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Synthesis and Biological Activity of Some New Pyrazolin–5–one and Pyrazole Derivatives.

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ABSTRACT

N'–(2–hydroxybenzoyl)–3–methyl–4–(substituted arylhydrazono)pyrazolin–5–ones, N–(2–hydroxybenzoyl)–3,5–dimethyl–4–(substituted arylazo)pyrazoles, N–(p–toluenesulphonyl)–3–methyl–4–(substituted arylhydrazono)pyrazolin–5–ones, and N–(p–toluenesulphonyl)–3,5–dimethyl–4–(substituted arylazo)pyrazoles have been synthesized in good yields and characterized by IR, NMR and elemental analyses. The compounds were evaluated for their *in vitro* antibacterial activity against some Gram-positive bacteria, *Staphylococcus aureus* and Gram-negative bacteria, *Escherichia Coli.* The compounds showed moderate to good antibacterial activities.

Keywords: pyrazole, pyrazolin-5-ones, salicyloyl hydrazine, p-toluenesulphonyl hydrazine, antibacterial activity.

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INTRODUCTION

A wide range of antimicrobial agents have been discovered over the years which prolonged the lifespan of human. But at the same time emergence of bacterial resistance towards a number of antimicrobial agents is becoming a major problem worldwide. In the past few years, gram negative bacteria *E. coli* have become resistant to many antibiotics [1-3]. Another significant problem in clinical practice is increased isolation of methicillin resistant *S. aureus* [4]. Therefore, there is a need for development of new antimicrobial agents which may be effective against the resistant microbes.

Careful literature survey for functional groups which could be considered as pharmacophores for the antitubercular activities revealed that the hydrazine moiety is common among most of the antitubercular agents [5-6]. Pyrazole and pyrazolin-5-one derivatives are also an important class of heterocyclic compounds showing wide range of biological activities such as antimicrobial [7-8], antitubercular [9-10] and anti-inflammatory [11-12] activities. In view of the above mentioned and in continuation of our earlier work [13-15] on pyrazole and pyrazolin-5-one derivatives we report herein the synthesis, characterization and antimicrobial evaluation of some pyrazole and pyrazolin-5-one derivatives.

MATERIALS AND METHODS

All chemicals and reagents were procured from Merck India limited. Melting points were determined in open capillary on a Mel-Temp apparatus and are uncorrected. The progress of the reaction was monitored by TLC (silica gel H, BDH, ethyl acetate-hexane, 3:5). The IR spectra were recorded on IR 200 FT-IR spectrometer as KBr pellets. The wave numbers were given in cm^{-1}. The ^1H NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Jeol JNM λ-400 MHz machine. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. The microanalyses were performed on Perkin-Elmer 240C elemental analyzer. The antibacterial activity of newly synthesized compounds was studied against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. Coli* by cup-plate method. Sulphamethoxazole was used as the standard. 10 mg of Sulphamethoxazole was dissolved in 100 ml of DMF to get a final concentration of 10µg/0.1ml. 50 mg of each test compound was dissolved in 100 ml of DMF in labelled sterile test tubes, thus giving a final concentration of 50 µg/0.1ml.

EXPERIMENTAL PROCEDURES

Preparation of the substituted aryl diazonium chloride [16] **(2a-e):**

The required primary amine was dissolved in a suitable volume of water containing 2.5–3.0 equivalents of hydrochloride acid (or sulphuric acid) by the application of heat if necessary. The solution thus obtained was cooled to 0^o C where the amine hydrochloride (or sulphate) usually crystallizes. The temperature was maintained at 0 to 5°C and the aqueous sodium nitrite solution was added portion wise till there was free nitrous acid. The solution was tested for the later with an external indicator paper (moist potassium iodide–starch paper). An excess of acid was maintained to stabilize the diazonium salt solution. However, in those cases where a large excess of acid was harmful, the concentration of the acid was reduced to optimum value (Scheme 1).

Preparation of 2-(substituted arylhydrazono)-ethyl-2,3-dioxobutyrates [17] **(3a-e) :**

A solution of sodium acetate (l00g) in 1000ml of aqueous alcohol (50%) was added to a solution of acetoacetic ester(l00g) in 500ml of ethanol and the mixture was cooled. To this cold mixture, the corresponding diazonium chloride was added gradually till a turbidity was observed. The addition was continued till yellow crystals separated out. These crystals were filtered washed with water and dried. The product formed was recrystallised from ethanol (Scheme 1).

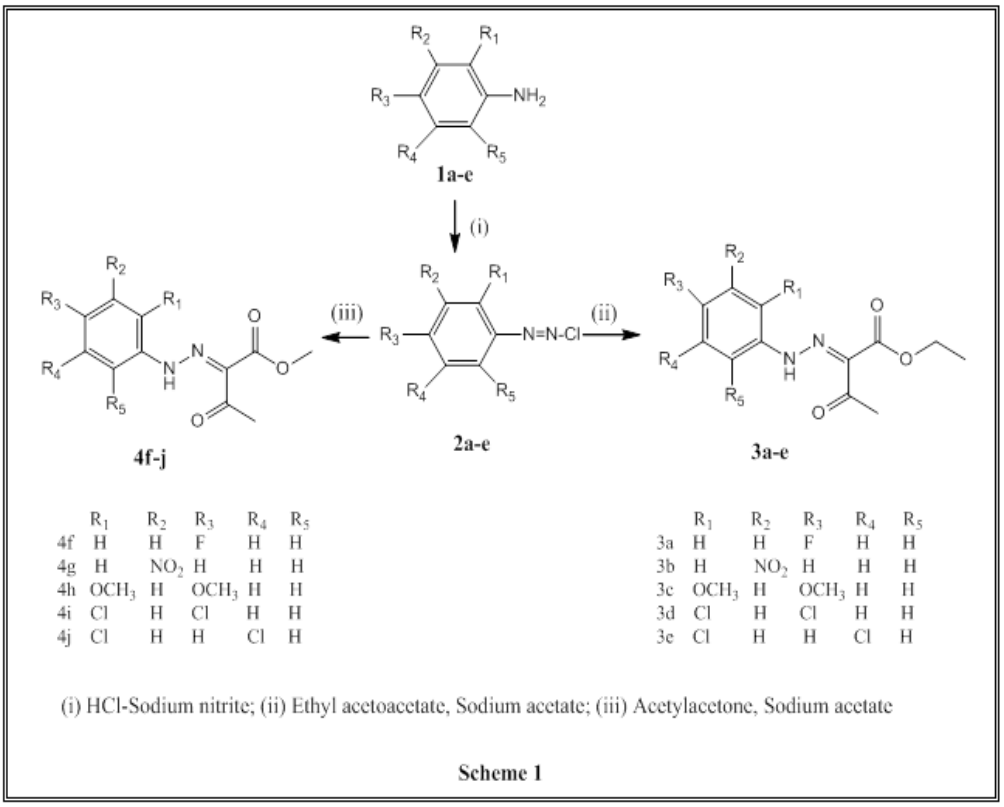
3a yield 70%, Colour light yellow, M.p. 90 – 94°C. IR (KBr) ν_{max}**:** 3450 (NH), 1690 (CO), 1520 (NHN=C) cm^{-1}. Analysis $C_{12}H_{13}FN_2O_3$. Found C,57.14; H,5.19; N,11.81 (Calculated C,57.96; H,5.85; N,12.15).

3b yield 60%, Colour yellow, M.p. 120–124°C. IR (KBr) ν_{max}**:** 3410 (NH), 1668 (CO), 1600 (NHN=C)cm^{-1}. Analysis $C_{12}H_{13}N_3O_5$. Found C,51.61; H,4.69; N,15.05 (Calculated C,51.75; H,4.78; N,15.65).

3c yield 58%. Colour Turmeric yellow, M.p. 118–122°C. IR (KBr) ν_{max}: 3443 (NH), 1680 (CO), 1596 (NHN=C)cm^{-1}. Analysis $C_{14}H_{18}N_2O_5$. Found C,57.13; H,6.10; N,9.32 (Calculated C,57.85; H,6.72; N,9.88).

3d yield 65%. Colour Orange, M.p. 108–112°C. IR (KBr) ν_{max}: 3376 (NH), 1690 (CO), 1586 (NHN=C) cm^{-1}. Analysis $C_{12}H_{12}Cl_2N_2O_3$: Found C,47.54; H,3.39; N,9.24 (Calculated C,47.82; H,3.56; N,9.56).

3e yield 62%. Colour Light brown, M.p. 96–100°C. IR (KBr) ν_{max}: 3364 (NH), 1689 (CO), 1590 (NHN=C) cm^{-1}. Analysis $C_{12}H_{12}Cl_2N_2O_3$: Found C,47.54; H,3.82; N,9.56 (Calculated C,47.82; H,3.96; N,9.50).



	R_1	R_2	R_3	R_4	R_5
4f	H	H	F	H	H
4g	H	NO_2	H	H	H
4h	OCH_3	H	OCH_3	H	H
4i	Cl	H	Cl	H	H
4j	Cl	H	H	Cl	H

	R_1	R_2	R_3	R_4	R_5
3a	H	H	F	H	H
3b	H	NO_2	H	H	H
3c	OCH_3	H	OCH_3	H	H
3d	Cl	H	Cl	H	H
3e	Cl	H	H	Cl	H

(i) HCl-Sodium nitrite; (ii) Ethyl acetoacetate, Sodium acetate; (iii) Acetylacetone, Sodium acetate

Scheme 1

Preparation of 2,3,4-pentanetrione-3-substituted arylhydrazones [18-19] **(4f-j) :**

The filtered diazonium solution was added drop wise into a well–cooled stirred mixture of sodium acetate (0.1M) and 2,3,4–pentanetrione (0.1M) in ethanol (25ml) and water (25ml). The product formed was recrystallised from ethanol (Scheme 1).

4f yield 60%, Colour Golden yellow, M.p.102–106°C. IR (KBr) ν_{max}: 3321 (NH), 1671 (CO), 1626(NHN=C)cm^{-1}. Analysis $C_{11}H_{11}FN_2O_2$: Found C, 71.58; H, 6.72; N, 12.81 (Calculated C,71.63; H,6.93; N,12.83).

4g yield 65%, Colour Yellow, M.p.130–134°C. IR (KBr) ν_{max}: 3392 (NH), 1686 (CO), 1596 (NHN=C) cm^{-1}. Analysis $C_{11}H_{11}N_3O_4$: Found C, 62.12; H, 5.96; N, 17.11 (Calculated C, 63.66; H, 6.16; N, 17.13).

4h yield 68%. Colour Orange red, M.p.112–114°C. IR (KBr) ν_{max}: 3443 (NH), 1672 (CO), 1625 (NHN=C) cm^{-1}. Analysis $C_{13}H_{16}N_2O_4$: Found C,69.12; H,7.25; N,10.42 (Calculated C,69.20; H,7.74; N,10.76).

4i yield 66%. Colour Yellow, M.p.158–162°C. IR (KBr) ν_{max}: 3283 (NH), 1686 (CO), 1631 (NHN=C)cm^{-1}. Analysis $C_{11}H_{10}Cl_2N_2O_2$: Found C, 57.89; H, 5.16; N, 9.56 (Calculated C, 58.01; H, 5.24; N, 10.40).

4j yield 72%. Colour Light green, M.p.108–112°C. IR (KBr) ν_{max}: 3292 (NH), 1686 (CO), 1632 (NHN=C)cm^{-1}. Analysis $C_{11}H_{10}Cl_2N_2O_2$: Found C, 57.86; H, 5.12; N, 9.56 (Calculated C, 58.01; H, 5.24; N, 10.40).

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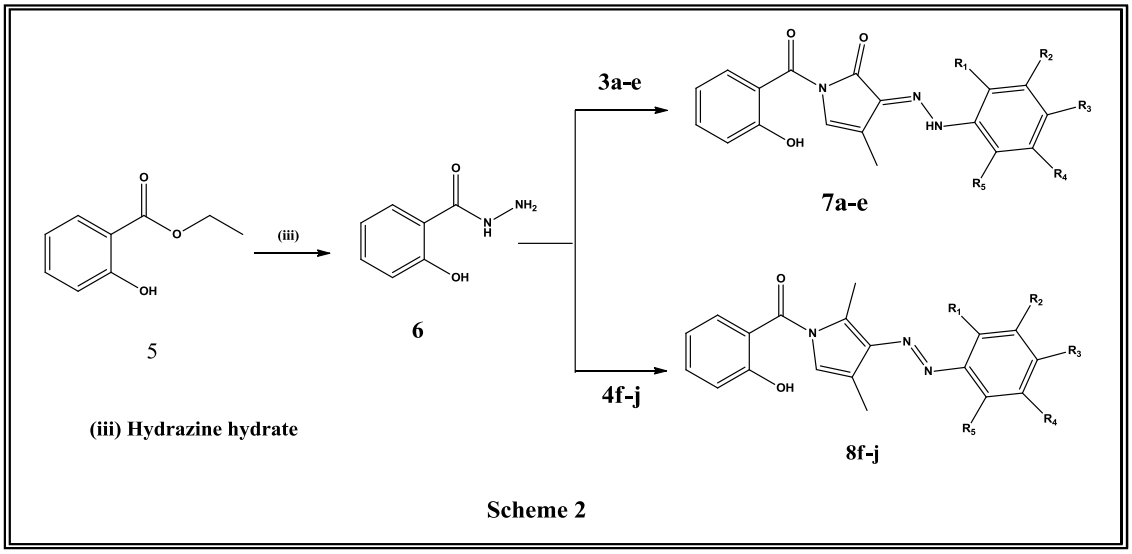
Preparation of Salicyloyl hydrazine (6):

Salicyloyl hydrazine was prepared by slowly adding 1 mole of ethylsalicylate (5) into a one litre round bottom flask containing 1.10 M of hydrazine hydrate (99%) in 500 ml of ethyl alcohol and refluxing the mixture for 3 hrs. The solid formed was collected and recrystallised from ethyl alcohol. The homogeneity and purity of the compound was tested through thin layer chromatography (TLC). Yield: 78%, melting point 150°C (Literature melting point: 147–150°C) (Scheme 2).



Scheme 2

Preparation of N'–(2–hydroxybenzoyl)–3–methyl–4–(substituted arylhydrazono)pyrazolin–5–ones (7a-e):

Equimolar concentrations of salicyloyl hydrazine (6) and ethyl–2,3–dioxobutyrate–2–arylhydrazone(0.0075M) (3a-e) dissolved in methanol(50 ml) were refluxed for 6 hours. The solid obtained on cooling was filtered, washed with water and recrystallised from glacial acetic acid. The purity of the compound was tested through TLC and the structure confirmed by chemical analysis and spectral data (Scheme 2).

7a yield 68%, Colour yellow, M.p. 78-82°C. IR (KBr) ν_{max}**:** 3210 (NH), 1672 (C=O), 1580 (C=C), 1540 (NHN=C) cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 2.43(3H,s,CH_3), 6.62–7.33 (8H,m, ArH), 7.39(1H,br, NH), 14.79(1H, s, OH) ppm. Analysis $C_{17}H_{13}N_4O_3F$. Found C,59.56; H,3.72; N,16.32 (Calculated C,60.00; H,3.85; N,16.46).

7b yield 68%, Colour Orange, M.p. 144–146°C. IR (KBr) ν_{max}**:** 3240 (NH), 1655 (C=O), 1582 (C=C), 1542 (NHN=C)cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 2.61(3H,s,CH_3), 6.73–7.37 (8H,m, ArH), 7.41(1H,br, NH), 14.38(1H, s, OH) ppm. Analysis $C_{17}H_{13}N_5O_5$. Found C,55.32; H,3.48; N,18.76 (Calculated C,55.59; H,3.57; N,19.07).

7c yield 62%. Colour Merwen Red, M.p. 138–140°C. IR (KBr) ν_{max}**:** 3447 (NH), 1683 (C=O), 1599 (C=C), 1523 (NHN=C)cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 2.70(3H,s,CH_3), 3.81(6H,s,Ar–OCH_3), 6.61–7.32 (7H,m, ArH), 7.45(1H,br, NH), 13.13(1H, s, OH) ppm. Analysis . $C_{19}H_{18}N_4O_5$; Found C,58.92; H,4.71; N,14.62 (Calculated C,59.68; H,4.74; N,14.65).

7d yield 61%. Colour Yellow, M.p. 150–152°C. IR (KBr) ν_{max}**:** 3255 (NH), 1657 (C=O), 1582 (C=C), 1522 (NHN=C)cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 2.49(3H,s,CH_3), 6.79–7.31 (7H,m, ArH), 7.41(1H,br, NH), 14.83(1H, s, OH) ppm. Analysis $C_{17}H_{12}N_4O_3Cl_2$; Found C,51.89; H,2.86; N,14.23 (Calculated C,52.19; H,3.09; N,14.32).

7e yield 74%. Colour Brown, M.p. 148–150°C. IR (KBr) ν_{max}**:** 3152 (NH), 1689 (C=O), 1606 (C=C), 1589 (NHN=C) cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 2.28(3H,s,CH_3), 6.94–7.12 (7H,m, ArH), 7.25(1H,br, NH), 13.43(1H, s, OH) ppm. Analysis $C_{17}H_{12}N_4O_3Cl_2$; Found C,51.94; H,2.84; N,14.32 (Calculated C,52.19; H,3.09; N,14.32).

Preparation of N'–(2–hydroxybenzoyl)–3,5–dimethyl–4–(substituted arylazo)pyrazoles (8f-j):

Equimolar concentrations of salicyloyl hydrazine(6) and 1,3–dimethyl–2–arylhydrazono– 1,2,3–propanetrione (0.004M) **(4f-j)** were dissolved in methanol (30 ml) and the mixture was refluxed for 6 hours. The solid obtained on cooling was filtered, washed with water and recrystallized from glacial acetic acid. The purity of the compound was tested by TLC and the structure confirmed by spectral data and chemical analysis (Scheme 2).

8f yield 58%, Colour Yellow, M.p. 86–88°C. IR (KBr) ν_{max}**:** 3319 (OH), 3066 (ArCH), 1669 (C=O), 1625 (C=N), 1605 (C=C), 1511 (N=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.48 (3H,s,CH_3), 2.60 (3H,s,CH_3), 7.09–7.40 (8H,m, ArH), 14.80 (1H,s, OH) ppm. Analysis $C_{18}H_{15}N_4O_2F$; Found C, 63.45; H, 4.24; N, 16.32 (Calculated C, 63.90; H, 4.47; N, 16.56).

8g yield 58%, Colour Brown, M.p. 128–130°C. IR (KBr) ν_{max}**:** 3294 (OH), 3064 (ArCH), 1672 (C=O), 1618 (C=N), 1560 (C=C), 1530 (N=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.48 (3H,s,CH_3), 2.61 (3H,s,CH_3), 6.83–8.25 (8H,m, ArH), 14.81 (1H,s, OH) ppm. Analysis $C_{18}H_{15}N_5O_4$; Found C, 58.43; H, 3.87; N, 19.02 (Calculated C, 59.17; H, 4.10; N, 19.17).

8h yield 60%, Colour Brown, M.p. 108–110°C. IR (KBr) ν_{max}**:** 3443 (OH), 2853, 2924 (Aliphatic CH), 1677 (C=O), 1513 (N=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.50 (3H,s,CH_3), 2.61 (3H,s,CH_3), 3.91 (6H,s,Ar–OCH_3), 6.67–7.70 (7H,m, ArH), 14.72 (1H,s, OH) ppm. Analysis $C_{20}H_{20}N_4O_4$; Found C, 63.10; H, 5.16; N, 14.52 (Calculated C, 63.15; H, 5.30; N, 14.73).

8i yield 65%, Colour Yellow, M.p. 122–124°C. IR (KBr) ν_{max}**:** 3280 (OH), 3056 (ArCH), 1670 (C=O), 1630 (C=N), 1570 (C=C), 1528 (N=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.61 (3H,s,CH_3), 2.52 (3H,s,CH_3), 6.84–7.64 (7H,m, ArH), 14.75 (1H,s, OH) ppm. Analysis $C_{18}H_{14}N_4O_2Cl_2$; Found C, 54.79; H, 3.12; N, 14.29 (Calculated C, 55.54; H, 3.63; N, 14.39).

8j yield 55%, Colour Lemon yellow, M.p. 84–86°C. IR (KBr) ν_{max}**:** 3317 (OH), 3069 (ArCH), 1668 (C=O), 1620 (C=N), 1582 (C=C), 1526 (N=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.49 (3H,s,CH_3), 2.61 (3H,s,CH_3), 6.83–7.66 (7H,m, ArH), 14.71 (1H,s, OH) ppm. Analysis $C_{18}H_{14}N_4O_2Cl_2$; Found C, 55.25; H, 3.59; N, 14.25 (Calculated C, 55.54; H, 3.63; N, 14.39).

Preparation of p–toluenesulphonyl hydrazine (10)

A solution of p-toluenesulphonyl chloride (20g) in acetone(100ml) was added to a solution of the hydrazine hydrate (H_2N $NH_2.H_2O$) (19.4ml) in 20ml pyridine. The reaction mixture was heated on a water bath for 20 minutes. The solution is cooled and poured into 500ml dil.HC1 (5%). The precipitate is filtered off. Washed with cold water and recrystallised from aqueous alcohol. The homogeneity and purity of the compound was tested through thin layer chromatography (TLC). Yield: 70%, melting point: 110°C (d) (Literature melting point: 108–110° C (d)) (Scheme 3).

Preparation of N'–(p–toluenesulphonyl)–3–methyl–4–(substituted arylhydrazono)pyrazolin–5–ones (11a-

A mixture of the appropriate ethyl-2,3-dioxobutyrate-2-aryl hydrazone (0.005M) (3a-e) and p–toluene sulphonyl hydrazine (0.005M) was dissolved in methanol (100 ml) and DMF (30 ml). The resulting mixture was refluxed for 6 hours. Yellow crystals obtained on cooling were recrystallised from ethanol (Scheme 3).

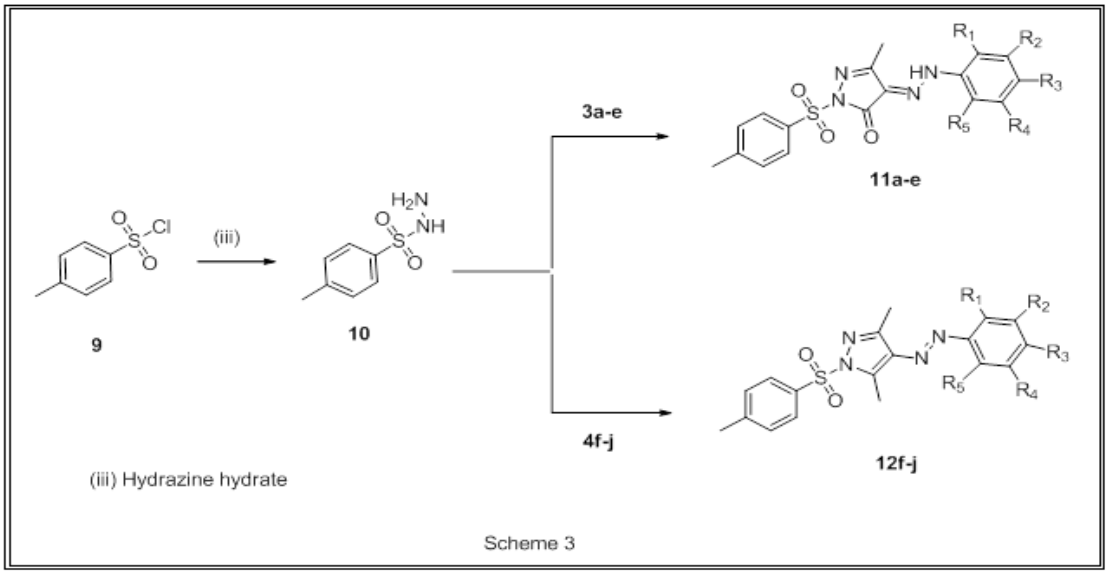
11a yield 68%, Colour Golden yellow, M.p. 124–126°C. IR (KBr) ν_{max}**:** 3410 (NH), 3076 (ArCH), 2960 (CH), 1685 (C=O cyclic), 1591 (C=N), 1547 (Heterocyclic five membered ring), 1176 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.95 (3H,s,Ar–CH_3), 2.51 (3H,s,CH_3), 6.67–7.32 (8H,m, ArH), 7.40(1H,s, NH) ppm. Analysis $C_{17}H_{15}N_4O_3SF$; Found C, 54.25; H, 3.96; N, 14.62 (Calculated (C, 52.19; H, 3.09; N, 14.32).

11b yield 62%, Colour Yellow, M.p. 104–107°C. IR (KBr) ν_{max}**:** 3400 (NH), 3095 (ArCH), 2935 (CH), 1697 (C=O cyclic), 1597 (C=N), 1541 (Heterocyclic five membered ring), 1165 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.95 (3H,s,Ar–CH_3), 2.51 (3H,s,CH_3), 6.75–7.36 (8H,m, ArH), 7.42(1H,s, NH) ppm. Analysis $C_{17}H_{15}N_5O_5S$; Found C, 50.16; H, 3.34; N, 17.04 (Calculated C, 50.84; H, 3.74; N, 17.40).

11c yield 70%, Colour Brich red, M.p. 100–103°C. IR (KBr) ν_{max}: 3445 (NH), 3057 (ArCH), 2958 (CH), 1680 (C=O cyclic), 1616 (C=N), 1596 (Heterocyclic five membered ring), 1162 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 2.12 (3H,s,Ar–CH_3), 2.50 (3H,s,CH_3), 3.81 (6H,s,Ar–OCH_3), 6.67–7.32 (7H,m, ArH), 7.46 (1H,s, NH) ppm. Analysis $C_{19}H_{20}N_4O_5S$; Found C, 54.35; H, 4.56; N, 13.28 (Calculated C, 54.80; H, 4.84; N, 13.40).

11d yield 64%, Colour Turmeric yellow, M.p. 110–112°C. IR (KBr) ν_{max}: 3444 (NH), 3085 (ArCH), 2980 (CH), 1700 (C=O cyclic), 1625 (C=N), 1568 (Heterocyclic five membered ring), 1165 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 1.95 (3H,s,Ar–CH_3), 2.52 (3H,s,CH_3), 6.85–7.32 (7H,m, ArH), 7.37 (1H,s, NH) ppm. Analysis $C_{17}H_{14}N_4O_3SCl_2$; Found C, 47.89; H, 3.16; N, 13.10 (Calculated C, 48.01; H, 3.32; N, 13.17).

11e yield 55%, Colour Orange, M.p. 80–83°C. IR (KBr) ν_{max}: 3410 (NH), 3076 (ArCH), 2960 (CH), 1685 (C=O cyclic), 1591 (C=N), 1547 (Heterocyclic five membered ring), 1176 (SO_2)cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ) 1.95 (3H,s,Ar–CH_3), 2.52 (3H,s,CH_3), 7.01–7.32 (7H,m, ArH), 7.47 (1H,s , NH) ppm. Analysis $C_{17}H_{14}N_4O_3SCl_2$; Found C, 47.86; H, 3.15; N, 13.12 (Calculated C, 48.01; H, 3.32; N, 13.17).



Scheme 3

Preparation of N'–(p–toluenesulphonyl)–3,5–dimethyl–4–(substituted arylazo)pyrazoles (12f-j)

A mixture of the appropriate 2,3,4-pentanetrione-3-arylhydrazones (4a-e) (0.005M) and p–toluene sulphonyl hydrazine (0.005M) was dissolved in methanol (100ml) and the mixture was refluxed for 6 hours. The crystals obtained on cooling were filtered and recrystallised from acetic acid. The homogeneity and purity of the compound was tested through TLC and the structure confirmed by spectral and chemical analysis (Scheme 3).

12f yield 55%, Colour Orange red, M.p. 78–81°C. IR (KBr) ν_{max}: 1670, 1625 (C=N or C=C), 1521 (N=N), 1315 (S=O anti symmetric str), 1185 (S=O symmetric str.) cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ) 1.93 (3H,s,SO_2–C_6H_4–CH_3), 2.48 (3H,s,CH_3), 2.60 (3H,s,CH_3), 7.09–7.92 (8H,m, ArH) ppm. Analysis $C_{18}H_{17}N_4O_2SF$; Found C, 57.96; H, 4.34; N, 14.96 (Calculated C, 58.05; H, 4.60; N, 15.04).

12g yield 58%, Colour Turmeric yellow, M.p. 96–99°C. IR (KBr) ν_{max}: 1680, 1596 (C=N or C=C), 1515 (N=N), 1329 (S=O anti symmetric str), 1172 (S=O symmetric str.) cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ) 1.95 (3H,s,SO_2–C_6H_4–CH_3), 2.53 (3H,s,CH_3), 2.63 (3H,s, CH_3), 7.19–8.26 (8H,m, ArH) ppm. Analysis $C_{18}H_{17}N_5O_4S$; Found C, 53.75; H, 4.12; N, 17.26 (Calculated C, 54.14; H, 4.26; N, 17.54).

12h yield 58%, Colour Yellow, M.p. 87–90°C. IR (KBr) ν_{max}: 1670, 1616 (C=N or C=C), 1517 (N=N), 1320 (S=O anti symmetric str),1195 (S=O symmetric str.) cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 1.94(3H,S,SO_2–C_6H_4–CH_3), 2.49(3H,s,CH_3), 2.62(3H,s,CH_3), 3.81(3H,s,OCH_3), 3.90(3H,s,OCH_3), 6.67–7.86(7H,m, ArH) ppm. Analysis $C_{20}H_{22}N_4O_4S$; Found C, C, 57.91; H, 5.26; N, 13.24 (Calculated C, 57.96; H, 5.35; N, 13.52).

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12i yield 56%, Colour Yellow, M.p. 101–104°C. IR (KBr) ν_{max}**:** 1667, 1623 (C=N or C=C), 1496 (N=N), 1318 (S=O anti symmetric str), 1195 (S=O symmetric str.) cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 1.93 (3H,s,SO_2–C_6H_4–CH_3), 2.52 (3H,s,CH_3), 2.61 (3H,s,CH_3), 7.12–7.85 (7H,m, ArH) ppm. Analysis $C_{18}H_{16}N_4O_2SCl_2$; Found C, 50.89; H, 3.46; N, 12.96 (Calculated C, 51.07; H, 3.81; N, 13.24).

12j yield 54%, Colour Orange red, M.p. 91–94°C. IR (KBr) ν_{max}**:** 1670, 1595 (C=N or C=C), 1547 (N=N), 1335 (S=O anti symmetric str), 1154 (S=O symmetric str.)cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 1.93 (3H,s,SO_2–C_6H_4–CH_3), 2.54 (3H,s,CH_3), 2.64 (3H,s,CH_3), 7.16–7.83 (7H,m, ArH) ppm. Analysis $C_{18}H_{16}N_4O_2SCl_2$; Found C, 50.92; H, 3.78; N, 13.12 (Calculated C, 51.07; H, 3.81; N,13.24).

RESULTS AND DISCUSSION

Twenty new compounds **7a-e, 8f-j, 11a-e and 12f-j** were prepared as depicted in Scheme 1, 2 and 3. The reaction of aryldiazonium chloride with ethyl acetoacetate and acetylacetone yielded the corresponding 2-(substituted arylhydrazono)-ethyl-2,3-dioxobutyrates **(3a-e)** and 2,3,4-pentanetrione-3-substituted arylhydrazones **(4f-j).** Salicyloyl hydrazine **(6)** and p-toluenesulphonyl hydrazine **(10)** were prepared by the reaction of ethylsalicylate and p-toluenesulphonyl chloride with hydrazine hydrate respectively. Reaction of compounds **3a-e** with **(6)** and **(10)** separately resulted in the formation of N'–(2–hydroxybenzoyl)–3–methyl–4–(substituted arylhydrazono)pyrazolin–5–ones **(7a-e)** and N'–(p–toluenesulphonyl)–3–methyl–4–(substituted arylhydrazono)pyrazolin–5–ones **(11a-e)** respectively. Whereas reaction of compounds **4f-j** with **(6)** and **(10)** separately resulted in the formation of N'–(2–hydroxybenzoyl)–3,5–dimethyl–4–(substituted arylazo)pyrazoles **(8f-j)** and N'–(p–toluenesulphonyl)–3,5–dimethyl–4–(substituted arylazo)pyrazoles **(12f-j)** respectively. The structures of the compounds were established by IR, proton NMR spectral data and elemental analysis. The spectral data revealed that compounds **7a-e** and **11a-e** exist in hydrazo form while compounds **8f-j** and **12f-j** exist in azo form.

Antibacterial Activity

The antibacterial activity of the compounds 7a–7e, 8f-8j, 11a-11e and 12f–12j was done against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli.* The results are presented in Table 1.

Table 1: Antibacterial activity results

S. No.	Compound	Diameter of zone of inhibition (mm)	
		S. aureus	*E. coli*
1	7a	1	2
2	7b	5	4
3	7c	NA*	NA*
4	7d	3	2
5	7e	3	1
6	8f	1	1
7	8g	5	4
8	8h	NA*	NA*
9	8i	5	6
10	8j	2	1
11	11a	2	1
12	11b	7	6
13	11c	NA*	NA*
14	11d	4	3
15	11e	3	2
16	12f	5	4
17	12g	6	5
18	12h	NA*	NA*
19	12i	3	2
20	12j	2	1
21	Sulphamethoxazole	13	14

*No Activity

Among Pyrazolin-5-one derivatives compound (7a –7e and 11a–11e), 7b and 11b exhibited moderate activity against both *S. aureus* and *E. coli* with a zone of inhibition 4-7mm. Compounds 7a, 7d, 7e, 11a, 11d & 11e exhibited feeble activity against *S. aureus* and *E. coli* with a zone of inhibition 1–4 mm. Rest of the compounds 7c, 11c were found to be inactive against both *S. aureus* and *E. coli.* Among the Pyrazole derivatives (8f-8j and 12f-12j) compounds 8g, 8i, 12f and 12g exhibited moderate activity against *S. aureus* and *E. coli* with a zone of inhibition 4–6 mm. 8f, 8j, 12i and 12j exhibited feeble activity against both organisms with a zone of inhibition 1–2 mm. Compound 8h and 12h were found to be inactive. The Pyrazolin–5–one and Pyrazole derivatives having substituents like $–NO_2$ and halogens in the benzene ring showed high activity in comparison with other substituents.

CONCLUSIONS

From the results it can be concluded that cyclization of hydrazines into its pyrazolin-5-one and pyrazole derivatives resulted in moderate to feeble antibacterial activities. Further, compounds having hybrid pharmacophore of 2-hydroxybenzoyl/p-toluenesulphonyl and pyrazolin-5-one/pyrazole seems to be responsible for antibacterial activity when there is an electron withdrawing group in the phenyl ring. Such compounds would provide a new opportunity for possible modification of pharmacophoric requirements.

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REFERENCES

[1] The Antibiotic Paradox: How Miracle Drugs are Destroying the Miracle, edited by S. B. Levy (Plenum Publishing, New York), 205, 1992.
[2] Piddock LJV. Drugs 1995; 49: 29.
[3] Salyers AA, Amiable-Cuevas CF. Antimicrob Agents Chemother 1997; 41: 2321.
[4] Chu DTW, Plattner JJ, Katz L. J Med Chem 1996; 39: 3853.
[5] Bijev, Arzneim. Forsch/Drug Res 2006; 56: 96.
[6] Sriram D, Yogeeswari P, Madhu K. Biorg Med Chem Lett 2006; 16: 876.
[7] Gouda MA, Berghot MA, Shoeib AI, Khalil AM. Eur J Med Chem 2010; 4:1843.
[8] Ragavan RV, Vijayakumar V, Kumari NS. Eur J Med Chem 2010; 4: 1173.
[9] Kucukguzel SG, Rollas S. Il Farmaco 2002; 57: 583.
[10] Daniele C, Fabrizio M, Marco R, Beatrice B, Mafalda P, Alessandro DL, Rita M, Manuela S, Maurizio B. Bioorg Med Chem 2009; 17: 5716.
[11] Kelekci NG, Yabanoglu S, Kupeli E, Salgin U, Ozgen O, Ucar G, Yesilada E, Kendi E, Yesilada A, Bilgin AA, Bioorg Med Chem 2007; 15: 5775.
[12] Amir M, Kumar H, Khan SA. Bioorg Med Chem Lett 2008;18: 918.
[13] Venkata Ramana P, Ravindranath LK. J Indian Chem Soc 1999; 76: 112.
[14] Nagaraju V, Srinivasulu R, Doraswamy K, Venkata Ramana P. J Indian Chem Soc 2011; 88: 293.
[15] Srinivasulu R, Nagaraju V, Doraswamy K, Venkata Ramana P. Journal of Chemical and Pharmaceutical Sciences 2010; 3: 141.
[16] Vogel's Text Book of Practical Organic Chemistry, Fifth edition (Addition Wesley Longman Limited, England) 1989.
[17] Garg HG, Joshi SS. J Indian Chem Soc 1960; 37: 626.
[18] Garg HG, Singh PP. 1968 J Mednl Pharm Chem 1968; 11 : 1103.
[19] Malik WU, Garg HG, Singh PP, Arora Veena. J Mednl Pharm Chem 1970; 13: 750.