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# Synthesis, Characterization and antimicrobial Evaluation of 2-{(2*E*)-2-[(1-aryl-3-phenyl-1*H*-pyrazol-4-yl)methylidene]hydrazinyl}-4-phenyl-1,3-thiazoles

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## ABSTRACT

*A series of 2-{(2E)-2-[(1-aryl-3-phenyl-1H-pyrazol-4-yl)methylidene]hydrazinyl}-4-phenyl-1,3-thiazoles were synthesized by the condensation of 4-substituted phenacyl bromides with substituted pyrazoline thiosemicarbazones. The thiosemicarbazones were in turn prepared by the reaction of 1-aryl-3-phenyl-1H-pyrazole-4-carbaldehyde with thiosemicarbazide in the presence of sodium acetate. The structures of newly synthesized compounds were characterized by elemental analysis, IR, $^1$H-NMR and mass spectral data. All compounds were screened for their antibacterial and antifungal studies. Compounds containing chloro and nitro groups showed promising activity.*

**Keywords:** Pyrazoles, thiosemicarbazones, thiazoles, antibacterial activity, antifungal activity.

## INTRODUCTION

The thiazole chemistry has been developed extensively and is still developing. Presently there are a number of drugs used clinically, which comprise of thiazole moiety substituted at various positions and in association with various other heterocyclic rings. They are mainly used as chemotherapeutic agents, diuretics and antihistamines. Pyrazoles are prominent nitrogen containing heterocyclic compounds play important role in medicinal chemistry. Considerable attention has been focused on pyrazole derivatives due to their interesting biological activities. They have found to possess antifungal [1], antibacterial [2], antidepressant [3], anticonvulsant [4,5], anti-inflammatory [6], anti-tumor [7], antidiabetic, anesthetic and analgesic [8,9] properties. Encouraged by the wide spectrum of activities exhibited these heterocycles and in continuation of our study on biologically active nitrogen heterocycles [10-12], we report herein the synthesis of a new series of thiazole derivatives in combination with the pyrazole nucleus along with their antibacterial and antifungal activity studies.

## MATERIALS AND METHODS

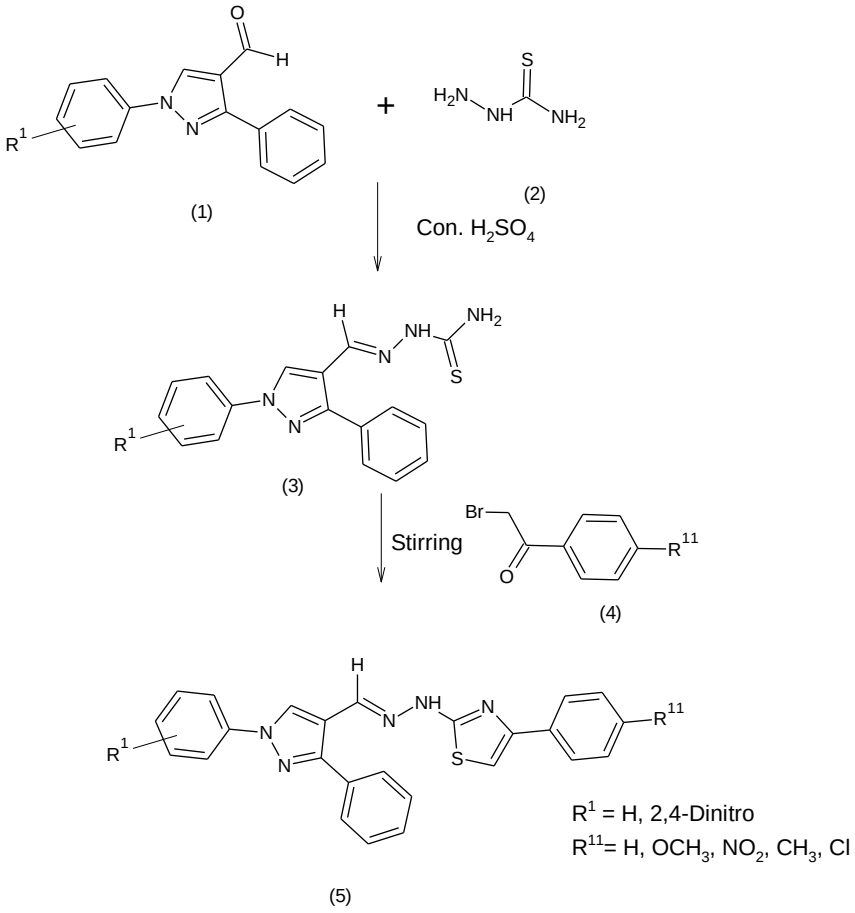
All chemicals used were of laboratory grade (Merck and Aldrich) and were purified by distillation or crystallization. The melting points of the newly synthesized compounds were determined in open capillaries and are uncorrected. The IR spectra were recorded on a JASCO FT IR 430 spectrophotometer

in KBr pellet. The $^1$H NMR spectra were recorded on a Bruker AV-III 400(L) (400MHz) NMR spectrometer using DMSO-$d_6$ and $CDCl_3$ as solvent and TMS as an internal standard. All chemical shift values are expressed in δ, scale downfield from TMS and proton signals are indicated as s = singlet, d = doublet, t = triplet, m = multiplet. Mass spectra were recorded on a Jeol JMS-D300 mass spectrometer operating at 70eV. The purity of all compounds was confirmed by TLC.

Among the various methods available for the synthesis of thiazoles, the Hantzch synthesis [13] is the method of choice, which involves the reaction of an α-halo carbonyl compound with an appropriate thiocarboxamide.

1-aryl-3-phenyl-1H-pyrazole-4-carbaldehyde (**1)** were prepared by the formylation of corresponding phenylhydrazones with dimethyl formamide in the presence of $POCl_3$ following method of Siddhartha Tarun et al. [14]. The 4-substituted phenacyl bromides (**4)** were obtained by the bromination of 4-substituted acetophenones with bromine in acetic acid.

**General method for the synthesis of (2*E*)-2-{[1-aryl-3-phenyl-1*H*-pyrazol-4-yl]methylidene} hydrazinecarbothioamide (3) :** To a solution of 1-aryl-3-phenyl-1H-pyrazole-4-carbaldehyde (**1**) (0.1 mol) in ethanol (25ml), thiosemicarbazide (0.1mol, 9.1g) in ethanol (30ml) and sodium acetate (0.05mol, 4.1g) in minimum amount of water was added. The reaction mixture was refluxed on a water bath for 4-6h. The solid obtained after cooling was separated and recrystallized from ethanol-dioxane mixture.



**(2*E*)-2-[(1,3-diphenyl-1*H*-pyrazol-4-yl)methylidene]hydrazinecarbothioamide (3a):** IR(KBr, $cm^{-1}$): 3410(NH str.); 3150 (NH str. of $NH_2$); 1610 (C=N); 1275(C=S str.); 1H NMR (400 MHz, $CDCl_3$): δ, 11.42 (s, 1H, N=CH); 7.80 (s, 1H, NH); 7.46–7.62 (m, 10H, Ar-H); 6.48 (s, 2H, $NH_2$); Nitrogen analysis: Found, 21.75; calculated, 21.81.

**(2*E*)-2-{[1-(2,4-dinitrophenyl)-3-phenyl-1*H*-pyrazol-4-yl]methylidene}hydrazinecarbothioamide (3b):** IR (KBr, $cm^{-1}$): 3409(NH str.); 3158 (NH str. of $NH_2$); 1595(C=N); 1270(C=S str.); 1H NMR (400 MHz, $CDCl_3$): δ, 11.46 (s, 1H,N=CH); 8.1 (s, 1H, NH); 7.46–7.84 (m, 8H, Ar-H); 6.42 (s, 2H, $NH_2$); MS m/z; 411 ($M^+$); Nitrogen analysis: Found, 23.64; calculated, 23.84.

**General procedure for the synthesis of 2-{(2*E*)-2-[(1-aryl-3-phenyl-1*H*-pyrazol-4-yl)methylidene]hydrazinyl}-4-phenyl-1,3-thiazoles (5) :** A mixture of (2*E*)-2-[(1,3-diphenyl-1*H*-pyrazol-4-yl)methylidene]hydrazine carbothioamide **(3)** (0.01 mol) in dioxane (20 mL) and 4-substituted phenacyl bromides (**4)** (0.01 mol) in ethanol (20 mL) was stirred at room temperature for 1 to 2 h. The solid separated was filtered, dried and recrystallised from ethanol-dioxane mixture. The yield, melting point and other characterization data of the newly synthesized compounds are given in Table **1**.

**Compound (5a):** IR (KBr) ν $cm^{-1}$: 3375 (N-H str.); 2923 (C-H str.); 1615 (C=N str.). 1H NMR (300MHz); solvent DMSO-$d_6$: δ, 7.180(s, 1H, NH); 7.810 (s, 1H, thiazole-5H) 7.710-7.820 (m, 14H, Ar-H); 9.000 (s, 1H, pyrazole-5H). 11.103 (s, 1H, N=CH). MS m/z; 421 ($M^+$);

**Compound (5b):** IR (KBr) ν $cm^{-1}$: 3375 (N-H str.); 2920 (C-H str.); 1600 (C=N str.); 1H NMR (400MHz); solvent DMSO-$d_6$: δ, 7.303 (s, 1H, NH); 7.424 (d, 2H, ortho protons of p-chlorophenyl); 7.655 (s, 1H, thiazole-5H); 7.708-7.828 (m, 12H, aromatic protons); 9.01 (s, 1H, pyrazole-5H)11.10 (s, 1H, N=CH). MS m/z; 455/457 ($M^+$).

**Compound (5c):** IR (KBr) ν $cm^{-1}$: 3385 (N-H str.); 2928 (C-H str.); 1620 (C=N str.); 1H NMR (400MHz): solvent DMSO-$d_6$: δ, 3.900(s, 3H, $OCH_3$); 7.100 (d, 2H, ortho protons of p-anisyl); 7.610 (d, 2H, meta protons of p-anisyl); 7.352(s, 1H, NH); 7.870 (s, 1H, thiazole-5H); 7.908-8.100(m, 10H, aromatic protons); 9.100 (s, 1H, pyrazole-5H);11.120 (s, 1H, N=CH). MS m/z; 451 ($M^+$);

**Compound (5d):** IR (KBr) ν $cm^{-1}$: 3380 (N-H str.); 2930 (C-H str.); 1620 (C=N str.): 1H NMR (400MHz); solvent DMSO-$d_6$: δ, 2.289 (s, 3H, $CH_3$); 7.223 (s, 1H, NH); 7.280 (d, 2H, ortho protons of p-tolyl); 7.460 (d, 2H, meta protons of p-tolyl); 7.652 (s, 1H, thiazole-5H); 7.708-8.100 (m, 10H, aromatic protons); 9.110 (s, 1H, pyrazole-5H); 11.100 (s, 1H, N=CH). MS m/z; 435 ($M^+$);

**Compound (5e):** IR (KBr) ν $cm^{-1}$: 3370 (N-H str.); 2910 (C-H str.); 01611 (C=N str.). ; 1H NMR (400MHz): solvent DMSO-$d_6$: δ, 7.250(s, 1H, NH); 7.640 (s, 1H, thiazole-5H); 7.760(d, 2H, ortho protons of p-nitrophenyl); 8.180 (d, 2H, meta protons of p-nitrophenyl); 7.680-7.710 (m, 10H, aromatic protons); 9.020 (s, 1H, pyrazole-5H); 11.120 (s, 1H, N=CH). MS m/z; 466 ($M^+$);

**Compound (5f):** IR (KBr) ν $cm^{-1}$: 3372 (N-H str.); 2922 (C-H str.); 1621 (C=N str.); 1H NMR (400MHz): solvent DMSO-$d_6$: δ, 7.233 (s, 1H, NH); 7.282-7.462 (m, 10H, aromatic protons); 7.650 (s, 1H, thiazole-5H); 7.610-7.810 (m, 3H, 2,4-dinitrophenyl protons); 9.150 (s, 1H, pyrazole-5H); 11.010 (s, 1H, N=CH). MS m/z; 511 ($M^+$).

**Compound (5g):** IR (KBr) ν $cm^{-1}$: 3375 (N-H str.); 2928 (C-H str.); 1625 (C=N str.).; 1H NMR (400MHz): solvent DMSO-$d_6$: δ, 7.333 (s, 1H, NH); 7.621 (s, 1H, thiazole-5H); 7.480- 8.120(m, 12H, aromatic protons); 9.000 (s, 1H, pyrazole-5H); 11.056 (s, 1H, N=CH). MS m/z; 545/547 ($M^+$).

**Compound (5h):** IR (KBr) ν $cm^{-1}$: 3362 (N-H str.); 2942 (C-H str.); 1635 (C=N str.).; 1H NMR (400MHz); solvent DMSO-$d_6$: δ, 2.860 (s, 3H, $OCH_3$); 7.330 (s, 1H, NH); 6.910 (d, 2H, ortho protons of p-anisyl); 7.400 (d, 2H, meta protons of p-anisyl); 7.500-7.880 (m, 8H, aromatic protons); 7.580 (s, 1H, thiazole-5H); 9.110 (s, 1H, pyrazole-5H);11.532 (s, 1H, N=CH). MS m/z; 541 ($M^+$).

**Compound (5i):** IR (KBr) ν $cm^{-1}$: 3342 (N-H str.); 2980 (C-H str.); 1631 (C=N str.); 1H NMR (400MHz); solvent DMSO-$d_6$: δ, 2.282 (s, 3H, $CH_3$); 7.252 (s, 1H, NH); 7.110 (d, 2H, ortho protons of p-tolyl); 7.400 (d, 2H, meta protons of p-tolyl);7.620-7.750 (m, 8H, aromatic protons); 7.612 (s, 1H, thiazole-5H); 9.100 (s, 1H, pyrazole-5H); 11.102 (s, 1H, N=CH). MS m/z; 525 ($M^+$).

**Compound (5j):** IR (KBr) ν $cm^{-1}$: 3368 (N-H str.); 2928 (C-H str.); 1600 (C=N str.). 1H NMR (400MHz); solvent DMSO-$d_6$: δ, 7.620 (s, 1H, NH); 7.782 (s, 1H, thiazole-5H); 7.760- 8.162(m, 12H, aromatic protons); 9.200 (s, 1H, pyrazole-5H); 11.092 (s, 1H, N=CH). MS m/z; 556 ($M^+$).

**Table-1:**- Characterization data of 2-{(2*E*)-2-[(1-aryl-3-phenyl-1*H*-pyrazol-4-yl)methylidene] hydrazinyl}-4-phenyl-1,3-thiazoles (5)

| Comp. No. | $R^1$ | $R^{11}$ | M.P.(°C) Yield (%) | Molecular formula | Colour and crystal nature | N Analysis (%) Found (calc) |
|---|---|---|---|---|---|---|
| **5a** | H | H | 213-215 75 | $C_{25}H_{19}N_5S$ | Red crystals | 16.50 (16.63) |
| **5b** | H | Cl | 195-196 70 | $C_{25}H_{18}N_5SCl$ | Brick red crystals | 15.25 (15.37) |
| **5c** | H | $OCH_3$ | 217-218 72 | $C_{26}H_{21}ON_5S$ | Red crystals | 15.66 (15.52) |
| **5d** | H | $CH_3$ | 212-214 70 | $C_{26}H_{21}N_5S$ | Red crystals | 16.05 (16.09) |
| **5e** | H | $NO_2$ | 215-217 65 | $C_{25}H_{18}O_2N_6S$ | Yellow crystals | 18.10 (18.03) |
| **5f** | 2,4 dinitro | H | 202-04 70 | $C_{25}H_{17}O_4N_7S$ | White crystals | 19.20 (19.18) |
| **5g** | 2,4 dinitro | Cl | 168-170 80 | $C_{25}H_{16}O_4N_7SCl$ | White crystals | 17.88 (17.97) |
| **5h** | 2,4 dinitro | $OCH_3$ | 178-180 65 | $C_{26}H_{19}O_5N_7S$ | Pale brown crystals | 18.14 (18.11) |
| **5i** | 2,4 dinitro | $CH_3$ | 144-146 73 | $C_{26}H_{19}O_4N_7S$ | Pale Yellow crystals | 18.72 (18.67) |
| **5j** | 2,4 dinitro | $NO_2$ | 178-80 75 | $C_{25}H_{16}O_6N_8S$ | White crystals | 20.12 (20.14) |

Solvent of crystallization: EtOH + Dioxane

**Antimicrobial activity:** All the newly synthesized compounds (**5**) were screened for their antibacterial activity in vitro against both Gram-positive and Gram-negative bacteria. *Staphylococcus aureus* (NCIM 2794), *Bacillius subtilis* (NCIM 2708), *Escherichia coli* (NCIM 2575), and *Pseudomonas aeruginosa* (NCIM 2053) were the microorganisms employed. Furacin was used as standard. The antifungal activity was screened in vitro against *Candida albicans* (NCIM 3466) and Flucanazol was the standard. The investigation was carried out by Minimum inhibitory concentration (MIC) by serial dilution method[15]. For this the compound whose MIC has to be determined is dissolved in serially diluted Dimethylformamide. Then a standard drop of the culture prepared for the assay is added to each of the dilutions and incubated for 16-18hrs at $37^0$C. MIC is the highest dilution of the compound, which shows clear fluid with no development of turbidity (Table-2).

**Table-2:** Antibacterial and antifungal Activity data of Thiazoles (5)

| Comp. No. | Antibacterial data in MIC (µg/ml) | | | | Antifungal data in MIC (µg/ml) |
|---|---|---|---|---|---|
| | *S. aer.* | *E. coli* | *P. aur.* | *B. subt.* | *C. albicans* |
| **5a** | 12.5 | 12.5 | 12.5 | 12.5 | 6 |
| **5b** | 6 | 6 | 6 | 6 | 6 |
| **5c** | 12.5 | 12.5 | 6 | 12.5 | 6 |
| **5d** | 12.5 | 12.5 | 12.5 | 12.5 | 6 |
| **5e** | 6 | 6 | 6 | 6 | 6 |
| **5f** | 12.5 | 6 | 6 | 6 | 6 |
| **5g** | 3 | 3 | 3 | 3 | 3 |
| **5h** | 6 | 6 | 12.5 | 6 | 6 |
| **5i** | 6 | 6 | 6 | 12.5 | 6 |
| **5j** | 3 | 3 | 3 | 3 | 3 |
| Furacin(STD) | 12.5 | 12.5 | 12.5 | 12.5 | - |
| Flucunazol(STD) | - | - | - | - | 6 |
| DMF (control) | - | - | - | - | - |

**Index for antibacterial and antifungal activity:**
Method : Minimum Inhibitory Concentration by serial dilution method,
Sample concentration: 1µg $ml^{-1}$, Medium used: Nutrient agar, Incubation period: 16-17h at $37^0$C,
Control : Dimethyl formamide, Standard for antibacterial: Furacin, Antifungal: Flucanazol.
Abbreviation used: *P. aer.: Pseudomonas aeruginosa, B. subt.: Bacillus subtilis, S. aur.: Staphylococcus aureus, E. coli.: Escherichia coli, C. albicans: Candida albicans.*

## RESULTS AND DISCUSSION

The structures of newly synthesized thiazoles (**5a-j**) were confirmed by analytical, IR, 1H NMR and mass spectral data. The characterization data of thiazoles (**5a-j**) are given in table **1.** The elemental analysis of newly synthesized compounds are in agreement with the theoretical values within the limits of experimental error.

The IR absorption bands corresponds to the $NH_2$ and C=S group of the thiosemicarbones were absent in the IR spectra of thiazoles (**5**) indicating the formation of the product. The evidence for the proposed thiazole structure was obtained by recording their 1H-NMR spectra. The thiazole -5H proton was observed as a sharp singlet at aroundδ, 7.5 to 7.7 which is in agreement with the earlier observation[16]. Further evidence is obtained by recording the mass spectra of thiazoles. The spectra were in agreement with respective molecular formula and proposed structure.

Antibacterial study reveals that compound **5g** and **5j** having chloro substituent and nitro groups exhibited maximum inhibition against all the tested microorganisms. Compounds **5b, 5e, 5f, 5h,** and **5i** showed activity at concentration lower than that of standard against almost all the four organisms. All remaining compounds showed moderate inhibition. Similarly compounds containing chloro and nitro substituents

exhibited significant antifungal activity against tested microorganism. Structure activity relationship (SAR) study of the substitution pattern of the aryl group towards antibacterial and antifungal activity have shown that electron withdrawing groups causes more activity.

## APPLICATIONS

The present study has shown that the selected compounds were possesses remarkable microbial activity against human pathogens. This research strongly supports the effectiveness of these drugs against different microbial diseases.

## CONCLUSIONS

The new thiazoles were synthesized in good yield and structures were confirmed by spectral and analytical data. The antibacterial activities of the synthesized thiazole derivatives were effective against gram positive and gram negative organisms respectively. The antifungal activity showed good activity against tested fungi. The results of antimicrobial screening data revealed that all the compounds 5a-j showed considerable and varied activity against the selected microorganisms. Structure activity relationship (SAR) study of the substitution pattern of the aryl group towards antibacterial and antifungal activity have shown that electron withdrawing and donating groups causes, respectively more and less activity.

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