SYNTHESIS OF NOVEL PYRAZOLINES AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

A series of novel 4-nitroaniline derived pyrazolines have been synthesized and characterized by data arrived from the analytical methods such as FT-IR, ¹H NMR, ¹³C NMR and Mass spectral analysis. X-ray crystallographic structure elucidation of a crystalline compound 4-fluoro benzaldehyde derived chalcone is presented. The impact of electronegativity and extension of conjugation offered by the nitro group in the synthesized molecule on its biological activity was analyzed. Title compounds were subjected to antimicrobial activity.

Keywords: Chalcones, Pyrazolines, XRD, Antimicrobial activity. 4-nitro aniline derived triazoles, electronegativity, extend of conjugation.

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INTRODUCTION

The diverse pharmacological activity of pyrazoline linked heterocyclic compounds was well established. A modified pyrazoline ring linked to other heterocyclic moieties has shown potent biological activity with lesser toxicity. The 2-pyrazolines constituted heterocyclic linked derivatives possess anticancer¹, antidepressant and anticonvulsant², Cannabinoid receptor antagonist activity³, COX-2 inhibitor activity⁴, antihepatotoxic activity⁵, antihyperglycemic activity⁶, anti-inflammatory and analgesic activity^{7,8,9}, antimalarial activity¹⁰, antioxidation property¹¹, MAO inhibitors^{12, 13}, antitubercular activity^{14,15}, antimicrobial activity.^{16, 17} From literature review it was found that pyrazolines linked to different chemical moieties viz., benzofuran, benzene sulphonamide, pyrimidine, coumarine, carboxamide and benzo pyrrole, 1, 2, 4-triazol, 1, 4-dioxane, quinoxaline, benzene sulphonyl urea and thiourea, carbohydrazone and thiazole have been studied to some extent with their biological activity. Hitherto only a very few compounds of this class like 4-methyl-5-(5-phenyl-4, 5-dihydro-1H-pyrazole-3-yl)-1-(p-tolyl)-1H-1, 2, 3-triazole have been reported in the literature.¹⁶ We, herein, report the synthesis and antimicrobial activity of a series of 1, 2, 3-triazole linked chalcone and their pyrazolines following Scheme-1. Out of ten chalcones prepared, 4-F benzaldehyde derived chalcone was induced to crystallize and its XRD pattern is presented.

EXPERIMENTAL

Solvents available commercially were used after purification by the distillation process. Reaction completion was monitored by both silica gel coated glass plates and pre-coated Aluminium TLC plates procured from Merck (Germany). Purified compounds were characterized by elemental analysis IR, ¹H NMR, ¹³C NMR and an EI-mass spectrometry. IR spectra were recorded in KBr on a Perkin-Elmer model

1620 FTIR spectrophotometer, ^1H NMR and ^{13}C NMR spectra were recorded in Bruker AvanceII 300 MHz spectrophotometer using $\text{CDCl}_3/\text{DMSO}$ as a solvent and mass spectra were recorded on a JEOL mass spectrometer. XRD was measured using CrysAlisPro (CrysAlisPro 1.171.39.46 (Rigaku Oxford Diffraction, 2018).

Scheme-1: Synthesis of 4-Nitro Aniline derived Pyrazolines

Antimicrobial Activity

Bacterial and fungal strains collected from IMTECH, Chandigarh, India. Antimicrobial studies for pyrazoline derivatives performed by serial dilution method.¹⁸ The test organisms used were (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *E. faecalis*, *Pseudomonas fluorescense*, *Pseudomonas aeruginosa*, *Klebsiella* and *Salmonella typhi*) and the fungal cultures of (*Aspergillus niger*, *Aspergillus terreus* and *Trichoderma harzanium*). A stock solution of $100\mu\text{g/mL}$ of the tested compounds was serially diluted to get the concentration of $50\mu\text{g/mL}$ and inoculated with $100\mu\text{L}$ of suspension of the respective organism in sterile saline. DMSO was used as a solvent. All the inoculated test tubes of synthesized compounds and the standard drugs ciprofloxacin, Ketoconazole, Fluconazole and Tetracycline incubated for 24 h at 37°C and the results were recorded as minimum inhibition concentration (MIC).

General Procedure for the Synthesis of p-Nitroazidobenzene¹⁹ (1)

p-nitro aniline (0.138g, 1 mmol) was dissolved in 1: 1 HCl taken in a round bottom flask equipped with stirrer and an ice bath to maintain the temperature between $0-5^\circ\text{C}$. Sodium nitrite (0.069g, 1 mmol) dissolved in 25 mL of ice-cold water was added in portions to the flask with constant stirring followed by (0.065g, 1 mmol) cooled sodium azide solution. The pale yellow color precipitate was washed with sodium carbonate solution and water until the filtrate was neutral to litmus paper and dried. The yield was 98%.

1-Azido-4-nitrobenzene (yellow colored powder) (1): MP 80°C ; IR (KBr, cm^{-1}): 3110, 1593 2127; ^1H NMR ($\text{DMSO}-d_6$, 300MHz): δ 7.38 (2H, d, $J = 3\text{Hz}$, Ar), 8.24 (2H, d, $J = 3\text{Hz}$ Ar); ^{13}C NMR ($\text{DMSO}-d_6$, 300 MHz) 147.12, 144.50, 125.96, 120.51; MS: m/z 164 [M^+].

General Procedure for the Synthesis of 1-(4-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazole-5-yl) ethanone²⁰ (2)

p-nitroazidobenzene (0.164 g, 1 mmol) was mixed with acetylacetone (1 g, 1 mmol) and K_2CO_3 (0.414 g, 3 mmol) in 20 mL of (95%) ethanol taken in a round bottom flask equipped with a stirrer. The reaction mixture was heated at 80°C for 6 hours with constant stirring and the reaction was monitored by TLC. After completion of the reaction, the excess solvent was removed under vacuum and quenched in ice, neutralized with 10% HCl and washed with water until the filtrate was neutral to litmus paper and dried. The yield was 94 %.

Yellow Colored Amorphous Powder (2): MP 147°C; IR (KBr, cm⁻¹): 3078, 1558, 1521, 1680; ¹H NMR (DMSO-d₆, 300 MHz): 7.97 (2H, d, J= 3Hz Ar), 8.47 (2H, d, J= 3Hz Ar), 2.66 (3H, s, COCH₃), 2.602 (3H, s, CH₃); ¹³C NMR (DMSO-d₆, 300 MHz): 193.71, 148.34, 143.55, 140.26, 138.64, 126.91, 125.55, 40.81, 39.97, 28.11, 10.26; MS: m/z 246.1 [M⁺].

General Procedure for 1-(4-methyl)-1-(4-nitrophenyl)-1H-1,2,3-triazole-5-yl)-3-phenylprop-2-en-1-one ²¹ (1)

1-(4-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazole-5-yl) ethanone (0.246g, 1mmol) mixed with substituted aromatic aldehydes, 10 mL of ethanol, 20 mL of 25% NaOH were added and was stirred for 5h at room temperature. The completion of the reaction was identified with the help of TLC analysis and the content in the flask was neutralized with 10% HCl and washed with water and dried. Yield was 98%.

1. 1-(4-methyl)-1-(4-nitrophenyl)-1H-1, 2, 3-triazole-5-yl)-3-phenylprop-2-en-1-one (Colorless, Amorphous Solid)

MP 207°C; IR (KBr, cm⁻¹): 3089, 1656, 1593, 1519, 1342; ¹H NMR (DMSO-d₆, 300 MHz): 8.52 (2H, d, J=8.5Hz, Ar), 8.04 (2H, d, J=9.5Hz Ar), 7.92 (1H, d, J=9.0Hz H_α), 8.0 (1H, d, J=9.0Hz H_β), 2.69 (3H, s, CH₃), 7.5- 7.84 (5H, m, Ar); ¹³C NMR (DMSO-d₆, 300 MHz): 183.75, 148.43, 143.90, 143.80, 140.31, 140.0, 134.78, 131.38, 129.59, 129.25, 127.05, 125.91, 125.60, 123.09, 10.52; MS: m/z 335.09 [M⁺].

2. 3-(4-methoxyphenyl)-1-(4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-5-yl)prop-2-en-1-one (Pale Yellow Crystalline Solid)

MP 212°C; IR (KBr, cm⁻¹): 3078, 1680, 1597, 1520, 1343; ¹H NMR (DMSO-d₆, 300 MHz): 8.52 (2H, d, J=8.5Hz Ar), 8.04 (2H, d, J=9.5Hz Ar), 7.94 (1H, d, J=15.6 Hz H_α), 7.87 (1H, d, J=9.0Hz, H_β), 7.06 (2H, d, J=8.7Hz Ar), 7.84 (2H, d, J=8.1Hz Ar), 2.68 (3H, s, CH₃), 3.84 (3H, s, -OCH₃); ¹³C NMR (DMSO-d₆, 300 MHz): 183.71, 162.07, 148.43, 143.88, 140.38, 139.74, 131.21, 127.42, 127.07, 125.60, 120.66, 115.10, 55.91, 10.50; MS: m/z 364.12 [M⁺].

3. 1-(4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-5-yl)-3-(4-nitrophenyl)prop-2-en-1-one, (Brown Amorphous Solid)

MP 218°C; IR (KBr, cm⁻¹): 3082, 166, 1601, 1523, 1346; ¹H NMR (DMSO-d₆, 300 MHz): 8.38 (2H, d, J=8.1Hz Ar), 7.94 (2H, d, J=9Hz Ar), 8.04 (1H, d, J=8.8Hz H_α), 8.38 (1H, d, J=8.8Hz H_β), 8.17 (2H, d, J=2.8Hz Ar), 8.11 (2H, d, J=2.8Hz dAr), 2.66 (3H, s, CH₃); ¹³C NMR (DMSO-d₆, 300 MHz): 182.40_{C=O}, 163.30, 151, 149, 143, 143, 142, 135, 134, 125, 123.3, 117, 10.43_{CH₃}. MS: m/z 378.77 [M⁺].

4. 1-(4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-5-yl)-3-(3-nitrophenyl)prop-2-en-1-one (Brown Amorphous Solid)

MP 195°C; IR (KBr, cm⁻¹): 3091, 1667, 1598, 1521, 1346; ¹H NMR (DMSO-d₆, 300 MHz): 8.53 (2H, d, J=9.0Hz Ar), 8.33 (2H, d, J=9.0Hz Ar), 8.05 (1H, d, J=6.6Hz H_α), 7.78 (1H, d, J=7.8 Hz H_β), 7.997-8.147 (4H, m, Ar), 2.66 (3H, s, CH₃); ¹³C NMR (DMSO-d₆, 300 MHz): 183.42, 150, 148, 148.37, 144, 143.65, 141, 140, 136.57, 134, 126.92, 126.73, 124, 123, 118, 10.53; MS: m/z 378.77 [M⁺].

5. 1-(4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-5-yl)-3-(2-nitrophenyl)prop-2-en-1-one (Brown Amorphous Solid)

MP 208°C; IR (KBr, cm⁻¹): 3082, 168, 1602, 1527, 1348; ¹H NMR (DMSO-d₆, 300 MHz): 8.53 (2H, d, J=8.7Hz Ar), 8.18 (2H, d, J=9.0Hz Ar), 8.13 (1H, d, J=10.5Hz H_α), 8.02 (1H, d, J=9.3Hz H_β), 7.88 (1H, t, J=7.5Hz, 7.2Hz Ar), 7.77 (1H, t, J=7.8Hz, 7.5 Hz Ar), 2.70 (3H, s, CH₃); ¹³C NMR (DMSO-d₆, 300 MHz): 183.37, 163.5, 149.25, 148.50, 143.56, 140.56, 140.23, 138.87, 134.56, 130.10, 129.81, 127.25, 127.13, 125.63, 124.34, 10.54; MS: m/z 379.1 [M⁺].

6. 3-(2-chlorophenyl)-1-(4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-5-yl)prop-2-en-1-one (Colorless Solid)

MP 202°C; IR (KBr, cm⁻¹): 3091 (C-H str): 1683, 1595, 1525, 1344; ¹H NMR (DMSO-d₆, 300 MHz): 8.49

(2H, d, J=9.0Hz Ar), 8.32 (2H, d, J=9.0Hz Ar), 7.63 (1H, d, J=9.0Hz H α), 7.72 (1H, d, J=9.0Hz H β), 7.5- 8.5 (4H, (m, Ar), 2.69 (3H, s, CH₃): ¹³C NMR (DMSO-d₆, 300 MHz):183.49, 148, 144, 143, 141, 143.80, 140.63, 140.33, 140.20, 138.68, 138.60, 134.94, 132.98, 129.59, 127.05, 125.91, 123.09, 10.5; MS: *m/z* 368.1[M⁺], 370.1(M+2) (1:3 ratio).

7. 3-(3-chlorophenyl)-1-(4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-5-yl)prop-2-en-1-one (Pale Yellow Solid)

MP 203°C; IR(KBr, cm⁻¹):3091(C-H str):3078, 169, 1596), 1515, 1342; ¹HNMR(DMSO-d₆,300MHz): 8.51 (2H, d, J=9.0Hz Ar), 7.78 (2H, d, J=9.0Hz Ar), 8.1 (1H, d, J=16.2Hz H α), 7.89 (1H, d, J=16.2Hz H β), 7.4-7.7 (4H, m, Ar), 2.78 (3H, s, CH₃): ¹³C NMR (DMSO-d₆, 300 MHz):183.4, 142.04, 136.25, 130.18, 129.94, 127.96, 126.70, 125.63, 124.91, 123.61, 10.21; MS: *m/z* 368.1 [M⁺], 370.1 [M+2] (1:3ratio).

8. 3-(4-chlorophenyl)-1-(4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-5-yl)prop-2-en-1-one, (Colorless Amorphous Solid)

MP189°C; IR(KBr, cm⁻¹):2966, 1680, 1597, 1525, 1344 ; ¹HNMR(DMSO-d₆,300MHz):8.58 2H, (d, J=5.7Hz Ar), 8.03 (2H, d, J=5.4Hz Ar), 8.1 (1H, d, J=10.5Hz H α), 7.97 (1H, d, J=8.1Hz H β), 7.62 (2H, d, J=5.3Hz Ar), 8.03 (2H, d, J=5.2Hz Ar), 2.71(3H, s,(CH₃): ¹³C NMR (DMSO-d₆, 300 MHz):183.66, 167, 148, 143, 140.2, 140, 135, 133, 131, 130, 128.64, 129, 125, 123.80, 123.0, 10.52; MS: *m/z* 368.1 [M⁺], 371.18 [M+2] (1:3 ratio).

9. 3-(2-hydroxyphenyl)-1-(4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-5-yl)prop-2-en-1-one, (Pale Yellow Amorphous Solid)

MP 209°C; IR(KBr, cm⁻¹):2885, 1681, 1575, 1523, 1344; ¹HNMR(DMSO-d₆,300MHz): 8.58 (2H, d, J=9.0Hz Ar), 8.10 (2H, d, J=9.0Hz Ar), 7.77 (1H, d, J=7.8Hz H α), 6.94 (1H, d, J=10.5Hz H β), 7.02-7.3 (4H, m, Ar), 2.74 (3H, s, CH₃): ¹³C NMR (DMSO-d₆, 300 MHz):183.71, 162.07, 148.43, 143.88, 140.38, 139.74, 131.21, 127.42, 127.07, 125.60, 120.66, 115.00, 10.50; MS: *m/z* 350.1 [M⁺].

10. 3-(4-fluorophenyl)-1-(4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-5-yl)prop-2-en-1-one (Colorless Crystalline Solid)

MP 206°C; IR(KBr, cm⁻¹):3094, 1668, 1597), 1523, 1343 ; ¹HNMR(DMSO-d₆,300MHz): 8.52 (2H, d, J=9.0Hz Ar), 7.36 (2H, d, J=8.7Hz Ar), 8.04 (2H, d, J=4.2Hz Ar), 7.93 (2H, d, J=6.0Hz, Ar), 7.91 (1H, d, J=16.2Hz, H α), 8.04 (1H, d, J=11.4Hz H β), 2.68 (3H, s, CH₃): ¹³C NMR (DMSO-d₆, 300 MHz): 183.71, 165.6, 162.4, 148.4, 143.7, 142.7, 140.3, 140, 131.75, 131.6, 131.00, 127.07, 125.60, 122.07, 116.7, 116.40, 10.52; MS: *m/z* 352.1 [M⁺].

11. 3-(4-bromophenyl)-1-(4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-5-yl)prop-2-en-1-one (Pale Yellow Solid)

MP 192°C;IR(KBr, cm⁻¹):3090, 1681, 1587, 1527, 1345; ¹HNMR(DMSO-d₆,300MHz): 8.52 (2H, d, J=9.0Hz Ar), 7.70 (2H, d, J=8.4Hz Ar), 8.08 (1H, J=13.2Hz H α), 7.82 (1H, d, J=26.4Hz H β), 8.03 (2H, d, J=6.3Hz Ar), 7.88 (2H, d, J=8.4Hz Ar), 2.68 (3H, s, CH₃): ¹³C NMR (DMSO-d₆, 300 MHz): 183.60, 167.08, 148.45, 143, 140.29, 140, 134.9, 136.5, 131.7, 131.1, 130.5, 127.35, 125.61, 123.8, 10.53; MS: *m/z* 413.02 [M⁺], 415.02 [M+2] (1:1 ratio).

General procedure for the synthesis of 4-Methyl-1-(4-nitrophenyl)-5-(5-phenyl-4, 5-dihydro-1H-pyrazole-3-yl)-1H-1, 2, 3 triazole (4)

1(4-methyl) -1-(4-nitrophenyl)-1H-1, 2, 3-triazole -5-yl)-3-phenylprop-2-en-1-one (0.334g, 1mmol), hydrazine hydrate (0.75g, 1.5 mmol), 20mL of ethanol, 5 mL of 20% glacial acetic acid were mixed and heated with stirring for 8 hours at 80°C. The content in the flask was neutralized with Na₂CO₃ and washed with water and dried. The yield obtained was 85%.

1. 4-Methyl-1-(4-nitrophenyl)-5-(5-phenyl-4,5-dihydro-1H-pyrazole-3-yl)-1H-1,2,3-triazole (Yellow Colour Solid)

MP 208°C; IR(KBr, cm⁻¹): 3327, 3089, 1609, 1519, 1343; ¹HNMR(DMSO-d₆,300MHz):8.46 (2H, d, J=9.0Hz Ar), 8.01 (2H, d, J=9.0Hz Ar), 3.01 (1H, dd, J=10.2Hz, 10.4Hz C-H), 3.04 (1H, dd,

J=10.5Hz, 10.8Hz C-H), 4.86 (1H, t, J=10.5Hz C-H), 7.402 (1H, s, N-H), 7.2 to 7.5 (5H, m, Ar), 2.62 (3H, s, CH₃): ¹³C NMR (DMSO-d₆, 300 MHz): 147.98, 144.05, 143.09, 140.98, 139.98, 132.54, 128.91, 127.68, 127.08, 126.36, 125.54, 63.09 C-H, 42.40, 10.58; MS: *m/z* 348.13 [M⁺].

2. 5-(5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazole (Pale Yellow Amorphous Solid)

MP 210°C; IR(KBr, cm⁻¹): 3327, 3089, 1610, 1519, 1342; ¹HNMR(DMSO-d₆, 300MHz): 8.48 (2H, d, J=8.5Hz Ar), 8.00 (2H, d, J=8.5Hz Ar), 3.00 (1H, dd, J=10.5Hz, 10.5Hz C-H_{2a}), 3.59 (1H, dd, J=10.5Hz, 10.8Hz C-H_{2b}), 4.83 (1H, t, J=10.5Hz C-H), 7.63 (1H, s, N-H), 6.93 (2H, d, J=8.0Hz Ar), 7.33 (2H, d, J=8.0Hz Ar), 2.62 (3H, s, CH₃), 3.75 (3H, s, OCH₃): ¹³C NMR (DMSO-d₆, 300 MHz): 148.12, 145.7, 145.2, 141.5, 136.12, 135.05, 134.1, 129.9, 129.2, 127.05, 125.1, 124.9, 65.9 C-H, 44.4, 11.9; MS: *m/z* 378.14 [M⁺].

3. 4-methyl-1-(4-nitrophenyl)-5-(5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-1H-1,2,3-triazole (Yellow Color Amorphous Solid)

MP 211°C; IR(KBr, cm⁻¹): 3317, 3087, 1599, 1517, 1345; ¹HNMR(DMSO-d₆, 300MHz): 8.48 (2H, d, J=9.2Hz Ar), 8.0 (2H, d, J=8.8Hz Ar), 3.04 (1H, dd, J=10.4Hz, 10.5Hz C-H_{2a}), 3.76 (1H, dd, J=10.2Hz, 10.5Hz C-H_{2b}), 5.04 (1H, t, J=7.6Hz C-H), 7.98 (1H, s, N-H), 8.25 (2H, d, J=8.4Hz Ar), 7.70 (2H, d, J=8.8Hz Ar), 2.62 (3H, s, CH₃): ¹³C NMR (DMSO-d₆, 300 MHz): 151.2, 148, 147.1, 143.7, 140.9, 139.0, 132.0, 128.4, 126.4, 125.5, 124.1, 62.35, 42.45, 10.56; MS: *m/z* 393.12 [M⁺].

4. 4-methyl-1-(4-nitrophenyl)-5-(5-(3-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-1H-1,2,3-triazole (Yellow Colored Amorphous Solid)

MP 211°C; IR(KBr, cm⁻¹): 3284, 3112, 1690, 1516, 1345; ¹HNMR(DMSO-d₆, 300MHz): 8.23 (2H, d, J=9.0Hz Ar), 7.78 (2H, d, J=7.8Hz Ar), 3.73 (1H, dd, J=10.2Hz, 10.5Hz C-H_{2a}), 3.01 (1H, dd, J=10.5Hz, 10.8Hz C-H_{2b}), 5.04 (1H, t, J=10.5Hz C-H), 8.81 (1H, s, N-H), 7.90 (2H, d, J=8.1Hz Ar), 8.01 (2H, d, J=9.0Hz Ar), 2.62 (3H, s, CH₃): ¹³C NMR (DMSO-d₆, 300 MHz): 167.20, 166.61, 150.77, 148.30, 147.98, 145.70, 140.0, 139.0, 134.0, 132.0, 130.0, 129.05, 125.91, 123.09, 62.16, 42.4, 10.56; MS: *m/z* 393.12 [M⁺].

5. 4-methyl-1-(4-nitrophenyl)-5-(5-(2-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-1H-1,2,3-triazole (Yellow Color Amorphous Solid)

MP. 210°C; IR(KBr, cm⁻¹): 3317, 3087, 1599, 1518, 1345; ¹HNMR(DMSO-d₆, 300MHz): 8.27 (2H, d, J=8.0Hz Ar), 7.79 (2H, d, J=7.9Hz Ar), 3.73 (1H, dd, J=9.2Hz, 10.8Hz C-H_{2a}), 3.18 (1H, dd, J=10.0Hz, 9.8Hz C-H_{2b}), 4.80 (1H, t, J=10.1Hz C-H), 8.75 (1H, s, N-H), 8.65-8.7 (4H, m, Ar), 2.62 (3H, s, CH₃): ¹³C NMR (DMSO-d₆) 167.20, 165.6, 151.7, 149.98, 149.30, 143.71, 142.7, 140.8, 139.5, 134.5, 132.9, 130.4, 129.05, 125.91, 123.09, 63.6, 42.8, 10.63; MS: *m/z* 393.12 [M⁺].

6. 5-(5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazole (Yellow Color Amorphous Solid)

MP 212°C; IR(KBr, cm⁻¹): 3325, 3078, 1599, 1523, 1345; ¹HNMR(DMSO-d₆, 300MHz): 8.47 (2H, d, J=9.0Hz Ar), 7.77 (2H, d, J=9.0Hz Ar), 3.25 (1H, dd, J=9.3Hz, 9.3Hz C-H_{2a}), 3.87 (1H, dd, J=10.8Hz, 2.4Hz C-H_{2b}), 5.36 (1H, t, J=10.5Hz C-H), 6.76 (1H, s, N-H), 7.4-7.6 (4H, m, Ar), 2.69 (3H, s, CH₃): ¹³C NMR (DMSO-d₆): 147.84, 146.05, 140.80, 139.59, 138.75, 132.56, 131.32, 129.77, 128.83, 127.26, 127.24, 127.02, 126.96, 125.40, 125.14, 60.01, 40.77, 10.72; MS: *m/z* 382.1 [M⁺], 384.1 [M+2].

7. 5-(5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazole (Yellow Color Amorphous Solid)

MP 215°C; IR(KBr, cm⁻¹): 3324, 3086, 1597, 1528, 1346; ¹HNMR(DMSO-d₆, 300MHz): 8.47 (2H, d, J=9.0Hz Ar), 7.78 (2H, d, J=8.7Hz Ar), 3.8 (1H, dd, J=7.5Hz, 10.8Hz C-H_{2a}), 3.33 (1H, dd, J=8.7Hz, 9.0Hz C-H_{2b}), 4.91 (1H, t, J=6.3Hz C-H), 7.2 (4H, m, Ar), 7.39 (1H, s, N-H), 2.70 (3H, s, CH₃): ¹³C

NMR (DMSO- d_6): 148.11, 147.99, 145.91, 144.82, 140.32, 139.50, 138.12, 133.86, 131.98, 129.63, 128, 127.26, 127.24, 127.02, 126.96, 125.40, 125.14, 62.27, 41.98, 10.16; MS: m/z 382.1 [M^+], 384.1 [$M+2$].

8. 5-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazole (Yellow Color Amorphous Solid)

MP 213°C; IR (KBr, cm^{-1}): 3359, 2928, 1609, 1522, 1346; 1H NMR (DMSO- d_6 , 300 MHz): 7.92 (2H, d, $J=8.7$ Hz Ar), 7.58 (2H, d, $J=8.7$ Hz Ar), 8.51 (2H, d, $J=3.9$ Hz Ar), 8.49 (2H, d, $J=8.4$ Hz Ar), 3.09 (1H, dd, $J=10.1$ Hz, 9.0 Hz C- H_{2a}), 3.86 (1H, dd, $J=10.1$ Hz, 9.0 Hz C- H_{2b}), 5.09 (1H, t, $J=6.3$ Hz C-H), 7.21 (1H, s, N-H), 2.66 (3H, s, CH_3); ^{13}C NMR (DMSO- d_6): 148.11, 146.99, 144.91, 143.82, 139.32, 139.0, 137.1, 132.8, 130.9, 127.6, 127, 127.86, 127.24, 126.0, 125.9, 125.4, 124.1, 61.2, 40.9, 11.6; MS: m/z 382.1 [M^+], 384.1 [$M+2$].

9. 2-(3-(4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-5-yl)-4,5-dihydro-1H-pyrazol-5-yl)phenol (Yellow Color Amorphous Solid):

MP 203°C; IR (KBr, cm^{-1}): 3337, 3091, 1626, 1520, 1346; 1H NMR (DMSO- d_6 , 300 MHz): 7.19 (2H, d, $J=8.4$ Hz Ar), 6.70 (2H, d, $J=8.8$ Hz Ar), 6.8-7.4 (4H, m, Ar), 2.89 (1H, dd, $J=10.0$ Hz, 9.6 Hz C- H_{2a}), 3.57 (1H, dd, $J=10.4$ Hz, 10.4 Hz C- H_{2b}), 4.96 (1H, t, $J=10.0$ Hz C-H), 5.59 (1H, s, N-H), 9.7 (1H, s, O-H), 2.43 (3H, s, CH_3); ^{13}C NMR (DMSO- d_6): 149.2, 147.5, 146.4, 143.9, 136.5, 131.9, 129.2, 128.5, 127.1, 126.9, 61.8, 46.1, 13.2; MS: m/z 364.13 [M^+].

10. 5-(5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazole (Pale Yellow Color Amorphous Solid)

MP 209°C; IR (KBr, cm^{-1}): 3465, 3071, 1606, 1520, 1344; 1H NMR (DMSO- d_6 , 300 MHz): 8.50 (2H, d, $J=9.0$ Hz Ar), 8.60 (2H, d, $J=8.4$ Hz Ar), 8.51 (2H, d, $J=8.1$ Hz Ar), 7.79 (2H, d, $J=8.4$ Hz Ar), 3.15 (1H, dd, $J=10.1$ Hz, 9.6 Hz C- H_{2a}), 3.82 (1H, dd, $J=10.1$ Hz, 9.6 Hz C- H_{2b}), 5.15 (1H, t, $J=7.4$ Hz C-H), 7.99 (1H, s, N-H), 2.62 (3H, s, CH_3); ^{13}C NMR (DMSO- d_6): 152.13, 148.9, 146.1, 145.2, 143.3, 141.8, 137.1, 137.8, 132.9, 129.6, 128, 128.86, 128.24, 127.0, 126.9, 126.4, 125.1, 63.2, 43.9, 14.6; MS: m/z 366.79 [M^+].

11. 5-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazole (Pale Yellow Color Amorphous Solid)

MP 210°C; IR (KBr, cm^{-1}): 3326, 3084, 1597, 1531, 1348; 1H NMR (DMSO- d_6 , 300 MHz): 8.52 (2H, d, $J=10.2$ Hz Ar), 8.01 (2H, d, $J=9.0$ Hz Ar), 7.57 (2H, d, $J=8.4$ Hz Ar), 7.38 (2H, d, $J=8.4$ Hz Ar), 3.01 (1H, dd, $J=10.2$ Hz, 10.2 Hz C- H_{2a}), 3.67 (1H, dd, $J=10.8$ Hz, 10.8 Hz C- H_{2b}), 4.85 (1H, t, $J=7.4$ Hz C-H), 7.80 (1H, s, N-H), 2.61 (3H, s, CH_3); ^{13}C NMR (DMSO- d_6): 148.0, 143.69, 142.75, 140.95, 139.91, 132.55, 131.77, 129.36, 126.38, 125.56, 120.63, 62.40, 42.33, 10.56; MS: m/z 426.1 [M^+], 428.1 [$M+2$].

RESULTS AND DISCUSSION

The *p*-amido aniline derived chalcones have shown appreciable antimicrobial activity compared to the standard drugs.^{22,23} Literature review reveals that only very few 1, 2, 3- triazole linked pyrazolines have been synthesized. As per the procedure given in the literature¹⁹ 4- nitro aniline was converted into its corresponding 4- methyl-5-acetyl-1, 2, 3- triazole. In the present work pyrazoline was synthesized as given in the literature.^{24,25} The conversion of 4- nitro aniline into azide **1** was confirmed by N = N stretching absorption band at 2127 cm^{-1} in its IR spectrum. The formation of 4- methyl- 1- 4- nitrophenyl- 1 *H*- 1, 2, 3- triazole- 5-yl ethanone **2** was ensured from a three proton singlet that appeared at δ value 2.60 for the methyl group. The chalcones **3.1- 3.11** were synthesized from 5- acetyl- triazole. Two doublets at δ 7.92 and 8.0 for H_α and H_β protons revealed the formation of chalcone. A series of target compounds pyrazolines **4.1- 4.11** were synthesized by following the literature.^{24, 25} The stereochemically non- equivalent proton signals at δ 3.01 & 3.04 and a triplet at δ 4. 86 confirm the formation of pyrazoline. The compounds 3-(4-methoxyphenyl)-1-(4-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-5-yl) prop-2-en-1-one, 3-(2-chlorophenyl)-1-(4-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-5-yl) prop-2-en-1-one and 3-(4-

nitrophenyl)-1-(4-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-5-yl) prop-2-en-1-one have shown zone of inhibition against *Klebsiella*, *E. faecalis*, *Pseudomonas fluorescence* 14,16,16/20mm and the compounds 5-(5-(4-methoxyphenyl)-4, 5-dihydro-1H-pyrazol-3-yl)-4-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazole, 4-methyl-1-(4-nitrophenyl)-5-(5-(2-nitrophenyl)-4, 5-dihydro-1H-pyrazol-3-yl)-1H-1, 2, 3-triazole have shown moderate inhibition against *Klebsiella*, *E. faecalis*, *Pseudomonas fluorescence* and *S.aures* 18,18,17, 16/20mm. Appreciable antimicrobial activity was shown by the Methoxy, Chloro and Nitro substituted chalcones and pyrazolines against the standard drugs infer that polarity variation among the functional groups in the derived compounds also plays some vital role in addition to that of extending of conjugation.

Fig.-1: XRD Molecular Structure of (E)-3-(4-fluorophenyl)-1-(4-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-5-yl) prop-2-en-1-one

A single crystal of compound **3.10** was obtained by slow evaporation method at room temperature using an alcohol-chloroform mixture. The crystal structure was solved and refined using CrysAlisPro 1.171.39.46 (Rigaku Oxford Diffraction, 2018). Mo K α radiation ($\lambda = 0.71073$ Å) and a graphite monochromator were used for data collection. The chemical formula and labeling scheme is shown in Fig.-1. Crystal data for compound **3.10**: C₁₈H₁₃N₄O₃F; Mr. 352.32; system, triclinic; space group, 'P -1'; unit cell dimensions, a, 7.4413(8) Å; b, 7.5604(6) Å; c, 15.4915(10) Å; α , 87.980(6); β , 78.021(7)°; γ , 67.884(9)°. V, 788.95(11) Å³; Z, 2; μ , 0.112 mm⁻¹; Density, 1.483 g cm⁻³; θ range for data collection 2.714 - 29.801°; T, 298 K; independent reflections, 7413; measured reflections, 4391; used reflections, 1921; R_{int}, 0.0766; R (all), 0.1318; Goodness of fit, 1.126.

CONCLUSION

4-Nitro aniline derived 1, 2, 3 triazole linked pyrazolines were synthesized and evaluated for their antimicrobial activity. All the chalcones and pyrazolines have shown only moderate antimicrobial activity compared to the standard drugs streptomycin and flucanazole. It was assumed that the extend of conjugation and electronegativity were the reasons for the better activity of the compounds. To conclude the above-said reason, the present work was designed with the precursor 4-nitro aniline. In our previous work, antimicrobial activity of 4-amino benzamide derived chalcones and pyrazolines were presented²³. Comparison of antimicrobial activity of chalcones and pyrazolines of 4-nitro aniline derived compounds revealed that greater extend of conjugation and electronegativity is not reflected on the microbial activity of the synthesized compounds. So that incorporation of structural modification in the 1,2,3-triazole-chalcone hybrid by incorporating the pyrazoline ring may not be a suitable chemical environment to achieve better anti-microbial compound was concluded.

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