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Synthesis and Antibacterial Evaluation of Some Novel Pyrazoline Derivatives

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ABSTRACT

New pyrazoline derivatives **4 (a -l)** were prepared from commercially available 2-Hydroxy-Aceto-Napthanone and substituted vanillin derivative. These compounds were evaluated for their antibacterial activity against *Escherichia coli* (MTCC-443), *Staphylococcus aureus* (MTCC-96), *Pseudomonas aeruginosa* (MTCC-424) and *Streptococcus pyogenes* (MTCC-442) bacterial strains. Most of the compounds were found to be active compared to the standard drug ampicillin. In general it is observed that the compounds having amide functionality **4i-4l** exhibited excellent antibacterial activity with zone of inhibition 19-22 mm towards all the tested bacterial strains.

Keywords: Pyrazoline derivatives, 2-Hydroxy-Aceto-Napthanone, Aldehydes, Antibacterial activity, Synthesis, E.coli, Ampicillin.

INTRODUCTION

The development of antimicrobial agents to treat infectious diseases has been one of the most notable achievements of the past century. The increased use of antimicrobial agents available in the market has resulted in the development of resistance to the commonly used drugs with important implications for morbidity, mortality [1-2] and health care costs. The treatment of bacterial infections remains a challenging therapeutic problem because of emerging infectious diseases and the increasing number of multidrug-resistant microbial pathogens. Despite the many antibiotics and chemotherapeutics available, the emergence of old and new antibiotic-resistant bacterial strains in the last decades constitutes a substantial need for new classes of anti-bacterial agents [3].

In recent years, the synthesis of pyrazoline derivatives remains a main focus of medicinal research. Pyrazolines are heterocyclic compounds which possess wide range of biological activities such as anti-inflammatory [4] anti-tuberculosis [5-6], kinase inhibitor [7], oxidase inhibitor [8], anti-cancer [9], antiproliferative activity [10], MAO inhibitors [11,12], anti-hepatotoxic activity [13], antibacterial [14-15], anti-analgesic [16-17], anti-coagulant [18], anti-tumor [19] and anti-diabetic activities [20]. Pyrazoline is a five-membered heterocyclic ring having two adjacent nitrogen atoms within the ring. It has only one endocyclic double bond and is basic in nature [21] and plays a crucial role in the development of theory in heterocyclic chemistry and is also extensively used as useful synthons in organic synthesis [22]. Among its various derivatives, 3, 5-diaryl-pyrazolines [23] seem to be the most frequently studied pyrazoline type compounds. Encouraged by these interesting biological activities associated with pyrazoline derivatives, in this paper we report here the synthesis and antimicrobial activity of some new pyrazoline

derivatives screened against *Escheria.Coli*, *Pseudomonas.aeruginosa*, *Staphylococcus.aureus* and *Streptococcus.pyogenes*, bacterial strains using ampicillin as standard.

MATERIALS AND METHODS

Chemical and solvents used were purchased either from Fluka or Merck. All the reagents were of analytical grade. Thin-layer chromatography (TLC) was performed on E.Merck AL silica gel 60 F254 plates and visualized under UV light. The IR spectra were recorded on a Perkin Elmer FT-IR spectrometer. The ^1H NMR spectra were recorded in CDCl_3 on a Varian EM-360 spectrometer (400MHz). The ^{13}C NMR spectra recorded in CDCl_3 on a Varian EM-360 spectrometer operating at 100MHz. All the chemical shifts were reported in δ (ppm) using TMS as an internal standard. The mass spectra were recorded on Agilent ion trap MS. All the reactions were carried out under argon atmosphere. All the alkyl bromides, phenyl hydrazines and benzoyl chlorides used for the preparation of **4a-4l** were purchased from commercial sources.

Experimental methods

The reaction sequence for the synthesis of pyrazoline derivatives is outlined in **Scheme-1**. The key intermediate, chalcone **3a** was prepared by treating 2-Hydroxy-Aceto-Naphthanone (**1**) with aldehyde **2**, in the presence of sodium hydroxide in methanol at r.t. for 12 h. After work-up, the chalcone **3** was utilized as such in the next step without further purification. The aldehyde **2** was prepared according to the literature procedure [24]. Chalcone **3** upon treatment with hydrazine hydrate in presence of sodium acetate in ethanol at reflux for 6 h gave compound **4a**. Alkylation of **4a** with requisite alkyl bromide such as methyl iodide, ethyl bromide and n-propyl bromide afforded compounds **4b-4d**. Further treatment of chalcone **3** with respective substituted hydrazine hydrates in presence of sodium acetate in ethanol at reflux for 10 h resulted in the formation of compounds **4a- 4l**.

General Procedure for the Preparation of Pyrazoline derivatives (4a-4l)

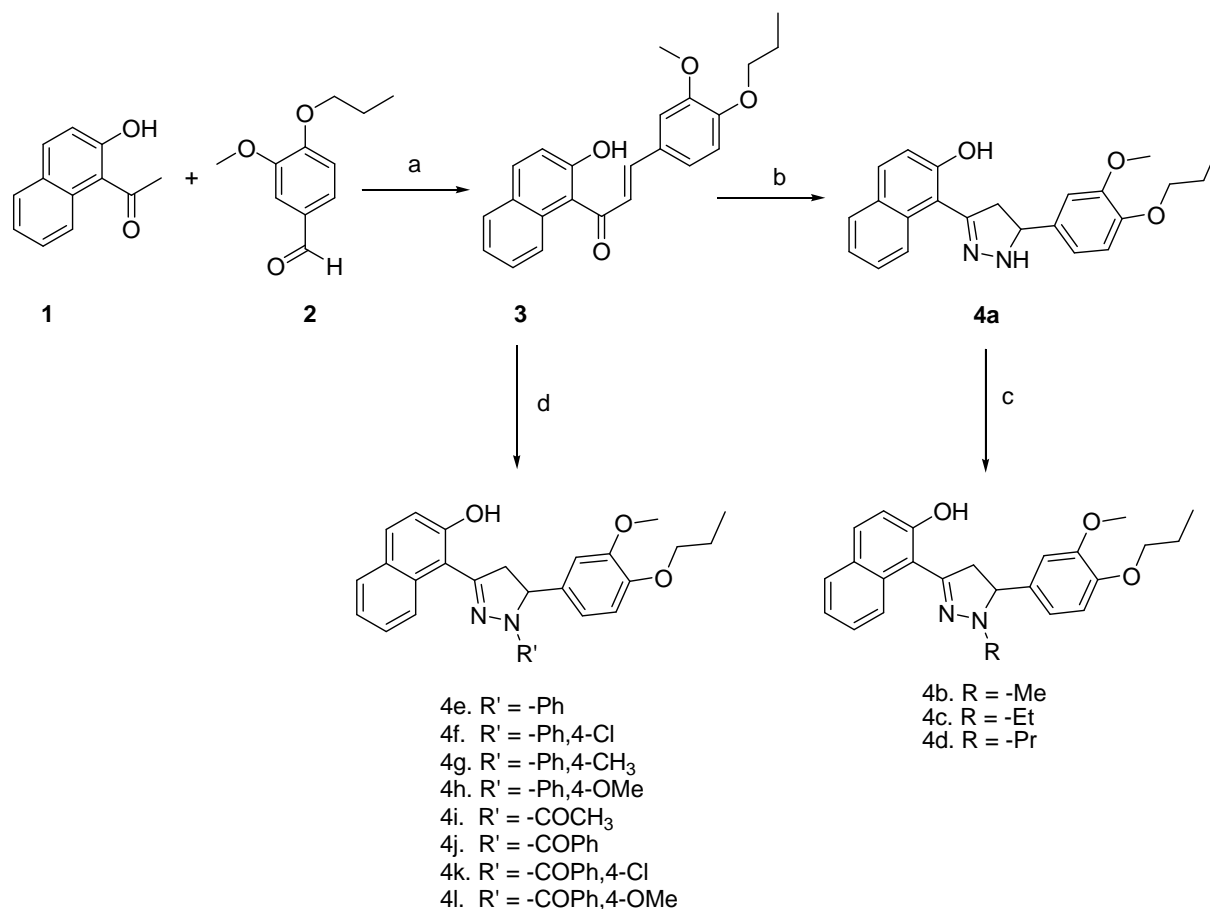
To a ethanol solution containing **3** (348 mg, 1.0 mmol) was added hydrazine hydrate (2.0 mmol), and sodium acetate (6.0 mmol). The contents were stirred at reflux temperature for 4 h under argon atmosphere; the reaction medium was poured into water and extracted with ethyl acetate. The organic layer was washed with water followed by brine solution, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure, to obtain the crude compounds. The crude compounds were purified by column chromatography using silica gel (60-120 mesh). Yields of the products varied between 58.0 and 78%.

Preparation of 1-(4,5-dihydro-5-(3-methoxy-4-propoxyphenyl)-1H-pyrazol-3-yl)naphthalen-2-ol (**4a**)

Yellow liquid; Yield: 300 mg, 80%; IR (neat): ν_{max} 3610.2, 3422.4, 1513.2, 1597.4, 1521,1155, cm^{-1} ; ^1H -NMR (400 MHz, DMSO-d_6): δ 1.12 (t, 3 H, $J = 7.4$ Hz), 1.75 (q, 2 H, $J = 7.4$ Hz), 3.12 (dd, 1 H, $J = 16.3$ & 10.6 Hz), 3.58 (dd, 1 H, $J = 16.5$ & 10.4 Hz), 3.81 (s, 3 H), 3.98 (t, 2 H, $J = 7.4$ Hz), 4.95 (dd, 1 H, $J = 15.4$ & 7.9 Hz), 7.82-6.98 (m, 7 H), 7.82 (t, 2 H, $J = 7.8$ Hz), 8.16 (d, 1H, $J = 12.0$ Hz), 10.62 (s, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6): 10.32, 22.20, 43.60, 49.22, 56.6, 71.88, 111.20, 112.24, 115.82, 118.72, 120.20, 124.20, 127.40, 127.81, 129.22, 130.22, 132.62, 134.34, 136.82, 144.54, 157.81, 152.21, 150.10; EI-MS: m/z (rel.abund.%) 377 (M^+ , 100).

1-(4,5-dihydro-5-(3-methoxy-4-propoxyphenyl)-1-methyl-1H-pyrazol-3-yl)naphthalen-2-ol (**4b**)

Brown liquid; Yield: 300 mg, 78%; IR (neat): ν_{max} 3615, 1506.4, 1514.5, 1599.5, 1145, cm^{-1} ; ^1H -NMR (400 MHz, CDCl_3): δ 1.08 (t, 3 H, $J = 7.6$ Hz), 1.74 (q, 2 H, $J = 7.6$ Hz), 2.54 (s, 3 H), 2.98 (dd, 1 H, $J = 16.3$ & 10.4 Hz), 3.44 (dd, 1 H, $J = 16.4$ & 10.4 Hz), 3.79 (s, 3 H), 3.98 (t, 2 H, $J = 7.6$ Hz), 4.92 (dd, 1 H, $J = 15.5$ & 7.6 Hz), 7.79 – 6.92 (m, 8 H), 8.12 (d, 1H, $J = 12.0$ Hz), 9.46 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3): 10.62, 22.61, 40.24, 41.62, 53.68, 56.42, 71.61, 112.82, 112.61, 115.42, 118.64, 121.21, 124.21, 127.22,127.44, 128.42, 129.32, 132.82, 131.32, 135.41, 144.82, 149.62, 151.21, 158.20; EI-MS: m/z (rel.abund.%) 391 (M^+ , 100).



Scheme 1. Reagents and Conditions: a) NaOH, Methanol, rt, 12 h b) NH₂NH₂·H₂O, NaOAc, ethanol, reflux, 6 h; c) R-Br, Et₃N, DCM, rt, 24 h; d) R'-NH₂-NH₂, NaOAc, ethanol, reflux, 10 h.

1-(1-ethyl-4,5-dihydro-5-(3-methoxy-4-propoxyphenyl)-1H-pyrazol-3-yl)naphthalen-2-ol (4c)

yellow liquid; Yield: 310 mg, 77%; IR (neat): ν_{\max} 3618, 1521, 1513.5, 1610, 1160, cm⁻¹; ¹H-NMR (400 MHz, CDCl₃): δ 1.06 (t, 3 H, *J* = 7.6 Hz), 1.21 (t, 3 H, *J* = 6.9 Hz), 1.77 (q, 2 H, *J* = 7.4 Hz), 2.52 (q, 2 H, *J* = 8.0 Hz), 3.78 (s, 3 H), 2.84 (dd, 1 H, *J* = 15.9 & 9.8 Hz), 3.44 (dd, 1 H, *J* = 16.2 & 9.4 Hz), 4.05 (t, 2H, *J* = 8.2 Hz), 4.95 (dd, 1H, *J* = 16.2 & 10.4 Hz), 6.89-7.69 (m, 8 H), 8.05 (d, 1 H, *J* = 11.8 Hz), 9.46 (s, 1H); ¹³C NMR (100 MHz, CDCl₃): 10.42, 11.28, 22.82, 41.40, 46.62, 51.82, 56.62, 71.81, 112.21, 112.41, 115.20, 118.44, 121.18, 124.22, 127.18, 127.34, 128.40, 129.28, 132.78, 131.22, 135.31, 144.78, 149.52, 151.18, 158.26; EI-MS: *m/z* (rel.abund.%) 405 (M⁺, 100).

1-(4,5-dihydro-5-(3-methoxy-4-propoxyphenyl)-1-propyl-1H-pyrazol-3-yl)naphthalen-2-ol (4d)

Colorless liquid; Yield: 313 mg, 75%; IR (neat): ν_{\max} 3120, 1620, 1590, 1514, 1150 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃): δ 1.08 (t, 6 H, *J* = 8.2 Hz), 1.76 (q, 4 H, *J* = 8.2 Hz), 2.98 (dd, 1H, *J* = 16.4 & 10.2 Hz), 3.22 (t, 2 H, *J* = 8.2 Hz), 3.34 (dd, 1H, *J* = 16.4 & 9.8 Hz), 3.84 (s, 3 H), 3.98 (t, 2 H, *J* = 8.0 Hz), 4.86 (dd, 1H, *J* = 16.0 & 10.4 Hz), 7.72-6.92 (m, 8 H), 8.12 (d, 1H, *J* = 12.2 Hz), 9.46 (s, 1 H); ¹³C NMR (100 MHz, CDCl₃): 10.42, 11.78, 19.62, 22.42, 41.72, 51.82, 53.44, 56.62, 71.61, 111.98, 112.21, 114.98, 118.26, 120.98, 123.88, 127.38, 127.64, 128.60, 129.32, 132.98, 130.92, 135.62, 143.88, 149.72, 151.48, 158.56; EI-MS: *m/z* (rel.abund.%) 419 (M⁺, 100).

1-(4,5-dihydro-3-(2-hydroxynaphthalen-1-yl)-5-(3-methoxy-4-propoxyphenyl)pyrazol-1-yl)ethanone (4e)

Light yellow liquid; Yield: 305 mg, 73%; IR (neat): ν_{\max} 3110, 1682.6, 1615, 1535, 1516.4, 1145 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃): δ 1.06 (t, 3 H, *J* = 7.9 Hz), 1.74 (q, 2 H, *J* = 7.6 Hz), 2.44 (s, 3 H), 3.24 (dd, 1 H, *J* = 16.2 & 10.4 Hz), 3.62 (dd, 1H, *J* = 16.0 & 9.8 Hz), 3.78 (s, 3 H), 4.05 (t, 2 H, *J* = 7.9 Hz), 5.12 (dd, 1H, *J* = 16.3 & 10.2 Hz),

7.70 -7.10 (m, 8 H), 8.18 (d, 1H, $J = 12.2$ Hz), 9.46 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3): 10.63, 22.38, 23.82, 40.44, 56.22, 60.29, 71.62, 111.38, 112.26, 114.48, 119.26, 121.38, 123.28, 127.40, 127.34, 128.44, 130.32, 133.24, 131.28, 135.42, 143.68, 150.42, 151.68, 158.60; 168.62; EI-MS: m/z (rel.abund.%) 419 (M^+ , 100).

1-(4,5-dihydro-5-(3-methoxy-4-propoxyphenyl)-1-phenyl-1H-pyrazol-3-yl)naphthalen-2-ol (4f)

Brown liquid; Yield: 325 mg, 72%; IR (neat): ν_{max} 3100, 1599.5, 1530, 1513.5, 1145, cm^{-1} ; ^1H -NMR (400 MHz, DMSO-d_6): δ 1.08 (t, 3H, $J = 10.8$ Hz), 1.92 (q, 2H, $J = 7.2$ Hz), 2.95 (dd, 1H, $J = 16.0$ & 6.8 Hz), 3.32 (dd, 1H, $J = 16.8$ & 8.0 Hz), 3.98 (s, 3H), 4.04 (t, 2H, $J = 7.2$ Hz), 5.22 (dd, 1H, $J = 12.0$ & 8.0 Hz), 7.20-6.88 (m, 7H), 7.29 (t, 2H, $J = 12.0$ Hz), 7.37 (m, 1H), 7.46 (t, 1H, $J = 7.6$ Hz), 7.62 (t, 1H, $J = 6.8$ Hz), 7.69 (d, 1H, $J = 8.8$ Hz), 7.82 (d, 1H, $J = 8.0$ Hz), 9.6 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 10.64, 22.78, 40.20, 53.90, 56.44, 71.60, 112.38, 112.42, 113.46 (2C), 115.28, 117.28, 118.46, 121.68, 124.28, 127.68, 127.84, 128.94, 129.34, 129.82 (2C), 133.20, 131.42, 135.72, 144.21, 144.38, 150.42, 151.68, 158.43; EI-MS: m/z (rel.abund.%) 453 (M^+ , 100).

1-(1-(4-chlorophenyl)-4,5-dihydro-5-(3-methoxy-4-propoxyphenyl)-1H-pyrazol-3-yl)naphthalen-2-ol (4g)

Yellow liquid; Yield: 270 mg, 66%; IR (neat): ν_{max} 3600, 1596.6, 1521.5, 1514.6, 1145, 790, cm^{-1} ; ^1H -NMR (400 MHz, DMSO-d_6): δ 1.07 (t, 3H, $J = 8$ Hz), 1.94 (q, 2H, $J = 7.6$ Hz), 2.95 (dd, 1H, $J = 15.6$ & 8.0 Hz), 3.22 (dd, 1H, $J = 16.2$ & 8.0 Hz), 3.92 (s, 3H), 4.04 (q, 2H, $J = 8$ Hz), 5.16 (dd, 1H, $J = 12.2$, 9.2 Hz), 6.82 (d, 2H, $J = 6.8$ Hz), 7.18 (d, 1H, $J = 8.8$ Hz), 7.2 (m, 2H), 7.35 (d, 2H, $J = 7.8$ Hz), 7.38 (d, 2H, $J = 10.8$ Hz), 7.46 (d, 1H, $J = 6.8$ Hz), 7.65 (d, 1H, $J = 7.2$ Hz), 7.76 (d, 1H, $J = 7.2$ Hz), 7.95 (d, 1H, $J = 8.4$ Hz), 9.4 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 10.60, 22.72, 40.42, 53.42, 56.20, 71.38, 112.32, 112.48, 115.20 (2C), 117.28, 118.26, 122.28, 124.36, 127.48, 127.84, 129.92, 129.34, 130.24 (2C), 133.40, 131.72, 135.42, 142.21, 144.21, 144.44, 150.22, 151.48, 158.26; EI-MS: m/z (rel.abund.%) 487 (M^+ , 100).

1-(4,5-dihydro-5-(3-methoxy-4-propoxyphenyl)-1-p-tolyl-1H-pyrazol-3-yl)naphthalen-2-ol (4h)

Light yellow liquid; Yield: 298 mg, 62%; IR (neat): ν_{max} 3612, 1610, 1545, 1513.4, 1145, cm^{-1} ; ^1H -NMR (400 MHz, DMSO-d_6): δ 1.04 (t, 3H, $J = 7.8$ Hz), 1.79 (q, 2H, $J = 7.8$ Hz), 2.42 (s, 3H), 3.12 (dd, 1H, $J = 16.4$ & 10.8 Hz), 3.74 (dd, 1H, $J = 16.2$, 9.8 Hz), 3.88 (s, 3H), 4.12 (t, 2H, $J = 7.9$ Hz), 5.2 (dd, 1H, $J = 15.8$ & 8.0 Hz), 6.36 (d, 2H, $J = 6.8$ Hz), 7.16 (d, 1H, $J = 7.6$ Hz), 7.2 (m, 2H), 7.22 (d, 2H, $J = 12.4$ Hz), 7.26 (d, 2H, $J = 10.2$ Hz), 7.54 (d, 1H, $J = 7.8$ Hz), 7.68 (d, 1H, $J = 7.2$ Hz), 7.82 (d, 1H, $J = 7.2$ Hz), 7.95 (d, 1H, $J = 8.4$ Hz), 9.4 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 10.48, 22.60, 24.37, 40.64, 53.82, 56.41, 71.60, 112.92, 113.2, 113.64 (2C), 116.82, 118.36, 121.88, 124.20, 127.48, 127.64, 129.74, 130.78, 131.62 (2C), 133.70, 131.22, 135.82, 141.22, 144.32, 144.48, 150.62, 151.38, 158.24; EI-MS: m/z (rel.abund.%) 466 (M^+ , 100).

1-(4,5-dihydro-5-(3-methoxy-4-propoxyphenyl)-1-(4-methoxyphenyl)-1H-pyrazol-3-yl)naphthalen-2-ol (4i)

Brown liquid; Yield: 306 mg, 66%; IR (neat): ν_{max} 3618, 1614, 1540, 1514.2, 1155, cm^{-1} ; ^1H -NMR (400 MHz, DMSO-d_6): δ 1.16 (t, 3H, $J = 6.8$ Hz), 1.89 (q, 2H, $J = 6.8$ Hz), 2.97 (d, 1H, $J = 16.4$ Hz), 3.25 (t, 1H, $J = 15.2$ Hz), 2.97 (dd, 1H, $J = 16.4$ & 7.8 Hz), 3.25 (dd, 1H, $J = 15.8$ & 8.0 Hz), 3.76 (s, 3H), 3.91 (s, 3H), 4.01 (t, 2H, $J = 6.8$ Hz), 5.56 (dd, 1H, $J = 14.2$ & 7.8 Hz), 6.76 (d, 1H, $J = 6.8$ Hz), 6.92 (d, 2H, $J = 7.2$ Hz), 7.05 (d, 2H, $J = 11.2$ Hz), 7.18 (d, 1H, $J = 8.8$ Hz), 7.2 (m, 1H), 7.46 (d, 1H, $J = 6.8$ Hz), 7.65 (d, 1H, $J = 7.2$ Hz), 7.76 (d, 1H, $J = 7.2$ Hz), 7.95 (d, 1H, $J = 8.4$ Hz), 9.4 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 10.38, 22.76, 40.64, 53.34, 56.22, 55.68, 71.62, 112.74, 113.28, 114.42 (2C), 116.42, 118.62, 121.68, 124.36, 127.68, 127.44, 129.94, 130.58, 130.82 (2C), 133.68, 131.28, 136.42, 141.72, 144.42, 144.56, 150.82, 151.78, 158.20; EI-MS: m/z (rel.abund.%) 483 (M^+ , 100).

(4,5-dihydro-3-(2-hydroxynaphthalen-1-yl)-5-(3-methoxy-4-propoxyphenyl)pyrazol-1-yl)(phenyl)methanone (4j)

Yellow liquid; Yield: 302 mg, 63%; IR (neat): ν_{max} 3618, 1682.8, 1615, 1535, 1511.4, 1145, cm^{-1} ; ^1H -NMR (400 MHz, DMSO-d_6): δ 1.06 (t, 3H, $J = 9.8$ Hz), 1.9 (q, 2H, $J = 9.6$ Hz), 3.04 (dd, 1H, $J = 15.8$ & 7.8 Hz), 3.42 (dd, 1H, $J = 16.4$ & 8.0 Hz), 3.96 (s, 3H), 4.12 (t, 2H, $J = 9.4$ Hz), 5.24 (dd, 1H, $J = 16.2$ & 7.8 Hz), 7.18 (d, 2H, $J = 7.8$ Hz), 7.2 (m, 4H), 7.46 (d, 1H, $J = 7.2$ Hz), 7.56 (d, 1H, $J = 7.8$ Hz), 7.60 (d, 1H, $J = 7.2$ Hz), 7.64 (d, 2H, $J = 7.6$ Hz), 7.79 (d, 2H, $J = 7.8$ Hz), 7.85 (d, 1H, $J = 8.8$ Hz), 9.4 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 10.44, 22.72, 40.84, 56.62, 60.24, 71.42, 112.64, 113.38, 116.22, 119.22, 121.48, 124.66, 127.68 (2C), 129.22 (2C), 129.94, 130.58, 132.44, 133.46, 131.28, 134.86, 136.42, 141.22, 144.32, 144.46, 151.28, 151.88, 168.43, 158.20 EI-MS: m/z (rel.abund.%) 481 (M^+ , 100).

(4-chlorophenyl)(4,5-dihydro-3-(2-hydroxynaphthalen-1-yl)-5-(3-methoxy-4-propoxyphenyl)pyrazol-1-yl)methanone (4k)

Brown liquid; Yield: 313 mg, 61%; IR (neat): ν_{\max} 3610, 1642.0, 1610, 1530, 1513.4, 1155, 785.2 cm^{-1} ; $^1\text{H-NMR}$ (400 MHz, DMSO-d_6): δ 1.12 (t, 3H, $J = 9.8$ Hz), 1.9 (q, 2H, $J = 9.6$ Hz), 3.18 (dd, 1H, $J = 16.8$ & 9.8 Hz), 3.58 (dd, 1H, $J = 16.8$ & 9.4 Hz), 3.98 (s, 3H), 4.14 (t, 2H, $J = 9.8$ Hz), 5.21 (dd, 1H, $J = 16.2$ & 9.8 Hz), 7.24-7.36 (m, 5H), 7.48 (d, 1H, $J = 10.2$ Hz), 7.52 (d, 2H, $J = 7.8$ Hz), 7.68 (d, 1H, $J = 10.8$ Hz), 7.77 (d, 1H, $J = 8.8$ Hz), 7.89 (d, 2H, $J = 7.8$ Hz), 7.78 (d, 1H, $J = 8.8$ Hz), 9.40 (s, 1H); $^{13}\text{C NMR}$ (100 MHz, DMSO-d_6): 10.46, 22.66, 40.62, 56.28, 60.28, 71.82, 112.44, 113.78, 116.24, 119.64, 121.56, 124.78, 129.28 (2C), 129.42 (2C), 130.74, 130.58, 132.62, 133.26, 131.28, 134.86, 136.42, 141.20, 144.28, 144.68, 151.38, 152.68, 168.24, 158.20 EI-MS: m/z (rel.abund.%) 515 (M^+ , 100).

(4,5-dihydro-3-(2-hydroxynaphthalen-1-yl)-5-(3-methoxy-4-propoxyphenyl)pyrazol-1-yl)(4-ethoxyphenyl)methanone (4l)

Yellow liquid; Yield: 295 mg, 58%; IR (neat): ν_{\max} 3600, 1680.4, 1650, 1530, 1514.4, 1160, cm^{-1} ; $^1\text{H-NMR}$ (400 MHz, DMSO-d_6): δ 1.1 (t, 3H, $J = 8.8$ Hz), 1.88 (q, 2H, $J = 9.0$ Hz), 3.08 (dd, 1H, $J = 15.8, 8.8$ Hz), 3.48 (dd, 1H, $J = 16.4, 8.4$ Hz), 3.74 (s, 3H), 3.97 (s, 3H), 4.04 (t, 2H, $J = 9.8$ Hz), 5.21 (dd, 1H, $J = 16.4, 7.8$ Hz), 7.24-7.36 (m, 5H), 7.64 (d, 1H, $J = 10.2$ Hz), 7.76 (d, 1H, $J = 10.8$ Hz), 7.68 (d, 1H, $J = 8.8$ Hz), 7.58 (d, 2H, $J = 7.8$ Hz), 7.70 (d, 2H, $J = 7.8$ Hz), 7.84 (d, 1H, $J = 8.8$ Hz), 9.45 (s, 1H); $^{13}\text{C NMR}$ (100 MHz, DMSO-d_6): 10.44, 22.74, 40.58, 55.78, 56.34, 59.82, 71.38, 112.26, 112.32, 114.68 (2C), 115.42, 117.34, 118.66, 121.88, 124.48, 127.78, 128.22, 128.96, 129.44, 128.72 (2C), 133.38, 131.78, 135.92, 144.28, 144.46, 151.88, 163.42, 167.89, 158.43; EI-MS: m/z (rel.abund.%) 511 (M^+ , 100).









Antibacterial Bioassay

Pyrazoline analogues (4a – 4l) were dissolved in dimethyl sulphoxide at 250 $\mu\text{g/mL}$ concentration. The composition of nutrient agar medium was Bactotryptone (10 g), yeast extract (5 g), NaCl (10 g), final pH 7.4. After 18 h the exponentially growing cultures of the six bacteria in nutrient broth at 37 $^\circ\text{C}$ were diluted in sterile broth. From each of these diluted cultures, 1 mL was added to 100 mL sterilized and cooled nutrient agar media to give a final bacterial count of 1×10^6 cell/ml. The plates were set at room temperature and later dried at 37 $^\circ\text{C}$ for 20h. Paper discs (6mm, punched from whatmann no 41 paper) were ultraviolet sterilized and used for the assays. Discs were soaked in different concentration of the test solution and placed on the inoculated agar media at regular intervals of 6-7 cm, care was taken to ensure that excess solution was not on the discs. All the samples were taken in triplicates. The plates were incubated at 37 $^\circ\text{C}$ in an inverted fashion. Activity was determined by zones showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control.

RESULTS AND DISCUSSION

The antibacterial activity of the synthesized compounds 4 (a-l) was evaluated against two Gram negative strains viz., i) *Escherichia coli*, (ii) *Pseudomonas aeruginosa*, and two Gram positive strains viz. i) *Streptococcus pyogenes* and ii) *Staphylococcus aureus* using agar well diffusion method following the literature procedure [25]. The antibacterial activity of the pyrazoline derivatives 4 (a-l) (250 $\mu\text{g/mL}$ concentration) was compared with standard drug ampicillin and the results of investigation have been presented in Table 1. *E.coli*: The compounds 4i and 4k exhibited excellent activity, while the compounds 4j and 4l displayed equivalent activity with the standard drug and the remaining compounds also showed promising antibacterial activity. *P.aeruginosa*: Compounds 4a-4d displayed moderate activity, while the compounds 4e-4h showed good activity and the compounds 4i-4l exhibited excellent activity. *S.aureus*: The compounds 4i, 4j and 4k displayed equivalent activity with the standard drug and the compound 4l exhibited excellent activity and remaining compounds in the series showed moderate to good activity. *S.pyogenes*: The compounds 4i-4l exhibited excellent activity and the compounds 4a-4h displayed moderate to good activity. In general it is observed that the compounds having amide functionality 4i-4l exhibited excellent antibacterial activity with zone of inhibition 19-20 mm towards all the tested bacterial strains. As all the compounds showed antibacterial activity against the bacteria tested, it indicates that this basic moiety can be a potential scaffold for anti bacterial drugs. It may be suggested that the pyrazoline derivative with a suitable R group may lead to a good antibacterial agent for all the *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Staphylococcus aureus* bacterial strains.

Table 1: Results of Antibacterial Bioassay of Compounds 4a-4l (concentration used 250 µg/mL of DMSO) Zones of inhibition of compounds 4a-4l

Compound no.	Name of the Bacteria				
	R	<i>E.coli</i>	<i>P. aeruginosa</i>	<i>S.aureus</i>	<i>S.pyogenes</i>
4a	H	15	14	14	15
4b	Methyl	16	12	16	13
4c	Ethyl	15	16	12	15
4d	Propyl	13	15	14	16
4e		18	17	18	17
4f		19	18	14	16
4g		17	16	14	15
4h		15	16	17	13
4i		22	21	19	19
4j		20	20	19	21
4k		22	21	20	19
4l		20	19	21	20
Standard drug	Ampicillin (250 µg/mL)	20	20	19	18

CONCLUSION

We have synthesized new pyrazoline derivatives **4 (a-l)** from commercially available 2-Hydroxy-Aceto-Napthanone and screened for the antibacterial activity against Escherichia coli (MTCC-443), Staphylococcus aureus (MTCC-96), Pseudomonas aeruginosa (MTCC-424) and Streptococcus pyogenes (MTCC-442) bacterial strains. All of the screened compounds exhibited good to excellent activity when compared to the standard drug ampicillin. In general it is observed that the compounds having amide functionality **4i-4l** exhibited excellent antibacterial activity with zone of inhibition 19-22 mm towards all the tested bacterial strains. Thus further lead optimization is required to get wide spectrum of activity.

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