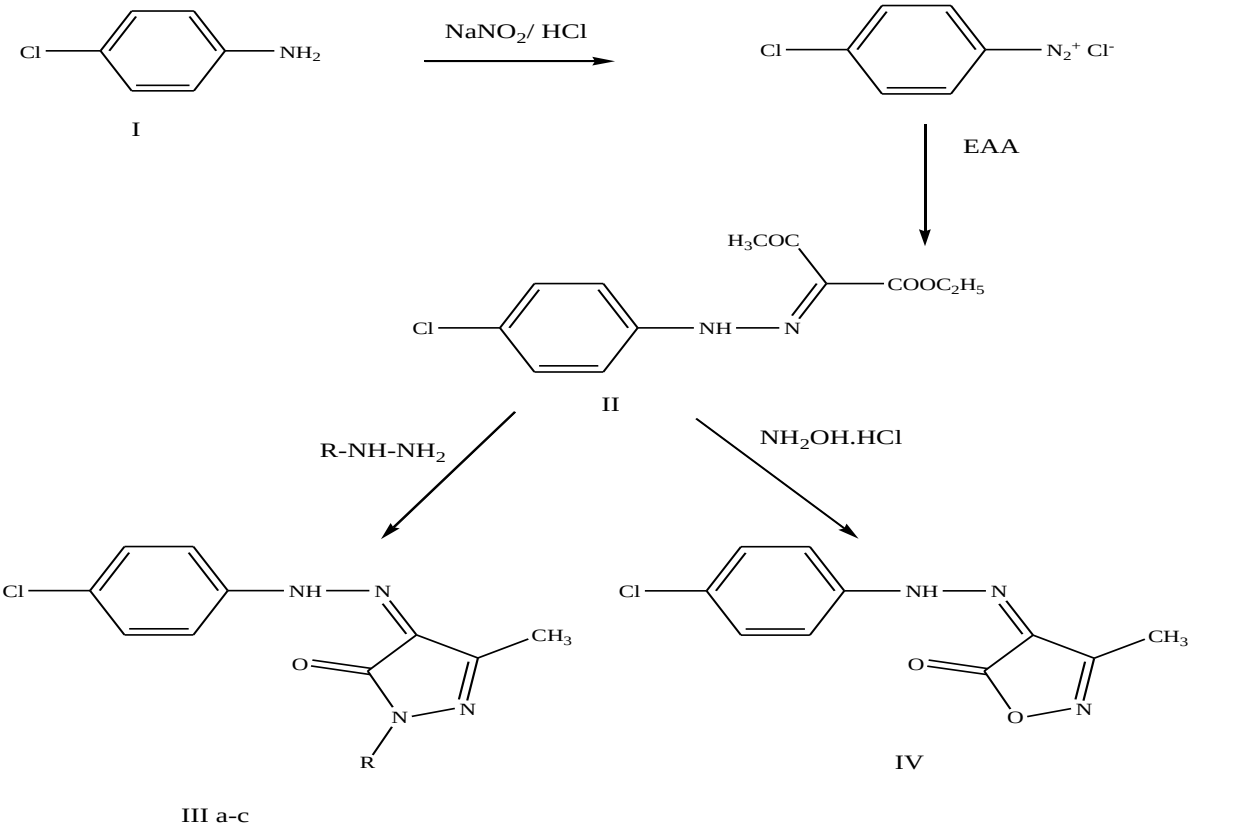
MATERIALS AND METHODS

All the reactions were carried out under prescribed laboratory conditions. The solvents and reagents used in the synthetic work were of laboratory reagent grade and were purified by distillation and crystallization techniques. Melting points of synthesized compounds were determined by open capillary method and were uncorrected. IR spectra of compounds were recorded on FT-IR spectrometer using KBr pellet. NMR spectra were recorded in JMR spectrometer using TMS as internal standard. Mass spectra were recorded in LC-MS spectrometer.

SCHEME.I



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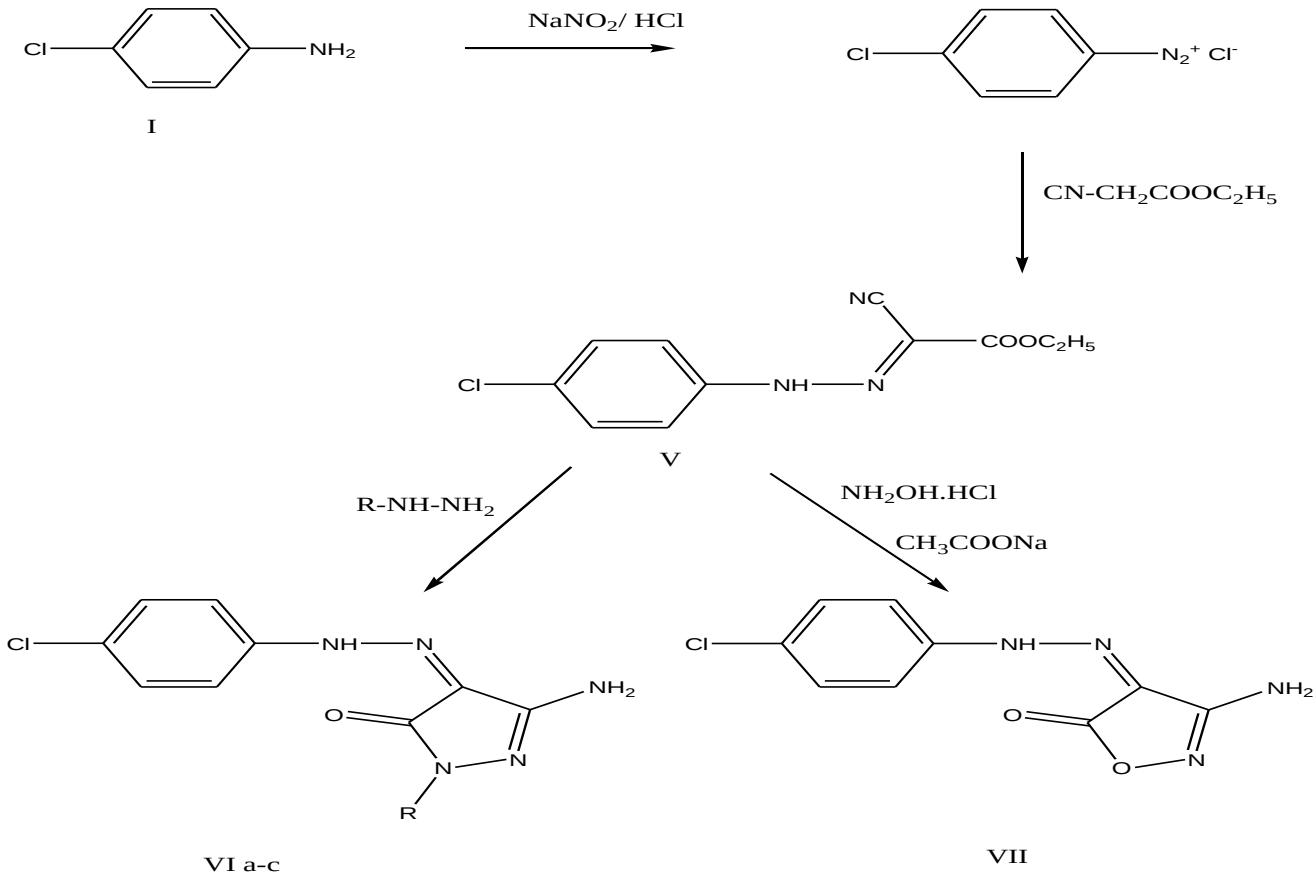

Experimental

Scheme-I

Step- 1: Synthesis of ethyl 2-[(4-chlorophenyl) hydrazono]-3-oxobutanoate (II)

p-Chloroaniline (0.1 mole) was dissolved in dilute hydrochloric acid and cooled to 0˚c in an ice. To this a cold solution of sodium nitrite (0.2 mole) was added. The diazonium salt solution was filtered in cold solution of ethyl acetoacetate (0.1mole) and in sodium acetate in ethanol, yellow solid product was filtered and recryastallized from ethanol (Padmavathi *et al.,* 2009).

Step-2: Synthesis of 3-methyl-1H-pyrazole-4,5-dione 4-[(4-chlorophenyl) hydrazone] (IIIa-c & IV)

Compound II (0.01 mole) was dissolved in glacial acetic acid and 0.02 mole of substituted compound was added. The mixture was refluxed for 4 hours. The mixture was then cooled by pouring in to 100ml of chilled water for (IIIa-c) the resulting solution then poured to crushed ice to get solid product (IV). The solid was filtered, dried and recrystallized with ethanol.

Scheme II

Step-1: Synthesis of ethyl[(4-chlorophenyl) hydrazono] cyano acetate (V)

p-Chloroaniline (0.1 mole) was dissolved in dilute hydrochloric acid and cooled to 0˚c in an ice. To this a cold solution of sodium nitrite (0.2 mole) was added. The diazonium salt solution was filtered in cold solution of ethyl cyanoacetate (0.1mole) and in acetate in ethanol, yellow solid product was filtered and recryastallized from ethanol.

Step-2: Synthesis of 3-amino-1H-pyrazole-4,5-dione 4-[(4-chlorophenyl) hydrazone] (VIa-c & VII)

Compound V (0.01 mole) was dissolved in glacial acetic acid and 0.02 mole of substituted compound was added. The mixture was refluxed for 4 hours. The mixture was then cooled by pouring in to 100ml of chilled water for (VIa-c) the resulting solution then poured to crushed ice to get solid product (VII). The solid was filtered, dried and recrystallized with ethanol.

Anti microbial activity

The antimicrobial activity can be evaluated by serial dilution test and disc diffusion test. To find the sensitivity of organism by measuring zone of inhibition and minimum inhibitory concentration respectively, the medium was prepared by dissolving the specified quantity of the dehydrated medium in purified water and was dispersed in 20 ml volumes into test tubes. The test were closed with cotton plugs and were sterilized by autoclaving at 121 °C (15lb psig) for 15 min. The contents of tubes were poured aseptically in to sterile petri plates (90mm) and allowed to solidify (Kiyoshi *et al.,* 1986).

1. Serial dilution method (Determination of MIC).

MIC of the synthesized compounds was determined by tube dilution techniques. Serial dilution of the substance under examination was placed into culture tubes containing suitable medium and inoculated with the test organism. After incubation, the minimum concentration of test compound that inhibited the growth of the organism was observed. The bacterial cultures used were *Bacillus subtilis* (G^{+ve}), *Staphylococcus aureus* (G^{+ve}), *Escherisia coli* (G^{-ve}), and *Pseudomonas aeruginosa* (G^{-ve}) (Mahesh *et al.,* 2010)The fungal cultures used were *Aspergillus niger* and *Candida albicans* against a control DMF (dimethyl methyl formamide) by using a standard drugs like Ampicillin (antibacterial) and Griseofulvin (anti fungal). All the test compounds were tested at 100µg/ml.

Table.1 Physico-Chemical data of synthesized compounds

Compound No.	Molecular Formula	R	Molecular Weight (gm)	%yield	Melting Point (°C)
IIIa	$C_{10}H_9ClN_4O$	H	237	75.5	189-190
IIIb	$C_{16}H_{13}ClN_4OS$	C_6H_5	312	60.0	129-130
IIIc	$C_{11}H_{10}ClN_5OS$	CS-NH_2	295	70.0	199-200
IV	$C_{10}H_8ClN_3O_2$	-	238	75.5	154-155
VIa	$C_9H_8ClN_5O$	H	237	75.0	118-119
VIb	$C_{15}H_{12}ClN_5O$	C_6H_5	313	80.0	129-130
VIc	$C_{10}H_9ClN_6OS$	CS-H_2	296	75.5	119-120
VII	$C_9H_7ClN_4O_2$	-	239	80.0	194-195

Table : 2 Anti microbial activity (Disc Diffusion method)

	Zone of inhibition (mm)					
	Anti bacterial activity				Anti fungal activity	
Compound	*B.subtilis*	*S.aureus*	*P.aeroginosa*	*E.coli*	*C.albicans*	*A.niger*
IIIa	11	10	13	10	10	12
IIIb	9	13	12	8	08	07
IIIc	17	15	16	12	13	17
Via	10	11	12	7	07	08
VIb	12	13	14	9	16	18
VIc	14	16	18	12	11	14
Standard (Ampicillin 1mg/ml)	16	14	17	13	-	-
Standard (Griseofulvin 1mg/ml)	-	-	-	-	14	17
DMF	-	-	-	-	-	-

PROCEDURE

All the synthesized compounds were dissolved separately to prepare a stock solution containing 1000µg/ml of DMF. 32 mg of different synthesized compounds were dissolved in 2 ml of DMF and 1 ml of this solution was aseptically transferred to the sterile nutrient broth medium and made up to 16 ml with sterile nutrient media, thus 1 ml of the resulted solution gives 1000µg/ml. 1 ml of the above solution was transferred to 1 ml of DMF to give half the concentration of the first. Thus successive concentrations like 250, 125, 62.5 and so were prepared in a similar manner up to 6 dilutions. The tubes were mixed well after each addition. All the tubes were inoculated with one loopful of one of the test organism. The process was repeated with different test organisms. A positive and negative control were also prepared to confirm the nutritive property and sterility, resp., of the prepared media. The tubes were incubated at 37°C for 24 hrs (Hassan *et al.,* 2011). The presence or absence of growth of organism was observed after incubation compared with that of the standard drug, Ampicillin.

Similar procedure was carried out for the evaluation of the anti fungal activity using Sabouraud's dextrose agar medium by standard drug, Griseofulvin.

RESULTS AND DISCUSSION

1. Spectral data of *3-methyl-1H-pyrazole-4,5-dione 4-[(4-chlorophenyl) hydrazone]* IIIa

I.R data **(cm^{-1})**: 3217.70(NH stretch), 3044.50(aromatic CH stretch), 1667.2(C=O), 1565.5(C=N), and 773(C-Cl), ***H^1 NMR(∂)*** : 2.6 (3H,S,-CH_3), 9.9 (1H,S,NH(pyrazolinone)), 11.1 (1H,S,NH(hydrazino)), 7.4- 7.6 (9H,M,Ar-H), ***Mass spectra***: 237 M^+ peak

2. Spectral data of *3-methyl-1-phenyl-1H-pyrazole-4,5-dione 4-[(4-chlorophenyl) hydrazone]* IIIb

I.R data **(cm^{-1})**: 3046.8(aromatic CH stretch), 1656.7(C=O), 1590.9(C=N), and 775.1(C-Cl), ***H^1 NMR(∂)*** : 2.4 (3H,S,-CH_3), 11.3 (1H,S,NH(hydrazino)), 7.2- 7.9 (9H,M,Ar-H), ***Mass spectra***: 312 M^+ peak.

3. Spectral data of *3-amino-1H-pyrazole-4,5-dione 4-[(4-chlorophenyl) hydrazone]* IVa

I.R data **(cm^{-1}):** 3217.1(NH stretch), 3192.1(NH_2 stretch), 1688.2(C=O), 1603.7(C=N), and 779.9 (C-Cl), ***H^1 NMR(∂)*** : 9.9 (1H,S,NH(pyrazolinone)), 6.2 (2H,S,NH_2(pyrazolinone)), 11.2 (1H,S,NH(hydrazino)), 7.3- 7.6 (4H,M,Ar-H), ***Mass spectra***: 237 M^+ peak

4. Spectral data of *3-methyl isoxazole-4,5-dione 4-[(4-chlorophenyl) hydrazone]* IV

I.R data **(cm^{-1})**: 3426.5 (NH stretch), 3209.3 (aromatic CH stretch), 1718.2(C=O), 1594.5(C=N), and 776.5 (C-Cl), ***H^1 NMR(∂)*** : 2.3 (3H,S,-CH_3), 11.1 (1H,S,NH(hydrazino)), 7.3- 7.8 (4H,M,Ar-H), ***Mass spectra***: 238 M^+ peak.

5. Spectral data of *3-amino isoxazole-4,5-dione 4-[(4-chlorophenyl) hydrazone]* VII

I.R data **(cm^{-1})**: 3475.8 (NH_2 stretch), 3217.1 (NH stretch), 1689.4(C=O), 1604.3(C=N), and 765.1 (C-Cl), ***H^1 NMR(∂)*** : 6.7 (2H,S,NH_2), 11.4 (1H,S,NH(hydrazino)), 7.2- 7.5 (4H,M,Ar-H), ***Mass spectra***: 239 M^+ peak.

Anti microbial activity

Disc diffusion activity

a) Anti bacterial activity: All the synthesized compounds have shown potent to weak antibacterial activity. Compounds IIIc, VIc showed potent antibacterial activity against B.*subtilis* and P. *aureginosa*. IIIa, VIb showed moderate and IIIb, VIa showed weak anti bacterial activity when compared to the standard (Table 2).

b) Anti fungal activity: From the results, the synthesized derivatives have shown potent to weak antifungal activity. Compounds IIIc, VIb showed potent antifungal activity against *C.albicans and A.niger*. IIIa, VIc showed moderate and IIIb, VIa showed weak anti fungal activity when compared to the standard (Table 2).

c) Discussion

d) A good yield of synthesized compounds of pyrazlinones were obtained and were confirmed by IR, NMR and Mass spectra. Derivatives shown antibacterial and antifungal activities with concentration at 100µg/ml(Vijay V Dabholkar *et al*.,2009). compounds IIIc, IVc shown potent antibacterial activity against *Bacillus subtilis* and *Pseudomonas aureoginosa* but have moderate activity on *Escherichia coli* and *Staphylcoccus aureus*. Compound IIIa exhibited moderate activity on all the bacterial strains under study. Compounds IIIc, IVb shown potent antifungal activity against *Candida albicans* and *Aspergillus niger*. Compound IIIc, IVc exhibited less activity on all bacterial and fungal strains when compared with other synthesized compounds.

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