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Synthesis of diverse pyrano[2,3-c]pyrazoles derivatives as potential antimicrobial agents

[a]Ranmal A. Varu, [b]Kailash S. Pancholi and [c]Denish C. Karia*

[a]Bahauddin Science College, Junagadh, Gujarat
[b]H. & H. B. Kotak Science College, Rajkot, Gujarat
[c]Patel J. D. K. Davolwala Science College, Borsad (Anand), Gujarat

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ABSTRACT

Synthesis of highly diverse pyrano[2,3-c]pyrazoles was achieved by one pot multicomponent reaction using piperidine as catalyst. The structures of synthesized derivatives were elucidated by various spectrometric techniques like FT-IR, Mass, and ^{1}H NMR spectroscopy. The synthesized derivatives were subjected to various antimicrobial activities like antibacterial and antifungal.

Keywords: Pyrano[2,3-*c*]pyrazoles, ^{1}H NMR spectroscopy, Antimicrobial activities, Multicomponent reaction.

INTRODUCTION

Pyrano[2,3-c]pyrazoles are associated with various biologicalactivities. These compounds are known as different biologically activesubstances, including antibiotics [1-3] enzyme inhibitors [4-6] antifungal [6] andanticancer [2] drugs. In this regard, the development of simple and effectivemethods of obtaining functionally substituted pyrano[2,3-c]pyrazoles is animportant task.

Though methods of pyrano[2,3-c]pyrazoles synthesis have long beendocumented, so far, all of them consist of two main groups: (1) two-step synthesisand (2) “one-pot” multicomponent condensation This way of synthesis includes Knoevenagelcondensation of aromatic aldehyde and malononitrile, Michael reaction offormed product with 3-methyl-2-pyrazolin-5-one and final cyclization ofKnoevenagel-Michael adduct to appropriate pyrano[2,3-c]pyrazole[8].

Prompted by this finding we approached a simple and efficient synthesis of pyrano[2,3-*c*]pyrazoles. The synthesized compounds were subjected to antimicrobial activities, and some of the compounds exhibited microbial species to a significant extent.

MATERIALS AND METHODS

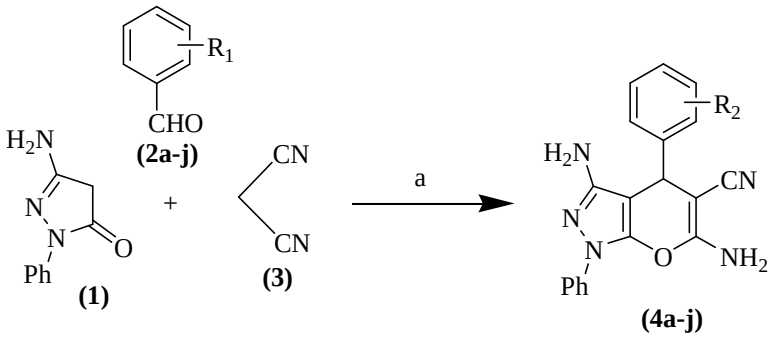
Experimental

Melting points were determined in open glass capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. IR spectra were recorded Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreements with the structures assigned.

General procedure for the 3,6-diamino-4-(aryl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (4a-j)

A mixture of the malononitrile (0.01 mol), 1-phenyl-3-amino-1*H*-pyrazol-5(4*H*)-one (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) in 8-10 mL of MeOH with catalytic amount of piperidine were refluxed for 10-12 h.After completion of the reaction, the reaction mixture was filtered to give the solid products **(4a-j)**, which were recrystallized from ethanol.

Reaction Scheme



Reagents and conditions: (a) Piperidine, MeOH, Reflux

Table 1: Physical data for pyrano[2,3-*c*]pyrazoles (4a-j)

Code	R_1	R_2	M.F.	M.W.	M.P. °C	Yield %
4a	C_6H_5	H	$C_{19}H_{15}N_5O$	329	157-159	75
4b	C_6H_5	4-F	$C_{19}H_{14}FN_5O$	347	162-164	79
4c	C_6H_5	4-Cl	$C_{19}H_{14}ClN_5O$	363	142-144	83
4d	C_6H_5	4-Br	$C_{19}H_{14}BrN_5O$	407	200-202	67
4e	C_6H_5	4-NO_2	$C_{19}H_{14}N_6O_3$	374	187-189	79
4f	C_6H_5	4-CH_3	$C_{20}H_{17}N_5O$	343	135-137	85
4g	C_6H_5	4-OH	$C_{19}H_{15}N_5O_2$	345	217-219	71
4h	C_6H_5	4-OCH_3	$C_{20}H_{17}N_5O_2$	359	196-198	77
4i	C_6H_5	3,4-OCH_3	$C_{21}H_{19}N_5O_3$	389	208-210	73
4j	C_6H_5	3-Cl	$C_{19}H_{14}ClN_5O$	363	233-235	69

3,6-diamino-1,4-dihydro-1,4-diphenylpyrano[2,3-c]pyrazole-5-carbonitrile (4a)

IR (cm^{-1}): 3448 (N-H stretching of free primary amine), 3321 (N-H stretching of pyrazolo ring), 3057 (C-H stretching of aromatic ring), 2196 (C≡N stretching of the nitrile group), 1660 (C=N stretching of pyrazolo ring), 1589 (N-H deformation pyrazolo ring), 1329 (C-N stretching of pyrazolo ring), 1176 (N-N deformation of pyrazolo ring), 1016 (C-H in plane bending of aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 4.62 (s, 1H), 6.48 (s, 2H), 6.88 (s, 2H), 7.23-7.34 (m, 6H), 7.43-7.47 (t, 2H), 7.79-7.81 (d, 2H); MS: *m/z* 329; Anal. Calcd. for $C_{19}H_{15}N_5O$: C, 69.29; H, 4.59; N, 21.26. Found: C, 69.15; H, 4.42; N, 21.02%.

3,6-diamino-4-(4-fluorophenyl)-1,4-dihydro-1-phenylpyrano[2,3-c]pyrazole-5-carbonitrile(4b)

IR (cm^{-1}): 3454 (N-H stretching of free primary amine), 3338 (N-H stretching of pyrazolo ring), 2995 (C-H stretching of aromatic ring), 2193 (C≡N stretching of the nitrile group), 1654 (C=N stretching of pyrazolo ring), 1591 (N-H deformation pyrazolo ring), 1388 (C-N stretching of pyrazolo ring), 1186 (N-N deformation of pyrazolo ring), 1070 (C-H in plane bending of aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 4.66 (s, 1H), 6.90 (s, 2H), 7.10 (s, 2H), 7.25-7.30 (m, 3H), 7.41-7.48 (m, 4H), 7.80-7.82 (d, 2H); MS: *m/z* 347; Anal. Calcd. for $C_{19}H_{14}FN_5O$: C, 65.70; H, 4.06; N, 20.16. Found: C, 65.52; H, 3.91; N, 20.01%.

3,6-diamino-4-(4-chlorophenyl)-1,4-dihydro-1-phenylpyrano[2,3-c]pyrazole-5-carbonitrile (4c)

IR (cm^{-1}): 3450 (N-H stretching of free primary amine), 3340 (N-H stretching of pyrazolo ring), 2990 (C-H stretching of aromatic ring), 2195 (C≡N stretching of the nitrile group), 1653 (C=N stretching of pyrazolo ring), 1590 (N-H deformation pyrazolo ring), 1390 (C-N stretching of pyrazolo ring), 1180 (N-N deformation of pyrazolo ring), 1071 (C-H in plane bending of aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 4.67 (s, 1H), 6.92 (s, 2H), 7.15 (s, 2H), 7.27-7.32 (m, 3H), 7.40-7.47 (m, 4H), 7.82-7.84 (d, 2H); MS: *m/z* 363; Anal. Calcd. for $C_{19}H_{14}ClN_5O$: C, 62.73; H, 3.88; N, 19.25. Found: C, 62.58; H, 3.73; N, 19.09%.

3,6-diamino-4-(4-bromophenyl)-1,4-dihydro-1-phenylpyrano[2,3-c]pyrazole-5-carbonitrile (4d)

IR (cm^{-1}): 3455 (N-H stretching of free primary amine), 3341 (N-H stretching of pyrazolo ring), 2992 (C-H stretching of aromatic ring), 2190 (C≡N stretching of the nitrile group), 1650 (C=N stretching of pyrazolo ring), 1592 (N-H deformation pyrazolo ring), 1390 (C-N stretching of pyrazolo ring), 1180 (N-N deformation of pyrazolo ring), 1071 (C-H in plane bending of aromatic ring);^{1}H NMR (DMSO-d_6) δ ppm: 4.65 (s, 1H), 6.94 (s, 2H), 7.16 (s,

2H), 7.20-7.25 (m, 3H), 7.45-7.52 (m, 4H), 7.85-7.87 (d, 2H); MS: *m/z* 407; Anal. Calcd. for $C_{19}H_{14}BrN_5O$: C, 55.90; H, 3.46; N, 17.15. Found: C, 55.78; H, 3.29; N, 17.01%.

3,6-diamino-1,4-dihydro-4-(4-nitrophenyl)-1-phenylpyrano[2,3-c]pyrazole-5-carbonitrile (4e)
IR (cm^{-1}): 3452 (N-H stretching of free primary amine), 3346 (N-H stretching of pyrazolo ring), 3012 (C-H stretching of aromatic ring), 2191 (C≡N stretching of the nitrile group), 1657 (C=N stretching of pyrazolo ring), 1591 (N-H deformation pyrazolo ring), 1395 (C-N stretching of pyrazolo ring), 1180 (N-N deformation of pyrazolo ring), 1074 (C-H in plane bending of aromatic ring);^{1}H NMR (DMSO-d_6) δ ppm: 4.64 (s, 1H), 6.89 (s, 2H), 7.12 (s, 2H), 7.29-7.34 (m, 3H), 7.46-7.53 (m, 4H), 7.84-7.86 (d, 2H); MS: *m/z* 374; Anal. Calcd. for $C_{19}H_{13}N_6O_3$: C, 60.96; H, 3.77; N, 22.45. Found: C, 60.81; H, 3.61; N, 22.31%.

3,6-diamino-1,4-dihydro-1-phenyl-4-p-tolylpyrano[2,3-c]pyrazole-5-carbonitrile (4f)
IR (cm^{-1}): 3457 (N-H stretching of free primary amine), 3349 (N-H stretching of pyrazolo ring), 3019 (C-H stretching of aromatic ring), 2195 (C≡N stretching of the nitrile group), 1657 (C=N stretching of pyrazolo ring), 1591 (N-H deformation pyrazolo ring), 1390 (C-N stretching of pyrazolo ring), 1185 (N-N deformation of pyrazolo ring), 1074 (C-H in plane bending of aromatic ring);^{1}H NMR (DMSO-d_6) δ ppm: 2.33 (S, 3H), 4.60 (s, 1H), 6.87 (s, 2H), 7.14 (s, 2H), 7.24-7.29 (m, 3H), 7.43-7.50 (m, 4H), 7.86-7.92 (d, 2H); MS: *m/z* 343; Anal. Calcd. for $C_{20}H_{17}N_5O$: C, 69.96; H, 4.99; N, 20.40. Found: C, 69.79; H, 4.86; N, 20.28%.

3,6-diamino-1,4-dihydro-4-(4-hydroxyphenyl)-1-phenylpyrano[2,3-c]pyrazole-5-carbonitrile (4g)
IR (cm^{-1}): 3453 (N-H stretching of free primary amine), 3348 (N-H stretching of pyrazolo ring), 3023 (C-H stretching of aromatic ring), 2196 (C≡N stretching of the nitrile group), 1651 (C=N stretching of pyrazolo ring), 1590 (N-H deformation pyrazolo ring), 1392 (C-N stretching of pyrazolo ring), 1188 (N-N deformation of pyrazolo ring), 1072 (C-H in plane bending of aromatic ring);^{1}H NMR (DMSO-d_6) δ ppm: 3.56 (S, 1H), 4.70 (s, 1H), 6.95 (s, 2H), 7.15 (s, 2H), 7.30-7.35 (m, 3H), 7.46-7.51 (m, 4H), 7.84-7.86 (d, 2H); MS: *m/z* 345; Anal. Calcd. for $C_{19}H_{15}N_5O_2$: C, 66.08; H, 4.38; N, 20.28. Found: C, 65.91; H, 4.21; N, 20.16%.

3,6-diamino-1,4-dihydro-4-(4-methoxyphenyl)-1-phenylpyrano[2,3-c]pyrazole-5-carbonitrile (4h)
IR (cm^{-1}): 3449 (N-H stretching of free primary amine), 3340 (N-H stretching of pyrazolo ring), 3029 (C-H stretching of aromatic ring), 2194 (C≡N stretching of the nitrile group), 1657 (C=N stretching of pyrazolo ring), 1590 (N-H deformation pyrazolo ring), 1391 (C-N stretching of pyrazolo ring), 1189 (N-N deformation of pyrazolo ring), 1071 (C-H in plane bending of aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 4.00 (S, 3H), 4.69 (s, 1H), 6.94 (s, 2H), 7.12 (s, 2H), 7.27-7.32 (m, 3H), 7.44-7.51 (m, 4H), 7.87-7.93 (d, 2H); MS: *m/z* 359; Anal. Calcd. for $C_{20}H_{17}N_5O_2$: C, 66.84; H, 4.77; N, 19.49. Found: C, 66.64; H, 4.60; N, 19.32%.

3,6-diamino-1,4-dihydro-4-(3,4-dimethoxyphenyl)-1-phenylpyrano[2,3-c]pyrazole-5-carbonitrile (4i)
IR (cm^{-1}): 3447 (N-H stretching of free primary amine), 3342 (N-H stretching of pyrazolo ring), 3039 (C-H stretching of aromatic ring), 2191 (C≡N stretching of the nitrile group), 1655 (C=N stretching of pyrazolo ring), 1591 (N-H deformation pyrazolo ring), 13961 (C-N stretching of pyrazolo ring), 1189 (N-N deformation of pyrazolo ring), 1071 (C-H in plane bending of aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 3.59 (S, 3H), 4.00 (S, 3H), 4.69 (s, 1H), 6.87 (s, 2H), 7.17 (s, 2H), 7.22-7.28 (m, 2H), 7.46-7.54 (m, 4H), 7.88-7.97 (d, 2H); MS: *m/z* 389; Anal. Calcd. for $C_{21}H_{19}N_5O_3$: C, 64.77; H, 4.92; N, 17.98. Found: C, 64.56; H, 4.78; N, 17.76 %.

3,6-diamino-4-(3-chlorophenyl)-1,4-dihydro-1-phenylpyrano[2,3-c]pyrazole-5-carbonitrile (4j)
IR (cm^{-1}): 3450 (N-H stretching of free primary amine), 3340 (N-H stretching of pyrazolo ring), 2990 (C-H stretching of aromatic ring), 2195 (C≡N stretching of the nitrile group), 1653 (C=N stretching of pyrazolo ring), 1590 (N-H deformation pyrazolo ring), 1390 (C-N stretching of pyrazolo ring), 1180 (N-N deformation of pyrazolo ring), 1071 (C-H in plane bending of aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 4.61 (s, 1H), 6.85 (s, 2H), 7.14 (s, 2H), 7.29-7.34 (m, 3H), 7.42-7.47 (m, 4H), 7.90-7.97 (d, 2H); MS: *m/z* 363; Anal. Calcd. for $C_{19}H_{14}ClN_5O$: C, 62.73; H, 3.88; N, 19.25. Found: C, 62.57; H, 3.72; N, 19.07%.

Antimicrobial evaluation
All the synthesized compounds (4a-j) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [9, 10] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and greseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using microdilution broth method according to NCCLS standards. Serial dilutions of the test compounds and reference drugs were prepared in Muellere-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Muellere-Hinton agar were performed to obtain the required concentrations. In primary screening 1000 μg mL^{-1}, 500 μg mL^{-1} and 250 μg mL^{-1} concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution at 200 μg mL^{-1}, 100 μg mL^{-1}, 50 μg mL^{-1}, 25 μg mL^{-1}, 12.5 μg mL^{-1}, and 6.25 μg mL^{-1} concentration against all microorganisms. The tubes were inoculated with 10^8 cfu mL^{-1} (colony forming unit/mL) and incubated at 37 ºC for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied.

The results obtained from antimicrobial susceptibility testing are depicted in Table 2.

Table 2. Antibacterial and antifungal activity of synthesized compounds (4a-j)

Code	Minimum inhibition concentration (μg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S.a.	***S. p.***	***E.c.***	***P.a.***	***C. a.***	***A. n.***	***A.c.***
4a	200	500	250	500	1000	500	>1000
4b	50	75	62.5	62.5	100	50	50
4c	62.5	50	75	50	250	>1000	>1000
4d	75	62.5	100	500	50	100	500
4e	200	100	100	500	500	1000	200
4f	250	250	250	250	500	500	1000
4g	100	500	500	1000	250	500	500
4h	500	100	500	100	500	500	>1000
4i	250	500	500	500	200	500	200
4j	500	250	500	500	1000	1000	1000
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Ciprofloxacin	50	50	25	25	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Greseofulvin	-	-	-	-	500	100	100

RESULTS AND DISCUSSION

For pyrano[2,3-*c*]pyrazoles**(4a-j)**, confirmatory bands for primary amine (-NH_2) and nitrile (C≡N) stretching band was observed at 3400-3500 cm^{-1} and 2190-2220 cm^{-1} respectively. Another characteristic band for N-H deformation was observed at 1580-1620 cm^{-1}, which suggested the formation of pyranopyrazoles ring system.. ^{1}H NMR spectra confirmed the structures of pyrano[2,3-*c*]pyrazoles**(4a-j)** on the basis of following signals: singlet for primary amino groups proton was observed at 5.91-6.62 δ ppm and a singlet for the methine proton of pyran ring at 4.52-4.64 δ ppm. The aromatic ring protons and *J* value were found to be in accordance with substitution pattern on phenyl ring.
On evaluation of biological screening data, it can be observed that biological activity is largely affected by the substitution on phenyl ring. The compounds **4b, 4c,** and **4e**exhibited significant antibacterial and antifungal activity as compare to standard drugs. It can be observed that electron donating group at p-position of the phenyl ring increases the biological activity whereas in rest of the compound the activity was much limited as compare to standard drugs.

CONCLUSION

In this paper, we have synthesized diverse pyrano[2,3-*c*]pyrazole derivatives with potential antimicrobial activities. The observed activities follow a specific pattern according to substitution on phenyl ring. Thus the study contributes a lot to structure activity relationship of the synthesized pyrano[2,3-*c*]pyrazole derivatives.

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