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# SYNTHESIS AND BIOLOGICAL EVALUATION OF 1-ACETYL-5-SUBSTITUTED ARYL-3-(β-AMINO NAPHTHYL) - 2-PYRAZOLINES

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## ABSTRACT

The pyrazoline is an important five membered heterocyclic ring system which has inspired many researchers to carry out structural variations in the ring, which has propelled the development of varied pyrazolines with an array of pharmacological activities. On the other hand naphthalene has been identified as a new range of potent antimicrobials effective against wide range of human pathogens. The present work is aimed to synthesize the novel derivatives of naphthalene substituted pyrazolines and to explore the potentiality of the derivatives as antioxidant and antibacterial activities. The intermediate β-amino naphthyl substituted chalcones were prepared by refluxing β-acetylamino naphthalene with various aromatic aldehydes in presence of NaOH. The intermediate is treated with hydrazine hydrate in presence of glacial acetic acid and ethanol to yield the title compounds 1-acetyl-5-substituted aryl-3-(β-aminonaphthyl)-2-pyrazolines (4a-j). The synthesized compounds were screened for their antibacterial activity against four strains of bacteria namely *Bacillus subtilis and Staphylococcus aureus* (gram-positive), *Escherichia coli* and *Staphylococcus aureus* (gram negative) by agar well diffusion method. The antioxidant activity was also evaluated by reduction of DPPH and Nitric oxide scavenging activity methods. Among the synthesized compounds, 4-methoxy (4c) derivative exhibited moderate activity against all the strains of the bacteria except towards *Escherichia coli*. 4-methoxy (4c), 3,4-dimethoxy (4d), 3,4,5-trimethoxy (4e) and 4-hydroxy (4j) derivatives showed moderate antioxidant activity.

**KEYWORDS:** *Pyrazolines, chalcones, antibacterial, antioxidant, nitric oxide scavenging activity*

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# INTRODUCTION

Synthetic heterocyclic compounds occupy a major part of the research in chemistry owing to their wide range of biological activities. The heterocyclic compounds like pyrazolines has been considered as one of the most prominent heterocyclic nucleus and very useful intermediates for the development of molecules of pharmaceutical or biological interests. They exhibited distinct pharmacological activities like antimicrobial,[1-3] anti-inflammatory,[2,3] analgesic,[3] antiamoebic,[4] anticonvulsant,[5] antidepressant,[5] etc. Naphthalene is another important pharmacophore known for its anti-microbial,[6,7] antioxidant,[7] and anticonvulsant[8] activities. Considering the potentiality of the above pharmacophores, the present work is aimed to synthesize the compounds with these two pharmacophores i.e. naphthalene and pyrazolines in hope of obtaining synergistic activity in antioxidant and antibacterial activities. The derivatives synthesized were screened for antibacterial and antioxidant activities by the standard procedures.

# MATERIALS AND METHODS

### *Chemistry*

All chemicals and solvents are of AR grade. All the melting points reported in this series were determined in open capillaries using Thermonik precision melting point cum boiling point apparatus model C-PMB-2 and are uncorrected. The progress of all reactions was monitored by using pre-coated TLC plates (E. Merck Kieselgel 60 F254) with Toluene and Ethyl acetate (9:1 v/v) as mobile phase and the spots were visualized by iodine vapour. The IR spectra were recorded using KBr pellets on a Perkin-Elmer 1760 Spectrophotometer ($cm^{-1}$). $H^1$ NMR spectra were recorded on GE Omega 400 MHz spectrometer or Bruker Avance 300 MHz spectrometer, using Tetramethyl -silane (TMS) as an internal standard. Mass spectra were recorded on a JEOL-JMS-D-300 spectrometer

### *EXPERIMENTAL*

### *Synthesis of β-acetyl amino naphthalene*

A mixture of 1.43gm (0.01mol) of β-amino naphthalene with acetic anhydride 0.15ml, and glacial acetic acid 0.57ml were refluxed for 6hrs on a water bath. The completion of the reaction was monitored by TLC. Then the reaction mixture was poured on to ice, the solid residue thus obtained was separated using Whatman filter paper, dried and recrystallized with methanol.[9]

### *Synthesis of β-amino naphthyl-substituted chalcones (3a-3j)*

The β-acetyl amino naphthalene (0.01mol) obtained in the earlier step was dissolved in ethanol (50ml) and refluxed with various aromatic aldehydes in presence of 30% NaOH for about 6hrs, cooled and poured in to ice. The solid was separated by filtration and recrystallized from methanol to obtain the chalcones.[9]

### *Synthesis of 1-acetyl-5-substituted aryl-3-(β-amino naphthyl) 2-pyrazolines (4a-4j)*

To a solution of compound (0.02mol), ethanol 99%, hydrazine hydrate (0.04ml) and a few drops of glacial acetic acid were added and the reaction mixture was refluxed for 6 hrs. The excess of solvent was distilled off and cooled. The separated solid was washed with cool water and recrystallized with methanol.[9]

### *Antibacterial activity*

Cultures of two gram positive bacteria, i.e *Bacillus subtilis*, *Staphylococcus aureus* and two gram negative bacteria, *Escherichia coli* and *Proteus vulgaris* were used to investigate the antimicrobial activity of the compounds (4a-j). The antimicrobial activity was assayed biologically using agar well diffusion method.[10,11] In this method, wells of standard diameter are made in the nutrient agar medium, containing standard bacterial inoculums. The test compounds were introduced into the wells and diameter of the zone of inhibition was measured by antibiotic zone reader. The standard drug used was streptomycin.

### *Assay of nitric oxide (NO) scavenging*

Sodium nitroprusside (10μM) in phosphate buffer pH 7.4 was incubated with 100μM concentrations of drug dissolved in a suitable solvent (dioxan /methanol) and tubes were incubated at 25°C for 120 min. Then 2mL of incubation solution was removed and diluted with 2mL of Griess reagent (Sulphanilamide: 4g, N-Napthylethylene diamine dihydrochloride: 0.2g, 10% Orthophosphoric acid:10 mL, Distilled water:100 mL). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and on subsequent coupling with N-napthylethylene diamine was read at 546 nm.[12] Control experiments without test compound were also conducted in an identical manner.

### *Interaction with stable free radical DPPH*

DPPH assay was performed as described.[13] Solutions of various drugs at 100 μM concentration were added to 100 μM DPPH in 95% ethanol and tubes were kept at an ambient temperature for 20 min and absorbance was measured at 517nm. Ethanol was used as a blank solution and DPPH solution in ethanol served as the control.

# RESULTS AND DISCUSSION

### *Synthesis of 1-acetyl -5-substituted aryl-3-(β-aminonaphthyl)-2-pyrazolines (2)*

β-Naphthylamine was used as starting material. The intermediate β-aminonaphthyl substituted chalcones were prepared by refluxing β-acetylamino naphthalene with various aromatic aldehydes in presence of NaOH. The intermediate was refluxed with hydrazine hydrate in presence of glacial acetic acid and ethanol underwent nucleophilic addition to yield the title compounds (Figure1). A total of ten compounds were synthesized in this series. The compounds were obtained in yield ranging from 40-80%. The physical and spectral data such as melting point, yield, IR and NMR are given in table 1 the physicochemical data of compounds.

***SCHEME OF SYNTHESIS OF TITLE COMPOUNDS***

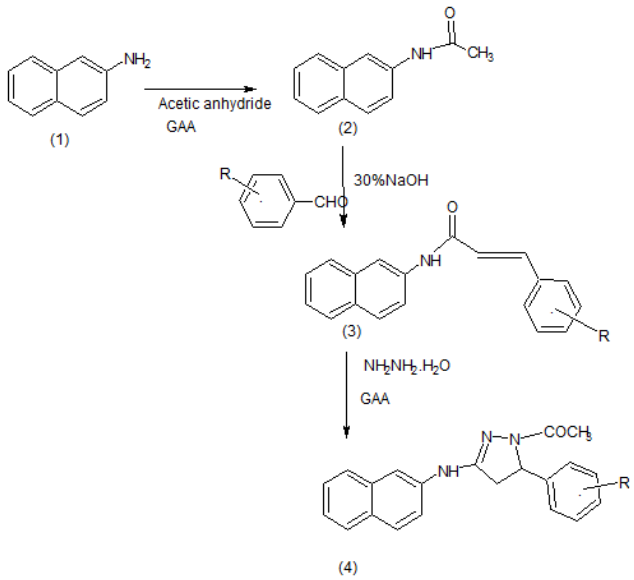


**Table 1**
***The physicochemical data of compounds***

| Compound | Substituent | Yield (%) | Melting Point (°C) | IR ($cm^{-1}$) | NMR (ppm,$CDCl_3$) |
|---|---|---|---|---|---|
| **4a** | -H | 60 | 192 | 3400 (N-H); 1710(C=O); 1580(C=N), 1560(C=C of aromatic ring); 1510 (C-N). | δ2.60(s, 3H, CO-$CH_3$), δ 7.65(d,2H, pyrazoline $CH_2$), δ7.8-8.2 (m, 12H, Ar-H), 9.3(s,1H,NH) |
| **4b** | 4-$CH_3$ | 70 | 133 | 3228 (N-H); 2935 (C-H); 1723 (C=O); 1597 (C=N); 1528 (C=C of aromatic ring); 1470 (C-N). | δ2.25 (s, 1H, $CH_3$ ); δ2.98 (d,3H,CO-$CH_3$), 7.51-7.52 (d, 2H, pyrazoline $CH_2$) δ 7.61- 7.61 (m, 11 H, Ar- CH); δ9.28(s, 1H, NH). |
| **4c** | 4-O $CH_3$ | 50 | 134 | 3430(N-H); 1720(C=O); 1560(C=N); 1560(C=C of aromatic ring); 1500(C-N). | δ2.70(s, 3H, CO-$CH_3$), δ 3.76 (s,3H,Ar-OCH3), δ 7.75(d,2H, pyrazoline $CH_2$), δ7.60-8.15 (m, 12H, Ar-H), 9.25 (s, 1H, NH) |
| **4d** | 3,4- $(OCH_3)_2$ | 45 | 155 | 3388(N-H); 1679(C=O); 1586( C=N); 1512(C=C of aromatic ring); 1459(C-N) ; 1021(O-$CH_3$). | - |
| **4e** | 3,4,5-$(OCH_3)_2$ | 52 | 151 | 3410 (N-H); 1710(C=O); 1580(C=N); 1560(C=C of aromatic ring); 1510 (C-N). | - |
| **4f** | 4-CH-( $CH_3)_2$ | 55 | 146 | 3420 (N-H); 1710(C=O); 1580 (C=N); 1540(C=C of aromatic ring); 1520(C-N). | - |
| **4g** | 4-N$(CH_3)_2$ | 47 | 142 | 3400 (N-H); 1710(C=O); 1580(C=N); 1560 (C=C of aromatic ring) 1510(C-N). | δ2.60(s, 3H, CO-$CH_3$), δ 2.90 (s, 6H,-N(CH3)2), δ 7.70(d,2H, pyrazoline $CH_2$), δ7.60-8.20 (m, 12H, Ar-H), 9.20 (s, 1H, NH) |
| **4h** | 4-F | 42 | 155 | 3410(N-H); 1710(C=O); 1580(C=N); 1560(C=C of aromatic ring); 1510(C-N). | δ2.60(s, 3H, CO-$CH_3$), δ 7.65(d,2H, pyrazoline $CH_2$), δ7.8-8.2 (m, 12H, Ar-H),9.3(s,1H, NH) |
| **4i** | 4-$NO_2$ | 49 | 110 | 3400 $cm^{-1}$ (N-H); 1710 $cm^{-1}$ (C=O); 1580 $cm^{-1}$ (C=N); 1560 $cm^{-1}$ (C=C of aromatic ring); 1510 $cm^{-1}$ (C-N). | δ2.60(s, 3H, CO-$CH_3$), δ 7.65(d,2H, pyrazoline $CH_2$), δ7.8-8.2 (m, 12H, Ar-H), 9.3(s,1H, NH) |
| **4j** | 4- OH | 42 | 135 | 3400(N-H); 1710(C=O); 1580(C=N); 1560(C=C of aromatic ring); 1510(C-N). | - |

***Biological evaluation***

The compounds synthesized (4a-4j) were evaluated for antibacterial activity against two gram-positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus* and two gram-negative bacteria, *Escherichia coli* and *Klebsiella pneumoniae*. The data is given in Table 2. 4-methoxy derivative (4c) and 4- nitro derivative (4i) exhibited significant antibacterial activity. The compounds 4h, 4d, 4e, 4j showed moderate antibacterial activity. All compounds were found to be less active against *Escherichia coli*. The synthesized compounds were also evaluated for their antioxidant activity by NO scavenging method and interaction with stable free radical method. Results from both the methods suggested that 4-methoxy (4c), 3,4-dimethoxy (4d), 3,4,5-trimethoxy (4e), 4-dimethyl amino (4g) and 4-hydroxy (4j) derivatives showed significant activity.

**Table 2**
***Antibacterial activity of 1-acetyl -5-substituted aryl-3-(β-amino naphthyl)-2-pyrazolines***

| Compound | Substituent | Average Diameter of Zone of Inhibition in mm | | | |
|---|---|---|---|---|---|
| | | *S.aureus* | *B.subtilis* | *E.coli* | *K.pneumoniae* |
| **4a** | -H | 10 | 10 | 11 | 09 |
| **4b** | 4-$CH_3$ | 10 | 12 | 12 | 12 |
| **4c** | 4-O $CH_3$ | 17 | 18 | 13 | 12 |
| **4d** | 3,4- $(OCH_3)_2$ | 13 | 12 | 12 | 13 |
| **4e** | 3,4,5-(O $CH_3)_3$ | 13 | 14 | 13 | 12 |
| **4f** | 4-CH-( $CH_3)_2$ | 13 | 10 | 10 | 10 |
| **4g** | 4-N$(CH_3)_2$ | 10 | 10 | 10 | 12 |
| **4h** | 4-F | 14 | 10 | 09 | 09 |
| **4i** | 4-$NO_2$ | 18 | 16 | 11 | 10 |
| **4j** | 4- OH | 12 | 12 | 05 | 11 |
| Streptomycin | | 25 | 26 | 23 | 22 |

**Zone of inhibition of test compounds at a concentration of 100µg/mL was measure *B. subtilis: Bacillus subtilis E.coli*: *Escherichia coli K. pneumoniae*: *Klebsiella pneumoniae S.aureus*: *Staphylococcus aureus***

**Table 3**
***Anti-oxidant activity of the title compounds***

| Compound | Substituent | NO Scavenging activity at 100µM | %Inhibition of DPPH at 100µM |
|---|---|---|---|
| **4a** | -H | 29 | 20 |
| **4b** | 4-$CH_3$ | 22 | 22 |
| **4c** | 4-O $CH_3$ | 57 | 53 |
| **4d** | 3,4- $(OCH_3)_2$ | 52 | 50 |
| **4e** | 3,4,5-(O $CH_3)_3$ | 53 | 54 |
| **4f** | 4-CH-( $CH_3)_2$ | 32 | 37 |
| **4g** | 4-N$(CH_3)_2$ | 49 | 48 |
| **4h** | 4-F | 40 | 32 |
| **4i** | 4-$NO_2$ | 35 | 32 |
| **4j** | 4- OH | 60 | 60 |
| Ascorbic acid | | 70 | 70 |

## CONCLUSION

The research study reports the successful synthesis of various 1-acetyl -5-substituted aryl-3-(β-amino naphthyl)-2-pyrazoline derivatives and screened for anti-bacterial and antioxidant activities. All the compounds were obtained in good yield. The compounds showed moderate to good antibacterial and antioxidant activities. Among the synthesized compounds 4c, 4d, 4e,4j showed good antibacterial & antioxidant activities. Further, we are planning to carry out the SAR studies of these compounds and also to exploit the various biological activities. So pyrazolines certainly holds great promise towards good active leads in medicinal chemistry.

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## CONFLICT OF INTEREST

Conflict of interest declared none.

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