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Synthesis and antimicrobial activity of some new pyrazole containing cyanopyridone derivatives

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ABSTRACT

*A series of new 4,6-disubstituted-3-cyano-2-pyridone derivatives **(4a-o)** were synthesized. The structures of all target molecules **(4a-o)** have been confirmed by various spectral techniques and elemental analyses. The newly synthesized compounds were screened for antibacterial and antifungal activity and most of the compounds showed significant activity comparable with that of the standard drug. The results revealed that **4b**, **4c**, **4d**, **4g**, **4m**, **4n** and **4o** showed good antibacterial activity towards all bacterial strains (Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa) when compared to standard drug streptomycin. Amongst all the compounds, **4c** showed moderate antifungal activity against Aspergillus flavus. The acute toxicity study has also been carried out for biologically active compounds and the experimental studies revealed that compounds were safe up to 2000 mg/kg and no deaths of animals were recorded.*

Keywords: Cyanopyridone, pyrazole, antimicrobial activity.

INTRODUCTION

Functionalized nitrogen and oxygen containing heterocycles play a predominant role in medicinal chemistry and they have been intensively used as scaffolds for drug development. The rapid assembly of molecular diversity is an important goal of synthetic organic chemistry and is one of the key paradigms of modern drug discovery. The synthesis of the pyridine containing

heterocyclic systems occupies an important place in the realm of synthetic organic chemistry, due to their therapeutic and pharmacological properties [1-3]. They have emerged as integral backbones of over 7000 existing drugs [4,5]. The pyridine nucleus is an integral part of anticancer and anti-inflammatory agents [6,7] too. On the other hand, cyanopyridone and cyanopyridine derivatives have shown to posses promising antimicrobial [8] and anticancer activities [9]. The interest in 3-cyano-2(1H)-pyridone and their derivatives is due to their wide range of practical uses as medicinal compounds [10]. In addition, the pharmacological and physiological activity of 3-cyanopyridines has attracted much attention in recent years with the synthesis and the study of the nonglycosidic cardiotonic agent milrinone.

Furthermore, pyrazoles represent a key motif in heterocyclic chemistry and occupy a prime place in medicinal and pesticide chemistry due to their capability to exhibit a wide range of bioactivities such as antimicrobial [11-14], anticancer [15], anti-inflammatory [16,17], antidepressant [18], anticonvulsant [19], antipyretic [20] and selective enzyme inhibitory activities [21]. Against this background, to extend our research work on synthesis of biologically active molecules [14,22], we have designed and synthesized a series of 4,6-disubstituted-3-cyano-2-pyridone derivatives **(4a-o)** via one-pot multicomponent reaction using 3-substituted-1*H*-pyrazole-4-carbaldehydes **(1a-e)**, various acetyl compounds **(2a-c),** ethyl cyanoacetate **(3)** and ammonium acetate. All the target molecules were screened for their antimicrobial activity against various microorganisms.

MATERIALS AND METHODS

2.1. Chemistry

Melting points were determined by open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Thermo Nicolet avatar 330-FT-IR spectrophotometer. ^{1}H NMR spectra were recorded (DMSO-d_6) on a Varian (400 MHz) spectrometer. Chemical shift values are given in δ scales. The mass spectra were recorded on API 2000 LC/MS system. 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). Commercial grade solvents and reagents were used without further purification.

2.2.General procedure for the synthesis of 4,6-disubstituted-3-cyano-2-pyridones (4a-o)

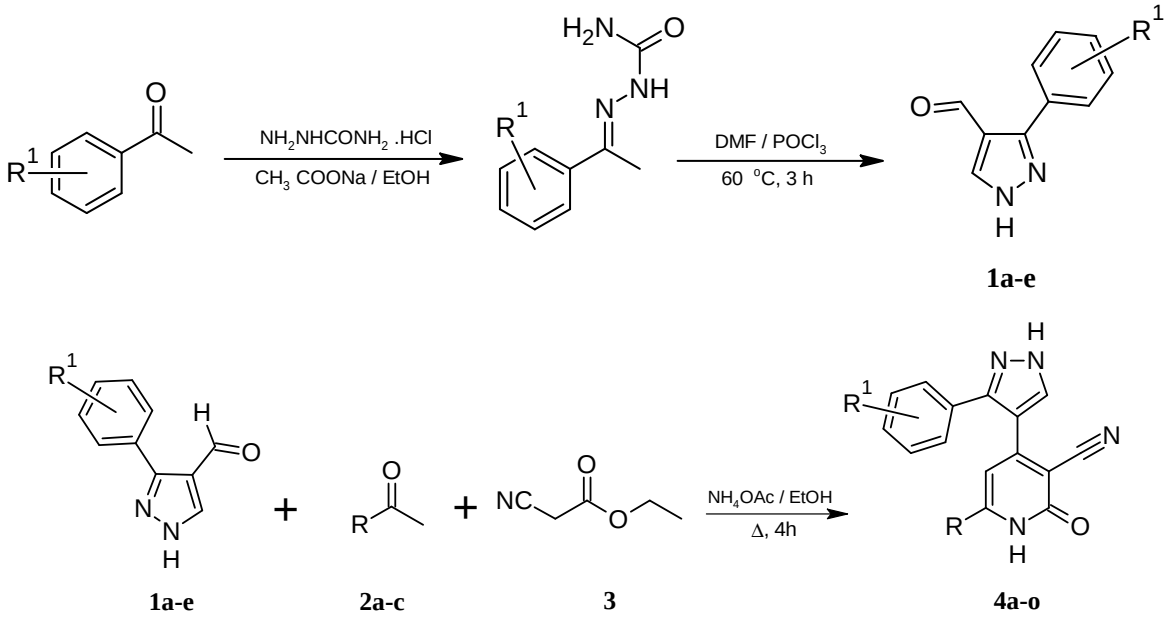
A mixture of 3-substituted-1*H*-pyrazole-4-carbaldehydes **1a-e** (0.001 mol), corresponding acetyl compounds **2a-c** (0.001 mol), ethyl cyanoacetate (0.0012 mol) and ammonium acetate (0.008 mol) were refluxed in ethanol for 4 h. The reaction mixture was cooled to obtain precipitate which was filtered, washed with ethanol and recrystallized from suitable solvent to get pure product.

2.2.1. 2-oxo-4-(3-phenyl-1H-pyrazol-4-yl)-6-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile (4a)

Yield 53 % ; M.p. >300 ^{o}C; IR (KBr, υ_{max} cm^{-1}): 3229, 3090 (N-H), 2211 (C≡N), 1635 (C=O), 1594 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.53 (bs,1H, pyrazole-NH), 12.66 (s, 1H, pyridone ring NH), 8.32 (s, 1H, pyrazole-5H), 7.18-7.89 (m, 8H, Ar-H), 6.23 (s, 1H, pyridone-5H); MS: m/z = 345 (M+1). Anal. calcd. for $C_{19}H_{12}N_4OS$: C, 66.26; H, 3.51; N, 16.27. Found: C, 66.20; H, 3.55; N, 16.24%.

2.2.2. 4-[3-(4-fluorophenyl)-1H-pyrazol-4-yl]-2-oxo-6-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile (4b)

Yield 56 %; M.p. >300 ^{o}C; IR (KBr, υ_{max} cm^{-1}): 3245, 3088 (N-H), 2205 (C≡N), 1637 (C=O), 1594 (C=N); MS: m/z = 363 (M+1). ^{13}C NMR: 163.7, 163.4, 161.3, 152.0, 134.7, 131.5, 130.48, 130.4, 129.3, 117.1, 116.2, 116.0, 114.3, 106.6; Anal. calcd. for $C_{19}H_{11}FN_4OS$: C, 62.97; H, 3.06; N, 15.46. Found: C, 62.91; H, 3.02; N, 15.41 %.



4a: *R= Thienyl, R^1=H;* ***4b****: R= Thienyl, R^1= 4-F;* ***4c****: R= Thienyl, R^1= 4-Cl;* ***4d****: R= Thienyl, R^1= 2,4-Cl_2;* ***4e****: R= Thienyl, R^1= 4-CH_3;* ***4f****: R= 1-napthyl, R^1= H;* ***4g****: R= 1-napthyl, R^1= 4-OCH_3;* ***4h****: R=1-napthyl, R^1= 4-F;* ***4i****: R= 1-napthyl, R^1= 4-Cl;* ***4j****: R= 1-napthyl, R^1= 2,4-Cl_2;* ***4k****: R=1-napthyl, R^1= 4-CH_3;* ***4l****: R= 5-Cl-thienyl, R^1= H;* ***4m****: R= 5-Cl-thienyl, R^1= 4-F;* ***4n****: R= 5-Cl-thienyl, R^1= 4-Cl;* ***4o****: R= 5-Cl-thienyl, R^1= 4-CH_3*

Scheme 1: **Synthetic route for 4,6-disubstituted-3-cyano-2-pyridone derivatives**

2.2.3. 4-[3-(4-chlorophenyl)-1H-pyrazol-4-yl]-2-oxo-6-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile (4c)

Yield 51 %; M.p. >300 ^{o}C; IR (KBr, υ_{max} cm^{-1}): 3224, 3080 (N-H), 2216 (C≡N), 1638 (C=O), 1594 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.56 (bs,1H, pyrazole-NH), 12.67 (s, 1H, pyridone ring NH), 8.24 (s, 1H, pyrazole-5H), 7.20-7.92 (m, 7H, Ar-H), 6.30 (s, 1H, pyridone-5H); MS: m/z = 379 (M+1). Anal. calcd. for $C_{19}H_{11}ClN_4OS$: C, 60.24; H, 2.93; N, 14.79. Found: C, 60.19; H, 2.89; N, 14.75 %.

2.2.4. 4-[3-(2,4-dichlorophenyl)-1H-pyrazol-4-yl]-2-oxo-6-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile (4d)

Yield 49 %; M.p. >300 ^{o}C; IR (KBr, υ_{max} cm^{-1}): 3199, 3088 (N-H), 2214 (C≡N), 1649 (C=O), 1606 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.54 (bs,1H, pyrazole-NH), 12.65 (s, 1H, pyridone ring NH), 8.38 (s, 1H, pyrazole-5H), 7.17-7.82 (m, 6H, Ar-H), 6.15 (s, 1H, pyridone-5H); MS: m/z = 412 (M^+). Anal. calcd. for $C_{19}H_{10}Cl_2N_4OS$: C, 55.22; H, 2.44; N, 13.56. Found: C, 55.18; H, 2.39; N, 13.51 %.

2.2.5. 4-[3-(4-methylphenyl)-1H-pyrazol-4-yl]-2-oxo-6-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile (4e)

Yield 56 %; M.p. >300 ^{o}C; IR (KBr, υ_{max} cm^{-1}): 3321, 3088 (N-H), 2807 (Aliphatic C-H), 2213 (C≡N), 1632 (C=O), 1596 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.56 (bs,1H, pyrazole-NH), 12.67 (s, 1H, pyridone ring NH), 8.11 (s, 1H, pyrazole-5H), 7.17-7.85 (m, 7H, Ar-H), 6.42 (s, 1H, pyridone-5H), 2.32 (s, 3H, -CH_3); ^{13}C NMR: 163.2, 152.4, 145.9, 138.2, 136.9, 135.7, 131.5, 129.7, 129.3, 128.1, 117.1, 114.0, 106.7, 96.7, 21.0; MS: m/z = 359 (M+1). Anal. calcd. for $C_{20}H_{14}N_4OS$: C, 67.02; H, 3.94; N, 15.63. Found: C, 67.06; H, 3.91; N, 15.59 %.

2.2.6. 6-(naphthalen-1-yl)-2-oxo-4-(3-phenyl-1H-pyrazol-4-yl)-1,2-dihydropyridine-3-carbonitrile (4f)

Yield 58 %; M.p. >300 ^{o}C; IR (KBr, υ_{max} cm^{-1}): 3121, 3088 (N-H), 2221 (C≡N), 1634 (C=O), 1594 (C=N); ^{13}C NMR: 162.1, 153.2, 150.3, 133.4, 131.1, 130.9, 130.2, 129.3, 128.8, 128.6, 128.0, 127.7, 126.9, 117.7, 114.2, 109.7, 100.05; MS: m/z = 389 (M+1). Anal. calcd. for $C_{25}H_{16}N_4O$: C, 77.30; H, 4.15; N, 14.42. Found: C, 77.26; H, 4.12; N, 14.38 %.

2.2.7. 4-[3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-6-(naphthalen-1-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (4g)

Yield 60 %; M.p. >300 ^{o}C; IR (KBr, υ_{max} cm^{-1}): 3217, 3088 (N-H), 2220 (C≡N), 1638 (C=O), 1586 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.55 (bs,1H, pyrazole-NH), 12.65 (s, 1H, pyridone ring NH), 8.15 (s, 1H, pyrazole-5H), 7.02-8.05 (m, 11H, Ar-H), 6.0 (s, 1H, pyridone-5H) 3.79 (s, 3H, -OCH_3). MS: m/z = 419 (M+1). Anal. calcd. for $C_{26}H_{18}N_4O_2$: C, 74.63; H, 4.34; N, 13.39. Found: C, 74.59; H, 4.31; N, 13.34 %.

2.2.8. 4-[3-(4-fluorophenyl)-1H-pyrazol-4-yl]-6-(naphthalen-1-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (4h)

Yield 57 %; M.p. >300 ^{o}C; IR (KBr, υ_{max} cm^{-1}): 3291, 3066 (N-H), 2211 (C≡N), 1657 (C=O), 1599 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.56 (bs,1H, pyrazole-NH), 12.78 (s, 1H, pyridone ring NH), 8.36 (s, 1H, pyrazole-5H), 7.31-8.06 (m, 11H, Ar-H), 6.02 (s, 1H, pyridone-5H). ^{13}C NMR: 163.8, 162.0, 153.1, 150.5, 133.4, 130.8, 130.3, 128.9, 128.0, 127.5, 126.9, 125.5, 125.0, 117.1, 116.3, 116.1, 114.3, 109.6; MS: m/z = 407 (M+1). Anal. calcd. for $C_{25}H_{15}FN_4O$: C, 73.88; H, 3.72; N, 13.79. Found: C, 73.81; H, 3.65; N, 13.74 %.

2.2.9. 4-[3-(4-chlorophenyl)-1H-pyrazol-4-yl]-6-(naphthalen-1-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (4i)

Yield 50 %; M.p. >300 ^{o}C; IR (KBr, υ_{max} cm^{-1}): 3217, 3058 (N-H), 2207 (C≡N), 1655 (C=O), 1600 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.53 (bs,1H, pyrazole-NH), 12.76 (s, 1H, pyridone ring NH), 8.26 (s, 1H, pyrazole-5H), 7.47-8.06 (m, 11H, Ar-H), 6.03 (s, 1H, pyridone-5H). ^{13}C NMR: 162.1, 152.9, 150.6, 133.4, 130.9, 130.3, 129.3, 128.9, 128.0, 127.5, 126.9, 125.6, 125.0, 117.1, 114.5, 109.7, 100.1; MS: m/z = 423 (M+1). Anal. calcd. for $C_{25}H_{15}ClN_4O$: C, 71.01; H, 3.58; N, 13.25. Found: C, 71.05; H, 3.53; N, 13.20 %.

2.2.10. 4-[3-(2,4-dichlorophenyl)-1H-pyrazol-4-yl]-6-(naphthalen-1-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (4j)

Yield 48 %; M.p. >300 ^{o}C; IR (KBr, υ_{max} cm^{-1}): 3145, 3058 (N-H), 2215 (C≡N), 1647 (C=O), 1592 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.55 (bs,1H, pyrazole-NH), 12.66 (s, 1H, pyridone ring NH), 8.40 (s, 1H, pyrazole-5H), 7.44-8.06 (m, 11H, Ar-H), 5.87 (s, 1H, pyridone-5H). MS: m/z

= 457 (M^+). Anal. calcd. for $C_{25}H_{14}Cl_2N_4O$: C, 65.66; H, 3.09; N, 12.25. Found: C, 65.61; H, 3.01; N, 12.19 %.

2.2.11. 4-[3-(4-methylphenyl)-1H-pyrazol-4-yl]-6-(naphthalen-1-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (4k)

Yield 56 %; M.p. >300 ^oC; IR (KBr, υ_{max} cm^{-1}): 3215, 3069 (N-H), 2221 (C≡N), 1653 (C=O), 1612 (C=N); MS: m/z = 403 (M+1). Anal. calcd. for $C_{26}H_{18}N_4O$: C, 77.59; H, 4.51; N, 13.92. Found: C, 77.53; H, 4.45; N, 13.88 %.

2.2.12. 6-(5-chlorothiophen-2-yl)-2-oxo-4-(3-phenyl-1H-pyrazol-4-yl)-1,2-dihydropyridine-3-carbonitrile (4l)

Yield 45 %; M.p. >300 ^oC; IR (KBr, υ_{max} cm^{-1}): 3217, 3088 (N-H), 2210 (C≡N), 1646 (C=O), 1602 (C=N); ^1H NMR (DMSO-d_6): ^1H NMR (DMSO-d_6): δ 13.52 (bs,1H, pyrazole-NH), 12.72 (s, 1H, pyridone ring NH), 8.26 (s, 1H, pyrazole-5H), 7.23-7.98 (m, 7H, Ar-H), 6.24 (s, 1H, pyridone-5H). MS: m/z = 379 (M+1). Anal. calcd. for $C_{19}H_{11}ClN_4OS$: C, 60.24; H, 2.93; N, 14.79. Found: C, 60.21; H, 2.97; N, 14.74 %.

2.2.13. 6-(5-chlorothiophen-2-yl)-4-[3-(4-fluorophenyl)-1H-pyrazol-4-yl]-2-oxo-1,2-dihydropyridine -3-carbonitrile (4m)

Yield 48 %; M.p. >300 ^oC; IR (KBr, υ_{max} cm^{-1}): 3224, 3089 (N-H), 2213 (C≡N), 1646 (C=O), 1602 (C=N); ^1H NMR (DMSO-d_6): ^1H NMR (DMSO-d_6): δ 13.55 (bs,1H, pyrazole-NH), 12.72 (s, 1H, pyridone ring NH), 8.18 (s, 1H, pyrazole-5H), 7.18-7.87 (m, 6H, Ar-H), 6.47 (s, 1H, pyridone-5H). MS: m/z = 397 (M+1). Anal. calcd. for $C_{19}H_{10}ClFN_4OS$: C, 57.51; H, 2.54; N, 14.12. Found: C, 57.47; H, 2.50; N, 14.08 %.

2.2.14. 4-[3-(4-chlorophenyl)-1H-pyrazol-4-yl]-6-(5-chlorothiophen-2-yl)-2-oxo-1,2-dihydropyridine -3-carbonitrile (4n)

Yield 51 %; M.p. >300 ^oC; IR (KBr, υ_{max} cm^{-1}): 3254, 3085 (N-H), 2212 (C≡N), 1630 (C=O), 1583 (C=N); ^1H NMR (DMSO-d_6): ^1H NMR (DMSO-d_6): δ 13.53 (bs,1H, pyrazole-NH), 12.72 (s, 1H, pyridone ring NH), 8.18 (s, 1H, pyrazole-5H), 7.09-7.75 (m, 6H, Ar-H), 6.24 (s, 1H, pyridone-5H). MS: m/z = 412 (M^+). Anal. calcd. for $C_{19}H_{10}Cl_2N_4OS$: C, 55.22; H, 2.44; N, 13.56. Found: C, 55.17; H, 2.39; N, 13.50 %.

2.2.15. 6-(5-chlorothiophen-2-yl)-4-[3-(4-methylphenyl)-1H-pyrazol-4-yl]-2-oxo-1,2-dihydropyri - dine-3-carbonitrile (4o)

Yield 53 %; M.p. >300 ^oC; IR (KBr, υ_{max} cm^{-1}): 3257, 3083 (N-H), 2213 (C≡N), 1627 (C=O), 1585 (C=N); ^1H NMR (DMSO-d_6): ^1H NMR (DMSO-d_6): δ 13.55 (bs,1H, pyrazole-NH), 12.66 (s, 1H, pyridone ring NH), 8.07 (s, 1H, pyrazole-5H), 7.21-7.66 (m, 6H, Ar-H), 6.72 (s, 1H, pyridone-5H) 2.32 (s, 3H, -CH_3). MS: m/z = 393 (M+1). Anal. calcd. for $C_{20}H_{13}ClN_4OS$: C, 61.14; H, 3.34; N, 14.26. Found: C, 61.11; H, 3.29; N, 14.20 %.

2.3.Antimicrobial activity

The following bacteria and fungi were used for the experiment. Bacteria: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853. All bacterial strains were maintained on nutrient agar medium at ±37°C. Fungi: *Aspergillus flavus, Chrysosporium keratinophilum* and *Candida albicans MTCC 227* are used in this study. These

cultures are obtained from the Department of Microbiology, Kuvempu University, Shimoga, India. All fungi strains were maintained on potato dextrose agar (PDA) at ±25 °C.

Table 1. Antibacterial activity of compounds 4a-o (*Zone of inhibition in mm*).

Compound code	*Escherichia coli*		*Staphylococcus Aureus*		*Pseudomonas Aeruginosa*	
Conc. in µg/ml	1000	500	1000	500	1000	500
4a	00	00	00	00	00	00
4b	07±0.1	05±0.2	08±0.2	06±0.2	06±0.2	04±0.1
4c	09±0.2	06±0.2	06±0.1	04±0.2	07±0.2	05±0.2
4d	08±0.2	06±0.2	09±0.2	07±0.1	07±0.2	05±0.2
4e	00	00	00	00	00	00
4f	00	00	00	00	00	00
4g	06±0.2	04±0.1	07±0.1	05±0.1	08±0.2	04±0.2
4h	00	00	00	00	00	00
4i	03±0.2	01±0.1	05±0.1	03±0.1	04±0.2	02±0.2
4j	04±0.2	01±0.1	02±0.1	00	03±0.2	01±0.2
4k	00	00	00	00	00	00
4l	00	00	00	00	00	00
4m	07±0.1	05±0.2	08±0.2	06±0.2	06±0.2	04±0.1
4n	06±0.2	04±0.1	07±0.1	05±0.1	08±0.2	04±0.2
4o	06±0.1	04±0.2	07±0.3	04±0.2	09±0.2	07±0.1
Streptomycin (Std.)	16±0.2	10±0.1	15±0.2	10±0.2	16±0.2	13±0.2

2.3.1. Antibacterial activity

The antibacterial activity of newly synthesized compounds (**4a-o**) were determined by well plate method in Mueller-Hinton Agar [23,24]. The compounds were tested against a panel of pathogenic microorganisms, including *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Microorganism strains were maintained on nutrient agar medium at 37°C. The cultures were inoculated in fresh 10 mL Nutrient Broth to yield an initial suspension of approximately 10–100 cfu/mL. All broths were then incubated statically at the aforementioned temperatures for microorganisms, for 18-24 h so that all cells were in the stationary phase. Susceptibility of the test organism to the compounds was determined by employing in the well plate technique. The bacterial suspensions were diluted tenfold in distilled water, and 0.1 mL from the appropriate dilution was spread plated on nutrient agar in order to give a population of approximately 10^6 cfu/plate. The wells were dug in each Petri plate by sterilized cork borer. The compounds were dissolved in DMSO and appropriate dilutions were made (1mg/mL and 0.5mg/mL). The same procedure was repeated for different micro-organisms. Each experiment was carried out in triplicate. After the inoculation of organism and compound, the Petri plates were incubated for 24 hrs at 37^oC. After the incubation, the inhibition zone was measured and the values for Dimethylsulphoxide (DMSO) were subtracted to get the actual values. Streptomycin was used as standard drug.

Antifungal activity

The fungal strains used in this study were *Aspergillus flavus*, *Chrysosporium Keratinophilum* and *Candida albicans*. The required amounts of each fungal strain were removed from the stock and suspended in 5mL of distilled water with 2 drops of Tween 80. This suspension was uniformly spread on Petri plates containing Potato dextrose agar media using sterile swabs. After applying the samples into the wells formed by using the same technique for tests on bacteria, the plates were incubated at 25 °C for 3 days. The plates were then examined for the presence of

zones of inhibition and the results were recorded. Fluconazole was used as a standard drug [25, 26].

Table 2. Antifungal activity of compounds 4a-o (*Zone of inhibition in mm*).

Compound code	*Aspergillus Flavus*		*Chrysosporium Keratinophilum*		*Candida Albicans*	
Con in µg/ml	1000	500	1000	500	1000	500
4a	00	00	00	00	00	00
4b	05±0.1	03±0.1	04±0.2	03±0.1	05±0.1	02±0.1
4c	06±0.1	05±0.2	04±0.1	03±0.1	05±0.1	04±0.1
4d	03±0.2	01±0.1	04±0.1	03±0.1	04±0.1	03±0.1
4e	00	00	00	00	00	00
4f	00	00	00	00	00	00
4g	04±0.1	03±0.2	06±0.1	05±0.2	04±0.1	02±0.1
4h	00	00	00	00	00	00
4i	03±0.1	02±0.1	03±0.1	01±0.1	03±0.1	01±0.1
4j	04±0.1	02±0.1	05±0.1	02±0.1	04±0.1	02±0.1
4k	00	00	00	00	00	00
4l	00	00	00	00	00	00
4m	04±0.2	01±0.1	04±0.1	02±0.1	03±0.2	01±0.2
4n	00	00	00	00	00	00
4o	00	00	00	00	00	00
Fluconazole (Std.)	13±0.2	10±0.1	17±0.2	15±0.2	22±0.2	20±0.2

2.3.2. Acute toxicity and behavioral studies

All the experiments were carried out using male swiss albino mice (20-25g each). The animals had free access to food and water and they were housed under natural (12h each) light-dark cycle with access to standard pellet chow and water. The animals were acclimatized for 7 days to the laboratory conditions before performing the experiments. The experimental protocol was approved by the institutional animal ethics committee. In all experimental models, six animals were used in each group.

The acute oral toxicity study for the compounds **4b**, **4c**, **4d**, **4g**, **4m**, **4n** and **4o** was carried out by following the OECD guidelines No. 420. Swiss albino male mice weighing 25-30 g were used for the evaluation (OECD Guidelines 2008). Each group consisting of 6 male mice (overnight fasted) was kept in the colony cage at 25±2°C with 55% relative humidity and 12 h light/dark cycle was maintained. A specified fixed dose of 250, 500, 750, 1000, 1500, 2000, 3000 and 4000 mg/kg was selected and administered orally as a single dose as fine suspension prepared in saline using gum acacia powder. The acute toxic symptoms and the behavioral changes produced by the test compounds were observed continuously for 4 h and at 8 h, 12 h and 24 h onset of toxic symptoms and gross behavioral changes were also recorded [27].

RESULTS AND DISCUSSION

3.1. Chemistry

The starting material 3-substituted-1*H*-pyrazole-4-carbaldehydes **(1a-e)** were synthesized from corresponding acetophenones through multi-step reactions [28]. First step involves the reaction of various 4-substituted acetophenones with semicarbazide hydrochloride in presence of sodium acetate to form respective semicarbazones. Further these semicarbazones were subjected to Vilsmayer-Haack reaction to yield 3-substituted-1*H*-pyrazole-4-carbaldehydes **(1a-e)**. Further**,** 4,6-disubstituted-3-cyano-2-pyridone derivatives were synthesized via one-pot multicomponent

reaction of 3-substituted-1*H*-pyrazole-4-carbaldehydes **(1a-e)**, various acetyl compounds **(2a-c),** ethyl cyanoacetate **(3)** and ammonium acetate in ethanolic medium (**Scheme 1**). The structures of the desired compounds were characterized by IR, ^{1}HNMR, ^{13}C NMR, mass spectral and elemental analyses.

The IR spectrum of compound **4a** showed absorption bands at 3229 & 3090, 2211, 1635, 1594 cm^{-1} which corresponds to N-H, C≡N, C=O and C=N stretching respectively. Similarly, its ^{1}H NMR spectrum showed two singlets at δ 13.53 and 12.66 which were due to presence of pyrazole N-H and N-H of pyridone ring respectively. Further, the pyrazole-5H and aromatic proton on pyridone ring resonated at δ 8.32 and 6.23 which confirm the structure. The mass spectrum of **4a** showed molecular ion peak at m/z = 345 (M+1), which is in agreement with the molecular formula $C_{19}H_{12}N_4OS$.

3.2. Antimicrobial activity

The new compounds **4a-o** were tested for their antibacterial activity (*in vitro)* at a concentration of 1000 and 500 µg/mL against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and their activity was compared to a well-known commercial antibiotic, streptomycin. The results were recorded for each tested compound as the average diameter of inhibition zones of bacterial growth surrounding the well in millimetres. The newly synthesized compounds exhibited variable antibacterial activity against the above tested bacterial strains. The results indicated that among the tested compounds, **4b**, **4c**, **4d**, **4g**, **4m**, **4n** and **4o** showed good antibacterial activity towards all bacterial strains at concentrations of 1000 and 500 µg/mL when compared with standard drug. Rest of compounds showed fair or poor activity. Results of antibacterial studies have been presented in **Table 1**. All the synthesized compounds were also tested for its antifungal activity (*in vitro)* against *Aspergillus flavus*, *Chrysosporium keratinophilum* and *Candida albicans* by measuring its average zone of inhibition. Fluconazole was used as standard for antifungal activity. Among the synthesized, compound **4c** showed moderate activity against *Aspergillus Flavus* at concentrations of 1000 and 500 µg/mL compared to standard drug fluconazole **(Table 2)**.

The results of antimicrobial study reveals that presence of substituents on the phenyl ring attached to the 4-position of pyrazole ring plays important role. The enhanced activity of **4b**, **4c**, **4d**, **4g**, **4m**, **4n** and **4o** is due to the presence of groups like, Cl, F, CH_3 and OCH_3 attached to 4-position of phenyl rings of pyrazole ring. This is also supported by the previous reports [29]. However, in general, compounds containing a halogen substituents showed better antibacterial activity than the compounds with other substituents [30]. The absence of such pharmacophore on phenyl ring fails to exhibit both antibacterial as well as antifungal activity. From the antimicrobial results we can conclude that, synthesized compounds are specific antibacterial agents. A combination of two different heterocyclic systems namely pyrazole and 3-cyano-2-pyridone has enhanced the pharmacological effect and hence they are ideally suited for further modifications to obtain more efficacious antibacterial compounds.

The acute oral toxicity study for compounds **4b**, **4c**, **4d**, **4g**, **4m**, **4n** and **4o** was also carried out by following the OECD guidelines No. 420. The experimental studies revealed that the compounds were quite safe up to 2000 mg/kg and no deaths of animals were recorded. Further, no significant gross behavioral changes were observed in experimental animals except in the

3000 and 4000 mg/kg of all organic compounds, which showed depression on the first day and dead on second day.

CONCLUSION

In the present work, a series of novel 4,6-disubstituted-3-cyano-2-pyridone derivatives were synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR, mass and elemental analyses. All the compounds were screened for its antimicrobial activity. Antibacterial results indicated that the compounds **4b**, **4c**, **4d**, **4g**, **4m**, **4n** and **4o** showed good antibacterial activity towards all bacterial strains when compared to standard drug streptomycin. **4c** showed moderate antifungal activity whereas remaining compounds showed poor antifungal activity compared to other synthesized compounds. The acute oral toxicity study for compounds **4b**, **4c**, **4d**, **4g**, **4m**, **4n** and **4o** was also carried out. The experimental studies revealed that the compounds were quite safe up to 2000 mg/kg and no deaths of animals were recorded.

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REFERENCES

[1] G.D. Henry, *Tetrahedron*, **2004**, 60, 6043.
[2] M.C. Bagley, K. Chapaneri, D.W. Dale, X. Xiong, J. Bower, *J. Org. Chem.*, **2005**, 70, 1389.
[3] T.L. Gilchrist, *J. Chem. Soc., Perkin Trans.*, **2001**, 2491–2497.
[4] A.H. Li, S. Moro, N. Forsyth, N. Melman, X.-D. Ji, K.A. Jacobsen, *J. Med. Chem.*, **1999**, 42, 706.
[5] B. Vacher, B. Bonnand, F. Funes, N. Jubault, W. Koek, M.-B. Assie, C. Cosi, M. Kleven, *J. Med. Chem,*. **1999**, 42, 1648.
[6] J.K. Son, L.X. Zhao, A. Basnet, P. Thapa, R. Karki, Y. Na, Y. Jahng, T.C. Jeong, B.S. Jeong, C.S. Lee, E.S. Lee, *Eur. J. Med. Chem.*, **2008**, 43, 675.
[7] A.G. Amr, M.M. Abdulla, *Bioorg. Med. Chem.*, **2006**, 14, 4341.
[8] A.G. Hammam, N.A. Abdel Hafez, W.H. Midura, M.Z. Mikolajczyk, *Z. Naturforsch.*, **2000**, 55, 417.
[9] M. Abo-Ghalia, M.M.Z. Abdulla, A.E. Amr, *Z. Naturforsch.*, **2003**, 58, 903.
[10] P. Dorigo, R.M. Gaion, P. Beiiuco, D. Fraccarollo, I. Maragno, G. Bombieri, F. Benetollo, L. Mosti, F. Orsini, *J. Med. Chem.*, **1993**, 36, 2475.
[11] T.J. Sultivan, J.J. Truglio, M.E. Boyne, P. Novichenok, X. Zhang, C.F. Stratton, H.J. Li, T. Kaur, A. Amin, F. Johnson, R.A. Stayden, C. Kisker, P.J. Tonge, *ACS Chem. Biol*., **2007**, 1, 43.
[12] A.M. Gilbert, A. Failli, J. Shumsky, Y. Yang, A. Severin, G. Singh, W. Hu, D. Keeney, P.J. Petersen, A.H. Katz, *J. Med. Chem.* **2006**, 49, 6027.
[13] O. Prakash, R. Pundeer, P. Ranjan, K. Pannu, Y. Dhingra, K.R. Aneja, *Indian. J. Chem.*, **2009**, 48B, 563.
[14] A.M. Isloor, B. Kalluraya, P. Shetty, *Eur. J. Med. Chem.*, **2009**, 44, 3784.
[15] I.V. Magedov, M. Manpadi, S.V. Slambrouck, W.F.A. Steelant, E. Rozhkova, N.M. Przheval'skii, S. Rogelj, A. Kornienko, *J. Med. Chem.*, **2007**, 50, 5183.
[16] G. Szabo, J. Fischer, A. Kis-Varga, K. Gyires, *J. Med. Chem.*, **2008**, 51, 142.

[17] N. Benaamane, B. Nedjar-Kolli, Y. Bentarzi, L. Hammal, A. Geronikaki, P. Eleftheriou, A. Langunin, *Bioorg. Med. Chem*., **2008**, 16, 3059.
[18] Y.R. Prasad, A. Lakshmana Rao, L. Prasoona, K. Murali, K.P. Ravi, *Bioorg. Med. Chem. Lett.*, **2005**, 15, 5030.
[19] Z. Ozdemir, H.B. Kandilici, B. Gumusel, U. Calis, A.A. Bilgin, *Eur. J. Med. Chem*., **2007**, 42, 373.
[20] A. Sener, M.K. Sener, I. Bildmci, R. Kasimogullari, Y. Akcamur, *J. Heterocycl. Chem*., **2002**, 39, 869.
[21] G.A. Wachter, R.W. Hartmann, T. Sergejew, G.L. Grun, D. Ledergerber, *J. Med. Chem*., **1996**, 39, 834.
[22] A.M. Vijesh, A.M. Isloor, V, Prabhu, S, Ahmad, S, Malladi, *Eur. J. Med. Chem*., **2010**, 45, 5460.
[23] B.A. Arthington-Skaggs, M. Motley, D.W. Warnock, C.J. Morrison, *J. Clin. Microbiol*., **2000**, 38, 2254.
[24] L. Rocha, A. Marston, O. Potterat, M.A.C. Kaplan, H. Stoeckli-Evans, K. Hostettmann, *Phytochemistry*, **1995**, 40, 1447.
[25] D. J. Mac. Lowry, M. J. Jaqua, S. T. Selepak, *Appl. Microbiol*., **1970**, 20, 46.
[26] A. Portillo, R. Vila, B. Freixa, T. Adzet, S. Can-igueral, *J. Ethnopharmacol.*, **2001**, 76, 93.
[27] N. Segovia, G. Crovetto, P. Lardelli, M. Espigares, *J. Appl. Toxicol*., **2002**, 22, 353.
[28] A.V. Lebedev, A.B. Lebedeva, V.D. Sheludyakov, E.A. Kovaleva, O.L. Ustinova, I.B. Kozhevnikov, *Russ. J. Gen. Chem*., **2005**, 75, 782.
[29] B. P. Bandgar, S. S. Gawande, R. G. Bodade , N. M. Gawande, C. N. Khobragade, *Bioorg. Med. Chem.*, **2009**, 17, 8168.
[30] P. K. Sharma, N. Chandak, P. Kumar, C. Sharma, K. R. Aneja, *Eur. J. Med. Chem*., **2011**, 46, 1425.