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Synthesis, characterization of some new 1- (2, 5 - dichloro phenyl hydrazino)-3,5-dimethyl- 4-(substituted phenyl azo) pyrazoles and 3,5-dimethyl- 4-(substituted phenyl benzene azo) isoxazoles as anti-bacterial agents

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ABSTRACT

Pyrazole is an important class of organic molecules and containing two nitrogen-atoms in five membered parent ring. In this synthesis approach features, newly substituted pyrazoles has been synthesized by condensation reactions between various substituted benzene-azo acetyl acetone (1a-1t) and substituted phenyl hydrazine with few drops of glacial acetic acid used as catalyst in alcoholic medium and substituted isoxazoles have been synthesized by the condensation reactions of subs. benzene-azo acetyl acetone (1a-1b) with hydroxyl amine hydrochloride and sodium acetate in the alcoholic medium. The structures of newly synthesized substituted pyrazoles and substituted isoxazoles were established on the basis of their spectral studies like IR, ^{1}H NMR, Elemental Analysis, physical properties. The newly synthesized compounds were screened for their anti-bacterial activity against Staphylococcus aureus and Escherichia coli.

Key words: Subs. benzene-azo acetyl acetone, Subs. Phenylhydrazine, Pyrazoles, Isoxazoles, Antibacterial activity.

INTRODUCTION

The chemistry of pyrazoles and it's derivatives are an important class of organic molecules has been extensively studied for last few decades. Many of the pyrazole derivatives have been found to possess biological activity[1]. Pyrazole derivatives have been reported to possesses as anti-cancer[2], anti-diuretic[3], anti-helmentic[4], hypoglycaemic[5], fungicidal[6], anti-inflammatory[7], anti-diabetic[8], anti-microbial[9] activities .

Substituted pyrazoles have pronounced sedative action on the CNS[10]. Pyrazole derivativees are known to be therapeutically useful compounds[11] such as Celecoxib, diclofenac, non-steroidal anti-inflammatory (NSAID's) and anti-pyretic drugs.

Isoxazoles consist a class of five membered ring containing Nitrogen and Oxygen with diverse applications[12], isoxazoles are well known for their biological properties[13], 3,5-dimethyl isoxazole is a potential hypoglycaemic agent[14], isoxazole derivatives have been reported to possess as anti-bacterial[15], anti-tubercular[16], anti-viral[17], anti-tumor[18] activities. In continuation of our earlier work[19]. In the present communication we wish to report here the synthesis of some new pyrazoles and isoxazoles.

EXPERIMENTAL

Material and Methods

All chemicals are used in the synthesis were of analytical grade and obtained from Sigma-Aldrich Company. All the mentioned melting points were determined in open capillary tubes and are uncorrected. The purities of the newly synthesized compounds were checked on silica-gel-coated Al plates (E-Merck). IR spectra were recorded in KBr-

disc method on Perkin-Elmer spectrum RX-1 FT-IR spectrophotometer at ST. John's College, Agra. ^{1}H NMR spectra was measured on Advanced Bruker DRX-300, using solution in DMSO d_6. Chemical shifts are given in δ (ppm) and protons signals are indicated as: s = singlet, d = doublet, t = triplet, m = multiplet. Elemental analysis was performed on Elementor Vario EL III.

General procedure for the synthesis of substituted phenyl benzene-azo acetyl acetone (1a-1t)

The substituted aniline (0.025 mole) was diazotised by adding concentrated HCl (8ml) in distilled water (6ml), cooled the solution in an ice-bath at maintained temperature 0^{O}C, after completing first step the cold aqueous solution of $NaNO_2$ (0.025 mole) was added drop-wise in to the cooled diazotised solution, then this solution was added drop-wise in to the cooled maintained temperature 0^{O}C solution of Sodium acetate (0.12 mole) and acetyl acetone (0.025 mole) in ethyl alcohol (25 ml), during stirring substituted benzene-azo acetyl acetone was separated out, filtered, washed with distilled water, recrystallized by hot ethanol.

General procedure for the synthesis of 1-(2, 5-dichloro phenyl hydrazino)-3,5 dimethyl-4(substituted phenyl benzene-azo acetyl acetone) pyrazoles (2a-2t)

A mixture of (1a-1t ;0.001 mole) dissolved in absolute ethanol and (0.001 mole) of 2,5-dichloro phenyl hydrazine, then refluxed for 4-5 hours in the presence of 4 drops of glacial acetic acid. A coloured solid was separated after cooling the solution, filtered and purified by absolute ethanol 99% several times. It was identified to be 1-(2, 5-dichloro phenyl hydrazino) - 3, 5 dimethyl- 4(substituted phenyl benzene - azo acetyl acetone) pyrazoles.

General procedure for the synthesis of 4-(substituted benzene - azo) -3,5-dimethyl isoxazole (3a-3b)

To (1a,1b ; 0.001 mole) dissolved in excess of ethanol (25 ml) was treated with aqueous solution of hydroxyl amine hydrochloride (0.01 mole) and Sodium acetate, then the mixture was refluxed for 4-hours on steam-bath, a coloured crystalline product obtained on cooling, filtered, recrystallized from ethanol 99%. It was identified to be 4 -(substituted benzene-azo)- 3,5 -dimethyl isoxazoles.

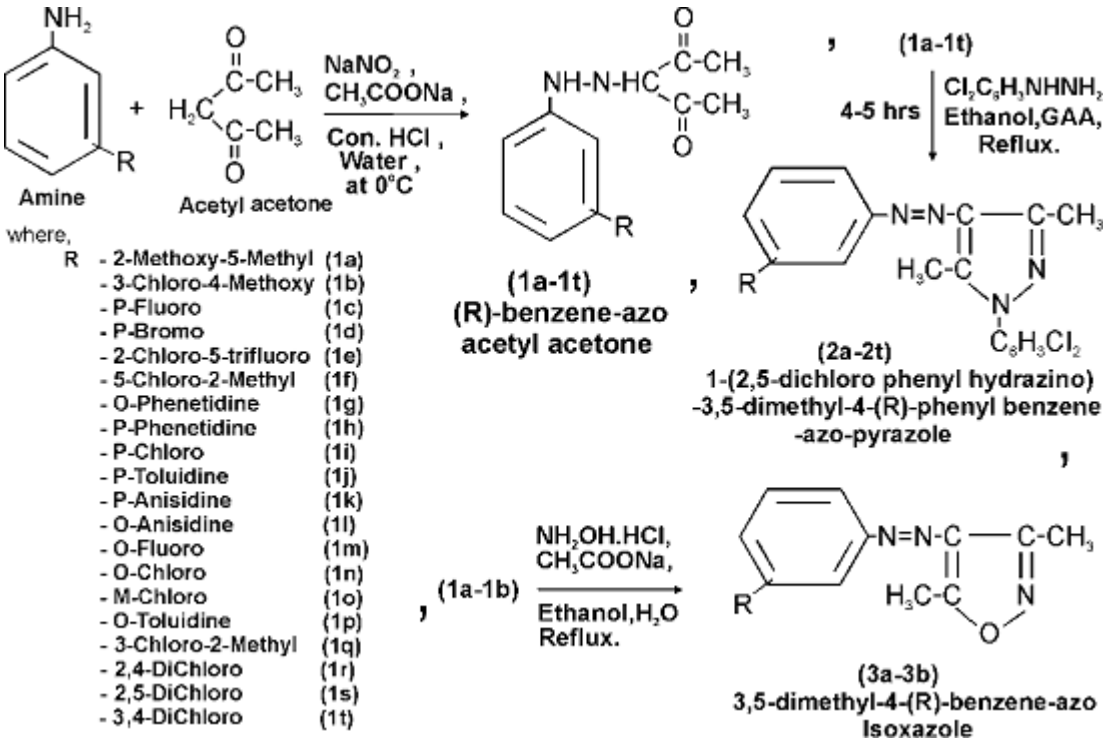

Table 1: Physical and Analytical data of the Synthesized Compounds

Codes	Molecular Formula	M.W	M.P 0°C	Yield %	colour	% Analytical data					
						C cal.%	(found)	H cal.%	(found)	N cal.%	(found)
1a	$C_{13}H_{17}N_2O_3$	249.29	138°	57.73	sunset	62.63	(62.65)	6.87	(6.88)	11.24	(11.26)
1b	$C_{12}H_{14}N_2O_3Cl_1$	269.71	131°	73.24	sporty yellow	53.44	(53.46)	5.23	(5.24)	10.38	(10.42)
2a	$C_{19}H_{18}N_4O_1Cl_2$	389.30	139°	53.52	orange	58.62	(58.57)	4.66	(4.82)	14.39	(14.72)
2b	$C_{18}H_{16}N_4O_1Cl_3$	409.72	151°	57.39	lihgt thar desert	52.76	(52.73)	3.69	(4.10)	13.67	(13.85)
2c	$C_{17}H_{13}N_4Cl_2F_1$	363.23	165°	56.25	yellow	56.21	(56.19)	3.60	(3.62)	15.42	(15.46)
2d	$C_{17}H_{13}N_4Cl_2Br_1$	424.15	137°	52.40	orange	48.14	(48.15)	3.09	(3.11)	13.21	(13.24)
2e	$C_{18}H_{12}N_4Cl_3F_3$	447.69	109°	40.49	light mango	48.29	(48.27)	2.70	(2.68)	12.51	(12.54)
2f	$C_{18}H_{15}N_4Cl_3$	393.72	134°	48.37	light desert	54.91	(54.89)	3.84	(3.81)	14.23	(14.26)
2g	$C_{19}H_{18}N_4O_1Cl_2$	389.30	103°	50.00	dirty yellow	58.62	(58.59)	4.66	(4.64)	14.39	(14.43)
2h	$C_{19}H_{18}N_4O_1Cl_2$	389.30	146°	58.21	sporty yellow	58.62	(58.61)	4.66	(4.68)	14.39	(14.44)
2i	$C_{17}H_{13}N_4Cl_3$	379.69	101°	52.88	dark desert	53.77	(53.79)	3.45	(3.46)	14.75	(14.79)
2j	$C_{18}H_{16}N_4Cl_2$	359.27	92°	55.80	light yellow	60.17	(60.19)	4.49	(4.51)	15.59	(15.63)
2k	$C_{18}H_{16}N_4O_1Cl_2$	375.27	99°	51.21	crystalline yellow	57.61	(57.63)	4.29	(4.31)	14.93	(14.96)
2l	$C_{18}H_{16}N_4O_1Cl_2$	375.27	126°	43.80	dirty yellow	57.61	(57.58)	4.29	(4.26)	14.93	(14.95)
2m	$C_{17}H_{13}N_4Cl_2F_1$	363.23	103°	65.50	light yellow	56.21	(56.19)	3.60	(3.58)	15.42	(15.46)
2n	$C_{17}H_{13}N_4Cl_3$	379.69	97°	48.07	light buff	53.77	(53.75)	3.45	(3.42)	14.75	(14.78)
2o	$C_{17}H_{13}N_4Cl_3$	379.69	102°	44.47	sporty yellow	53.77	(53.74)	3.45	(3.47)	14.75	(14.79)
2p	$C_{18}H_{16}N_4Cl_2$	359.27	109°	51.76	yellow	60.17	(60.15)	4.49	(4.51)	15.59	(15.63)
2q	$C_{18}H_{15}N_4Cl_3$	393.72	112°	41.62	yellow	54.91	(54.88)	3.84	(3.81)	14.23	(14.26)
2r	$C_{17}H_{12}N_4Cl_4$	414.14	117°	47.22	light orange	49.30	(49.31)	2.92	(2,94)	13.52	(13.56)
2s	$C_{17}H_{12}N_4Cl_4$	414.14	154°	38.13	light desert	49.30	(49.28)	2.92	(2.93)	13.52	(13.57)
2t	$C_{17}H_{12}N_4Cl_4$	414.14	97°	40.13	light yellow	49.30	(49.32)	2.92	(2.94)	13.52	(13.55)
3a	$C_{13}H_{15}N_3O_2$	245.28	94°	37.10	light orange	63.66	(63.68)	6.16	(6.18)	17.13	(17.17)
3b	$C_{12}H_{12}N_3O_2Cl_1$	265.71	115°	39.64	light sporty yellow	54.24	(54.25)	4.55	(4.57)	15.81	(15.85)

Elemental Analysis for C, H, N of Compound (2a) are as-58.62(58.57), 4.66(4.82), 14.39(14.72) and (2b)-52.76(52.73), 3.69(4.10), 13.67(13.85).

Anti-bacterial Activity

The substituted pyrazoles (2a,2b,2e,2o, 2j,2q,3a,3b) were screened for antibacterial activity against one Gram + ve *Staphylococcus aureus* and one Gram - ve E.coli applying filter paper disc method[20] at concentration of 25 µg ml^{-1} using Hi-Media Sterile disc SD-067 and Hi-Media Muller Hinton Agar Medium, using dimethyl formamide as a solvent, after 24 hours of incubation at 37°, the zone of inhibition were measured in mm.

The activity was compared with known antibiotic such as streptomycin and the results ofantibacterial activity is listed in the Table-3.

Most of the pyrazole and isoxazole showed significant antibacterial activity. The antibacterial activity is morderate to highly active of the compounds (2a,2b,3b) against gram positive S.aureus. The compounds 2a, 2b, showed morderate to highly anti-bacterial activity against gram -ve E.coli .

Table 2: Spectral data of Newly Synthesized Compounds

Compounds codes	IR Absorption Bands in cm^{-1}						
	-NH cm^{-1} stretching	-N=N cm^{-1} stretching	-C=C cm^{-1} stretching	-C-N cm^{-1} stretching	-N-N cm^{-1} stretching	$-CH_3$ cm^{-1} stretching	mono substitution
2a	3446.3	1466.1	1562.2	1219.7	1521.2	1420.8	668.2
2b	3457.8	1466.1	1554.1	1258.2	1499.7	1426.1	668.0
2c	3455.9	1466.0	1546.0	1219.7	1500.6	1425.9	668.0
2d	3453.7	1464.9	1542.0	1222.2	1500.9	1422.8	668.5
2e	3414.5	1467.6	1552.0	1178.8	1512.1	1426.1	668.3
2f	3448.4	1467.0	1552.0	-	1511.7	1421.1	668.3
3a	3451.6	1460.7	1548.3	1211.4	1509.4	1414.7	668.5
3b	3450.0	1466.3	1546.5	1239.1	1500.3	1414.2	668.4

codes	^{1}H-NMR Spectral data δ (ppm)
1a	2.306(s,3H,-CH_3),2.480(s,3H,-CH_3),3.340(s,1H,ring),7.046(m,1H,C=O)
1b	2.428(s,3H,-CH_3),2.500(s,3H,-CH_3),3.360(s,1H,ring),7.223(s,1H,C=O)
2b	2.417(s,3H,-CH_3),2.446(s,3H,-CH_3),3.334(s,1H,pyrazole ring),7.788(s,1H,C=O)

Table 3: Antibacterial data of synt. compounds

Codes	Zone of inhibition (in mm)	
	S.aureus **Gram +ve**	***E.coli*** **Gram -ve**
2a	+++	+++
2b	+++	+++
2e	++	++
2j	++	R
2o	++	++
2q	+	R
3a	++	+
3b	+++	++
Streptomycin	+++	+++

Key to symbols: Resistance=R; slighty active = + (inhibition zone 6-9 mm); moderately active = ++ (inhibition zone 9-12 mm);highly active = +++(inhibition zone >12).

RESULTS AND DISCUSSION

The IR Spectra of the newly synthesized compounds have been recorded in the frequency region 4000-500 cm^{-1}. The IR (KBr-disc method) Spectral data and ^{1}H NMR spectral data are recorded in the Table-2.

The IR Spectra of the compounds showed absorption bands in the range 3457.8-3414.5 cm^{-1} showed stretching vibrations of -NH, while absorption in the range 1467.6-1464.9 cm^{-1} indicates the pyrazole ring because of -N=N stretching vibrations, absorption bands in the range

1562.2-1542.0 shows the presence of -C=C stretching vibrations, absorption in the range 1258.2-1178.8 cm^{-1} indicates the -C-N stretching vibrations, stretching vibrations of -N-N in the range 1521.2-1499.7 cm^{-1}, stretching vibrations in the range 1426.1-1420.8 cm^{-1} indicates the $-CH_3$, stretching vibrations in the range 668.5-668.0 cm^{-1} reveals the mono substitution.

Thus the above observations are lent support to the assigned structure of compounds 2a-2f and other compounds 2g-2t.

IR spectrum of (3a,3b) shows absorption at 3451.6 cm^{-1}, 3450.0 cm^{-1} indicates -NH stretchi ng,absorptions at 1460.9 cm^{-1},1460.3 cm^{-1} reveals -N=N stretching vibrations, while absorption at 1548.3 cm^{-1} ,1546.5 cm^{-1} show aromatic -C=C, absorption at 1211.4 cm^{-1}, 1239.1 cm^{-1} indicates the presence of -CN, absorption at 1509.4 cm^{-1} ,1500.3 cm^{-1} indicates the presence of -N-N, absorption at 1414.7 cm^{-1}, 1414.2 cm^{-1} indicates the presence of $-CH_3$,stretching vibrations at 668.5 cm^{-1}, 668.4 cm^{-1} indicating the mono substitution .

The above observations are sufficient to support the assigned structure of the compound (3a,3b). The ^1H NMR spectra showed singlet at δ 2.306, 2.428(CH_3), 2.480,2.500($-CH_3$), 3.340,3.360(hydrazone ring), 7.046, 7.223(-C=O), these observations confirming the structures of compounds 1a,1b. The ^1H NMR spectra showed singlet at δ 2.417($-CH_3$), 2.446($-CH_3$), 3.334(-pyrazole ring), 7.778(-C=O), these results are confirming the structure of the compound 2b and other compounds 2a, 2c-2t.

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