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Synthesis of some new pyrazolone derivatives as potent antimicrobial agents

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ABSTRACT

*Invasive microbial infections are major problems around the world, especially in immuno compromised patients. The recent expansion of antimicrobial drug research has occurred because there is a critical need for new antimicrobial agents to treat these life threatening invasive infections. In the present study three series of new substituted pyrazolone derivatives (**5a-f, 6a-f** and **8a,b**) were synthesized by the Knoevenagel condensation reaction of pyrazolones (**3a,b**) with various substituted carbaldehydes (**4a-f, 7**). These newly synthesized compounds were characterized by IR, NMR, mass spectra and also by C, H, N analyses. New compounds were screened for their antimicrobial studies against S. aureus, B. subtilis, E. coli and P. aeruginosa. The results revealed that compounds **5c** and **6c** having 2,5-dichlorothiophene substituent showed significant antibacterial activity against all tested microorganisms as compared to the standard drug Ceftriaxone.*

Key words: pyrazolones, pyrazoles, 6-methoxy-2-naphthaldehyde, antimicrobial studies.

INTRODUCTION

Microbial development of resistance, as well as economic incentives, has resulted in research and development in the search for new antibiotics in order to maintain a pool of effective drugs at all time. It is important to find out newer, safer and more effective antibiotics with broad-spectrum of activity. Although several antifungal agents and the azole class of drugs are currently available there is clearly a critical need for the development of new specific antimicrobial agents. Heterocycles containing a pyrazole/pyrazolone ring system are found to exhibit a wide spectrum of biological activities, including antibacterial and antifungal activities.

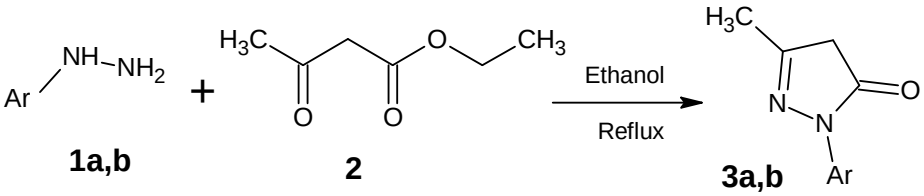
Pyrazolone derivatives are an important class of heterocyclic compounds that occur in many drugs and synthetic products [1-2]. These compounds exhibit remarkable analgesic [3], antitubercular [4], antifungal, antibacterial [5], anti-inflammatory [6], antioxidant and antitumor activities [7]. Due to their easier preparation and rich biological activity, pyrazolone framework plays an essential role and represents an interesting template for combinatorial and medicinal chemistry. Similarly, pyrazole derivatives have showed significant biological activities, such as anti-microbial [8], analgesic [9], anti-inflammatory [10] and anticancer [11] activities. This gave a great impetus to the search for potential pharmacologically active drugs carrying pyrazole substituents.

Increasing antibiotic resistance in microbial populations has necessitated the search for alternate cellular targets for new and existing antimicrobial agents. It is well established that small modifications in the structure of the targets are altering their biological character as well as their physiochemical properties. A detailed literature survey on antimicrobial activity of various types of compounds clearly indicates that presence of certain pharmacophore such as pyrazole in any molecule plays an important role in enhancing activity. Keeping in view of this and in continuation of our search on biologically potent molecules [12-15], we hereby report the synthesis and antimicrobial property of some new pyrazolone derivatives containing substituted pyrazole/ 6-methoxy-2-naphthyl moiety.

MATERIALS AND METHODS

2.1. Chemistry

Melting points were determined by open capillary method and were uncorrected. The IR spectra (in KBr pellets) were recorded on a JASCO FT/IR-4100 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded (DMSO-d_6) on a Bruker (400 MHz) and Varian (400 MHz) spectrometer using TMS as internal standard. Chemical shift values are given in δ (ppm) scales. The mass spectra were recorded on a JEOL JMS-D 300 spectrometer operating at 70 eV. Elemental analyses were performed on a Flash EA 1112 series CHNS-O Analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 F254) obtained from Merck. Commercial grade solvents and reagents were used without further purification.

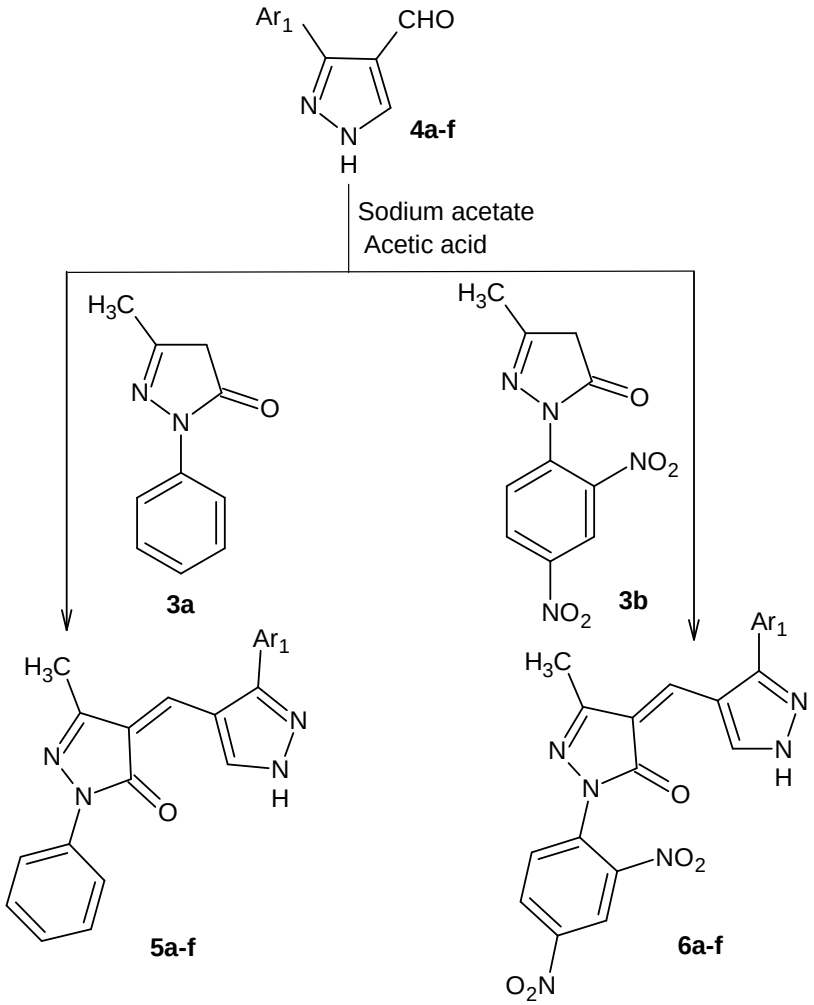


Where Ar = Phenyl, 2,4-Dinitrophenyl

Scheme-1. Synthetic route for the compounds 3a,b

2.2. General procedure for the synthesis of 2-Aryl-5-methyl-2,4-dihydro-3H-pyrazol-3-one (3a,b)

A mixture of phenyl hydrazine/ 2,4-dinitrophenyl hydrazine (0.01 mol) and ethylacetoacetate (0.01 mol) were taken in absolute alcohol (30 mL) and refluxed for 12 hrs. After completion of the reaction, excess of solvent was distilled off and the resultant residue was poured on crushed ice to obtain the yellow/ orange long needle shaped crystals. Solids precipitated were filtered and recrystallized using ethanol [16] (**Scheme-1**).



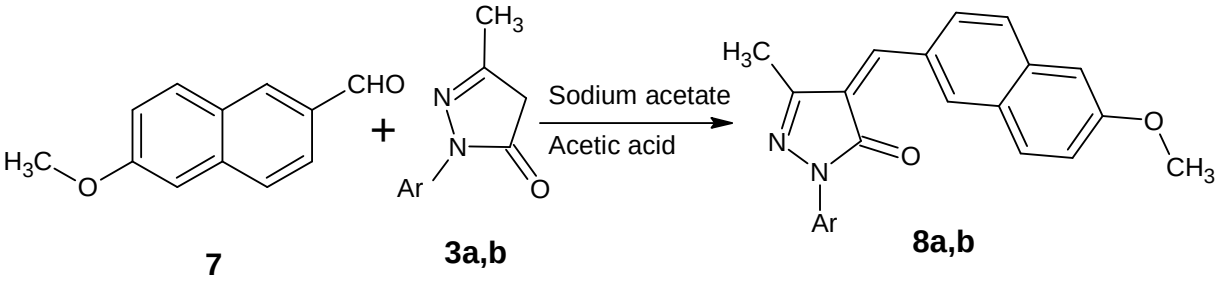
Ar_1= 2,4-Dichlorophenyl, Thioanisyl, 2,5-Diclorothiophene, Biphenyl, 4-Anisyl, 4-Chlorophenyl

Scheme-2. Synthetic route for the compounds 5a-f and 6a-f.

2.3 General procedure for the synthesis of (4Z)-4-{[3-(substituted)-1H-pyrazol-4-yl] methylidene}-5-methyl-2-aryl-2,4-dihydro-3H-pyrazol-3-one (5a-f and 6a-f)/ (4Z)-4-[(6-methoxynaphthalen-2-yl)methylidene]-5-methyl-2-aryl-2,4-dihydro-3H-pyrazol-3-one(8a,b)

An equimolar mixture of substituted carbaldehydes **4a-f, 7** (0.10 mol) and 2-Aryl-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one **3a,b** (0.10 mol) in acetic acid (20 mL) and sodium acetate (0.01 mol) were refluxed for 8 h. After completion of the reaction, the reaction mixture was allowed to cool,

filtered and poured on crushed ice. The solid thus separated was collected by filtration and recrystallized from acetic acid. (S**heme-2** and **Scheme-3)**.



7 **3a,b** **8a,b**

Where Ar = Phenyl, 2,4-Dinitrophenyl

Scheme-3. Synthetic route for the compounds 8a,b.

Table-1 Characterization data of the compounds 5a-f, 6a-f, and 8a,b

Comp. No.	Ar or Ar_1	Mol. Formula (Mol. wt.)	Yield (%)	M.p.(^{o}C)	Colour
5a	2,4-Dichlorophenyl	$C_{20}H_{14}Cl_2N_4O$ (397.25)	85	206- 208	Yellow
5b	4-SCH_3-C_6H_4	$C_{21}H_{18}N_4OS$ (374.45)	86	218-220	Yellow
5c	2,5-Dichloro thiophene	$C_{18}H_{12}Cl_2N_4OS$ (403.28)	84	170-172	Yellow
5d	Biphenyl	$C_{26}H_{20}N_4O$ (404.46)	85	242-244	Yellow
5e	4-OCH_3-C_6H_4	$C_{21}H_{18}N_4O_2$ (358.39)	82	240-242	Yellow
5f	4-Chlorophenyl	$C_{20}H_{15}ClN_4O$ (362.81)	87	236-238	Orange
6a	2,4-Dichlorophenyl	$C_{20}H_{12}Cl_2N_6O_5$ (487.25)	82	224-226	Orange
6b	4-SCH_3-C_6H_4	$C_{21}H_{16}N_6O_5S$ (464.45)	84	222-224	Red
6c	2,5-Dichloro thiophene	$C_{18}H_{10}Cl_2N_6O_5S$ (493.28)	81	248-250	Red
6d	Biphenyl	$C_{26}H_{18}N_6O_5$ (494.45)	83	284-286	Red
6e	4-OCH_3-C_6H_4	$C_{21}H_{16}N_6O_6$ (448.38)	85	256-258	Red
6f	4-Chlorophenyl	$C_{20}H_{13}ClN_6O_5$ (452.80)	84	258-260	Red
8a	Phenyl	$C_{22}H_{18}N_2O_2$ (342.39)	81	144-146	Red
8b	2,4-Dinitrophenyl	$C_{22}H_{16}N_4O_6$ (432.38)	82	270-272	Red

2.3.1. (4Z)-4-{[3-(2,4-dichlorophenyl)-1H-pyrazol-4-yl]methylidene}-5-methyl-2-phenyl-2,4-dihydro -3H-pyrazol-3-one (5a)

IR (KBr, ν_{max} cm^{-1}): 3233 (N-H-str), 1673 (C=O), 1603 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.10 (s, 3H, CH_3), 7.11-7.60 (m, 7H, Ar-H), 7.80 (s, 1H, pyrazole-5H), 7.86 (d, 1H, Ar-H), 9.60 (s, 1H, C=CH), 13.97 (s, 1H, pyrazole-NH); ^{13}C-NMR: δ 191.77, 171.58, 130.94, 129.33, 123.58, 118.75, 21.54; MS: m/z = 397.2 (M^+), 399.2 (M+2), 401.2 (M+4); Anal. calcd. for $C_{20}H_{14}Cl_2N_4O$: C, 60.47; H, 3.55; N, 14.10; Found: C, 60.45; H, 3.52; N, 14.07.

2.3.2 (4Z)-5-methyl-4-({3-[4-(methylsulfanyl)phenyl]-1H-pyrazol-4-yl}methylidene)-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (5b)

IR (KBr, ν_{max} cm^{-1}): 3138 (N-H-str), 1677 (C=O), 1598 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.88 (s, 3H, CH_3), 2.56 (s, 3H, SCH_3), 7.12-7.60 (m, 9H, Ar-H), 7.87 (s, 1H, pyrazole-5H), 9.72 (s, 1H, C=CH), 13.95 (s, 1H, pyrazole-NH); MS: m/z = 375.2 (M+1); Anal. calcd. for $C_{21}H_{18}N_4OS$: C, 67.36; H, 4.85; N, 14.96; Found: C, 67.33; H, 4.82; N, 14.94.

2.3.3 (4Z)-4-{[3-(2,5-dichlorothiophen-3-yl)-1H-pyrazol-4-yl]methylidene}-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (5c)

IR (KBr, ν_{max} cm^{-1}): 3298 (N-H-str), 1672 (C=O), 1611 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.19 (s, 3H, CH_3), 7.12-7.86 (m, 6H, Ar-H), 7.88 (s, 1H, pyrazole-5H), 9.57 (s, 1H, C=CH), 14.01 (s, 1H, pyrazole-NH); MS: m/z = 404.1 (M+1), 405.2 (M+2), 407.2 (M+4); Anal. calcd. for $C_{18}H_{12}Cl_2N_4OS$: C, 53.61; H, 3.00; N, 13.89; Found: C, 53.58; H, 2.96; N, 13.85.

2.3.4 (4Z)-4-{[3-(4-biphenyl)-1H-pyrazol-4-yl]methylidene}-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (5d)

IR (KBr, ν_{max} cm^{-1}): 3255 (N-H-str), 1663 (C=O), 1600 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.04 (s, 3H, CH_3), 7.13-7.79 (m, 14H, Ar-H), 7.93 (s, 1H, pyrazole-5H), 9.51(s, 1H, C=CH), 13.96 (s, 1H, pyrazole-NH); MS: m/z = 405.4 (M+1); Anal. calcd. for $C_{26}H_{20}N_4O$: C, 77.21; H, 4.98; N, 13.84; Found: C, 77.18; H, 4.96; N, 13.81.

2.3.5 (4Z)-4-{[3-(4-methoxyphenyl)-1H-pyrazol-4-yl]methylidene}-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (5e)

IR (KBr, ν_{max} cm^{-1}): 3305 (N-H-str), 1666 (C=O), 1595 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.17 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 6.93-7.88 (m, 7H, Ar-H), 7.90 (s, 1H, pyrazole-5H), 9.23 (s, 1H, C=CH), 13.76 (s, 1H, pyrazole-NH); ^{13}C-NMR: δ 193.54, 169.86, 138.83, 131.64, 130.94, 122.02, 118.66, 115.60, 113.28, 55.96, 21.99; MS: m/z = 357.4 (M-1); Anal. calcd. for $C_{21}H_{18}N_4O_2$: C, 70.38; H, 5.06; N, 15.63; Found: C, 70.35; H, 5.02; N, 15.61.

2.3.6 (4Z)-4-{[3-(4-chlorophenyl)-1H-pyrazol-4-yl]methylidene}-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (5f)

IR (KBr, ν_{max} cm^{-1}): 3130 (N-H-str), 1677 (C=O), 1602 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.86 (s, 3H, CH_3), 7.11-7.87 (m, 9H, Ar-H), 7.89 (s, 1H, pyrazole-5H), 9.59 (s, 1H, C=CH), 13.89 (s, 1H, pyrazole-NH); ^{13}C-NMR: δ 194.52, 172.46, 131.39, 129.32, 124.38, 118.69, 21.52; MS: m/z = 363.8 (M+1); Anal. calcd. for $C_{20}H_{15}ClN_4O$: C, 66.21; H, 4.17; N, 15.44; Found: C, 66.18; H, 4.15; N, 15.41.

2.3.7. (4Z)-4-{[3-(2,4-dichlorophenyl)-1H-pyrazol-4-yl]methylidene}-2-(2,4-dinitrophenyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (6a)

IR (KBr) ν/cm^{-1}: 3282 (N-H-str), 1682 (C=O), 1618 (C=N); ^{1}H NMR (DMSO-d_6) δ: 1.85 (s, 3H, CH_3), 7.31-8.17 (m, 5H, 2,4-dichlorophenyl, 2,4-dinitrophenyl), 8.56 (s, 1H, pyrazole-5H), 8.87 (d, 1H, *J* = 2.8, 2,4-dinitrophenyl), 9.70 (s, 1H, C=CH), 13.55 (s, 1H, pyrazole-NH); ^{13}C NMR: δ 194.06, 172.63, 144.82, 143.35, 136.94, 134.67, 133.82, 129.56, 123.76, 21.52; MS: m/z = 487.0 (M^+); Anal. calcd. for $C_{20}H_{12}Cl_2N_6O_5$ (%): C, 49.30; H, 2.48; N, 17.25; Found: C, 49.27; H, 2.46; N, 17.22.

2.3.8. (4Z)-2-(2,4-dinitrophenyl)-5-methyl-4-({3-[4-(methylsulfanyl)phenyl]-1H-pyrazol-4-yl}methyl-idene)-2,4-dihydro-3H-pyrazol-3-one (6b)

IR (KBr) ν/cm^{-1}: 3265 (N-H-str), 1671 (C=O), 1606 (C=N); ^{1}H NMR (DMSO-d_6) δ: 1.91 (s, 3H, CH_3), 2.55 (s, 3H, SCH_3), 7.42-7.60 (m, 4H, thioanisyl), 8.01 (d, 1H, *J* = 7.2, 2,4-dinitrophenyl), 8.34 (dd, 1H, *J* = 2.0, 2,4-dinitrophenyl), 8.79 (s, 1H, pyrazole-5H), 8.86 (d, 1H, *J* = 2.8, 2,4-dinitrophenyl), 9.85 (s, 1H, C=CH), 13.52 (s, 1H, pyrazole-NH); MS: m/z = 465.4 (M+1); Anal. calcd. for $C_{21}H_{16}N_6O_5S$ (%): C, 54.31; H, 3.47; N, 18.09; Found: C, 54.27; H, 3.43; N, 18.06.

2.3.9. (4Z)-4-{[3-(2,5-dichlorothiophen-3-yl)-1H-pyrazol-4-yl]methylidene}-2-(2,4-dinitrophenyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (6c)

IR (KBr) ν/cm^{-1}: 3262 (N-H-str), 1705 (C=O), 1604 (C=N); ^{1}H NMR (DMSO-d$_6$) δ: 1.91 (s, 3H, CH_3), 7.34 (s, 1H, 2,5-dichlorothiophene), 7.77-8.28 (m, 2H, 2,4-dinitrophenyl), 8.65 (s, 1H, pyrazole-5H), 8.86 (d, 1H, *J* = 2.8, 2,4-dinitrophenyl), 9.64 (s, 1H, C=CH), 13.64 (s, 1H, pyrazole-NH); MS: m/z = 493.2 (M^+), 495.2 (M+2); Anal. calcd. for $C_{18}H_{10}Cl_2N_6O_5S$ (%): C, 43.83; H, 2.04; N, 17.04; Found: C, 43.80; H, 2.01; N, 17.01.

2.3.10. (4Z)-4-{[3-(4-biphenyl)-1H-pyrazol-4-yl]methylidene}-2-(2,4-dinitrophenyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (6d)

IR (KBr) ν/cm^{-1}: 3275 (N-H-str), 1674 (C=O), 1609 (C=N); ^{1}H NMR (DMSO-d$_6$) δ: 1.85 (s, 3H, CH_3), 7.34-7.78 (m, 9H, biphenyl), 7.96-8.80 (m, 4H, 2,4-dinitrophenyl, pyrazole-5H), 9.75 (s, 1H, C=CH), 13.43 (s, 1H, pyrazole-NH); MS: m/z = 495.4 (M+1); Anal. calcd. for $C_{26}H_{18}N_6O_5$ (%): C, 63.16; H, 3.67; N, 17.00; Found: C, 63.14; H, 3.65; N, 16.95.

2.3.11. (4Z)-2-(2,4-dinitrophenyl)-4-{[3-(4-methoxyphenyl)-1H-pyrazol-4-yl]methylidene}-5-methyl-2,4-dihydro-3H-pyrazol-3-one (6e)

IR (KBr) ν/cm^{-1}: 3274 (N-H-str), 1688 (C=O), 1611 (C=N); ^{1}H NMR (DMSO-d$_6$) δ: 1.85 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 7.04-7.55 (m, 4H, anisyl), 7.93 (d, 1H, *J* = 9.6, 2,4-dinitrophenyl), 8.25 (dd, 1H, *J* = 2.4, 2,4-dinitrophenyl), 8.70 (s, 1H, pyrazole-5H), 8.80 (d, 1H, *J* = 2.4, 2,4-dinitrophenyl), 9.60 (s, 1H, C=CH), 13.32 (s, 1H, pyrazole-NH); ^{13}C NMR: δ 195.69, 172.46, 144.84, 136.82, 130.11, 123.64, 117.16, 114.78, 113.75, 55.80, 21.52; MS: m/z = 447.0 (M-1); Anal. calcd. for $C_{21}H_{16}N_6O_6$ (%): C, 56.25; H, 3.60; N, 18.74; Found: C, 56.22; H, 3.57; N, 18.72.

2.3.12. (4Z)-4-{[3-(4-chlorophenyl)-1H-pyrazol-4-yl]methylidene}-2-(2,4-dinitrophenyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (6f)

IR (KBr) ν/cm^{-1}: 3266 (N-H-str), 1695 (C=O), 1600 (C=N); ^{1}H NMR (DMSO-d$_6$) δ: 1.85 (s, 3H, CH_3), 7.55-7.64 (m, 4H, 4-chlorophenyl), 7.92 (d, 1H, *J* = 6.8, 2,4-dinitrophenyl), 8.28-8.80 (m, 3H, 2,4-dinitrophenyl, pyrazole-5H), 9.67 (s, 1H, C=CH), 13.47 (s, 1H, pyrazole-NH); MS: m/z = 452.8 (M^+); Anal. calcd. for $C_{20}H_{13}ClN_6O_5$ (%): C, 53.05; H, 2.89; N, 18.56; Found: C, 53.02; H, 2.87; N, 18.53.

2.3.13. (4Z)-4-[(6-methoxynaphthalen-2-yl)methylidene]-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (8a)

IR (KBr) ν/cm^{-1}: 1667 (C=O), 1576 (C=N); ^{1}H NMR (DMSO-d$_6$) δ: 2.32 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3) 7.20-8.76 (m, 11H, Ar-H), 9.01 (s, 1H, C=CH); ^{13}C NMR: δ 192.55, 136.85, 129.32, 127.34, 120.13, 118.90, 106.84, 56.03; MS: m/z = 343.3 (M+1); Anal. calcd. for $C_{22}H_{18}N_2O_2$ (%): C, 77.17; H, 5.30; N, 8.18; Found: C, 77.14; H, 5.27; N, 8.15.

2.3.14. (4Z)-2-(2,4-dinitrophenyl)-4-[(6-methoxynaphthalen-2-yl)methylidene]-5-methyl-2,4-dihydro-3H-pyrazol-3-one (8b)

IR (KBr) ν/cm^{-1}: 1677 (C=O), 1599 (C=N); ^{1}H NMR (DMSO-d$_6$) δ: 2.35 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3) 7.16-8.83 (m, 9H, Ar-H), 9.38 (s, 1H, C=CH); MS: m/z = 433.3 (M+1); Anal. calcd. for $C_{22}H_{16}N_4O_6$ (%): C, 61.11; H, 3.73; N, 12.96; Found: C, 61.07; H, 3.71; N, 12.93.

2.4. Antimicrobial studies

All the newly synthesized compounds were screened for their antibacterial activity. For this, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* microorganisms were employed. Antimicrobial study was assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method [17]. Several colonies of *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* were picked off a fresh isolation plate and inoculated in corresponding tubes containing 5 mL of trypticase soya broth. The broth was incubated for 6 h at 37 ^{0}C until there was visible growth. Mc Farland No.5 standard was prepared by adding 0.05 mL of 1% w/v $BaCl_2.2H_2O$ in Phosphate Buffered saline (PBS) to 9.95 mL of 1% v/v H_2SO_4 in PBS. The growth of all the four cultures was adjusted to Mc Farland No.5 turbidity standard using sterile PBS. This gives a 108 cfu/mL suspension. The working inoculums of aforementioned four different microorganisms containing 105 cfu/mL suspension was prepared by diluting the 108 cfu/mL suspension, 103 times in trypticase soya broth.

2.4.1. Preparation of Anti-microbial Suspension (1mg/mL)

Dissolved 10 mg of each compound in 10 mL of Dimethyl formamide to get 1 mg/mL concentration.

Table 2: Antibacterial activity data in MIC (µg/mL) of the compounds 5a-f, 6a-f and 8a-b

Comp. No.	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*
5a	12.5	12.5	12.5	6.25
5b	6.25	6.25	12.5	6.25
5c	1.6125	1.6125	1.6125	1.6125
5d	6.25	6.25	6.25	6.25
5e	6.25	6.25	12.5	6.25
5f	3.125	6.25	6.25	6.25
6a	3.125	3.125	3.125	1.6125
6b	3.125	3.125	1.6125	3.125
6c	1.6125	1.6125	1.6125	1.6125
6d	6.25	3.125	6.25	6.25
6e	6.25	6.25	12.5	6.25
6f	6.25	3.125	3.125	3.125
8a	6.25	12.5	12.5	6.25
8b	12.5	3.125	3.125	3.125
Ceftriaxone (Standard)	3.125	1.6125	1.6125	1.6125
Inoculum control	***	***	***	***
Broth control	No growth	No growth	No growth	No growth

********* *Indicates growth in all concentrations*

2.4.2 Preparation of dilutions

In all, for each of the 12 anti-microbial compounds and standard antimicrobial i.e Ceftriaxone, 24 tubes of 5 mL capacity were arranged in 4 rows with each row containing 6 tubes. Then 1.9 mL of trypticase soya broth was added in the first tube in each row and then 1 mL in the remaining tubes. Now, 100µL of anti microbial suspension dissolved in Dimethyl formamide was added to the first tube in each row and then after mixing the content, 1 mL was serially transferred from these tubes to the second tube in each of the rows. Then the contents in the second tube of each of the rows were mixed and transferred to the third tube in each of the rows. This serial dilution was repeated till the sixth tube in each of the rows. This provided anti

microbial concentrations of 50, 25, 12.5, 6.25, 3.125, 1.6125 µg /mL in the first to sixth tube respectively in each row. Finally, 1 mL of 10^5 cfu/mL of *S. aureus, B. subtilis*, *E. coli* and *P. aeruginosa* suspension were added to the first, second, third and fourth rows of tubes respectively. Along with the test samples and Ceftriaxone (standard), the inoculums control (without antimicrobial compound) and broth control (without antimicrobial compound and inoculum) were maintained. All the test sample and control tubes were then incubated for 16 hrs at 37 ^oC.

2.4.3. Interpretation

After incubation, the tubes showing no visible growth were considered to be representing the MIC. The details of results are furnished in **Table-2**. Inoculums control showed visible growth, where as the broth control showed no growth.

RESULTS AND DISCUSSION

2-Aryl-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (**3a,b**) were synthesized by refluxing phenyl hydrazine or 2,4-dinitrophenyl hydrazine with ethylacetoacetate in ethanol media [16]. 3-Substituted-1*H*-pyrazole-4-carbaldehydes (**4a-f**) were synthesized by the Vilsmayer Haack reaction of semicarbazones [18]. The targeted pyrazolone derivatives (**5a-f, 6a-f,** and **8a,b**) were obtained in excellent yields by refluxing 2-Aryl-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (**3a,b**) with various substituted carbaldehydes (**4a-f, 7**) and anhydrous sodium acetate in acetic acid for 8 h [19]. The reaction pathway has been summarized in Scheme-1 and Scheme-2. Newly synthesized compounds (**5a-f, 6a-f,** and **8a,b**) were characterized by IR, NMR, mass spectral and C, H, N elemental analyses.

Formation of (4*Z*)-4-{[3-(substituted)-1*H*-pyrazol-4-yl]methylidene}-5-methyl-2-aryl-2,4-dihydro-3*H*-pyrazol-3-one (**5a-f** and **6a-f**) and (4*Z*)-4-[(6-methoxynaphthalen-2-yl) methylidene]-5-methyl-2-aryl-2,4-dihydro-3*H*-pyrazol-3-one (**8a,b**) were confirmed by recording their IR, ^1H NMR, ^{13}C NMR and mass spectra. For first series, IR spectrum of compound **5a** showed absorption at 3282 cm^{-1} which is due to the NH stretching. Bands at 1680 cm^{-1} is due to cyclic C=O. Similarly, band at 1618 cm^{-1} is due to C=N group. The ^1H NMR spectrum of **5a** showed a singlet at δ 2.10 is due to CH_3 protons. Aromatic protons appeared as multiplet at δ 7.12-7.60. Pyrazole 5H appeared as a singlet at δ 7.80. A doublet at δ 7.86 (J = 7.6 Hz) is due to aromatic protons of phenyl moiety. Similarly a singlet appeared at δ 9.60 is due to C=CH protons. A singlet at δ 13.97 is due to pyrazole-NH further confirms the structure. The mass spectrum of **5a** showed molecular ion peak at m/z = 397 (M^+), which is in agreement with the molecular formula $C_{20}H_{14}Cl_2N_4O$. Similarly the spectral values for all the compounds and C, H, N analyses are given in the experimental part and the characterization is provided in **Table-1**.

CONCLUSION

Three series of novel substituted pyrazolone derivatives were synthesized in reasonably good yields. They were characterized by ^1H-NMR, ^{13}C-NMR, mass spectrometry, IR studies and elemental analyses. All the newly synthesized compounds were screened for their antibacterial activity by MIC method. Among the screened samples, **5c** and **6c** have showed excellent antibacterial activity at 1.6125 µg/mL concentration against *S. aureus* bacteria as compared to

the standard drug Ceftriaxone which is active at 3.125 µg/mL concentration. They also showed similar activity as that of standard, against *B. subtilis*, *E. coli and P. aeruginosa*, at 1.6125 µg/mL concentration.

As regards the relationships between the structure of the heterocyclic scaffold and the detected antibacterial properties, it showed varied biological activity. Probably in this case the nature of the heterocyclic ring is not so important for anti-microbial activity. Moreover, the presence of different substituents causes a certain change of activity. Compounds **5c** and **6c** have 2,5-dichlorothiophene moiety, which is accounted for the enhanced antibacterial activity against all the four tested microorganisms. Similarly compound **6a** showed significant activity against *S. aureus* and *P. aeruginosa*, which is active at the same concentration as that of the standard drug. Compound **6a** has 2,4-Dichlorophenyl subsistent, which is accounted for the activity of the compound. Compound **6b** showed similar activity as that of standard, against *E. coli*, at 1.6125 µg/mL concentration. Compound **6b** has thioanisyl group on pyrazole ring. On the other hand, the remaining compounds showed moderately good activity against all of the four tested bacterial strains compared to standard, Ceftriaxone. From the obtained results, it is clear that the major role for antibacterial activity is played by the substituent present on pyrazole and aromatic rings bonded to pyrazolone moiety. It is evident that pyrazolones with 2,5-dichlorothiophene are the most active compounds.

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