Original Research Article

SYNTHESIS, ANTIBACTERIAL, AND ANTIFUNGAL EVALUATION OF NOVEL MANNICH BASES COMPOUNDS CONTAINING OXADIAZOLE AND PYRAZOLE MOIETIES

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

1. Sri Krishnadevaraya University, Anatapur, A.P., India.
2. ICFAI Foundation for higher Education, Hyderabad, A.P., India.

ABSTRACT

Purpose: To synthesize novel series of compounds containing 1,3,4-oxadiazole and pyrazole-3-one moieties in good yields and to evaluate the antimicrobial activity of title compounds.

Methods: Title compounds were characterized by IR, ^{1}H NMR and mass spectra and evaluated for their antimicrobial activities against certain selected gram-positive bacteria, gram-negative bacteria and fungi by disk diffusion method and broth dilution method.

Results: Fluoro, chloro, bromo, nitro, morphonilyl, piperizynyl, N-methylpiperizine substituted compounds (VII c, d, e, f, i, j and k) showed more antimicrobial activity against than other compounds of the series.

Conclusion: Antimicrobial activity was expressed as the corresponding minimum inhibitory concentration (MIC). The compounds have demonstrated considerable antibacterial and antifungal activities.

***Keywords*:** 1,3,4-oxadiazole moiety; Pyrazole-3-one moiety; Antibacterial activity; Antifungal activity; Minimum inhibitory concentration

***Corresponding author:* Maliki Reddy Dastagiri Reddy**, Sri Krishnadevaraya University, Anatapur, A.P., India. E.: drdastagirireddy@gmail.com

Running Title: Synthesis and antimicrobial activity of certain novel heterocycles

INTRODUCTION

Among the wide variety of heterocycles that have been explored for developing new therapeutic molecules, pyrazole-3-ones and 1,3,4-oxadiazoles attracted substantial attention of the medicinal chemists. The widespread use of 1,3,4-oxadiazoles and pyrazole-3-ones as scaffolds in medicinal chemistry establishes these moieties as an important bio-active class of heterocycles. 1,3,4-oxadiazoles have demonstrated versatile range of medicinal activities like antibacterial [1, 2], antifungal [3, 4], antitumor [5], antitubercular [6], anticonvulsant [7] and HIV-I inhibitory activities [8]. Same scale of medicinal importance is associated with compounds containing pyrazole-3-one moiety [9-15]. The present article is an effort to incorporate both these crucial functionalities in a single entity to exploit their collective medicinal potentials.

MATERIALS AND METHODS

Materials and instrumentations

Melting points were determined by using melting point apparatus procured from Bio Technics India Ltd. It consists of aluminum cylindrical block for heating, radial holes for a thermometer and glass capillary. All chemicals used were analytical grade obtained from Merck India Limited, India. The bacterial and fungal strains were procured from National Centre for Cell Science, Pune, India. UV/Visible spectrophotometer model 106 manufactured by Synstronics Instruments Ltd., Ahmadabad, India was used in the studies. The infrared spectra were recorded on Perkin–Elmer KBr spectrometer. ν values were expressed in cm^{-1}. ^{1}HNMR spectra were recorded on JEOL MODEL GSX 270 FT NMR Spectrometer and NMR 200 MHz Supercon machine, using $CDCl_3$ and DMSO–d_6 as solvents and TMS as an internal standard. Chemical shifts were expressed as δ values (ppm). Mass spectra of the compounds were recorded on a Jeol JMS-D300 mass spectrometer operating at 70 eV. . The amount of halogens present in the compound was determined by the procedure reported in the literature [16].

Synthesis of compounds of interest Synthesis of 2-[3-methyl-5-oxo-4-(phenylhydrazono)-4,5-dihydro-1H-pyrazol-1 yl]acetohydrazide (V) [17].

a. Synthesis of phenyl diazonium chloride (I)

The required primary amine was dissolved in a suitable volume of water containing 2.5 equivalents of hydrochloric acid (or sulphuric acid). The solution thus obtained was cooled to 0^{o}C to crystallize amine hydrochloride (or sulphate). To this, aqueous solution of sodium nitrite was added portion wise until there was excess of free nitrous acid.

b. Synthesis of ethyl 3-oxo-2-(phenylhydrazono) butanoate (II)

To an ice cold solution of a mixture of sodium acetate (1.0 g, 0.08 mol), 100 mL of aqueous alcohol (50%) and ethylacetoacetate (0.65 mL, 0.1 mol) in 50 mL of ethanol, the diazonium chloride I (0.72 g, 0.1 mol) was added till yellow crystals were separated out. These crystals were filtered, washed with water and dried under vacuum.

c. Synthesis of 3-methyl 4-(4'-substituted aryl hydrazono) pyrazoline-5-one (III)

A mixture of eqimolar quantities of 2-arylhydrazono acetoacetic ester (II), hydrazine and dimethyl formamide (0.5 mL) was subjected to microwave irradiation at 150 W intermittently at 30 s intervals for 2 min. After completion of the reaction, as indicated by TLC, the reaction mixture was cooled to room temperature and washed with cold water. The precipitated 3-methyl-4-(phenyl hydrazono)-pyrozoline-5-one was filtered and recrystallized from hot ethanol. To improve the recovery for reusing, Al_2O_3 (0.1 mmol) have been used in the reaction.

d. Synthesis of ethyl [(4E)-3-methyl-5-oxo-4-(phenylhydrazono)-4,5-dihydro-1H-pyrazol-1-yl]acetate (IV)

A mixture of III (0.2 mol), ethylchloloroacetate (0.2 mol), anhydrous K_2CO_3 (0.03 mol) and dimethylformamide was stirred at room temperature for 8 h. After the completion of reaction, reaction mixture was diluted with cold water. The precipitated solid was filtered and recrystallized from hot ethanol.

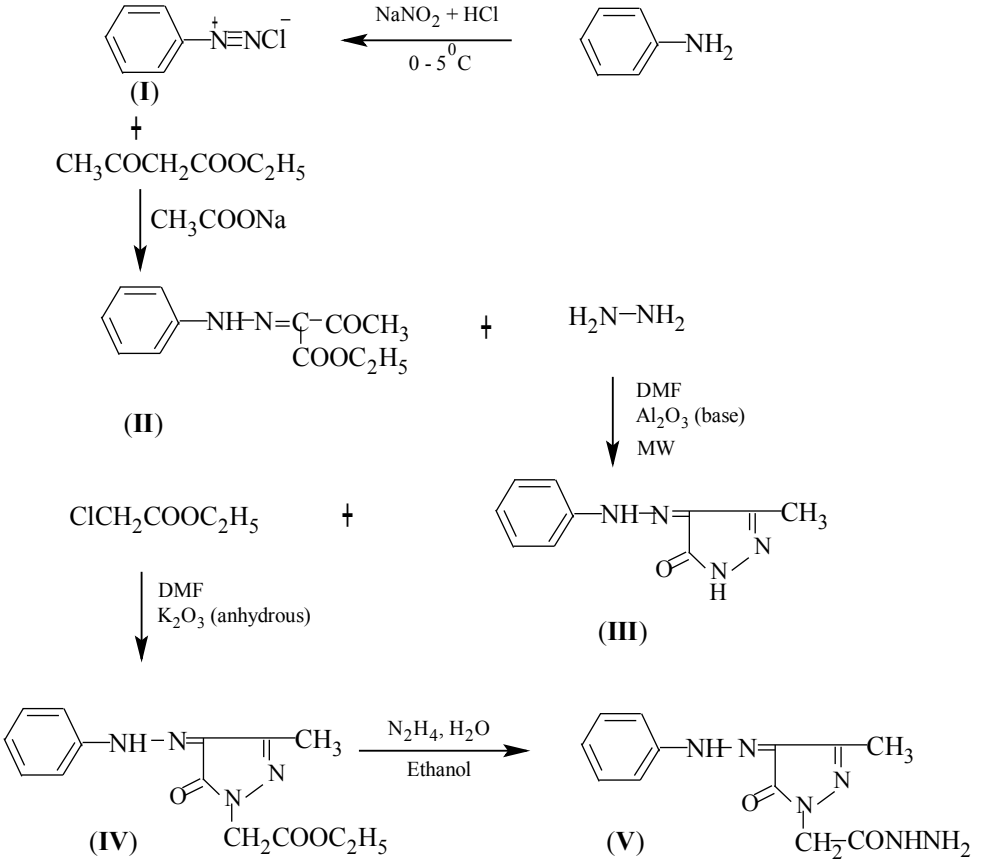
e. Synthesis of 2-[3-methyl-5-oxo-4-(phenylhydrazono)-4,5-dihydro-1H-pyrazol-1-yl]-acetohydrazide (V)

Equimolar solutions of IV (0.1 mol) and 99% hydrazine hydrate (0.1 mol) in ethanol (20 mL) was refluxed for five h. The reaction mixture was cooled and poured into cold water with continuous stirring. The precipitate so obtained was filtered, washed with water and recrystallized from ethanol to give V. The reaction sequence is shown in the Scheme 1.

Synthesis of certain novel Mannich bases bearing pyrazoline-3-one moiety.

a. Synthesis of 3-methyl-1-[(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-1H-pyrazole-4,5-dione 4-(phenylhydrazones)(VI)

A mixture of V (19.9 g, 0.1 mol), KOH (5.5 g, 0.1 mol) ethanol (100 mL) and carbon disulphide (6 mL, 0.1 mol) were refluxed in a round bottom flask for 2-3 h. After the completion of the reaction, excess of alcohol was removed by distillation. The reaction mixture was cooled, poured into cold water and neutralized with dilute hydrochloric acid. The solid precipitated (VI) was filtered, washed thoroughly with water and recrystallized from ethanol-dioxane mixture.

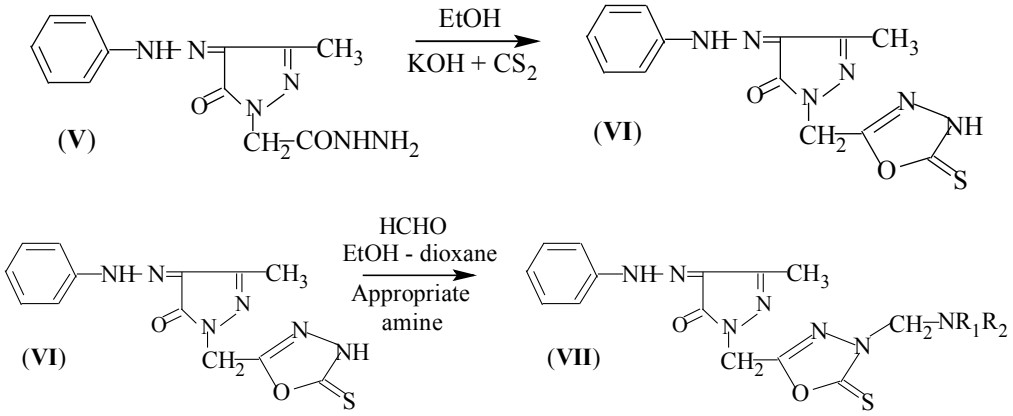


Scheme 1. General proedure for the synthesis of 2-[3-methyl-5-oxo-4-(phenylhydrazono)-4,5-dihydro-1*H*-pyrazol-1-yl]acetohydrazide (V).

b. S*ynthesis of 3-methyl-1-({4-[(N-substituted and N,N-disubstituted amino)methyl]-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl}methyl)-1H-pyrazole-4,5-dione-4-(phenylhydrazones) (VII)*

A solution of VI (0.01 mol) in ethanol and dioxan mixture (1:1, 20 mL) mixed with formaldehyde (40%, 1.5 mL) was stirred over night with the solution of appropriate amine (0.01 mol) in ethanol (10 mL). The precipitated Mannich base (like VIIa) was collected by filtration, dried and recrystallized from ethanol-DMF mixture. The reaction sequence is depicted in the Scheme 2.

The treatment of **VI** with *p*-anisylamine/ *p*-flurophenylamine/ *p*-chlorophenylamine/ *p*-bromophenylamine/ *p*-nitrophenylamine/ diethyl amine/ diphenyl amine/ piperazine/ morpholine/ N-methyl piperizine in the presence of formaldehyde in ethanol-dioxane mixture leads to the formation of respective Mannich base **VIIa–k**. The purity of all the compounds was confirmed by TLC.



Scheme 2. Synthesis of Mannich bases containing 1,3,4-oxadiazole and pyrazol-3-one moieties.

R_1=-H; R_2=*p*-Tolyl, *p*-anisyl, *p*-fluorophenyl, *p*-chlorophenyl, *p*-bromophenyl, *p*-nitrophenyl, diethyl, diphenyl, morpholinyl, piperazinyl, N-methylpiprrazinyl.

Antibacterial and antifungal activities

Antibacterial and antifungal activities of the synthesized compounds were studied by disc diffusion method [18]. The activity of the synthesized compounds was expressed as minimum inhibitory concentration (MIC). Broth dilution method [19] was used to determine the minimum inhibitory concentration of an antimicrobial agent. The general procedures are given below.

Disc diffusion method

A suspension of *the* microorganism was added to sterile nutrient agar at 45ºC. The mixture was transferred to sterile petri dishes to give a depth of 3 to 4 mm and allowed to solidify. Precautions were taken to get uniform layer of medium on the plate. Sterile discs 5 mm in diameter (made from Whatmann Filter paper) were immersed in the solutions of synthesized compounds (250 µg/ml). An untreated control sample was always kept for comparison.

After the plates were allowed to stand for 1 h at room temperature to minimize the effects of variations in different time, they incubated at 37ºC for 24 h and observed for antimicrobial activity. The diameter of the zone of inhibition was measured in each plate. The average zone of inhibition was calculated.

Similar procedure was adopted for studying the antimicrobial activity against the other organisms.

Broth dilution method

Standardized inoculum (matched to McFarland $BaSO_4$ standard) of suspension of organisms were prepared. A series of glass tubes containing different concentrations of test compounds dissolved in DMSO and spiller in nutrient broth were incubated with one drop of inoculum and mixed gently by shaking the rack. Two growth control tubes were also prepared without the addition of test compound and its optical density was determined.

Tubes were incubated for 24 h at 37^{o}C in air. The turbidity produced in each tube was recorded by UV/Visible spectrophotometer. The turbidity produced by the broth (without inoculum) was considered as 100% transparency. The minimum inhibitory concentration was noted as the concentration of the test substance, which completely inhibits the growth of the microorganism i.e. 100% transparency.

RESULTS AND DISCUSSION

Compounds of each series (**V, VI** and **VII**) were characterized by elemental analysis and respective IR, ^{1}H and NMR spectra. The compounds **V** and **VII** were also characterized by mass spectra. The details are given below.

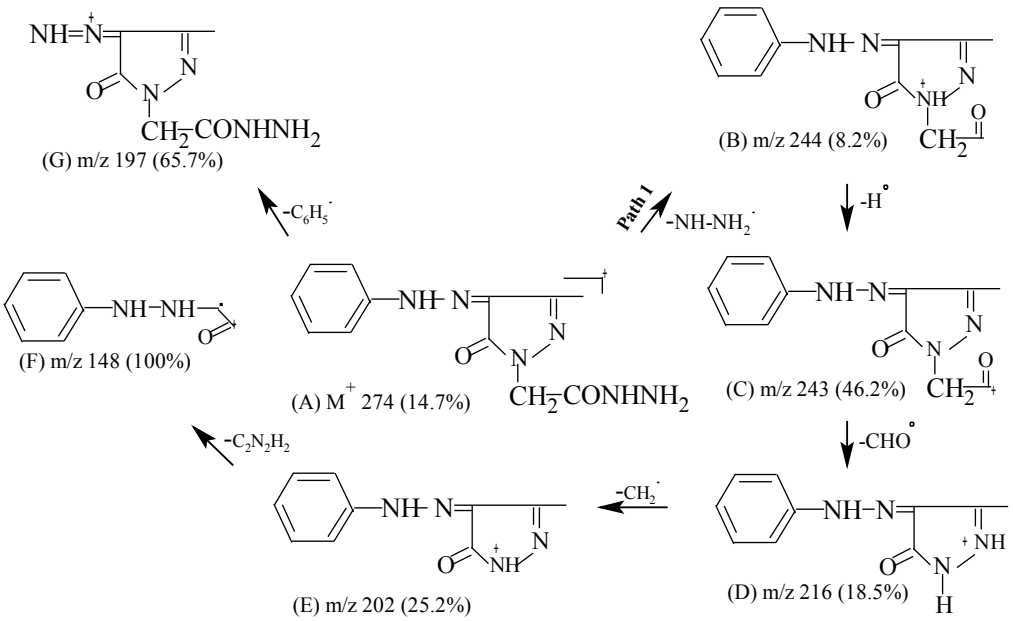
Characterization of compounds V, VI and VII

2-[3-methyl-5-oxo-4-(phenylhydrazono)-4,5-dihydro-1H-pyrazol-1-yl]-acetohydrazide (V)

Yeild 65%; m.p.152-153^{o}C; molecular formula $C_{12}H_{14}N_6O_2$; elemental analysis: C 52.63 (52.55), H 5.22 (5.14), N 30.71 (30.64), O 11.74 (11.67); IR (KBr) (ν_{max} in cm^{-1}): 3445, 3425 (NH_2), 3305 (NH), 1665 (C=O), 1620 (C=N); ^{1}H NMR (DMSO-d_6): δ 1.20 (s, 3H, CH_3), 2.10 (s, 2H, NH_2), 3.85 (s, 2H, N-CH_2-CO), 7.00 (s, 1H, Ar-NH), 7.30-7.40 (m, 5H, C_6H_5), 8.40 (s, 1H, NH).

Mass spectral data of V

The fragmentation process revealed by the mass spectrum of 2-[3-methyl-5-oxo-4-(phenylhydrazono)-4,5-dihydro-1*H*-pyrazol-1-yl]acetohydrazide V is depicted in Scheme 3. The spectrum showed the molecular ion (M^+) peak at m/z 274 with an intensity of 14.7%. Decomposition of molecular ion A along path I resulted in the formation of the fragment B at m/z 244 (8.2%), B loses a H radical atom to form cation C at m/z 243 (46.2%). Elimination of CHO molecule from C yielded cation D at m/z 216 (18.5%). Loss of methylene radical from D yielded cation E at m/z 202 (25.2%). Elimination of $C_2H_2N_2$ molecule from E resulted in the formation of radical cation F at m/z 148 (100%). Loss of C_6H_5 radical from molecular ion resulted in the formation of cation G at m/z 197 (65.7%).



Scheme 3. Mass spectral fragmentation of 2-[3-methyl-5-oxo-4-(phenylhydrazono)-4,5-dihydro-1*H*-pyrazol-1-yl]acetohydrazide (V).

3-methyl-1-[(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-1H-pyrazole-4,5-dione-4-(phenylhydrazone) VI

Yield 65%; m.p.150-151°C; molecular formula $C_{13}H_{12}N_6O_2S$; elemental analysis: C 49.50 (49.36), H 3.98 (3.82), N 26.66 (26.52), O 10.70 (10.12), S 10.32 (10.14); IR (KBr) (ν_{max} in cm^{-1}): 3126 (oxadiazole NH), 3180 (NH), 1670 (C=O), 1603 (C=N), 1134 (C=S); ^{1}H NMR (DMSO-d_6): δ 2.30 (s, 3H CH_3), 5.45 (s, 2H, N-CH_2-), 6.60–7.20 (m, 5H, Ar-H), 7.90 (s, H, Ar-NH), 14.70 (s, H, thiol–thione tautomeric proton NH)

Mannich bases (VII)

5-Methyl-2-{4-[(methyl-p-tolyl-amino)-methyl]-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-ylmethyl}-4-(phenyl-hydrazono)-2,4-dihydro-pyrazol-3-one (VIIa):

Yeild 75%; m.p. 240-241°C; molecular formula $C_{21}H_{21}N_7O_2S$; elemental analysis found % (Calc. %): C 57.82 (57.92), H 4.82 (4.86), N 22.45 (22.51), O 7.30 (7.35), S 7.25 (7.36); IR (KBr) (ν_{max} in cm^{-1}): 3250 (Ar-NH), 3140 (NH), 2939 (C-H), 1665 (C=O), 1608 (C=N), 1156 (C=S); ^{1}H NMR (DMSO-d_6): δ2.28 (s, 3H, CH_3), 2.36 (s, 3H CH_3), 5.00 (s, 2H, NCH_2), 5.64 (s, 2H, N-CH_2-N), 6.95 (d, 2H, Ar-H), 7.45 (d, 2H, Ar-H), 7.80 (s, H, Ar-NH), 11.20 (s, H, Ar-NH).

2-(4-{[(4-Methoxy-phenyl)-methyl-amino]-methyl}-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-ylmethyl}-5-methyl-4-(phenyl-hydrazono)-2,4-dihydro-pyrazol-3-one (VIIb):

Yeild 77%; m.p. 245-246°C; molecular formula $C_{21}H_{21}N_7O_3S$; elemental analysis found % (Calc. %): C 55.75 (55.86), H 4.60 (4.69), N 21.65 (21.72), O 10.60 (10.63), S 7.00 (7.10); IR (KBr) (ν_{max} in cm^{-1}): 3240 (Ar-NH), 3130 (NH), 2925 (C-H), 1660 (C=O), 1620 (C=N), 1150 (C=S); ^{1}H NMR (DMSO-d_6): δ2.40 (s, 3H CH_3), 3.82 (s, 3H, CH_3), 5.06 (s, 2H, N-CH_2), 5.62 (s, 2H, N-CH_2-N), 6.97 (d, 2H, Ar-H), 7.47 (d, 2H, Ar-H), 7.90 (s, H, Ar - NH), 11.10 (s, H, Ar-NH).

2-(4-{[(4-Fluoro-phenyl)-methyl-amino]-methyl}-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-ylmethyl}-5-methyl-4-(phenyl-hydrazono)-2,4-dihydro-pyrazol-3-one (VIIc):

Yeild 72%; m.p. 235-236ºC; molecular formula $C_{20}H_{18}FN_7O_2S$; elemental analysis found % (Calc. %): C 54.45 (54.66), H 4.10 (4.13), N 22.28 (22.31), O 7.25 (7.28), S 7.07 (7.30), Fl 4.26 (4.32); IR (KBr) (ν_{max} in cm^{-1}): 3255 (Ar-NH), 3145 (NH), 2945 (C-H), 1670 (C=O), 1610 (C=N), 1160 (C=S).

2-(4-{[(4-Chloro-phenyl)-methyl-amino]-methyl}-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-ylmethyl}-5-methyl-4-(phenyl-hydrazono)-2,4-dihydro-pyrazol-3-one (VIId):

Yield 73%; m.p. 250-251ºC; molecular formula $C_{20}H_{18}ClN_7O_2S$; elemental analysis found % (Calc. %): C 52.33 (52.69), H 4.01 (3.98), N 21.13 (21.51), O 7.11 (7.02), S 6.88 (7.03), Cl 7.98 (7.78); IR (KBr) (ν_{max} in cm^{-1}): 3253 (Ar-NH), 3172 (NH), 2940 (C-H), 1663 (C=O), 1608 (C=N), 1158 (C=S).

2-(4-{[(4-Bromo-phenyl)-methyl-amino]-methyl}-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-ylmethyl}-5-methyl-4-(phenyl-hydrazono)-2,4-dihydro-pyrazol-3-one (VIIe):

Yield 75%; m.p. 230-231ºC; molecular formula $C_{20}H_{18}BrN_7O_2S$; elemental analysis found % (Calc. %): C 47.67 (48.01), H 3.54 (3.63), N 20.01 (19.59), O 6.50 (6.40), S 6.55 (6.41), Br 16.11 (15.97); IR (KBr) (ν_{max} in cm^{-1}): 3254 (Ar-NH), 3143 (NH), 2943 (C-H), 1665 (C=O), 1609 (C=N), 1155 (C=S).

5-Methyl-2-(4-{[methyl-(4-nitro-phenyl)-amino]-methyl}-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-ylmethyl}-4-(phenyl-hydrazono)-2,4-dihydro-pyrazol-3-one (VIIf):

Yield 78%; m.p. 255-256ºC; molecular formula $C_{20}H_{18}N_8O_4S$; elemental analysis found % (Calc. %): C 52.00 (51.50), H 3.78 (3.98), N 23.88 (24.02), O 13.56 (13.72), S 7.01 (6.87); IR (KBr) (ν_{max} in cm^{-1}): 3245 (Ar-NH), 3135 (NH), 2930 (C-H), 1655 (C=O), 1605 (C=N), 1145 (C=S); ^{1}H NMR (DMSO-d_6): δ2.50 (s, 3H, CH_3), 4.96 (s, 2H, N-CH_2), 5.50 (s, 2H, N–CH_2-N), 6.80-7.20 (m, 5H, Ar-H), 7.48 (d, 2H, *o*-protons of *p*-nitrophenyl), 7.86 (d, 2H, protons of *p*-nitrophenyl), 7.89 (s, 1H, Ar-NH), 10.23 (s, 1H, NH).

2-(4-Diethylaminomethyl-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-ylmethyl)-5-methyl-4-(phenyl-hydrazono)-2,4-dihydro-pyrazol-3-one (VIIg):

Yield 72%; M.p. 260-261ºC; molecular formula $C_{18}H_{23}N_7O_2S$; elemental analysis found % (Calc. %): C 53.65 (53.85),H 5.67 (5.77), N 24.22 (24.42), O 7.65 (7.97) S 7.55 (7.99); IR (KBr) (ν_{max} in cm^{-1}): 3230 (Ar-NH), 3125 (NH), 2925 (C-H), 1645 (C=O), 1590 (C=N), 1135 (C=S).

2-{4-[Diphenylamino)-methyl]-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-ylmethyl}-5-methyl-4-(phenyl-hydrazono)-2,4-dihydro-pyrazol-3-one (VIIh):

Yield 76%; m.p. 265-266ºC; molecular formula $C_{26}H_{23}N_7O_2S$; elemental analysis found % (Calc. %): C 62.70 (62.76), H 4.60 (4.66), N 19.65 (19.71), O 6.35 (6.43), S 6.38 (6.44); IR (KBr) (ν_{max} in cm^{-1}): 3250 (Ar-NH), 3150 (NH), 2940 (C-H), 1667 (C=O), 1593 (C=N), 1107 (C=S); ^{1}H NMR (DMSO-d_6): δ2.67 (s, 3H CH_3), 5.26 (s, 2H, N-CH_2), 5.50 (s, 2H, N-CH_2-N), 6.60-7.20 (m, 5H, Ar-H), 7.26-7.56 (m, 10H, aromatic protons of both phenyl rings), 7.60 (s, H, Ar-NH), 7.80 (s, 1H, Ar-NH).

5-Methyl-2-(4-morpholin-4-ylmethyl-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-ylmethyl)-4-(phenyl-hydrazono)-2,4-dihydro-pyrazol-3-one (VIIi):

Yield 70%; m.p. 270-271^{o}C; molecular formula $C_{18}H_{21}N_7O_3S$; elemental analysis found % (Calc. %): C 52.08 (52.04), H 5.33 (5.09), N 23.75 (23.60), O 11.10 (11.55), S 7.50 (7.72); IR (KBr) (ν_{max} in cm^{-1}): 3265 (Ar-NH), 3150 (NH), 2955 (C-H), 1675 (C=O), 1610 (C=N), 1165 (C=S); ^{1}H NMR (DMSO-d_6): δ2.60 (s, 3H, CH_3), 2.62 (t, 4H CH_2-N-CH_2), 3.70 (t, 4H, CH_2-O-CH_2), 4.50 (s, 2H, N-CH_2-N), 5.24 (s, 2H, N-CH_2), 5.48 (s, 2H, NCH_2-N), 6.80-7.20 (m, 5H, Ar-H), 7.60 (s, H, Ar-NH),10.20 (s, 1H, NH).

5-Methyl-4-(phenyl-hydrazono)-2-(4-piperazin-1-ylmethyl-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-ylmethyl)-2,4-dihydro-pyrazol-3-one (VIIj):

Yield 72% m.p. 272-273^{o}C; molecular formula $C_{18}H_{22}N_8O_2S$; elemental analysis found % (Calc. %): C 52.20 (52.16), H 5.50 (5.35),n N 27.25 (27.03), O 7.68 (7.72), S 7.58 (7.74); IR (KBr) (ν_{max} in cm^{-1}): 3260 (Ar-NH), 3145 (NH), 2945 (C-H), 1670 (C=O), 1610 (C=N), 1160 (C=S); ^{1}H NMR (DMSO-d_6): δ2.58 (s, 3H, CH_3), 2.56 (t, 4H CH_2-N-CH_2), 4.45 (s, 2H, N-CH_2-N), 5.20 (s, 2H, N-CH_2), 5.45 (s, 2H, NCH_2N), 6.70-7.10 (m, 5H, Ar-H), 7.50 (s, H, Ar-NH), 10.19 (s, H, NH).

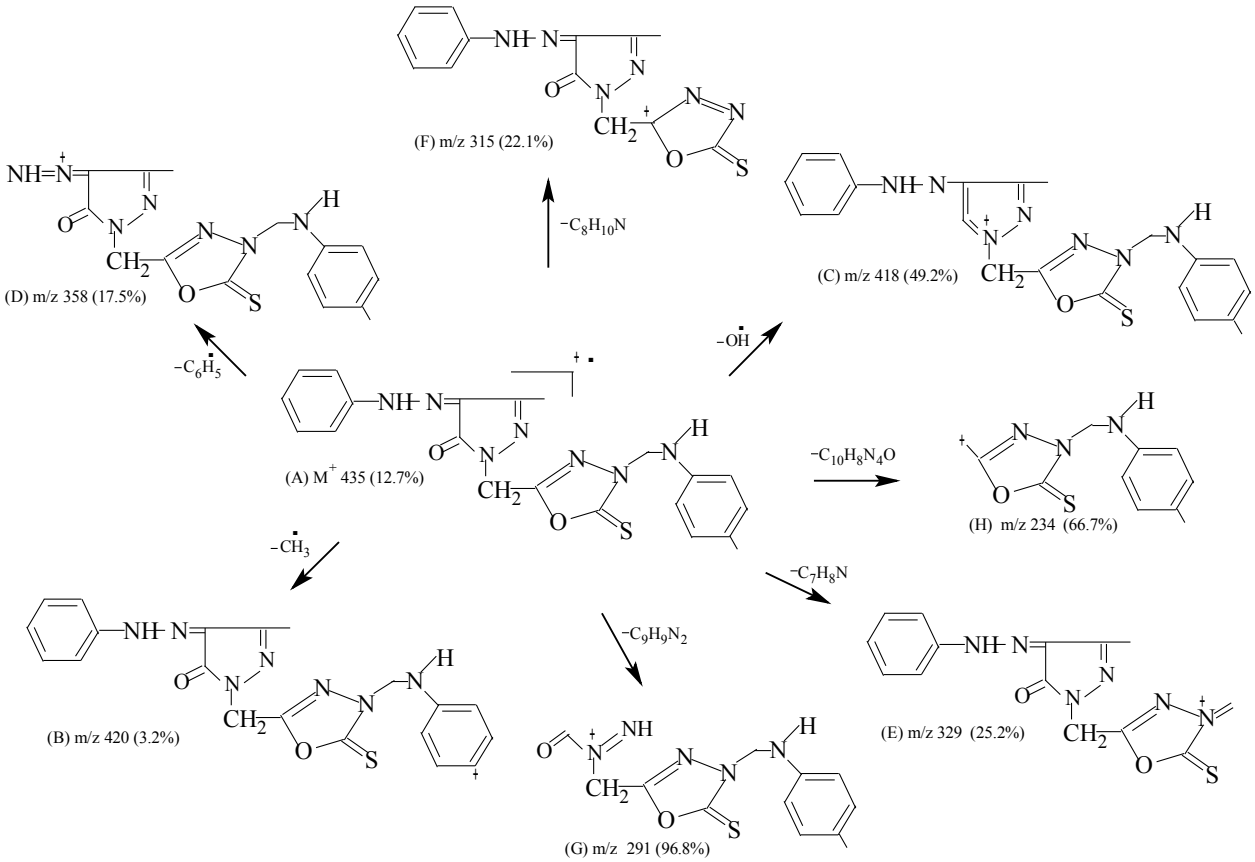
5-Methyl-2-[4-(4-methyl-piperazin-1-ylmethyl)-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-ylmethyl]-4-(phenyl-hydrazono)-2,4-dihydro-pyrazol-3-one (VIIk):

Yield 68%; m.p. 267-268^{o}C; molecular formula $C_{19}H_{24}N_8O_2S$; elemental analysis found % (Calc. %): C 53.65 (53.25), H 5.98 (5.65), N 25.81 (26.15), O 7.28 (7.47), S 7.36 (7.48); IR (KBr) (ν_{max} in cm^{-1}): 3255 (Ar-NH), 3140(NH), 2935 (C-H), 1655 (C=O), 1605 (C=N), 1145 (C=S); ^{1}H NMR (DMSO-d_6): δ2.55 (s, 3H, CH_3), 2.42 (t, 4H, CH_2-N-CH_2), 4.20 (s, 3H, N-CH_3), 4.52 (s, N-CH_2-N), 5.18 (s, 2H, N-CH_2), 5.40 (s, 2H, NCH_2N), 6.60-7.20 (m, 5H, Ar-NH), 7.40 (s, H, Ar – NH), 10.18 (s, H, NH).

Mass spectral data of VII

The mass spectrum of 5-Methyl-2-{4-[(methyl-p-tolyl-amino)-methyl]-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-ylmethyl}-4-(phenyl-hydrazono)-2,4-dihydro-pyrazol-3-one VIIa (R_1=H, R_2=*p*-$CH_3C_6H_4$) exploited the molecular ion (M^+) peaks at m/z 435.

The fragmentation pattern for VIIa is presented in Scheme 4. The spectrum showed the molecular ion (M^+) peak at m/z 435 with intensity 12.7%. The loss of a CH_3 radical resulted in the formation of the fragment B at m/z 420 (3.2%). Loss of OH radical from molecular ion A led to the formation of cation C at m/z 418 (49.2%). Loss of C_6H_5 radical from A yielded the cation D at m/z 358 (17.5%). Elimination of $C_8H_{10}N$ molecule from A has resulted in the formation of cation F at m/z 315 (22.2%). Elimination of C_7H_8N from molecular (M^+) ion resulted in the formation of cation E at m/z 329 (100%). Cleavage of molecular ion A led to the formation of cations G and H at m/z 291 (9.6%) and 234 (66.7%) respectively.



Scheme 4. Mass spectral fragmentation of 5-Methyl-2-{4-[(methyl-p-tolyl-amino)-methyl]-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-ylmethyl}-4-(phenyl-hydrazono)-2,4-dihydro-pyrazol-3-one (VIIa)

Antibacterial and antifungal activity

The Mannich bases were evaluated for their antimicrobial activities against certain selected gram-positive bacteria, gram-negative bacteria and fungi. Antimicrobial activity was expressed as the minimum inhibitory concentration. The details are given in Table 1.

Compounds of each series (V, VI and VII) were characterized by elemental analysis, IR, ^{1}H NMR spectral data. The elemental analysis data of each compound synthesized indicated that the reported data almost coincides with the theoretical data. In each case the characteristic signals in the spectra indicative of the compound were identified and reported. The compounds V and VII were also characterized by mass spectra. The details of mass spectral fragmentation pattern presented in the Scheme 3 and Scheme 4 were confirmative of corresponding structures.

Antibacterial and antifungal activity

The details of antimicrobial studies are shown in Table 1. The gram-positive bacteria screened were *Staphylococcus aureus* NCCS 2079 and *Bacillus cereus* NCCS 2106 and the gram negative bacteria screened were *Escherichia coli* NCCS 2065 and *Pseudomonas aeruginosa* NCCS 2200. Antifungal activity tests were also performed against *Aspergillus niger* NCCS 1196 and *Candida*

albicans NCCS 2106. The preliminary antimicrobial activity of synthesized compounds was studied by disc diffusion method. As shown in Table 1, fluoro, chloro, bromo, nitro, morphonilyl, piperizynyl, N-methylpiperizine substituted compounds (VII c, d, e, f, i, j and k) showed more antibacterial activity against *Staphylococus aureu* , *Bacillus cereus*, *Escherichia coli* and *Pseudomonos aeruginosa* than other compounds of the series. Similar observations were made against *Aspergillus niger* and *Candida albicans* in antifungal studies.

Table 1. **Antibacterial and antifungal activities of Mannich bases**

Compound	Zone of inhibition in mm and MIC in µg/mL*					
	Antibacterial activity				Antifungal activity	
	***Staphylococcus aureus* NCCS 2079**	***Bacillus cereus* NCCS 2106**	***Escherichia coli* NCCS2065**	***Pseudomonos aeruginosa* NCCS 2200**	***Aspergillus niger* NCCS 1196**	***Candida albicans* NCCS 2106**
VIIa	1.50 (39.0)	1.75 (35.0)	1.25 (40.0)	1.50 (40.0)	2.50 (38.0)	2.25 (28.0)
VIIb	1.75 (40.0)	1.50 (38.0)	1.50 (40.0)	1.25 (38.0)	2.25 (35.0)	2.00 (28.0)
VIIc	2.50 (33.0)	2.75 (30.7)	2.25 (28.0)	2.50 (30.7)	3.75 (25.0)	3.00 (20.0)
VIId	3.00 (33.0)	2.50 (30.8)	2.25 (25.0)	2.75 (30.8)	3.50 (25.0)	3.75 (25.0)
VIIe	2.50 (30.8)	2.75 (30.7)	2.00 (28.0)	2.25 (33.0)	3.50 (22.3)	3.00 (22.3)
VIIf	2.75 (33.0)	2.50 (33.0)	2.50 (35.0)	2.50 (35.0)	15.00 (20.0)	3.50 (20.0)
VIIg	1.75 (50.0)	2.00 (55.7)	1.50 (60.0)	1.25 (60.0)	8.00 (45.5)	1.75 (40.0)
VIIh	1.75 (45.5)	1.75 (40.0)	1.25 (40.0)	1.50 (47.5)	2.00 (40.0)	2.00 (35.3)
VIIi	2.50 (30.7)	3.00 (30.8)	2.50 (35.3)	2.75 (30.8)	3.75 (22.2)	3.50 (20.0)
VIIj	2.75(20.0)	2.50 (25.0)	2.25 (35.23)	2.50 (20.0)	3.50 (18.5)	3.00 (18.5)
VIIk	2.50 (22.2)	2.75 (27.2)	2.25 (35.3)	2.75 (27.3)	3.50 (20.0)	3.50 (20.0)

*Average of three determinations. Zone of inhibition in mm and MIC (in side the brackets) in µg/mL

As is evident from the literature, extensive reports pertaining to the antimicrobial screening of compounds containing 1,3,4-oxadiazole moiety [1-8] or pyrazole-3-one moiety are available [9-15]. The pharmacological applications of Mannich bases containing different moieties are also reported in the literature [20-29], but pharmacological applications of Mannich bases

incorporating 1,3,4-oxadiazole or pyrazole-3-one moieties have not beenexplored. Moreover the reported information witnesses disadvantages such as requirement of high concentration of the drug [20, 24, 26, 27, 29] or low zone of inhibition [25] or the compounds are active against bacteria only [20, 27, 29] or active against gram positive bacteria only [21] or active against fungi only [22, 23, 28]. The present article reports the antibacterial and antifungal activities of Mannich bases containing pharmacologically potential 1,3,4-oxadiazole and pyrazole-3-one moieties. The discern is clearly evident from the fact that the Mannich bases (VIIa-VIIk) demonstrated low magnitudes of MIC, higher magnitudes of zone inhibition and wide spectrum of activity against bacteria and fungi.

CONCLUSION

Series of new compounds containing 1,3,4 oxadiazole and pyrazole-3-one moiety were synthesized. The compounds were characterized by elemental analysis, IR, ^{1}H NMR and mass spectral data. All compounds were screened for antibacterial and antifungal activities. The activities of the compounds were assessed by the disc diffusion method and the minimum inhibitory concentration was established by broth dilution method. The results reveal that all the compounds exhibited promising antimicrobial activities.

REFERENCES

1. Kaur H, Kumar S, Verma RS, Garg A, Saxena KK, Lata S. Synthesis and antibacterial activity of various substituted oxadiazole derivatives. Arch Pharm. 2011; 344:466-473.
2. Chandrakantha B, Shetty P, Nambiyar V, Isloor N, Isloor AM. Synthesis, characterization and biological activity of some new 1,3,4-oxadiazole bearing 2-flouro-4-methoxy phenyl moiety. Eur J Med Chem. 2010; 45: 1206-1210.
3. Kadi AA, El-Brollosy NR, Al-Deeb OA, Habib EE, Ibrahim TM, El-Emam AA. Synthesis, antimicrobial, and anti-inflammatory activities of novel 2-(1-adamantyl)-5-substituted-1,3,4-oxadiazoles and 2-(1-adamantylamino)-5-substituted-1,3,4-thiadiazoles. Eur J Med Chem. 2007; 42: 235-242.
4. Xu W, He J, He M, Han F, Chen X, Pan Z. Synthesis and antifungal activity of novel sulfone derivatives containing 1,3,4-oxadiazole moieties. Molecules 2011; 16: 9129-9149.
5. Bezerra NMM, De Oliveira SP, Srivastava RM, Da Silva JR. Synthesis of 3-aryl-5-decapentyl-1,2,4-oxadiazoles possessing anti-inflammatory and antitumor properties. Farmaco 2005; 60: 955-960.
6. Ali M, Yar MS. Oxadiazole Mannich bases: Synthesis and antimycobacterial activity. Bioorg Med Chem Lett. 2007; 17:3314-3316.
7. Zarghi A, Tabatabai SA, Faizi M, Ahadian A, Navabi P, Zanganeh V. Synthesis and anticonvulsant activity of new 2-substituted-5-(2-benzyloxyphenyl)-1,3,4-oxadiazole. Bioorg Med Chem Lett. 2005; 15: 1863-1865.
8. Ravichandran V, Shalini S, Sundram K, Sokkalingam AD. QSAR study of substituted 1,3,4-oxadiazole naphthyridines as HIV-1 integrase inhibitors. Eur J Med Chem. 2010; 45: 2791-2797.
9. Al-Haiza MA, El-Assiery SA, Sayed GH. Synthesis and potential antimicrobial activity of some new compounds containing the pyrazol-3-one moiety. Acta Pharm. 2001; 51: 251-261.

10. Ibrahim MA, Saleh AM, Zohdi HF. Synthesis and antitumor activity of 5-trifluoromethyl-2,4-dihydropyrazol-3-one nucleosides. Molecules 2004; 9: 109-119.
11. Genin MJ, Biles C, Keiser BJ, Poppe SM, Swaney SM, Tarpley WG. Novel 1,5-diphenylpyrazole nonnucleoside hiv-1 reverse transcriptase inhibitors with enhanced activity versus the delavirdine-resistant p236l mutant: Lead identification and SAR of 3- and 4-substituted derivatives. J Med Chem. 2000; 43: 1034-1040.
12. Kalla R, Zablocki J. Recent advances in adenosine receptor (AR) ligands in pulmonary diseases. Annu Rep Med Chem. 2009; 44: 265-277.
13. Rostom SAF, Ashour HMA, El-Razik HAA, El-Fattah AEHA, El-Din NN. Azole antimicrobial pharmacophore-based tetrazoles: Synthesis and biological evaluation as potential antimicrobial and anticonvulsant agents. Bioorg Med Chem. 2009; 17: 2410-2422.
14. Rostom SAF. Synthesis of some 1-(4-chlorophenyl)-4- hydroxy-1H-pyrazol-3-carbonyl derivatives linked to nitrogenous heterocyclic ring systems as potential antitumor agents. Bioorg Med Chem. 2010; 18: 2767-2776.
15. Sujatha K, Shanthi G, Selvam NP, Manoharan S, Perumal PT, Rajendran M. Synthesis and antiviral activity of 4,4′-(arylmethylene)bis(1*H*-pyrazol-5-ols) against peste des petits ruminant virus (PPRV). Bioorg Med Chem Lett. 2009; 19: 4501-4503.
16. Elek A, Hill DW. The micro estimation of halogens in organic compounds. J Am Chem Soc. 1993; 55:2550–2554.
17. Ravindranath LK, Srikanth K, Dastagiri Reddy M, Ishrath Begum SD. Synthesis and antimicrobial activity of novel compounds containing pyrazoles and 1,3,4 oxadiazoles. Orient J Chem. 2009; 25: 993-998.
18. Arthington-Skaggs BA, Motley M, Warnock DW, Morrison CJ. Comparative evaluation of PASCO and National Committee for Clinical Laboratory Standards M27-A broth microdilution methods for antifungal drug susceptibility testing of yeasts. J Clin Microbiol. 2000; 38: 2254-2260.
19. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 2008; 3: 163-175.
20. Bespalova GV, Sedavkina VG, Kharchenko VG. Kulikova LK. Synthesis and antimicrobial properties of Mannich bases in the 2-pyrrolidone and 2-thiopyrrolidone series. Pharmaceutical Chemistry Journal. 1981; 15: 643-645.
21. Daisley RW, Shah VK. Synthesis and antibacterial activity of some 5-nitro-3-phenyliminoindol-2(3*H*)-ones and their *N*-mannich bases. J Pharm Sci. 1984; 3: 407-408.
22. Gul HI, Ojanen T, Vepsalainen J, Gul M, Erciyas E, Hanninen O. Antifungal activity of some mono, bis and quaternary Mannich bases derived from acetophenone. Arzneimittelforschung. 2001; 51: 72-75.
23. Gul HI, Ojanen T, Hänninen O. Antifungal evaluation of bis Mannich bases derived from acetophenones and their corresponding piperidinols and stability studies. Biol Pharm Bull. 2002; 25: 1307-1310.
24. Jumade PP, Wadher SJ, Chourasia AJ, Kharabe UV, Mude D, Yeole PG. Synthesis of newer mannich bases of quinoline derivative for antimicrobial activity. Int J Chem Sci. 2009; 7: 1518-1530.

25. Khare DK, Mandloi D. Synthesis and antimicrobial studies of some novel mannich bases derived from secondary amines. International Journal of Basic and Applied Chemical Sciences 2012; 2: 37-42.
26. Ravichandran V, Mohan S, Suresh Kumar K. Synthesis and antimicrobial activity of Mannich bases of isatin and its derivatives with 2-[(2,6-dichlorophenyl)amino]phenylacetic acid. Arkivoc 2007; 14: 51-57.
27. Seda F, Serap B, Hakan B, Meltem Y, Ahmet D. Reduction, Mannich reaction, and antimicrobial activity evaluation of some new 1,2,4-triazol-3-one derivatives. Turk J Chem. 2012; 36: 567-582.
28. Shivananda MK, Shet Prakash M. Antifungal activity studies of some mannich bases carrying nitrofuran moiety. J Chem Pharm Res. 2011; 3: 303-307.
29. Singh UK, Pandeya SN, Singh A, Srivastava BK, Pandey M. Synthesis and antimicrobial activity of Schiff's and N-Mannich bases of isatin and its derivatives with 4-amino-n-carbamimidoyl benzene sulfonamide. Int J of Phar Sci Drug Res. 2010; 2: 151-154.