SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL BIS-HETEROCYCLIC MOIETIES

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ABSTRACT

*In the present investigation malonyl dihydrazide **2** served as key intermediate for the synthesis of novel malonyl bis(3-methyl-4,5-dihydro-5-oxo-1-yl) pyrazoline moieties **3-6** were synthesized. The structures of these compounds were confirmed by their elemental analysis, IR, ^{1}H, ^{13}C NMR and mass spectral data. All the newly synthesized compounds were screened for their antioxidant and antimicrobial activities. The antioxidant activity results the compound **6a** showed good RSA (61.41, 71.53, 73.82 & 81.70%) at concentrations 25, 50, 75 & 100 µg/mL. Antibacterial activity **fig 2,** Compound **6** displayed moderate activity against all tested microorganisms. Antifungal activity **fig 3,** compound **6** have shown to possess moderate antifungal activity against both fungi, A.niger and C.albicans.*

Keywords: *Synthesis, bis-heterocycles, pyrazole moiety, antioxidant, antimicrobial activities*

1. INTRODUCTION

The natural occurrence and biological importance of compounds containing pyrazole ring system. In view of the significance of five membered heterocycles such as pyrazole, it was thought to investigate the synthesis of new series of bis heterocycles containing two pyrazole rings bridged via carbon atom. Currently bis heterocycles of pyrazole moiety synthesis from 20 years vast novel work is used for biological and pharmacological activities, these work helpful pharma industries to encourages. Compound containing $–CONHNH_2$ group can be converted in to pyrazoles as a precursor by reaction with following reagents as ethyl acetate, ethyl cyanoacetate, benzoyl acetone and acetyl acetone. In this work shows flaunty of biological activities like antibacterial [1–6], antiviral [7,8] antiproliferative, proapoptoic [9], antitumor, and anti-inflammatory [11,12] activities. Furthermore, thioazole bis- hetrocycles were exploded in several most important biologically dynamic drug molecules like Ritonavir as antiretroviral drugs, Sulfathiazole as antimicrobial drug. Tiazofurin as antineoplastic and antifungal drug.

All these synthesis and biological activities motivates us to synthesis new extension of our work [13-16], effective drug molecules which contain bis-hetrocylic pyrazole and evaluate their biological activities that these new moieties could be effective bis-heterocyclic in the library of confess drug molecule.

2. EXPERIMENTAL SECTION

Chemistry

2.1Materials and Methods

All the reagents were obtained commercially and used by further purification using standard procedures. Melting points were determined by an open capillary method and are uncorrected. Purity of the compounds was checked by thin layer chromatography using silica gel-G coated Al plates (Merck) and spots were visualized by exposing the dry plates in iodine vapors. The IR (KBr pellet) spectra were recorded on a Perkin-Elmer (Spectrum ONE) FT-IR Spectrometer. The ^{1}H and ^{13}C NMR (DMSO-d_6) spectra were recorded with a BRUKER NMR 500 and 125 MHz spectrometers, and the chemical shift values are expressed in ppm (δ scale) using tetramethylsilane as an internal standard. The mass spectral measurements were carried out by Electron Impact method on JEOL GC mate spectrometer at 70 eV. Elemental analyses were performed on flash EA 1112 series elemental analyzer.

2.2Experimental

General Procedure for the synthesis of Malonyl dihydrazide (2): A mixture of diethyl malonate **1** (8.0 g, 0.05 mol) and hydrazine hydrate (10 ml, 80%) was refluxed in ethanol (30 ml) on a steam bath for 4 h. The reaction mixture was cooled thoroughly and colourless solid that separated was collected by filtration. It was crystallized from ethanol. It was crystallized from ethanol as shining flakes yield, (80%), m.p. 160^0C (Lit. 160^0C)

General Procedure for the synthesis of Malonyl bis(3-methyl-4,5-dihydro-5-oxo-1-yl) pyrazoline (3): To a mixture of malonyl dihydrazide **2** (0.01 mol) and ethyl acetoacetate (0.02

mol) in ethanol (10 ml), few drops of concentrated hydrochloric acid was added and the reaction mixture was heated under reflux for 5h. The resulting solution was cooled to room temperature and the solid which separated was collected and crystallized from ethanol.
Yield 80%, m.p. 260-61°C, FT-IR (KBr) (cm^{-1}): 3290-3320 $NHNH_2$, 3295 OH group, 1734 C=O, 1663 C=O, 1596 C=N, ^{1}H NMR (DMSO-d_6) δ: 7.81 (s, 1H, 2X CH_2), 3.02 (s, 2H, CH_2), 2,05 (s, 2H, 2XCH_2), ^{13}C-NMR (DMSO-d6): δ 171.0, 162· 9, 154.8, 78.6, 28.9, Mass: m/z 249 $[M]^{+\cdot}$, Anal. Calcd. for $C_{10}H_9N_4O_4$ (249.062), C, 48.20, H, 3.64, N, 22.48. Found: C, 48.26, H, 3.60, N, 22.45%.

General Procedure for the synthesis of Malonyl bis (3-amino-4,5- dihydro-5-oxo-1-yl) pyrazoline (4): A mixture of malonyl dihydrazide **2** (0.01mol) and ethyl cyanoacetate (0.02mol) in anhydrous ethanol (10ml) was heated under reflux in presence of catalytic quantity of hydrochloric acid. After heating for 5h the reaction mixture was concentrated and cooled to room temperature. The solid separated was collected and crystallized from ethanol.
Yield 85%, m.p. 235-66°C, FT-IR (KBr) (cm^{-1}): 3200-3250 NH_2, 1643, 1610 C=O, 1538 C=N, ^{1}H NMR (DMSO-d_6) δ: 4.92 (s, 4H, 2XNH_2), 3.12 (s, 2H, CH_2), 2,09 (s, 4H, 2X CH_2), ^{13}C-NMR (DMSO-d6): δ 175.1, 161.9, 152.5, 79.8, 42.6, Mass: m/z 266 $[M]^{+\cdot}$, Anal. Calcd. for $C_9H_{10}N_6O_4$ (266.214), C, 40.61, H, 3.79, N, 31.57. Found: C, 40.60, H, 3.83, N, 31.62%.

General Procedure for the synthesis of Malonyl bis(3,5-dimethyl-1-yl) pyrazoline (5): A mixture of malonyl dihydrazide **2** (0.01mol) and acetylacetone (0.02mol) was heated in boiling ethanol in presence of catalytic amount of concentrated hydrochloric acid for 5h. The reaction mixture was cooled to room temperature and the product separated was collected and crystallized from ethanol.
Yield 90%, m.p. 120-21°C, FT-IR (KBr) (cm^{-1}): 3290-3320 $NHNH_2$, 1610 C=N, ^{1}H NMR (DMSO-d_6) δ: 5.90 (s, 4H, 2X CH_2), 3.42 (s, 2H, CH_2), 2.58 (s, 8H, 4XCH_2), ^{13}C-NMR (DMSO-d6): δ 196.2, 145· 9, 143.8, 105.4, 28.6, 17.8, 11.9, Mass: m/z 260 $[M]^{+\cdot}$, Anal. Calcd. for $C_{13}H_{16}N_4O_2$ (260.127), C, 59.99, H, 6.20, N, 21.52. Found: C, 59.98, H, 6.26, N, 21.57%.
General Procedure for the synthesis of Malonyl bis(5-methyl-3-phenyl-1-yl) pyrazoline (6): A mixture of malonyl dihydrazide **2** (0.01mol) and benzoyl acetone (0.02mol) was heated at reflux temperature in presence catalytic amount of concentrated hydrochloric acid for 5h. The products which separate on cooling was collected and crystallized from ethanol.
Yield 66, m.p. 122-23°C, FT-IR (KBr) (cm^{-1}): 1683 C=O, 1610 C=N, ^{1}H NMR (DMSO-d_6) δ: 7.21-7.61 (m, 12H, Ar-H), 3.51 (s, 2H, CH_2), 2,61 (s, 6H, 2XCH_2), ^{13}C-NMR (DMSO-d6): δ 196.2, 145· 9, 143.8, 105.4, 28.6, 17.8, 11.9, Mass: m/z 388 $[M]^{+\cdot}$, Anal. Calcd. for $C_{23}H_{24}N_4O_2$ (388.19), C, 71.11, H, 6.23, N, 14.42. Found: C, 71.14, H, 6.25, N, 14.47%.

3.Antioxidant Activity

3.1Free radical scavenging activity

Free radical scavenging activity was done by DPPH method [17]. Different concentrations (10µg, 50µg and 100µg) of samples and butylated hydroxy anisole (BHA) were taken in different test tubes. The volume was adjusted to 100µl by adding MeOH. Five

milliliters of 0.1mM methanolic solution of DPPH was added to these test tubes and shaken vigorously. The tubes were allowed to stand at 27°C for 20min. The control was prepared as above without samples. The absorbances of samples were measured at 517nm. Radical scavenging activity was calculated using the following formula:
% Radical scavenging activity = [(Control OD-Sample OD)/ (Control OD)] ×100.

3.2Antimicrobial activity

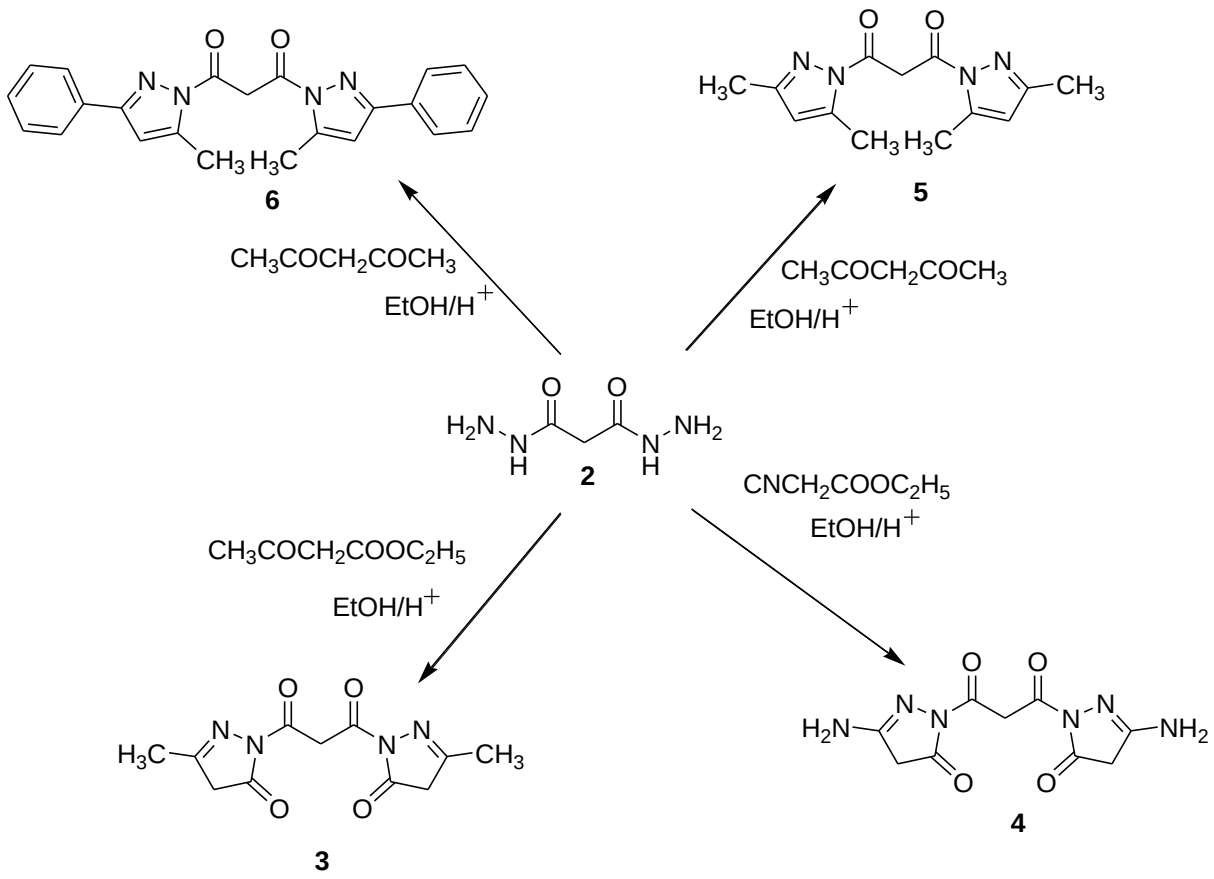
All the novel bis heterocyclic pyrazole moieties were tested for *in-vitro* antimicrobial activity against gram negative bacteria *E. Coli* and P. *Aeruginosa* and gram positive S. Aureus and *B.subtilis* against antifungal activity against *A.niger* and *Candida albicans* by applying cup plate method [18]. Dilution process was adopted at 25 μg, 50 μg, and 100 μg/mL concentrations, respectively. The activity is compared with reference drugs gentamycin for antibacterial and fluconazole for antifungal activity. The zone of inhibition after 24 hr of incubation at 37^0C in case of antibacterial activity and 48 hr in case of antifungal activity was compared with that of standards.

4. RESULTS AND DISCUSSION

4.1 Chemistry

In the present investigation malonyl dihydrazide **2** served as key intermediate. The reaction of diethyl malonate **1** with hydrazine hydrate (80%) in anhydrous ethanol at reflux temperature for 4-5 h afforded malonyl dihydrazide **2** in quantitative yield. The structure of **2** was confirmed by its physical constant and IR spectral data. Compounds containing –$CONHNH_2$ group can be conveniently converted into pyrazoles by reaction with different reagents such as ethyl acetoacetate, ethyl cyanoacetate, acetyl acetone and benzoyl acetone.

The reaction of malonyl dihydrazide **2** with ethyl acetoacetate in anhydrous ethanol in presence of catalytic amount of concentrated hydrochloric acid at reflux temperature for 5h resulted in the formation of a crystalline compound. The IR spectrum of **3** exhibited characteristic absorption band in the region 1663 cm^{-1} due to C=O, a band at 1734 cm^{-1} due to C=O stretching of pyrazole. Further, the presence of absorption band at 3295 cm^{-1} due to OH group indicated the existence of **3** as the enol tautomer. The absence of absorption band in the region 3290-3320 cm^{-1} due to $NHNH_2$ and appearance of band at 1596 cm^{-1} due to C=N confirmed pyrazole ring formation. The treatment of malonyl hydrazide **2** with ethyl cyanoacetate in boiling ethanol containing catalytic amount of concentrated hydrochloric acid for 5h gave malonyl bis (3-amino-4,5-dihydro-5-oxo-1-yl)pyrazoline **4** .



Scheme 1: Synthetic routes of bis-hetericyclic pyrazole moieties **3-6**

The IR spectrum of malonyl bis(3-amino-4,5-dihydro-5-oxo-1-yl)pyrazoline **4** exhibited characteristic IR absorption band in the region 3200-3250cm^{-1} due to NH_2, a band at 1643cm^{-1} and 1610cm^{-1} due to ring CO and CO malonyl groups. The appearance of absorption band at 1538 cm^{-1} due to C=N confirmed the formation of pyrazole ring. It was next thought to carry out the reaction of carbohydrazide **2** with acetyl acetone. Thus the condensation of malonyl di hydrazide with acetyl acetone in boiling ethanol containing catalytic amount of concentrated hydrochloric acid for 4h furnished the corresponding malonyl bis(3,5-dimethyl-1-yl)pyrazoline **5** in good yield. The structure of compound **5** was ascertained by IR, ^{1}H NMR and micro analytical data. The IR spectrum of **5** showed a strong absorption band at 1683 cm^{-1} due to C=O group. The disappearance of absorption band in the region 3290-3315cm^{-1} due to $NHNH_2$ and appearance of a band in the region 1610cm^{-1} due to C=N confirmed the involvement of hydrazino group in the pyrazole ring formation. The ^{1}H NMR spectrum of **5** displayed three signals. A singlet at δ 3.6 due to 12 protons of four methyl groups, another singlet at δ 3.6 due to two protons of CH_2 group and third singlet at δ 6.0 due to a methine proton of pyrazole ring. The mixture of malonyl dihydrazide **2** (0.01mol) and Benzoyl acetone (0.02mol) was heated at reflux temperature in presence catalytic amount of concentrated hydrochloric acid for 5h. The product which separate on cooling was collected and crystallized from Ethanol **6**. In IR spectrum the bispyrazole **6** displayed absorption band in the region 1683 cm^{-1} characteristic of C=O group of malonyl moiety. The absence of absorption band due to $NHNH_2$ and appearance of absorption band at 1610cm^{-1} due to C=N confirmed, the formation.

4.2 Biological Evaluation

4.2.1Antioxidant activities

1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity (RSA)

Numbers of methods are available for the determination of free radical scavenging activity (RSA) but the assay employing the stable DPPH· has received much attention owing to its ease of use and convenience. This assay is the most widely used *in vitro* test to access free radical scavenging capacities of test compounds. The RSA of synthesized compounds were carried out at 25, 50, 75 and 100 µg/ml concentrations in methanol using the DPPH method. All the test analyses were performed on three replicate and the results are averaged. Results are expressed as the percentage decrease with respect to the control values. The results are illustrated in the **fig-1.** The compound **6a** showed good RSA (61.41, 71.53, 73.82 & 81.70%) at concentrations 25, 50, 75 & 100 µg/ml. This higher RSA may be attributed to the presence of two phenyl groups group present in it, which may responsible for stabilization of free radical formed after donating a hydrogen atom to DPPH free radical.

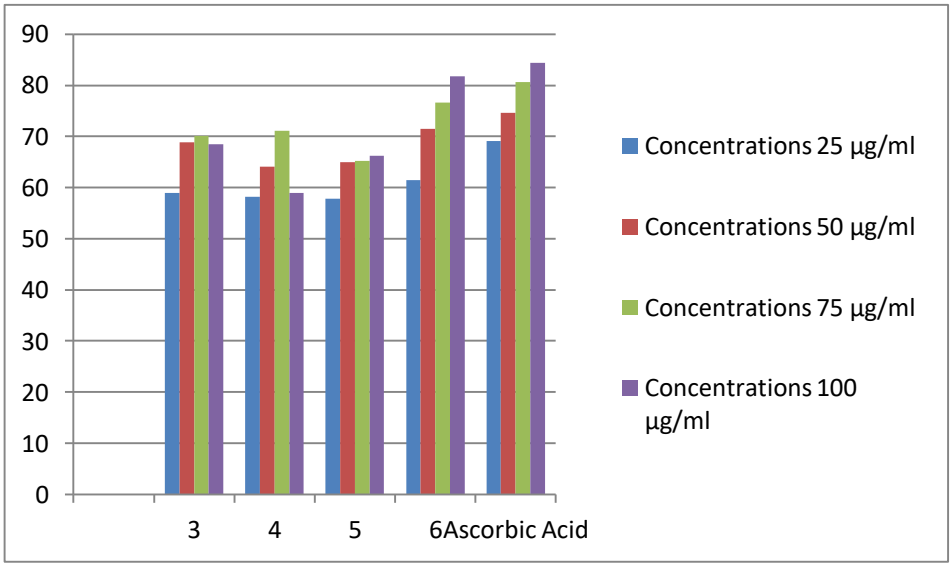


Figure-1: 1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity (RSA)

4.2.2 Antimicrobial activity

Antibacterial activity **fig 2,** Compound **6** displayed moderate activity against all tested microorganisms. Antifungal activity **fig 3,** compound **6** have shown to possess moderate antifungal activity against both fungi, *A.niger* and *C.albicans.* Rest of the compounds shows moderate to less activity.

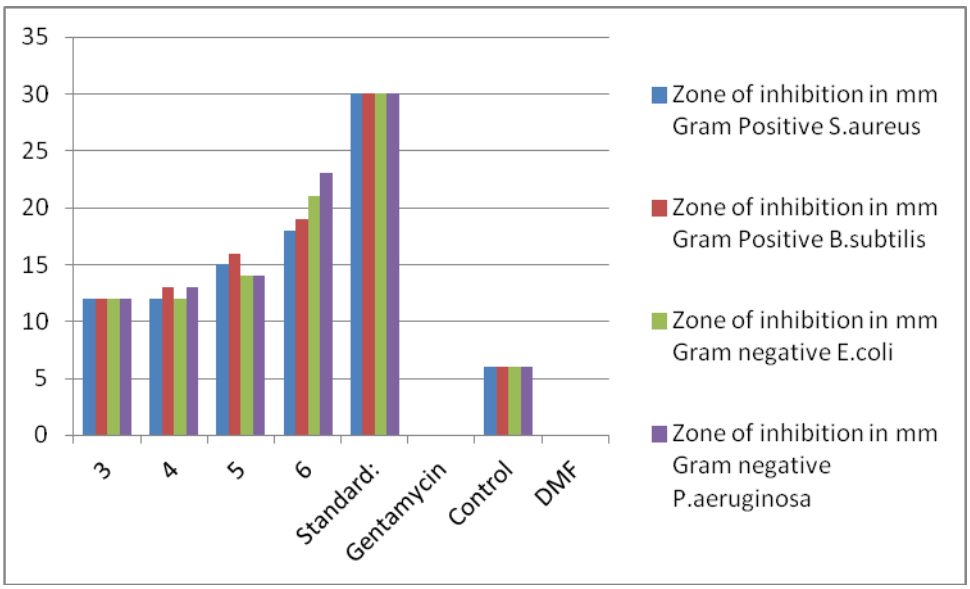


Figure-2: Antibacterial activity of bis-heterocycles **(3-6)**

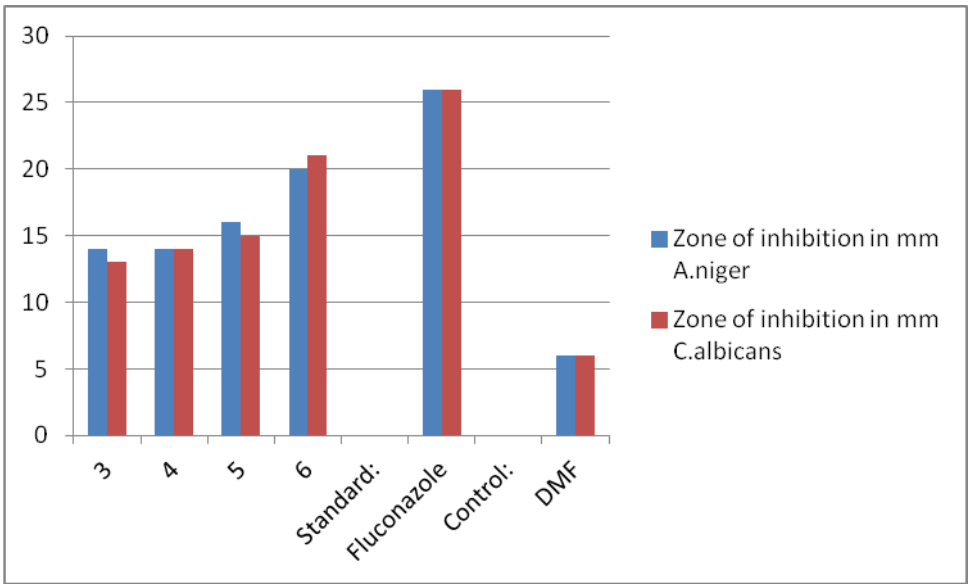


Figure-3: Antifungal activity of bis-heterocycles **(3-6)**

5. CONCLUSION

In the present investigation malonyl dihydrazide **2** served as key intermediate for the synthesis of novel malonyl bis(3-methyl-4,5-dihydro-5-oxo-1-yl) pyrazoline moieties **3-6** in good yield. The structures of these compounds were confirmed by their elemental analysis, IR, ^{1}H, ^{13}C NMR and mass spectral data. All the newly synthesized compounds were screened for their antioxidant and antimicrobial activities. The antioxidant activity result the compound **6a** showed good RSA (61.41, 71.53, 73.82 & 81.70%) at concentrations all the tested

concentrations. Compound **6** displayed moderate activity against all tested microorganisms. The compound **6** have possessed moderate antifungal activity against both fungi, *A.niger* and *C.albicans.*

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CONFLICT OF INTERESTS: The authors declare no conflict of interest

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