SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF NEW PYRAZOLE AND PYRAZOLINE-5-ONE DERIVATIVES

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ABSTRACT

A series of N'-(4-hydroxybenzoyl)-3-methyl-4-(4'-substituted arylhydrazono)pyrazoline-5-ones, N'-(2-hydroxybenzoyl)-3-methyl-5-phenyl-4-(2'-substituted arylazo)pyrazoles and N'-(p-toluenesulphonyl)-3,5-dimethyl-4-(4'-substituted arylazo)pyrazoles have been synthesized and characterized by chemical analysis, IR and ^{1}H-NMR spectral data. The compounds have been screened for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

KEY WORDS: Antibacterial activity, pyrazoles and pyrazoline-5-ones.

1. INTRODUCTION

Pyrazole and pyrazoline-5-ones represent very important classes of heterocyclic compounds possessing a wide spectrum of biological activities (Etman , 1990; Klemn , 1976; Anderson , 1964). Besides, the traditional interest in pyrazoline derivatives which have been the basis of numerous dyes and drugs a number of substituted pyrazoles are used as antidiabetic and antineoplastic agents (Harman , 1968; Garg and Prakash, 1971). Also incorporation of hydrazono (Ross and Warweek, 1961)/azo (Wiley and Clovenger, 1962; Messarans , 1971) group enhances the biological activity of heterocycles. In view of the biological acitivities displayed by arylazo pyrazoles (Rajeev , 2000) and arylhydrazono pyrazoline-5-ones (Amir and Rajesh, 1997), various pyrazoles and pyrazoline-5-one derivatives have been synthesized and screened for their antimicrobial activity.

2. MATERIALS AND METHODS

Melting points were taken in open capillaries and are uncorrected. The purity of the synthesized compounds was checked on silica gel-G TLC plates. FT-IR spectra (KBr) were recorded on a JASCO-FT-IR-5300 spectrophotometer. ^{1}H NMR spectra ($CDCl_3$) were recorded on a AVANCE spectrophotometer (400 MHz) using TMS as an internal standard. All the compounds gave satisfactory C, H, N analyses.

Antimicrobial activity

All the synthesized compounds were screened for antimicrobial activity against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E.coli* by agar diffusion method at a concentration 0.5 mg cm^{-3} using DMF as solvent. The zone of inhibition was measured in mm. Sulphamethaoxazole was used as the control.

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General methods for the synthesis

Synthesis of 2-(substituted arylhydrazono)-ethyl-2,3-dioxobutyrates (1a-e):

The starting substituted primary amine (0.01 mol) dissolved in a mixture of concentrated HCl (5 ml) and water (8 ml) was cooled to 0° C. Cold aqueous solution of sodium nitrite was then added to it and the diazonium salt so formed was filtered into a cooled solution of sodium acetate (8 g) and ethyl acetoacetate(0.01 mol) in ethanol (25 ml). The resulting solids were washed with water and crystallized from acetic acid to furnish 1a-e (65-75%): 1a, m.p. 184° C (Found: C, 62.15; H, 5.68; N, 11.47. $C_{12}H_{14}N_2O_3$ calcd. for: C, 61.53; H, 5.98; N, 11.96%); ν_{max} 3450 cm^{-1} (NH), 1690 cm^{-1} (C=O), 1520 cm^{-1} (NH-N=C); δ 1.40 (3H,t,CH_2CH_3), 2.73 (3H,s,$COCH_3$), 4.30 (2H,q,CH_2CH_3), 7.28 (5H,s,ArH), 14.71(1H,S,NH); 1b, m.p. 65-66° C; 1c, 178-179° C; 1d, 70-72° C; 1e, 119-120° C.

Synthesis of N'-(4-hydroxybenzoyl)-3-methyl-4-(4'-substituted arylhydrazono) pyrazoline-5-ones (2a-e):

Equimolar concentration of 4-hydroxybenzoic hydrazide and 2-(substituted arylhydrazono)-ethyl-2,3-dioxobutyrate(1a-e; 0.0075 mol) dissolved in methanol was refluxed for 6 h. The solid obtained on cooling was filtered and recrystallized from glacial acetic acid to yield 2a-e. The physical, chemical and spectral data of the compounds presented in Table 1 are consistent with the structure of the compounds. The steps involved in the synthesis of the compounds are presented in Scheme 1.

Synthesis of 1-methyl-3-phenyl-2-arylhydrazone-1,2,3-propanetriones (3a-e):

The starting substituted primary amine (0.01 mol) dissolved in a mixture of concentrated HCl (5 ml) and water (8 ml) was cooled to 0°C. Cold aqueous solution

of sodium nitrite was then added to it and the diazonium salt so formed was filtered into a cooled solution of sodium acetate (8 g) and benzoylacetone (0.01 mol) in ethanol (25 ml). The resulting solids were washed with water and crystallized from acetic acid to furnish 3a-e (58-74%): 3a, m.p. 89-91° C (Found: C, 71.8; H, 4.96; N, 10.29. $C_{16}H_{14}N_2O_2$ calcd. for: C,72.18; H, 5.26; N, 10.53%); ν_{max} 3230 cm^{-1}(NH), 1640 cm^{-1} (C=O), 1522cm^{-1} (NH-N=C); 750 cm^{-1} (substituted phenyl); δ 2.55(3H,s,$COCH_3$), 7.15-8.20(9H,m,Arh); 13.34(1H,s,NH hydrogen bonded); 3b, m.p. 82-84° C; 3c, 124-126° C ; 3d, 86-87° C; 3e, 120-122° C.

Synthesis of N'-(2'-hydroxybenzoyl)-3-methyl-5-phenyl-4-(2'-substituted arylazo)pyrazoles (4a-e):

Equimolar concentration of salicylic hydrazide and 1-methyl-3-phenyl-2-arylhydra-zone-1,2,3-propanetrione (3a-e; 0.004 mol) were dissolved in methanol and the mixture was refluxed for 6 h. The solid obtained on cooling was filtered and recrystallized from glacial acetic acid to yield 4a-e. The physical, chemical and spectral data of the compounds presented in Table 1 are consistent with the structure of the compounds. The steps involved in the synthesis of the compounds are presented in Scheme 1.

Synthesis of 2,3,4-pentanetrione-3-substituted arylhydrazones (5a-e):

The starting substituted primary amine (0.01 mol) dissolved in a mixture of concentrated HCl (5 ml) and water (8 ml) was cooled to 0° C. Cold aqueous solution of sodium nitrite was then added to it and the diazonium salt so formed was filtered into a cooled solution of sodium acetate (8 g) and acetylacetone (0.01 mol) in ethanol (25 ml). The resulting solids were washed with water and crystallized from acetic acid to furnish 5a-e (56-74%): 5a, m.p. 92° C (Found: C, 63.64; H, 5.57; N, 13.24. $C_{11}H_{12}N_2O_2$ calcd. for: C, 64.70; H, 5.88; N, 13.72%); ν_{max} 3380 cm^{-1}(NH), 1678 cm^{-1} (C=O), 1591 cm^{-1} (NH-N=C); δ 2.41 (3H,s,CH_3), 2.53 (3H,s,CH_3), 7.26 (5H,s,ArH), 14.61(1H,s,NH); 5b, m.p. 98° C; 5c, 100-101° C; 5d, 202° C; 5e, 233° C.

Synthesis of N'-(p-toluene sulphonyl)-3,5-dimethyl-4-(substituted arylazo) pyrazoles (6a-e):

A mixture of p-toluenesulphonyl hydrazide (0.001 mol) and 2,3,4-pentanetrione-3-substituted arylhydrazone (5a-e; 0.001 mol) dissolved in methanol was refluxed for 6 h. The solids that separated on cooling were dried and crystallized from acetic acid to yield 6a-e. The physical, chemical and spectral data of the compounds presented in Table 1 are consistent with the structure of the compounds. The steps involved in the synthesis of the compounds are presented in Scheme 1.

3. RESULTS AND DISCUSSION

Antimicrobial activity

Antimicrobial activity of the compounds 2a-e, 4a-e and 6a-e was tested *in vitro* against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli* by cup plate technique (Barry , 1976; Cavangh , 1963) at a concentration of 0.5 mg cm^{-3}. Sulphamethaxazole (zone of inhibition 17-19 mm against the selected bacteria) was used as the control. The results are presented in Table 2. Compounds 2d and 2e exhibited moderate activity (zone of inhibition 5-6 mm) against both the organisms. Compound 2c exhibited feable activity (zone of inhibition 2-3 mm). Rest of the compounds, 2a and 2b were found to be inactive against both organisms. Compound 4e exhibited moderate activity (zone of inhibition 5-6 mm) against *S. aureus and E. coli,* 4d exhibited feable acitivity (zone of inhibition 2-3 mm). Rest of the compounds, 4a, 4b and 4c were found to be inactive. Compounds 6d and 6e exhibited maximum activity(zone of inhibition 6-9 mm) against *S. aureus and E. coli*, 6b and 6c exhibited feable activity (zone of inhibition 2-3 mm). 6a was found to be inactive.

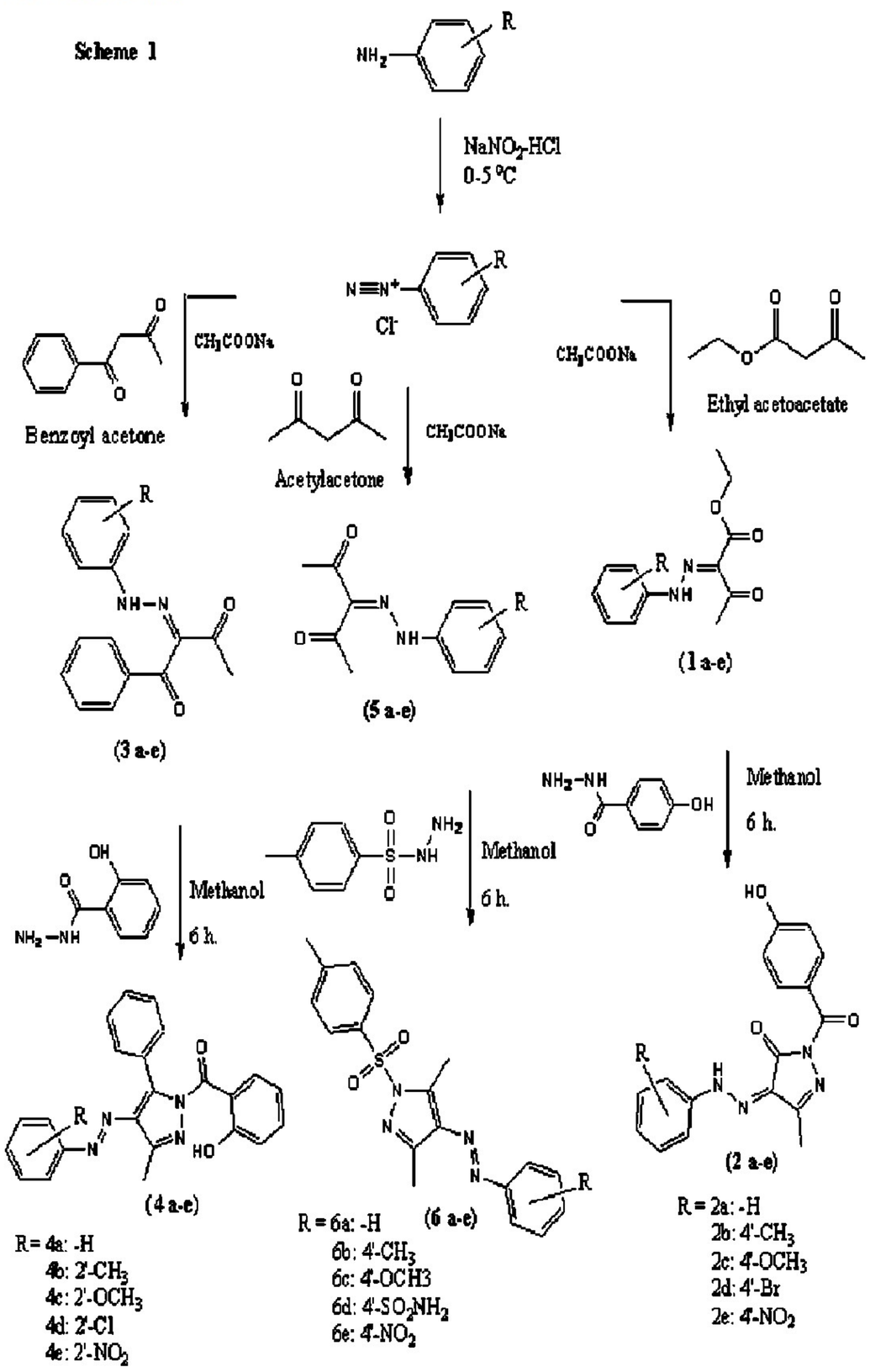

Table 1
Physical, Analytical and Spectral data of the compounds 2a-e, 4a-e and 6a-e

S.No.	R	m.p. °C	% yield	Colour	Molecular formula	Analytical data	IR (ν,cm^{-1})	^{1}H NMR (δ, ppm)
1	-H (2a)	206-208	61	Red	$C_{17}H_{14}N_4O_3$	Found: C,62.87; H,4.19 ; N,16.97. (Calculated for C,63.35; H,4.35; N,17.39%)	3255 (NH) 1657 (C=O) 1582 (C=C) 1522 (NHN=C)	2.30(3H,s,CH_3) 5.85(1H,s,OH) 6.80-7.80 (9H,m,ArH) 9.70(1H,br,NH)
2	4'-CH_3 (2b)	220-222	72	Yellow	$C_{18}H_{16}N_4O_3$	Found: C,63.75; H,4.57 ; N,16.45. (Calculated for C,64.29; H,4.76; N,16.67%)	3117 (NH) 1677 (C=O) 1603 (C=C) 1550(NHN=C)	2.30 (3H,s,CH_3) 2.33 (3H,s,Ar-CH_3) 5.82 (1H,s,OH) 6.82-7.85 (8H,m,ArH) 9.75 (1H,br,NH)
3	4'-OCH_3 (2c)	180-184	59	Dark brown	$C_{18}H_{17}N_4O_4$	Found: C,61.82; H,4.49; N,15.62. (Calculated for C,61.19; H,4.82; N,15.86%)	3215 (NH) 1662 (C=O) 1560 (C=C) 1525 (NHN=C)	2.32 (3H,s,CH_3) 3.86 (3H,s,Ar-OCH_3) 6.05 (1H,s,OH) 6.80-7.80 (8H,m,ArH) 9.71 (1H,br,NH)
4	4'-Br (2d)	204-206	70	Yellow	$C_{17}H_{14}N_4O_3Br$	Found: C,50.13; H,3.16; N,13.64. (Calculated for C,50.75 ; H,3.48; N,13.93%)	3210 (NH) 1672 (C=O) 1580 (C=C) 1540 (NHN=C)	2.31 (3H,s,CH_3) 6.14 (1H,s,OH) 6.90-7.80 (8H,m,ArH) 9.70 (1H,br,NH)
5	4'- NO_2 (2e)	296-298	68	Pale yellow	$C_{17}H_{14}N_5O_5$	Found: C,54.97; H,3.51; N,18.74. (Calculated for C,55.43; H,3.80; N,19.02%)	3240 (NH) 1655 (C=O) 1582 (C=C) 1542 (NHN=C)	2.31 (3H,s,CH_3) 6.12 (1H,s,OH) 6.80-7.90 (8H,m,ArH) 9.74 (1H,br,NH)
6	-H (4a)	152-154	58	Brown	$C_{23}H_{18}N_4O_2$	Found: C,71.84; H,4.53; N,14.25. (Calculated for C,72.25 ; H,4.71; N,14.66%)	3417 (OH) 1637 (C=O) 1603 (C=N) 1487 (N=N) 751 (substituted phenyl)	2.5(3H,s,CH_3) 6.8-8.2 (14H,m,ArH) 11.0 (1H,s,OH)
7	2'-CH_3 (4b)	160-162	53	Pale yellow	$C_{24}H_{20}N_4O_2$	Found: C,72.28; H,4.82; N,13.94. (Calculated for C,72.73; H,5.05; N,14.14%)	3416 (OH) 1637 (C=O) 1614 (C=N) 1485 (N=N) 753 (substituted phenyl)	2.4 (3H,s,CH_3) 2.5 (3H,s,Ar- CH_3) 6.8-8.2 (13H,m,ArH) 11.1 (1H,s,OH)
8	2'-OCH_3 (4c)	181-182	56	Yellow	$C_{24}H_{20}N_4O_3$	Found: C,69.35 ; H,4.67; N,13.46. (Calculated for C,69.90; H,4.85; N,13.59%)	3410 (OH) 1648 (C=O) 1624 (C=N) 1497 (N=N) 752 (substituted phenyl)	2.3 (3H,s,CH_3) 3.92 (3H,s,Ar-OCH_3) 6.8-8.2 (13H,m,ArH) 11.0 (1H,s,OH)
9	2'-Cl (4d)	100-102	56	Yellow	$C_{23}H_{17}N_4O_2Cl$	Found: C,65.82; H,3.87; N,13.26. (Calculated for C,66.19; H,4.08; N,13.43%)	3407 (OH) 1639 (C=O) 1598 (C=N) 1509 (N=N) 752 (substituted phenyl)	2.6 (3H,s,CH_3) 6.9-8.1 (8H,m,ArH) 11.8 (1H,s,OH)
10	2'- NO_2 (4e)	112-114	60	Yellow	$C_{23}H_{17}N_5O_4$	Found: C,64.13; H,3.76; N,16.18. (Calculated for C,64.64 ; H,3.98; N,16.39%)	3416 (OH) 1668 (C=O) 1602 (C=N) 1490 (N=N) 746 (substituted phenyl)	2.5(3H,s,CH_3) 6.9-8.2 (13H,m,ArH) 11.0 (1H,s,OH)
11	-H (6a)	77-78	52	Yellow	$C_{18}H_{18}N_4O_2S$	Found: C,59.48; H,4.86 ; N,15.65. (Calculated for C,61.01; H,5.08; N,15.81%)	1670,1618 (C=N or C=C) 1522 (N=N) 1305-(S=O antisymmetric str.) 1167 (S=O symmetric str.)	2.42 (3H,s,SO_2- C_6H_4-CH_3) 2.55 (6H,s,two heterocyclic CH_3 groups) 7.10-7.40 (9H,m, ArH)
12	4'- CH_3 (6b)	89-90	57	Brown	$C_{19}H_{20}N_4O_2S$	Found: C,61.57; H,5.08 ; N,14.92. (Calculated for C,61.95; H,5.43; N,15.22%)	1667,1623 (C=N or C=C) 1496 (N=N) 1318-(S=O antisymmetric str.) 1195 (S=O symmetric str.)	2.41 (3H,s,SO_2- C_6H_4-CH_3) 2.59 (3H,s,C_6H_5-CH_3) 2.78 (6H,s,two heterocyclic CH_3 groups) 7.10-7.90 (8H,m, ArH)
13	4'-OCH_3 (6c)	100-101	51	Yellow	$C_{19}H_{20}N_4O_3S$	Found: C,60.76; H,5.21; N,14.71 (Calculated for C,61.29 ; H,5.38; N,15.05%)	1670,1616 (C=N or C=C) 1517 (N=N) 1320 (S=O antisymmetric str.) 1195 (S=O symmetric str.)	2.40 (3H,s,SO_2- C_6H_4-CH_3) 2.82 (6H,s,two heterocyclic CH_3 groups) 3.91 (3H,s, C_6H_5-OCH_3) 7.10-7.80 (8H,m, ArH)
14	4'-SO_2NH_2 (6d)	111-112	53	Yellow	$C_{18}H_{19}N_5O_4S_2$	Found: C,49.52; H,4.08 ; N,15.94. (Calculated for C,49.88; H,4.39; N,16.17%)	1670, 1595 (C=N or C=C) 1547 (N=N) 1335-(S=O antisymmetric str.) 1154 (S=O symmetric str.)	2.41 (3H,s,SO_2-C_6H_4-CH_3) 2.56 (6H,s,two heterocyclic CH_3 groups) 3.42 (2H, hump, NH_2) 7.00-7.80 (8H,m, ArH)
15	4'- NO_2 (6e)	212-213	54	Pale yellow	$C_{18}H_{17}N_5O_4S$	Found: C,53.75; H,4.12; N,17.26. (Calculated for C,54.14 ; H,4.26; N,17.54%)	1671,1599 (C=N or C=C) 1508 (N=N) 1334-(S=O antisymmetric str.) 1162 (S=O symmetric str.)	2.42 (3H,s,SO_2-C_6H_4-CH_3) 2.63 (6H,s,two heterocyclic CH_3 groups) 7.10-7.80 (8H,m, ArH)

Table 2
Antimicrobial activity of the compounds 2a-e, 4a-e and 6a-e

S. No.	Compound No.	R	Diameter of zone of inhibition (mm)	
			S. aureus	*E. coli*
			50 µg/0.1ml	50 µg/0.1ml
1	2a	-H	NA*	NA*
2	2b	4'-CH_3	NA*	NA*
3	2c	4'-OCH_3	3	2
4	2d	4'-Br	6	5
5	2e	4'-NO_2	6	5
6	4a	-H	NA*	NA*
7	4b	2'-CH_3	NA*	NA*
8	4c	2'-OCH_3	NA*	NA*
9	4d	2'-Cl	3	2
10	4e	2'-NO_2	6	5
11	6a	-H	NA*	NA*
12	6b	4'-CH_3	3	2
13	6c	4'-OCH_3	3	2
14	6d	4'-SO_2NH_2	8	6
15	6e	4'-NO_2	9	6

*NA : No Activity

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