ARTÍCULO

Novel Compounds containing 1,3,4-oxadiazole and pyrazole-3-one Nuclei – Synthesis and *in vitro* Antimicrobial Evaluation

Krishna Naik[1], Aluru Raghavendra Guru Prasad[2*], Yadati Narasimha Spoorthy[1], Mokkapati Pariplavi[3], Lakshmana Rao Krishna Rao Ravindranath[1]

[1]Sri Krishnadevaraya University, Anantapur, A.P., India. [2]ICFAI Foundation for Higher Education, Hyderabad, A.P., India.[3]Osmania Medical College, Osmania University, Hyderabad, A.P., India.
*e-mail: guruprasadar@yahoo.co.in

An. Real Acad. Farm. Vol 80, Nº 1(2014), pág. 201-213

ABSTRACT

New series of heterocycles containing 1,3,4-oxadiazole and pyrazole-3-one moiety have been synthesized and evaluated *in vitro* against *Staphylococus aureus* NCCS 2079, *Bacillus cereus* NCCS 2106, *Escherichia coli* NCCS 2065, *Pseudomanas aeruginosa* NCCS 2200, *Aspergillus niger* NCCS 1196 and *Candida albicans* NCCS 2106. The compounds demonstrated moderate antimicrobial activity against selected fungal and bacterial strains.

Keywords: Synthesis; 1,3,4-Oxadiazoles; Pyrazole-3-ones; Antimicrobial activity.

RESUMEN

Nuevos compuestos que contienen núcleos 1,3,4-oxadiazol y pirazol-3-ona. Sintesis y actividad antimicrobiana "in vitro"

Nuevas series de compuestos heterocíclicos que contienen 1,3,4-oxadiazol y pyrazol-3-ona han sido sintetizadas y evaluadas in-vitro contra *Staphylococus aureus* NCCS 2079, *Bacillus cereus* NCCS 2106, *Escherichia coli* NCCS 2065, *Pseudomanas aeruginosa* NCCS 2200, *Aspergillus niger* NCCS 1196 y *Candida albicans* NCCS 2106. Los compuestos demostraron actividad antimicrobiana moderada contra estirpes fúngicas y bacterianas seleccionadas.

Palabras clave: Síntesis; 1,3,4-Oxadiazol; Pyrazol-3-uno; Actividad antimicrobiana.

1. INTRODUCTION

Heterocycles bearing nitrogen, oxygen and sulphur atoms in their structure have received remarkable attention because of their biological and pharmacological applications [1-3]. Among the wide variety of heterocycles that were explored for developing pharmaceutically important molecules, compounds containing 1,3,4-oxadiazole nucleus [4-7] and pyrazole nucleus [8-13] constitute the important class of compounds exhibiting diverse and extensive spectrum of biological activities. This scenario led us to synthesize the novel compounds VII and IX containing 1,3,4-oxadiazole and pyrazole nuclei and evaluate their antibacterial and antifungal activities.

2. MATERIALS AND METHODS

All chemicals used were analytical grade obtained from Merck India Limited, India. All the glass ware used were of borosilicate grade. The standard bacterial and fungal stains were procured from National Centre for Cell Science, Pune, India. UV-Visible spectrophotometer manufactured by Shimadzu Corporation, Japan was used for absorption measurements. The IR spectra were recorded on a Perkin-Elmer 983 IR spectrometer. The ^{1}H-NMR spectra were recorded on a Bruker AC 300F (200 MHz) NMR spectrometer using DMSO – d_6 as 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.

2.1. General Synthetic Procedures

The novel compounds were synthesized by specified procedures and the critical intermediate compounds were characterized by elemental analysis and spectral data.

2.1.1. Synthesis of {4-[3-Methyl-5-oxo-4-(4|-substituted phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid hydrazide (V).

a. Synthesis of substituted phenyl diazoniam chloride (I)

The required primary amine was dissolved in a suitable volume of water containing 2.5–3.0 equivalents of hydrochloric acid (or sulphuric acid). The solution was cooled to 0 ^{o}C. To the crystals of amine hydrochloride (or sulphate) so obtained, an aqueous solution of sodium nitrite was added portion wise. An excess of acid was necessary to stabilize the diazonium chloride. Similar procedure was adopted for the preparation of other substituted phenyl diazonium chlorides.

b. Synthesis of substituted phenyl diazonium ethyl acetoacetic ester (II)

To an ice-cold solution of mixture of sodium acetate (1.0 g) in 100 mL of aqueous alcohol and ethyl acetoacetate (0.1 mol) in 50 mL of ethanol, the corresponding diazonium chloride was added till yellow crystals were separated

out. These crystals were filtered, washed with water and dried.

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

3-methyl-4-(4|-substituted phenyl hydrazono)-pyrozoline-5-one (**III**) was synthesized by thecondensation of 4-substituted phenyl hydrazono acetoacetic ester (**II**) and hydrazine in the presence of required amounts of dimethylformamide. The mixture was subjected to microwave irradiation at 150W intermittently at 30 sec intervals for 2 minutes. After complete conversion as indicated by TLC, the reaction mixture was cooled and washed with cold water. The precipitated **III** was filtered recrystallized from ethanol.

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

A mixture of **III**, anhydrous K_2CO_3 and DMF was stirred at room temperature for 8 hours. The reaction mixture was diluted with ice cold water. The separated solid was filtered and recrystallized from ethanol.

e. Synthesis of {4-[3-Methyl-5-oxo-4-(4|-aryl-hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid hydrazides (V)

A mixture of **IV** and hydrazine hydrate in ethanol was refluxed for five hours. The reaction mixture was cooled to room temperature and poured in ice cold water with continuous stirring. The separated solid was filtered, washed with water and recrystallized from ethanol. Other members of the series **V**were prepared on the same lines. The reaction scheme is depicted in Scheme **1.**

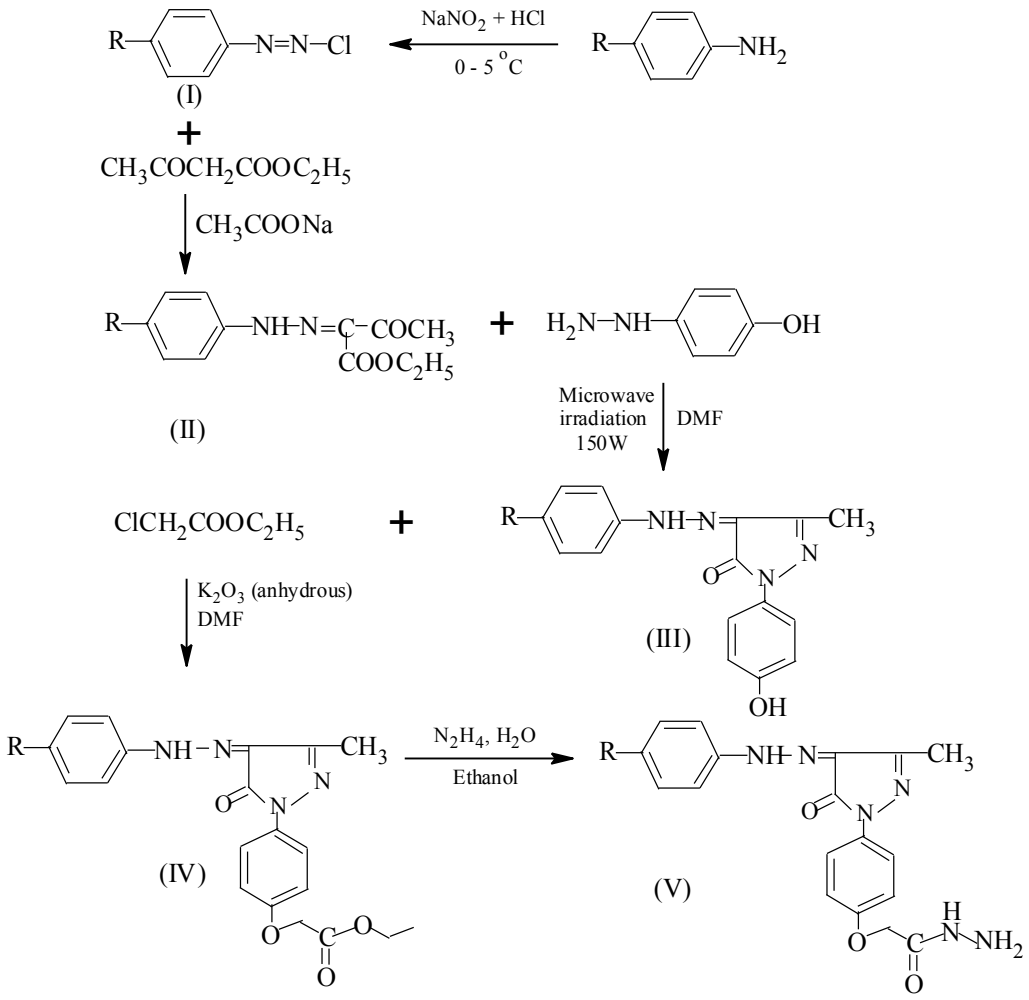
2.1.2. Synthesis of 2-(4-acetyl- -5,5-disubstituted 4,5-dihydro-[1,3,4]oxadiazole-2-yl(methyloxyphenyl)-5-methyl-4(aryl hydrazono)- -pyrazol-3-ones (VII)

a. Synthesis of {4-[3- methyl- 5- oxo- 4-(4|-phenyl hydrazono)- 4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid (1-phenyl-ethylidene)-hydrazide (VI)

A mixture of **V** (0.01 mol) in hot methanol (25 mL), acetophenone (0.01 mol) and a drop of glacial acetic acid were refluxed for 3 hours. The solid separated was filtered, washed with cold methanol and recrystallized from methanol to give **VI**a. Compounds **VI** b-h were synthesized on similar lines.

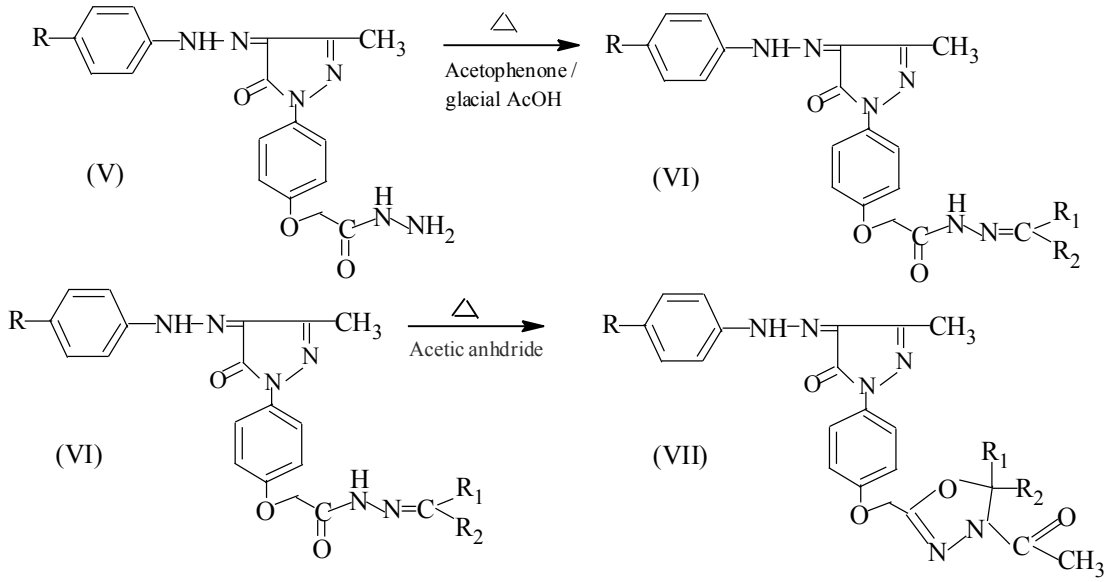
b. Synthesis of 2-(4-acetyl-5,5-disubstituted 4,5-dihydro-[1,3,4]oxadiazole-2-yl(methyloxyphenyl)-5-methyl-4(4|-substituted phenyl hydrazono)-pyrazol-3-ones (VII)

A mixture of **VI**a (0.01 mol) and an excess of acetic anhydride (10 mL) were refluxed for 2 hours. The excess acetic anhydride was distilled off and the residue was poured on to crushed ice. The solid obtained was filtered, washed with water and recrystallized from aqueous methanol to get **VII**a. The cyclization reaction was extended to other hydrazones **VI** b–j and in each case the respective compound was isolated. The reaction scheme is given in Scheme **2**.



R=H, CH_3, OCH_3, OC_2H_5, Cl, Br.

Scheme 1.-Synthesis of {4-[3-Methyl-5-oxo-4-(4ˈ-substituted phenyl hydrazono)-4,5-dihydro-pyrazol-1-yl]-phenoxy}-acetic acid hydrazide (**V**).



R = -H, $-CH_3$, $-OCH_3$, $-OC_2H_5$, -Cl, -Br, R_1 = $-CH_3$, R_2 = $-C_6H_5$, *p*-$CH_3C_6H_4$*p*-ClC_6H_4, *p*-$OCH_3C_6H_4$*p*-$NO_2C_6H_4$.

Scheme 2.- Synthesis of 2-(4-acetyl-5-methyl-5-phenyl-4,5-dihydro-[1,3,4]oxadiazole-2-yl(methyl)-5-methyl-4(4ˈ-phenyl hydrazono)-2,4-dihydrazono-pyrazol-3-one (VII).

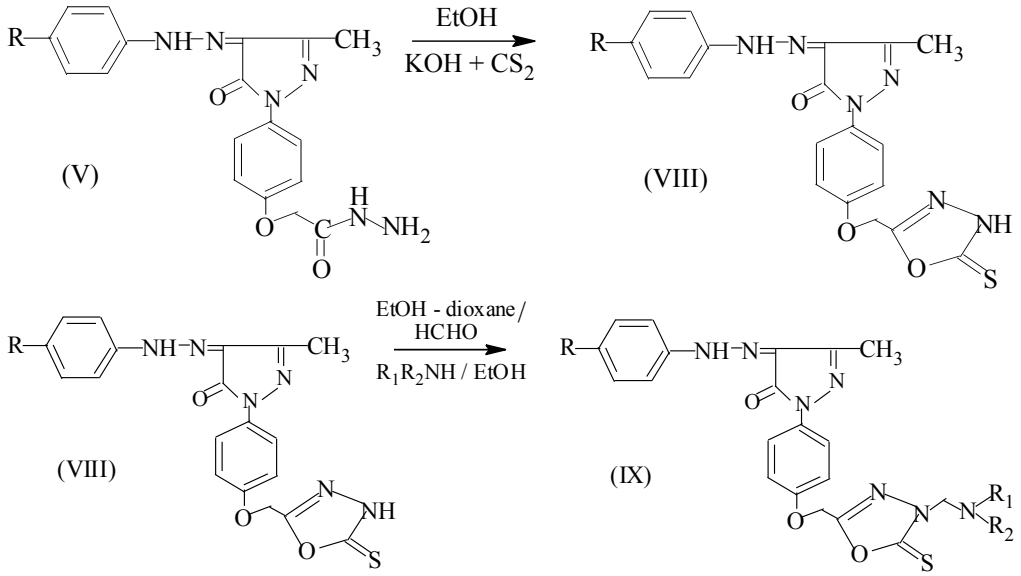
2.1.3. Synthesis of Mannich bases containing [1,3,4] oxadiazole and pyrazol-3 one nuclei (IX)

a. 5-methyl- 4-(4/-substituted phenyl hydrazono)-2-(5-thioxo-[1,3,4] oxadiazole-2-yl-methyloxyphenyl)-2,4-dihydro-pyrazol-3-one (VIII)

A mixture of **V** (19.9 g, 0.1 mol), KOH (5.5 g, 0.1 mol), ethanol (100 mL) and carbon disulphide (6.02 mL, 0.1 mol) was refluxed in a water bath till the evolution of hydrogen sulphide is ceased. The excess of alcohol was removed by distillation. The reaction mixture was cooled to room temperature, poured into ice cold water and neutralized with dilute hydrochloric acid. The precipitate so formed was filtered, washed with water, dried and recrystallized from ethanol-dioxane mixture (1:1) to give **VIII**.

b. 5-methyl-4-(4/-substituted phenyl hydrazono)-2-[5-thioxo-4-[alkyl/phenyl/heterocyclic amino methyl]-4,5-dihydro-[1,3,4] oxadiazol-2-yl-methyl]-2,4-dihydro-pyrazol-3-ones (IX)

A mixture of **VIII** (0.01 mol) in ethanol and dioxane (20 mL) was treated with formaldehyde (40%, 1.5 mL). Appropriate amine (0.01 mol) in ethanol (10 mL) was added to the reaction mixture and stirred over night. The precipitated Mannich base was filtered, dried and recrystallized from ethanol-DMF mixture (1:1). The reaction sequence is outlined in Scheme **3**.



NHR_1R_2 = morpholinyl, piperazinyl, *N*-methylpiprazinyl; R_1 = -H, R_2 = *p*-Tolyl, *p*-anisyl, *p*-fluorophenyl, *p*-chlorophenyl, *p*-bromophenyl, *p*-nitrophenyl; $R_1 = R_2$ = ethyl or phenyl.

Scheme 3. Synthesis of Mannich bases containing [1,3,4] oxadiazole and pyrazol-3-one moiety.

2.2. Antimicrobial activity

a. Antibacterial activity

The antibacterial activity of synthesized compounds (250 µg/mL in DMSO) was preliminarily studied by disc diffusion method. The procedure followed for the disc diffusion method is given below.

A suspension of *Staphylococcus aureus* was added to sterile nutrient agar at 45^{o}C. The mixture was transferred into sterile petri-dishes to a depth of 3 mm and allowed to solidify. Sterile discs of 5 mm in diameter (made of Whatmann Filter paper)were immersed in solutions of synthesized compounds. Sterile discs immersed in DMSO were used as control. Both chemical-treated and DMSO-treated discs were laid down onto bacteria mixed agar plates. The plates were allowed to stand for 1 hour at room temperature followed by incubation at 37^{o}C for 24 hours and observed for antibacterial activity. The diameter of the zone of inhibition was measured in each plate. The average zone of inhibition was calculated. A similar procedure was adopted for the antibacterial activity studies against other organisms.

b. Antifungal activity

The procedure described above was followed for antifungal activity against *Aspergillus niger* NCCS 1196 and *Candida albicans*. Compounds were treated at several different concentrations using DMSO as a solvent.

c. Determination of Minimum Inhibitor Concentration

The procedure followed to find out MIC by Broth Dilution Method is given below.

Standardized inoculum (matched to McFarland $BaSO_4$ standard) of suspension of organisms was 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 shaken gently to mix the contents. Two growth control tubes were also prepared by mixing 0.1 mL of control and 0.9 mL of sterile saline and its optical density was determined. The control contained 1×10^{-5} colony forming units /mL which is equivalent to 20 colonies.

Tubes were incubated for 24 hours at 37^{o}C in air. The turbidity developed 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 (MIC) was noted as the concentration of the test sample which completely inhibits the growth of the microorganism i.e. 100 % transparency.

3. RESULTS AND DISCUSSION

3.1. Characterization of synthesized compounds

a. Elemental analysis details of compounds V (-R, M.P., Yield, Molecular formula, Element: Found %, (Calc %))

Va: H, 152 °C, 65 %, $C_{18}H_{22}N_6O_3$, C:58.37(58.25), H:5.94(5.86), N:22.70(22.65), O:12.97(12.85).

Vb: CH_3, 153 °C, 60 %, $C_{19}H_{24}N_6O_3$, C:59.37(59.26), H:6.25 (6.17), N:21.87(21.76), O:12.23(12.14).

Vc: OCH_3, 156 °C, 75%, $C_{19}H_{24}N_6O_3$, C:57.00(55.85), H:6.00 (5.68), N:21.00 (19.85), O:16.00 (15.85).

Vd: OC_2H_5, 168 °C, 80 %, $C_{20}H_{26}N_6O_4$, C:57.97(57.85), H:6.28(6.18), N:20.28(20.20), O:15.45(15.35).

Ve: Cl, 174 °C, 75 %, $C_{18}H_{21}N_6O_3Cl$, C:53.39(53.29), H:5.19(5.10), N:20.76(20.66), O:11.86(11.76), Cl:8.77(8.66).

Vf: Br, 169 °C, 65 %, $C_{18}H_{21}N_6O_3Br$, C:48.11(47.98), H:4.71(4.60), N:18.17(18.07), O:10.89(10.78), Br:17.69(17.58).

b. IR (KBr) Spectral data (ν_{max} in cm^{-1})

Va: 3445, 3425 (NH_2), 3305 (NH), 1665 (C=O), 1620 (C=N).

Vb: 3400, 3420 (NH_2), 3285 (NH), 1665 (C=O), 1610 (C=N).

Vc: 3425, 3405 (NH_2), 3200 (NH), 1615 (C=N), 1555 (C=O).

Vd: 3435, 3415 (NH_2), 3300 (NH), 1615 (C=N), 1660 (C=O).

Ve: 3420, 3400 (NH_2), 3275 (NH), 1645 (C=O), 1610 (C=N).

Vf: 3444, 3424 (NH_2), 3290 (NH), 1650 (C=O), 1605 (C=N).

c. ^{1}H NMR (DMSO – d_6) Spectral data (δ in ppm)

Va: 1.2 (s, 3H, CH_3), 2.1 (s, 2H, NH_2), 3.85 (s, 2H, O-CH_2-CO) 6.8 (s, 1H, Ar-NH) 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,NH)

Vb: 0.9 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 2.06 (s, 2H, NH_2), 3.80(s, 2H, O-CH_2-CO) 6.8(s,1H,Ar-NH) 7.1-7.3 (m, 4H, C_6H_4),7.4 (d, 2H, C_6H_4), 7.7(d, 2H,C_6H_4), 8.36 (s, 1H, N, CONH)

Vc: 1.12 (s, 3H, CH_3), 2.02 (s, 2H, NH_2), 3.24 (s, 3H, OCH_3), 3.76 (s, 2H, O-CH_2-CO), 6.8 (s, 1H, Ar–NH), 7.1-7.3 (m, 4H, C_6H_4), 7.4 (d, 2H, C_6H_4), 7.7 (d, 2H, C_6H_4), 8.32 (s, 1H, NH)

Vd: 0.9 (s, 3H, CH_3), 1.11 (t, 3H, CH_3), 2.06 (s, 2H, NH_2), 3.14 (q, 2H, O–CH_2), 3.8 (s, 2H, O-CH_2-CO), 6.8 (s, H, Ar–NH), 7.1-7.3 (m, 4H, C_6H_4), 7.4 (d, 2H,C_6H_4), 7.7(d,2H,C_6H_4), 8.36 (s,1H,NH)

Ve: 1.08 (s, 3H, CH_3), 2.08 (s, 2H, NH_2), 3.82 (s, 2H, O-CH_2-CO) 6.8 (s, 1H, Ar–NH), 7.1-7.3 (m, 4H, C_6H_4), 7.4 (d, 2H, C_6H_4), 7.7 (d, 2H, C_6H_4), 8.38 (s, 1H, NH)

Vf: 1.04 (s, 3H, CH_3), 2.04 (s, 2H, NH_2), 3.78 (s, 2H, O-CH_2-CO), 6.8 (s, 1H Ar–NH), 7.1-7.3 (m, 4H, C_6H_4), 7.4 (d, 2H, C_6H_4), 7.7 (d, 2H, C_6H_4), 8.34 (s, 1H, NH)

d. Elemental analysis details of compounds VI (-R, -R_1, -R_2, M.P., Yield, Molecular formula)

VIa: -H, -H, -C_6H_5,240 °C, 75 %, $C_{26}H_{28}N_6O_3$, C:66.10(65.95), H:5.93(5.83), N:17.93(17.80), O:10.16(10.02).

VIb: -CH_3, -CH_3, -C_6H_5, 245 °C, 77 %, $C_{27}H_{30}N_6O_3$, C:66.66(66.50), H:6.17(6.05), N:17.28(17.12), O:9.87(9.72).

VIc: OCH_3, CH_3, C_6H_5, 35 °C, 72 %, $C_{27}H_{30}N_6O_4$, C:64.54(64.42), H:5.97(5.83), N:16.73(16.58), O:12.74(12.62).

VId: OC_2H_5, CH_3, C_6H_5, 250 °C, 73 %, $C_{28}H_{32}N_6O_4$, C:65.11(64.95), H:6.20(6.05), N:16.27(16.12), O:12.40(12.22).

VIe: Cl, CH_3, C_6H_5, 230 °C, 75, $C_{26}H_{27}N_6O_3Cl$, C:61.59(61.42), H:5.33(5.23), N:16.58(16.46), O:9.47(9.33), Cl:7.00(6.86).

VIf: Br, CH_3, C_6H_5, 255 °C, 78 %, $C_{27}H_{27}N_6O_3Br$, C:56.63(56.52), H:4.90(4.78), N:15.24(15.07), O:8.71(8.58), Br:14.50(14.37).

VIg: H, CH_3, $CH_3C_6H_4$, 260 °C, 72 %, $C_{27}H_{30}N_6O_3$, C:66.64(66.48), H:6.17(6.05), N:17.20(17.07), O:9.87(9.70).
VIh: H, CH_3, ClC_6H_4, 265 °C, 76 %, $C_{26}H_{27}N_6O_3Cl$, VIh: C:61.59(61.45), H:5.33(5.19), N:16.58(16.45), O:9.47(9.32), Cl:7.00(6.85).
VIi: H, CH_3, $OCH_3C_6H_4$, 270 °C, 70 %, $C_{27}H_{30}N_6O_4$,C:64.54(64.42), H:5.97(5.81), N:16.73(16.59), O:12.74(12.60).
VIj: H, CH_3, $NO_2C_6H_4$, 272 °C, 72 %, $C_{26}H_{27}N_7O_5$, C:60.34(60.20), H:5.22(5.07), N:18.95(18.82), O:15.47(15.30).

e. IR (KBr) Spectral data (ν_{max} in cm^{-1})

VIa: 3185 (NH), 1665 (C=O), 1600 (C=N)
VIb: 3175 (NH), 1670 (C=O), 1602 (C=N)
VIc: 3200 (NH), 1665 (C=O), 1605 (C=N)
VId: 3190 (NH), 1670 (C=O), 1604 (C=N)
VIe: 3210 (NH), 1650 (C=O), 1605 (C=N)
VIf: 3215 (NH), 1660 (C=O), 1602 (C=N)
VIg: 3195 (NH), 1670 (C=O), 1605 (C=N)
VIh: 3190 (NH), 1675 (C=O), 1604 (C=N)
VIi: 3205 (NH), 1660 (C=O), 1605 (C=N)
VIj: 3180 (NH), 1660 (C=O), 1604 (C=N)

f. ^{1}H NMR (DMSO-d6) Spectral data (δ in ppm)

VIa: 1.52 (s, 3H, CH_3), 2.35 (s, 3H, N=C-CH_3), 6.8 (s, H, Ar-NH), 7.0 (s, 2H, O-CH_2-CO), 7.1 – 7.3 (m, 10H, Ar–H), 7.4 (d, 2H, C_6H_4), 7.58 (d, 2H, Ar-H), 7.7 (d, 2H, C_6H_4), 10.92 (s, H, NH)
VIg: 1.50 (s,3H, CH_3), 2.26 (s, 3H, CH_3), 2.33 (s, 3H, N=C-CH_3), 6.8 (s, H, Ar-NH), 7.1-7.3(m, 9H, Ar-H), 7.23 (s, 2H, O-CH_2-CO), 7.4 (d, 2H,C_6H_4), 7.7 (d, 2H, Ar,C_6H_4), 10.90 (s, H, NH),
VIh: 1.58 (s, 3H, CH_3), 2.39 (s, 3H, N=C-CH_3), 6.8 (s, H, Ar-NH), 7.1-7.3 (m, 9H, Ar-H), 7.27 (s, 2H, O-CH_2-CO), 7.4 (d, 2H, C_6H_4), 7.7 (d, 2H, C_6H_4), 10.94 (s, H, NH)
VIi: 1.53 (s, 3H, CH_3), 2.37 (s, 3H, N=C-CH_3), 6.8 (s, H, Ar-NH), 7.1-7.3 (m, 9H, Ar-H), 7.26 (s, 2H, O-CH_2-CO), 7.4 (d, 2H, C_6H_4), 7.7 (d, 2H, C_6H_4), 10.93 (s, H, NH)
VIj: 1.48 (s, 3H, CH_3), 2.30 (s, 3H, N=C-CH_3), 6.8 (s, H, Ar-NH), 7.1-7.3 (m, 9H, Ar-H), 7.20 (s, 2H, O-CH_2-CO), 7.4 (d, 2H, C_6H_4), 7.7 (d, 2H, C_6H_4), 10.89 (s, H, NH)

g. Elemental analysis data of compounds VII (-R, -R_1=CH_3, -R_2, M.P., Yield, Molecular formula, Element: Found %, (Calc %))

VIIa: H, 4-C_6H_5, 240 °C, 75 %, $C_{28}H_{30}N_6O_4$, C:65.36(65.23), H:5.83(5.70), N:16.34(16.21), O:12.45(12.30).
VIIb: CH_3, 4-C_6H_5, 245 °C, 77 %, $C_{29}H_{32}N_6O_4$, C:65.90(65.72), H:6.06(5.92), N:15.90(15.75), O:12.12(12.00).
VIIc: OCH_3, 4-C_6H_5, 235 °C, 72 %, $C_{29}H_{32}N_6O_4$, C:63.91(63.76), H:5.88(5.74), N:15.44(15.30), O:14.70(14.56).\
VIId: OC_2H_5, 4-C_6H_5, 250 °C, 73 %, $C_{30}H_{34}N_6O_5$, C:64.51(64.37), H:6.09(5.94), N:15.05(14.92), O:14.33(14.18).
VIIe: Cl, 4-C_6H_5, 230 °C, 75 %, $C_{28}H_{29}N_6O_4Cl$, C:61.25(61.10), H:5.28(5.12), N:15.31(15.19), O:11.66(11.44), Cl:6.47(6.33).
VIIf: Br, 4-C_6H_5, 255 °C, 78 %, $C_{28}H_{29}N_6O_4Br$, C:56.67(56.50), H:4.89(4.66), N:14.16(14.02), O:10.79(10.52), Br:13.47(13.30).
VIIg: H, 4-$CH_3C_6H_4$, 260 °C, 72 %, $C_{29}H_{32}N_6O_4$, C:65.90(65.72), H:6.06(5.87), N:15.90(15.78), O:12.12(11.95).
VIIh: H, 4-ClC_6H_4, 265 °C, 76 %, $C_{28}H_{29}N_6O_4Cl$, C:61.36(61.21), H:5.11(5.00), N:15.34(15.20), O:11.68(11.47), Cl:6.17(6.02).
VIIi: H, 4-$OCH_3C_6H_4$, 270 °C, 70 %, $C_{27}H_{30}N_6O_4$, C:64.34(64.17), H:5.97(5.78), N:16.73(16.58), O:12.74(12.60).
VIIj: H, 4-NOC_6H_4, 272 °C, 72 %, $C_{26}H_{27}N_6O_5$, C:60.34(60.15), H:5.22(5.08), N:18.95(18.80), O:15.74(15.62).

h. IR (KBr) Spectral data (ν_{max} in cm^{-1})

VIIa: 3206 (NH), 1685 (C=O), 1620 (C=N)
VIIb: 3195 (NH), 1690 (C=O), 1622 (C=N)
VIIc: 3230 (NH), 1685 (C=O),1625 (C=N)
VIId: 3215 (NH), 1695 (C=O), 1624 (C=N)
VIIe: 3230 (NH), 1675 (C=O), 1630 (C=N)
VIIf: 3210 (NH), 1685 (C=O), 1627 (C=N)
VIIg: 3180 (NH), 1695 (C=O), 1627 (C=N)
VIIh: 3195 (NH), 1700 (C=O), 1629 (C=N)
VIIi: 3245 (NH), 1705 (C=O), 1630 (C=N)
VIIj: 3240 (NH), 1685 (C=O), 1630 (C=N)

i. ^{1}H NMR (DMSO-d_6) Spectral data (δ in ppm)

VIIa: 2.22 (s, 3H CH_3), 2.40 (s, 3H, CH_3), 2.46 (s, 3H, $COCH_3$), 5.26 (s, 2H, OCH_2), 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).
VIIg: 2.20 (s, 3H CH_3), 2.26 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.44 (s, 3H, $COCH_3$), 5.24 (s, 2H, OCH_2), 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).
VIIh: 2.19 (s, 3H, CH_3), 2.37 (s, 3H, $COCH_3$), 2.24 (s, 2H, CH_3), 4.92 (s, 2H, OCH_2), 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).
VIIi: 2.18 (s, 3H CH_3), 2.39 (s, 3 H, CH_3), 2.43 (s, 3H, $COCH_3$), 3.89 (s, 3H, OCH_3), 5.24 (s, 2H, OCH_2), 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).
VIIj: 2.16 (s, 3H CH_3), 2.35 (s, 3H, CH_3), 2.40 (s, 3H, $COCH_3$), 5.22 (s, 2H, OCH_2), 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).

j. Elemental analysis data of compounds VIII (M.P., Yield, Molecular formula, Element: Found %, (Calc %)

VIII: 150 °C, 65 %, $C_{19}H_{20}N_6O_3S$, C:55.33(55.19), H:4.85(4.68), N:20.38(20.21), O:16.50(16.35), S:7.76(7.62).

k. IR (KBr) Spectral data (ν_{max} in cm^{-1})

VIII: 3126 (oxadiazole NH), 3180 (NH), 1603 (C=N), 1670 (C=O), 1134 (C=S).

l. ^{1}H NMR (DMSO-d_6) Spectral data (δ in ppm)

VIII: 2.3 (s, 3H CH_3), 5.45 (s, 2H, OCH_2-), 6.8 (s, H, Ar - NH), 7.1–7.3 (m, 5H, Ar - H),7.4 (d, 2H, C_6H_4), 7.7 (d, 2H, C_6H_4), 14.7 (s, H, thiol-thione tautomeric proton NH)

m. Elemental analysis data of compounds IX (R_1=H, R_2, M.W., M.P., Yield, Molecular formula, Element: Found %, (Calc %)

IXa: *p*-tolyl, 240°C, 75 %, $C_{27}H_{25}N_7O_3S$, C:61.48(61.32), H:4.47(4.30), N:18.59(18.45), O:9.10(8.93), S:6.07(5.90).
IXb: *p*-anisyl, 245°C, 77 %, $C_{27}H_{25}N_7O_4S$, C:59.66(59.48), H:4.60(4.56), N:18.04(17.85), O:11.78(11.62), S:5.89(5.69).
IXc: *p*-flurophenyl, 235°C, 78 %, $C_{26}H_{22}N_7O_3SFl$, C:58.76(58.65), H:4.14(3.98), N:18.45(18.33), O:9.04(8.86), S:6.02(5.80), Fl:3.55 (3.38).
IXd: *p*-chlorophenyl, 250°C, 73 %, $C_{26}H_{22}N_7O_3SCl$, C:56.98(56.75), H:4.01(3.83), N:17.88(17.69), O:8.76(8.61), S:5.84(5.69), Cl:6.48(6.31).
IXe: *p*-bromophenyl, 230°C, 80 %, $C_{26}H_{23}N_7O_3Br$, C:52.71(52.58), H:3.71(3.59), N:16.55(16.38), O:8.10(7.92), S:5.40(5.23), Br:13.49(13.30).
IXf: *p*-nitrophenyl, 255°C, 83 %, $C_{26}H_{22}N_8O_5S$, C:55.91(55.74), H:3.94(3.78), N:20.07(19.87), O:14.33(14.21), S:5.73(5.57).
IXg: diethyl, 260°C, 72 %, $C_{28}H_{37}N_8O_3S$, C:59.46(59.28), H:6.54(6.39), N:19.82(19.70), O:8.49(8.32), S:5.66(5.44).
IXh: diphenyl, 266 °C, 70 %, $C_{32}H_{27}N_7O_3S$, C:65.29(65.18), H:4.52(4.62), N:17.01(16.63), O:8.03(8.14), S:5.27(5.44).
IXi: morpholinyl, 270°C, 70 %, $C_{20}H_{18}N_7O_4S$, C:53.09(52.89), H:3.98(3.76), N:21.68(21.52), O:14.15(13.98), S:7.07(6.84).
IXj: piperazinyl, 272°C, 72 %, $C_{22}H_{20}N_7O_3S$, C:57.14(56.98), H:4.32(4.17), N:21.21(21.07), O:10.38(10.23), S:6.92(6.76).

IXk: N-methyl piperizinyl, 267°C, 68 %, $C_{22}H_{21}N_7O_3S$, C:57.01 (56.63), H:4.53 (4.38), N:21.16 (21.02), O:10.36 (10.12), S:6.91 (6.83).

n. IR (KBr) Spectral data (ν_{max} in cm^{-1})

IXa: 1608 (C=N), 1665 (C=O), 1156 (C=S), 2939 (C-HStr), 3140 (NH), 3250 (Ar – NH).
IXb: 1620 (C=N), 1660 (C=O), 1150 (C=S), 2925 (C-HStr), 3130 (NH), 3240 (Ar – NH).
IXc: 1610 (C=N), 1670 (C=O), 1160 (C=S), 2945 (C-HStr), 3145 (NH), 3255 (Ar – NH).
IXd: 1608 (C=N), 1663 (C=O), 1158 (C=S), 2940 (C-HStr), 3172 (NH), 3253 (Ar – NH),
IXe: 1609 (C=N), 1665 (C=O), 1155 (C=S), 2943 (C-HStr), 3143 (NH), 3254 (Ar – NH).
IXf: 1605 (C=N), 1655 (C=O), 1145 (C=S), 2930 (C-HStr), 3135 (NH), 3245 (Ar – NH).
IXg: 1590 (C=N), 1645 (C=O), 1135 (C=S), 2925 (C-HStr), 3125 (NH), 3230 (Ar – NH).
IXh: 1593 (C=N), 1667 (C=O), 1107 (C=S), 2940 (C-HStr), 3150 (NH), 3250 (Ar – NH).
IXi: 1610(C=N), 1675 (C=O), 1165 (C=S), 2955 (C-HStr), 3150 (NH), 3265 (Ar – NH).
IXj: 1610 (C=N), 1670 (C=O), 1160 (C=S), 2945 (C-HStr), 3145 (NH), 3260 (Ar – NH).
IXk: 1605 (C=N), 1655 (C=O), 1145 (C=S), 2935 (C-HStr), 3140 (NH), 3255 (Ar – NH).

o. ^{1}H NMR (DMSO-d_6) Spectral data (δ in ppm)

IXa: 2.28 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 5.0 (s, 2H, NCH_2), 5.64 (s, 2H, N-CH_2-N), 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), 11.2 (s, H, Ar-NH).
IXb: 2.40 (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), 6.8 (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), 11.1 (s, H, Ar-NH).
IXf: 2.50 (s, 3H, CH_3), 4.96 (s, 2H, N-CH_2), 5.50 (s, 2H, N-CH_2-N), 6.8 (s, 1H, Ar-NH), 7.1-7.3 (m, 9H, Ar-H), 7.4 (d, 2H, C_6H_4), 7.7 (d, 2H, C_6H_4), 10.23 (s, 1H, NH).
IXi: 2.60 (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, N-CH_2-N), 6.8 (s, H, Ar-NH), 7.1-7.3 (m, 9H, Ar-H), 6.8 (s, H, Ar-NH), 7.4 (d,2H,C_6H_4), 7.7(d, 2H, C_6H_4), 10.20 (s, 1H, NH).
IXj: 2.56 (t, 4H CH_2-N-CH_2), 2.58 (s, 3H, CH_3), 4. 45 (s, 2H, N-CH_2-N), 5.20 (s, 2H, N–CH_2), 5.45 (s, 2H, N-CH_2-N), 4.45 (s, 2H, N-CH_2-N), 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), 10.19 (s, H, NH).

3.2. Antimicrobial activity

All synthesized compounds were subjected to preliminary antibacterial screening by disc diffusion method against *Staphylococus aureus* NCCS 2079, *Bacillus cereus* NCCS 2106, *Escherichia coli* NCCS 2065 and *Pseudomanas aeruginosa* NCCS 2200. As shown in Table 1, VII series showed moderate activity against tested organism. In this series chloro, bromo and nitro substituted compounds showed more activity than other compounds. Whereas among the compounds of **IX** series, fluoro, chloro, bromo, nitro, morphonilyl, piperizynyl, *N*-methylpiperizine showed more activity than other compounds.

All synthesized compounds were subjected to preliminary antifungal screening by disc diffusion method against *Aspergillus niger* NCCS 1196 and *Candida albicans* NCCS 2106. Among the compounds of **VII** series, Chloro, bromo and nitro substituted compounds showed more activity against *Aspergillus niger* NCCS 1196 and *Candida albicans* NCCS 2106. The compounds of **IX** series have demonstrated good antifungal activity particularly fluoro, chloro, bromo, nitro, morphonilyl, piperizynyl and *N*-methyl piperizine showed good antifungal activity. The details are given in Table 1 and 2**.**

Table 1.- Antifungal activity of novel compounds synthesized.

Compound	-R	-R_1	-R_2	Zone of inhibition in mm (MIC in µg/mL)	
				Aspergillus niger **NCCS 1196**	***Candida albicans*** **NCCS 2106**
VIIa	H	CH_3	C_6H_5	2.25 (55)	1.75(52.5)
VIIb	CH_3	CH_3	C_6H_5	1.75(45)	1.25(52.5)
VIIc	OCH_3	CH_3	C_6H_5	1.5(52.5)	1.5(52.5)
VIId	OC_2H_5	CH_3	C_6H_5	1.5(47.5)	1.25(50)
VIIe	Cl	CH_3	C_6H_5	1.75(40)	2(120)
VIIf	Br	CH_3	C_6H_5	1.75(40)	1.75(42.5)
VIIg	H	CH_3	$CH_6C_6H_5$	1.25(52.5)	1.25(50)
VIIh	H	CH_3	ClC_6H_4	2(37.5)	1.75(33.75)
VIIi	H	CH_3	$OCH_3C_6H_4$	1.5(195)	1.5(>50)
VIIj	H	CH_3	$NO_2C_6H_4$	1.75(41.25)	1.75(41.25)
IXa	H	H	*p*-tolyl	2.5(45)	2.25(45)
IXb	H	H	*p*-anisyl	2.25(47.5)	2(45)
IXc	H	H	*p*-fluoro phenyl	3.5(35)	3(37.5)
IXd	H	H	*p*-chloro phenyl	3.25(37.5)	3.5(36.25)
IXe	H	H	*p*-bromo phenyl	3.25(37.5)	3(37.5)
IXf	H	H	*p*-nitro phenyl	3.75(32.5)	3.25(36.25)
IXg	H	H	diethyl	2(47.5)	1.75(47.5)
IXh	H	H	di phenyl	2(47.5)	2(47.5)
IXi	H	H	morphonilyl	3.5(35)	3.25(35)
IXj	H	H	Piperizyinyl	3.25(37.5)	3(35)
IXk	H	H	*N*-methyl piperizine	3.25(35)	3.25(32.5)

Table 2.- Antibacterial activity of novel compounds synthesized.

Compound	Zone of inhibition in mm (MIC in µg/mL)			
	Staphylococus aureus **NCCS 2079**	***Bacillus Cereus*** **NCCS 2106**	***Escherichia coli*** **NCCS2065**	***Pseudomanas aeruginos*** **NCCS 2200**
VIIa	1.5(50)	1.25(52.5)	1.25(52.5)	1.75(45)
VIIb	1.5(50)	1.25(52.5)	1(55)	1.5(50)
VIIc	1(50)	1.25(50)	1(55)	1.25(52.5)
VIId	2(42.5)	1.5(47.5)	1(50)	1.75(47.5)
VIIe	2(31.25)	2.25(35)	1.5(47.5)	2(32.5)
VIIf	1.75(35)	2(35)	1.5(45)	1.25(47.5)
VIIg	1(55)	1.25(55)	1(60)	1.25(60)
VIIh	2(31.25)	2(36.25)	1.5(40)	1.75(32.5)
VIIi	1(50)	1.5(47.5)	1.5(45)	1.25(50)
VIIj	1.75(33.75)	2(35)	1.5(38.75)	1.75(41.25)
IXa	1.5(52.5)	1.75(57.5)	1.25(57.5)	1.5(60)
IXb	1.75(50)	1.5(52.5)	1.5(55)	1.25(57.5)
IXc	2.5(36.25)	2.75(38.75)	2.25(38.75)	2.5(38.75)
IXd	3(35)	2.5(38.75)	2.25(38.75)	2.75(36.25)
IXe	2.5(37.5)	2.75(38.75)	2(40)	2.25(37.5)
IXf	2.75(36.25)	2.5(38.75)	2.5(35)	2.5(35)
IXg	1.75(50)	2(52.5)	1.5(57.5)	5(57.5)
IXh	1.75(50)	1.75(50)	5(60)	1.5(55)
IXi	2.5(37.5)	3(35)	2.5(37.5)	2.75(35)

Compound	Zone of inhibition in mm (MIC in µg/mL)			
	Staphylococus aureus **NCCS 2079**	***Bacillus Cereus*** **NCCS 2106**	***Escherichia coli*** **NCCS2065**	***Pseudomanas aeruginos*** **NCCS 2200**
IXj	2.75(32.5)	2.5(37.5)	2.25(40)	2.5(37.5)
IXk	2.5(35)	2.75(35)	2.25(37.5)	2.75(35)

4. CONCLUSSION

All the novel compounds have demonstrated moderate antimicrobial activity against selected of fungal and bacterial stains.

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