Original Research Article

DESIGN, SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF NEW PYRAZOLINE-5-ONES

Krishna Naik*, Aluru Raghavendra Guru Prasad[2], Yadati Narasimha Spoorthy[1], Lakshmana Rao Krishna Rao Ravindranath[1]

1. Sri Krishnadevaraya Univerisity, Anantapur, Andhra Pradesh, India.
2. ICFAI Foundation for Higher Education, Hyderabad, Andhra Pradesh, India.

ABSTRACT

Purpose: To synthesise, charecterise phenyl hydrazones namely, {4-[3-methyl-5-oxo-4-(phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid N^{l}-(4-substituted-thiazole-2-yl)-hydrazide VII and to evaluate the antibacrial activity.

Methods: The synthesis of title compounds has been schemed elaborately and the structures of the compounds were established by elemental analysis, IR, ^{1}H NMR and mass spectra. The antibacterial activity of the title compounds were evaluated against *Staphylococcus aureus* NCCS 2079 and *Bacillus cereus* NCCS 2106, *Escherichia coli* NCCS 265 and *Pseudomonas aeruginosa* NCCS 2200 and antifungal activity evaluated against *Aspergillus niger* NCCS 1196, *Candida albicans NCCS* 2106 by disk diffusion method.

Results: The screened data reveal that the studied phenyl hydrazones under study exhibited promising antimicrobial activity against all the tested microbes. The antimicrobial activity of title compounds were compared with that of standadards. The title compounds with *p*-nitrophenyl, *p*-chlorophenyl, *p*-bromophenyl were more active agaist bacteria, where as the compounds with substituents namely phenyl, *p*-tolyl, *p*-anisyl, *p*-hydroxyphenyl, *p*-nitrophenyl were more active against fungi than the other compounds under investigation.

Conclusion: Among the ten novel phenyl hydrazones synthesised {4-[3-methyl-5-oxo-4-(phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid N^{l}-(4-(4-nitro-phenyl)-thiazole-2-yl)-hydrazide was found the most active than the others. The other compounds demonstrated moderate activity against the tested microorganisms.

Keywords: Phenyl hydrdrazones, Synthesis, Structural elucidation, Antibacterial activity, Antifungal activity.

***Corresponding Author*: Prof. Guru Prasad,** ICFAI Foundation for Higher Education, Hyderabad, Andhra Pradesh, India. Email: guruprasadar@yahoo.co.in

INTRODUCTION

Inspite of many significant advances in antibacterial therapy, infectious diseases caused by bacteria and microbes have increased dramatically in recent years. This is due to the increased resistance of disease causing microbes to antibiotic drug therapy. The antibiotic

prescription, whether appropriate or inappropriate, can contribute to the surfacing of antibiotic resistant bacteria. In case of appropriate priscriptions, the disadvantage of emergence of antibiotic resistant bacteria is offset by the necessity for the treatment of the concerned bacterial infection. The inappropriate priscriptions do not lead to any kind of benefit to the patient but only increase the spread of resistant bacteria. However in both the cases, use of antibiotics is directly related to the spread of antibiotic resistant bacteria (Ballow and Schentag, 1992, McGowan, 1983, Mouton *et al.,* 1990 Ringertz and Kronvall 1987). On the other hand this scenario has neccissiated the development of new drugs to deal with resistant bacteria.

Nitrogen and sulfur heterocyclic systems have gained great deal of importance because of their diverse pharmacological properties and hense are extensively integrated into new drugs. Particularly compounds containing the pyrzoline ring system and thiazole ring system are known to possess potent therapeutic properties (Andreani *et al.,* 1996, Kucukguzel *et al.,* 2006, Pattan *et al.,* 2006, Pushkal *et al.,* 2012, Prem *et al.,* 1977, Narayana *et al.,* 2006, Tapia *et al.,* 2003, Verma and Saraf 2008). Keeping these details and the need for the development of new drugs in view, we herein report a new series of compounds containing these multiactive nuclei to ensure wide spectrum of antimicrobial activity.

EXPERIMENTAL

Instuments and Chemicals

IR spectra were recorded on a Perkin-Elmer 983 IR spectrometer. ^{1}H NMR spectra were recorded on a Bruker AC 300F (200 MHz) NMR spectrometer using DMSO – d_6 as a solvent and TMS as an internal standard. Mass spectra of the compounds were recorded on a Jeol JMS-D300 mass spectrometer operating at 70 eV.

Nutrient broth, nutrient agar and 5 mm diameter antibiotic assay discs were obtained from Hi-Media Laboratories Limited, India. All the reagents and chemicals used were of analytical reagent grade procured from Ranbaxy Laboratories Ltd, India. The standard bacterial and fungal strains were procured from National Centre for Cell Science, Pune, India.

Synthesis of title compounds

The substituted aryl bromides were sysnthesysed by the procedure mentioned in the literature [13].

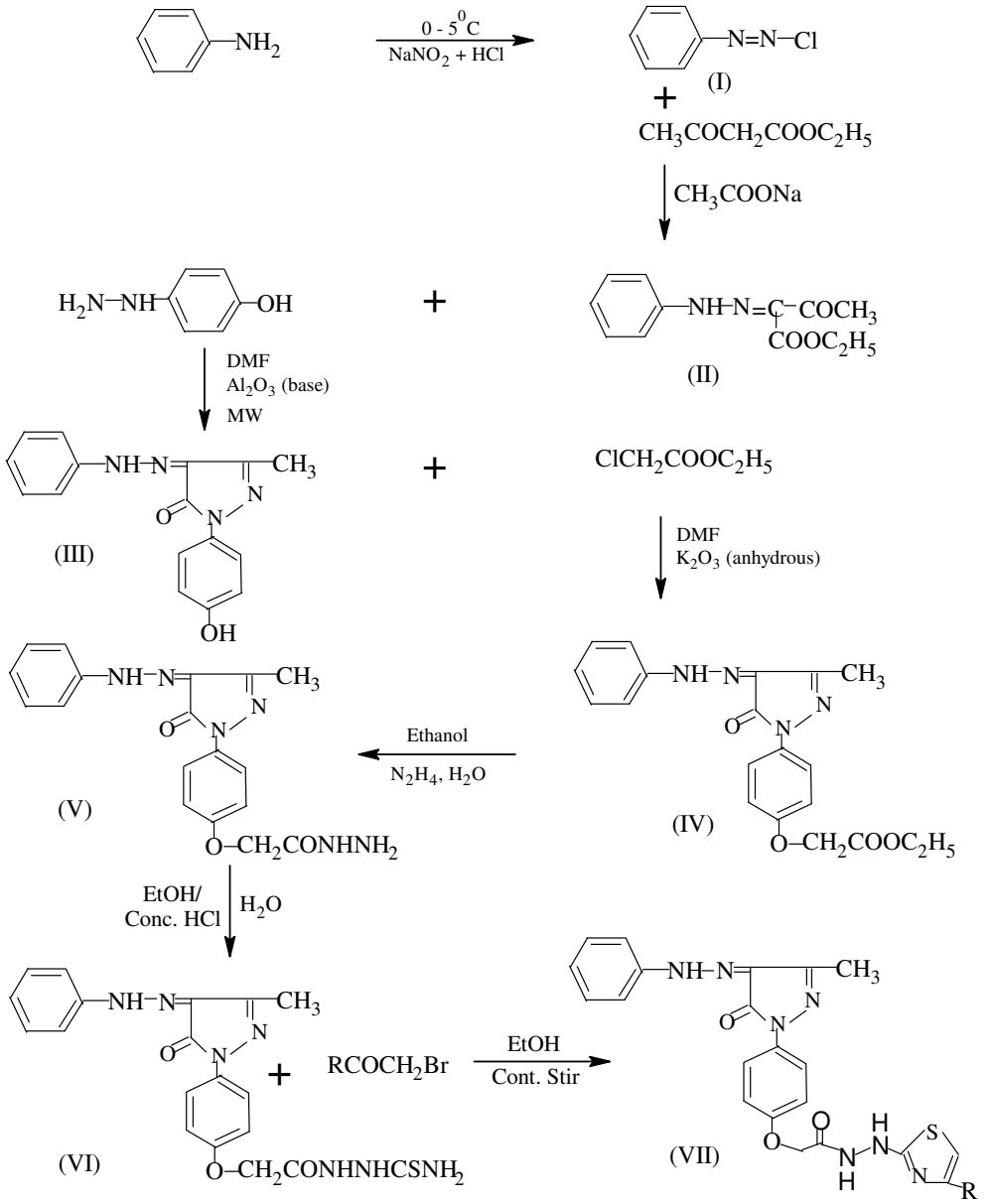
Synthesis of {4-[3-Methyl-5-oxo-4-(phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid ethyl ester (V)

Phenyl diazonium acetoacetic ester (II) was prepared by the procuedure mentioned in the literature (Dastagiri Reddy *et al.,* 2013)[13].

Phenyl hydrazono aceto acetic ester (II) and hydrazine in the presence of dimethyl formamide (10 drops) under microwave irradiation at 150W intermittently at 30 sec intervals for 2 minutes resulted in the formation of 4-[3-Methyl-4-(phenyl hydrazono)-pyrazoline-3-one III. The precipitate of 4-[3-Methyl-4-(hydrazono)-pyrazoline-3-one III was filtered and recrystallized from ethanol.

A mixture of III, anhydrous K_2CO_3 and dimethylformamide was continuously stirred at room temperature for 8 hours. The reaction mixture was diluted with ice cold water. The

solid separated was collected by filtration, recrystalized from ethanol and was identified as {4-[3-Methyl-5-oxo-4-(phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid ethyl ester (IV).



Scheme 1. **Synthesis of 4-[3-methyl-5-oxo-4-(phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid N'-(4-substituted-thiazole-2-yl)-hydrazide VII**

A solution of IV and hydrazine hydrate in ethanol was refluxed for 5 hours. The reaction mixture was cooled, poured on to ice cold water. The separated solid was filtered, washed with water and recrystalized from ethanol to get {4-[3-Methyl-5-oxo-4--(phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid hydrazide V.

Synthesis of {4-[3-methyl-5-oxo-4-(phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-aceto thiosemicarbazone VI.

A mixture of {4-[3-Methyl-5-oxo-4--(phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid hydrazide V (0.01 mole), potassium thiocyanate (0.02 mole), concentrated hydrochloric acid (1 mL), ethyl alcohol (10 mL) and water (20 mL) were refluxed for 3 hours. The solution was cooled, filtered, the precipitate was washed with water, dried and recrystallized from ethanol-DMF mixture to yield 3-methyl-5-oxo-4(phenylhydrazono)4,5-dihydropyrazol-1-ylacetothiosemicarbazone VI.

Synthesis of {4-[3-methyl-5-oxo-4-(phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid N^{I}-(4-substituted-thiazole-2-yl)-hydrazide VII.

A mixture of {4-[3-methyl-5-oxo-4(phenyl hydrazono)-4,5-dihydro pyrazol-1-yl]-phenoxyamino}-aceto thiosemicarbazone VI (0.01 mole) in dimethylformamide (10 mL) and bromo acetophenone (0.01 mol) in ethanol (10mL) was stirred at room temperature for 2 hours. The solid seperted was filtered, dried, recrystallized from ethanol-DMF mixture and found to be {4-[3-methyl-5-oxo-4-(phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid N^{I}-(4-phenyl-thiazole-2-yl)-hydrazide VIIa. The detailed steps leading to the formation of title compounds is given in Scheme 1.

The above reaction was extended to different bromo acetyl derivatives i.e. *p*-tolyl, *p*-anisyl, *p*-hydroxyphenyl, *p*-nitrophenyl, *p*-chlorohphenyl, *p*-bromophenyl, phenylsidnonyl, N-*p*-tolylsydnonyl, N-*p*-anisylsidnonyl.

RESULTS AND DISCUSSION

{4-[3-Methyl-5-oxo-4--(phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid hydrazide V was characterized by means of their elemental analysis, I.R, ^{1}H NMR and mass spectral data.

Characterization data of {4-[3-Methyl-5-oxo-4-(phenyl hydrazono)-4,5-dihydro-pyrayol-1-yl]-phenoxy}-acetic acid hydrazide V

m.p.: 152 ^{o}C, Yield: 65%, Molecular formula: $C_{18}H_{18}N_6O_3$, Molecular mass: 366, Elemental analysis Found % (Calc. %): C 59.20 (59.01), H 5.03 (4.95), N 22.99 (22.94), O 13.15 (13.10), IR (KBr) (ν_{max} in cm^{-1}): 3445, 3425 (NH_2), 3305 (NH), 1665 (C=O), 1620 (C=N), ^{1}HNMR (DMSO-d_6) (δppm): 1.2 (s, 3H, CH_3), 2.1(s, 2H, NH_2), 3.85 (s, 2H, N-CH_2-CO), 6.8 (s, 1H, Ar- NH), 7.0 (s, H, HN-N=C), 7.1-7.3 (m, 5H, C_6H_5), 7.4 (d, 2H, C_6H_4), 7.7 (d, 2H, C_6H_4), 8.4 (s, 1H, CO-NH).

The structure of 3-methyl-5-oxo-4(phenylhydrazono) 4,5-dihydropyrazol-1-ylacetothiosemicarbazone VI was conformed by IR, ^{1}HNMR and mass spectral data.

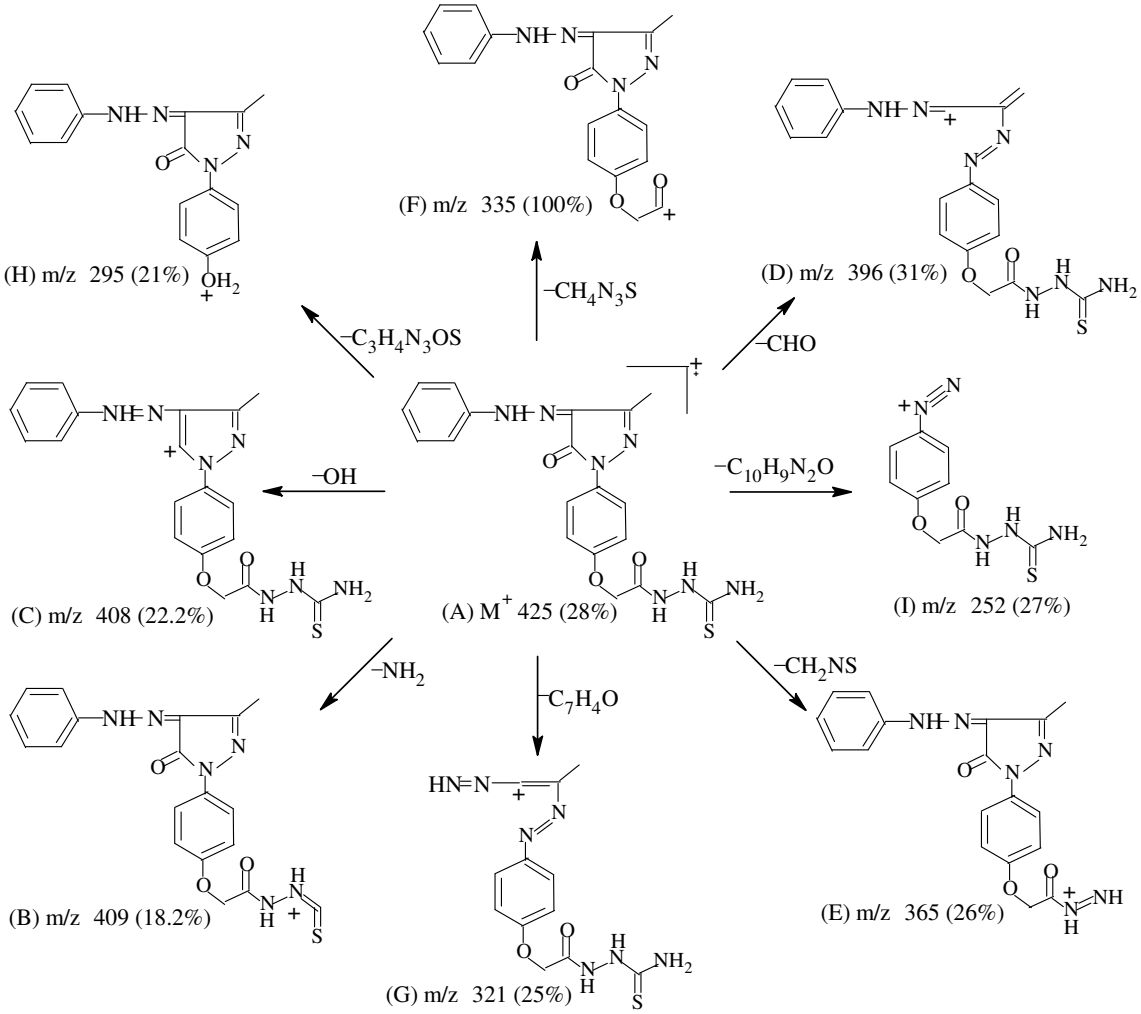
Characterisation of {4-[3-methyl-5-oxo-4-(phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-aceto thiosemicarbazone VI.

m.p.: 213^{0}C, Yield: 78%, Molecular formula: $C_{19}H_{19}N_7O_3S$, Molecular mass: 425, Elemental analysis Found % (Calc. %): C 53.60 (53.64), H 4.61 (4.50), N 22.96 (23.04), O 11.32 (11.28), S 7.48 (7.54), IR (KBr)(ν_{max} in cm^{-1}): 3260 (Ar-NH), 3180 (NH), 2959 (C–H), 1685 (C=O), 1622 (C=N) and 1176 (C=S), ^{1}HNMR (DMSO-d_6) (δppm): 2.29 (s, 3H, CH3), 3.3 (s, 2H, NH_2), 4.80 (s, 2H, N-CH_2), 6.8 (s, 1H, Ar-NH), 7.1-7.3 (m, 5H, Ar-H), 7.4 (d, 2H, C_6H_4), 7.7 (d, 2H, C_6H_4) and 9.36 and 10.27 due to (NH-NH),

Mass spectral details

The mass spectra of {4-[-3-methyl-5-oxo-4-(phenylhydrazono)-4,5-dihydro pyrazol-1-yl]-phenoxy}-aceto thiosemicarbazone VI exhibit the molecular ion peak (M^+) at m/z 425 (A, 27.8%)..

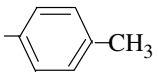
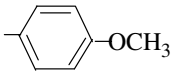
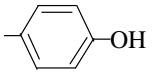
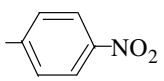
The fragmentation pattern noticed in mass spectrum of VI is presented in Scheme 2. Disintigration of molecular ion A yielded the cation at m/z 409 (B, 18.5%) by the loss of NH_2 radical. Elimination of OH radical from molecular ion resulted in the fragment C at m/z 408 (C, 22.2%). Expulsion of CHO radical from molecular ion produced the fragment D at m/z 396 (D, 30.8%). Elimination of CH_2NS radical afforded the cation E at m/z 365 (E, 25.5%). The loss of CH_4N_3S radical from the molecular ion yielded the cation F at m/z 335 (F, 100%) and has appeared as a base peak. The other fragmentations are noticed at m/z 321 (G, 24.9%), m/z 295 (H, 20.6%) and m/z 252 (I, 27.0%).

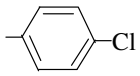
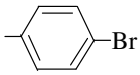
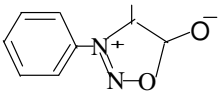
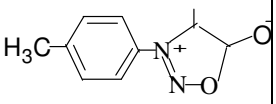
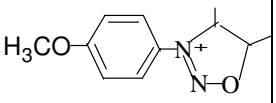


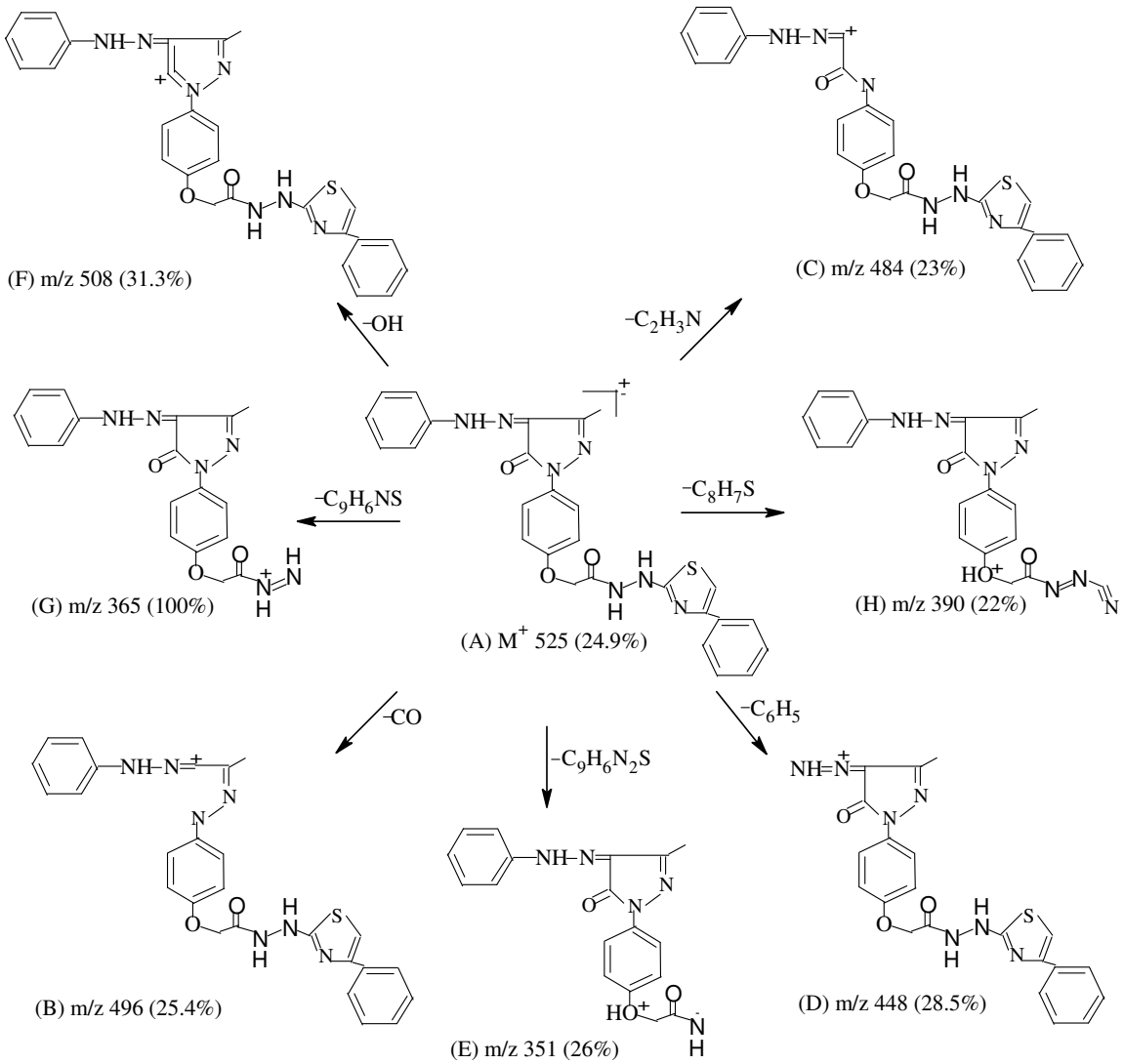
Scheme 2. Mass spectral fragmentation details {4-[3-methyl-5-oxo-4-(phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-aceto thiosemicarbazone VI.

The compounds synthesized 4-[3-methyl-5-oxo-4-(phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid N^l-(4-substituted thiazole-2-yl)-hydrazide VII a – j have been characterized by means of elemental analysis, IR, ^{1}H NMR and mass spectra

. **Characterization data of {-4-[3- methyl-5-oxo-4-(4[I]-phenyl- hydrazono)- 4,5-dihydro- pyrazol-1-yl]-phenoxy}-acetic acid- N-(4[I]phenyl-thiazol-2-yl)-hydrazide VII**

Compound	Substituent (-R)	Characterisation
VIIa	phenyl 	m.p.: 185 ^{O}C; Yield: 75%; Molecular formula: $C_{27}H_{23}N_7O_3S$; Molecular weight: 525; Elemental analysis Found % (Calc. %): C 61.83 (61.70), H 4.39 (4.41), N 18.70 (18.65), O 9.16 (9.13), S 6.01 (6.10); IR (KBr)(ν_{max} in cm^{-1}): 3230 (NH), 2962 (CH), 1692 (C=O), 1546 (C=N); ^{1}HNMR (DMSO-d_6) (δppm): 2.23 (s, 3H, CH_3), 4.90 (s, 2H, N-CH_2-CO), 6.8 (s, H, Ar-NH), 7.0 (s, H, thiazole-4H), 7.1-7.3 (m, 10H, Ar-H), 7.4 (d, 2H, C_6H_4), 7.7 (d, 2H, C_6H_4), 9.54 (s, H, NH), 10.65 (s, H, CONH).
VIIb	*p*-tolyl 	m.p.: 197 ^{O}C; Yield: 77%; Molecular formula: $C_{28}H_{25}N_7O_3S$; Molecular weight: 539; Elemental analysis Found % (Calc. %): C 62.42 (62.32), H 4.46 (4.67), N 18.21 (18.17), O 8.92 (8.90), S 5.97 (5.94); IR (KBr)(ν_{max} in cm^{-1}): 3235(NH), 2964 (CH), 1699 (C=O), 1551 (C=N).
VIIc	*p*-anisyl 	m.p.: 194 ^{O}C; Yield: 72%; Molecular formula: $C_{28}H_{25}N_7O_4S$; Elemental analysis Found % (Calc. %): C 60.64 (60.53), H 4. (4.54), N 17.68 (17.65), O 11.55 (11.52), S 5.67 (5.77); IR (KBr)(ν_{max} in cm^{-1}): 3240 (NH), 2868 (CH), 1687 (C=O), 1546 (C=N).
VIId	*p*-hydroxyphenyl 	m.p.: 190 ^{O}C; Yield: 73%; Molecular formula: $C_{27}H_{23}N_7O_4S$; Molecular weight: 541; Elemental analysis Found % (Calc. %): C 60.01 (59.88), H 4.19 (4.28), N 18.14 (18.10), O 11.85 (11.82), S 5.98 (5.92);, IR (KBr)(ν_{max} in cm^{-1}): 3250 (NH), 2972 (CH), 1710 (C=O), 1552 (C=N); ^{1}HNMR (DMSO-d_6) (δppm): 2.27 (s, 3H, CH_3), 4.2 (s,1H,-OH), 4.90 (s, 2H, N-CH_2-CO), 6.8 (s, H, Ar-NH), 7.1-7.3 (m, 9H, Ar-H), 7.4 (d, 2H, C_6H_4),7.5 (s, H, thiazole-4H), 7.7 (d, 2H, C_6H_4), 9.56 (s, H, NH), 10.67 (s, H, CO-NH).
VIIe	*p*-nitrophenyl 	m.p.: 187 ^{O}C; Yield: 75%; Molecular formula: $C_{27}H_{22}N_8O_5S$; Molecular weight: 570; Elemental analysis Found % (Calc. %): C 56.94 (56.84), H 3.69 (3.89), N 19.68 (19.64),

		O 14.05 (14.02), S 5.66 (5.62); IR (KBr)(ν_{max} in cm^{-1}): 3260 (NH), 2980 (CH), 1720 (C=O), 1560 (C=N):
VIIf	*p*-chlorophenyl 	m.p.: 188 ^{O}C; Yield: 78%; Molecular formula: $C_{27}H_{22}N_7O_3SCl$; Molecular weight: 560; Elemental analysis Found % (Calc. %): C 58.01 (57.91), H 3.76 (3.96), N 17.54 (17.51), O 8.59 (8.57), S 5.72 (5.73), Cl 6.35 (6.33); IR (KBr)(ν_{max} in cm^{-1}): 3222 (NH), 2960 (CH), 1687 (C=O), 1548 (C=N); ^{1}HNMR (DMSO-d_6) (δppm): 2.31 (s, 3H, CH_3), 4.92 (s, 2H, N-CH_2-CO), 6.8 (s, H, Ar-NH), 7.1-7.3 (m, 9H, Ar-H), 7.4 (d, 2H, C_6H_4), 7.7 (d, 2H, C_6H_4), 7.9 (s, H, thiazole-4H), 9.69 (s, H, NH), 10.71 (s, H, CO-NH).
VIIg	*p*-bromophenyl 	m.p.: 195 ^{O}C; Yield: 85%, Molecular formula: $C_{27}H_{22}N_7O_3SBr$, Molecular weight: 604; Elemental analysis Found % (Calc. %): C 53.74 (53.65), H 3.58 (3.67), N 16.25 (16.22), O 7.96 (7.94), S 5.33 (5.30), Br 13.25 (13.22); IR (KBr)(ν_{max} in cm^{-1}): 3232 (NH), 2956 (CH), 1690 (C=O), 1546 (C=N).
VIIh	phenyl sydnonyl 	m.p.: 192 ^{O}C; Yield: 80%, Molecular formula: $C_{29}H_{25}N_9O_5S$, Molecular weight: 611; Elemental analysis Found % (Calc. %): C 57.05 (56.95), H 3.98 (4.12), N 20.32 (20.61), O 13.12 (13.08), S 5.30 (5.24); IR (KBr)(ν_{max} in cm^{-1}): 3276 (NH), 2930 (CH), 1690 (C=O), 1560 (C=N).
VIIi	N-*p*-tolyl sydnonyl 	m.p.: 191 ^{O}C; Yield: 80%, Molecular formula: $C_{30}H_{27}N_9O_6S$, Molecular weight: 625; Elemental analysis Found % (Calc. %): C 57.65 (57.59), H 4.20 (4.35), N 20.26 (20.15), O 12.81 (12.79), S 5.19 (5.13); IR (KBr)(ν_{max} in cm^{-1}): 3276 (NH), 2941 (CH), 1689 (C=O), 1559 (C=N), 1739 (sydnone C=O str).
VIIj	N-*p*-anisyl sydnonyl 	m.p.: 186 ^{O}C; Yield: 82%; Molecular formula: $C_{30}H_{27}N_9O_6S$; Molecular weight: 641; Elemental analysis Found % (Calc. %): C 56.06 (56.15), H 4.13 (4.24), N 19.75 (19.65), 15.03 (14.96), S 5.07 (5.00); IR (KBr)(ν_{max} in cm^{-1}): 3286 (NH), 2951 (CH), 1678 (C=O), 1560 (C=N), 1745 (sydnone C=O str):



Scheme 3. **Mass spectral fragmentation details {4-[3-methyl-5-oxo-4-(phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid N^{l}-(4-phenyl-thiazole-2-yl)-hydrazide VIIa**

Mass spectral details

The mass spectrum of {4-[3-methyl-5-oxo-4-(phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid N^{l}-(4-phenyl-thiazole-2-yl)-hydrazide VIIa exhibited the molecular ion (M^+) peak at m/z 525 (A, 24.9%) indicating in the presence of odd number of nitrogens. The fragmentation pattern noticed in the mass spectrum of VIIa is presented in Scheme 3. Disintigration of molecular ion A resulted in cation B at m/z 496 (B, 25.4%). Loss of C_2H_3N radical from molecular ion afforded cation C at m/z 484 (C, 23.0%). Expulsion of C_6H_5 radical from molecular ion produced the fragment D at m/z 448 (D, 28.5%). The other important fragaments were noticed at m/z 351 (E, 26.0%), 508 (F, 31.3%), m/z 365 (G, 100%) and 390 (H, 22.0%). The base peak was noticed at m/z 365 (G, 100%).

Antimicrobial activity

The antimicrobial activity of title compounds was studied by disc diffusion method (Ericsson and Sherris, 1971)[14] against certain pathogenic organisms.

Table 1. Antibacterial/antifungal activity studies

Compound	Zone of inhibition (mm)*					
	Bacterial screened				Fungi screened	
	Staphylococus aureus NCCS 2079	*Bacillus Cereus* NCCS 2106	*Escherichia coli* NCCS 2065	*Pseudomanas aeruginos* NCCS 2200	*Aspergillus niger* NCCS 1196	*Candida albicans* NCCS 2106
VIIa	6	7	7	6	21	25
VIIb	5	6	6	7	18	22
VIIc	6	6	7	6	22	27
VIId	7	6	6	5	20	24
VIIe	11	12	13	11	20	24
VIIf	10	11	11	10	17	19
VIIg	10	9	11	10	14	18
VIIh	6	7	7	7	16	16
VIIi	6	6	7	6	15	17
VIIj	5	6	7	6	17	15
Amoxcycillin	21	27	24	22		
Cefaclor	19	22	19	20		
Ketoconazole					22	25

*Average of three determinations

The antibacterial activity of synthesized compounds (250 µg/mL using DMSO as a solvent) was studied against gram positive bacteria namely *Staphylococcus aureus* NCCS 2079 and *Bacillus cereus* NCCS 2106. The gram negative bacterial screened were *Escherichia coli* NCCS 265 and *Pseudomonas aeruginosa* NCCS 2200. Amoxicillin 10 µg/mL and cefaclor 30 µg/mL were used as a standard for assessing the antibacterial studies. The antifungal activity of synthesized compounds (100 µg/mL using DMSO as a solvent) was studied against *Aspergillus niger* NCCS 1196 and *Candida albicans* NCCS 3471. Ketaconazole 50 µg/mL was used as a standard for assessing the antifungal studies.

It can be seen from the Table 1that, VIIe, f and g with substituents namely *p*-nitrophenyl, *p*-chlorophenyl, *p*-bromophenyl were more active agaist bacteria, where as the compounds VIIa, b, c, d and e with substituents namely phenyl, *p*-tolyl, *p*-anisyl, *p*-hydroxyphenyl, *p*-nitrophenyl were more active against fungi than the other compounds under study. The compound VIIe with *p*-nitrophenyl substituent was found to exhibit significant activity against both bacteria and fungi.

COCLUSION

The article describes the synthesis and characterization of new N and S bearing heterocyclic compounds. The antimicrobial activity of these compounds was evaluated against gram positive, gram negative bacteria and fungi. The results reveal that all the compound showed significant antimicrobial activity against tested bacteria and fungi.

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