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Synthesis characterization and antimicrobial activity of pyrazolo pyrazolone derivatives (Mannich Reactions)

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ABSTRACT

Pyrazoleone pyrazoles are prepared by condensing 3-(2-phenylhydrazono)pentane-2,4-dione with hydrazezene hydrate in presence of suitable condensing agents. They undergo a variety of chemical reactions and are found useful in synthesis of variety of biologically active mannich derivatives of heterocyclic compounds. Mannich reaction is one of the versa tile reaction widely used in organic synthesis. This reaction is useful for synthesizing N-methyl derivatives and many drug molecules. Mannich base derivatives of pyrazolone pyrazoles play an important role in medical field with so many pharmaceutical importance such as antibacterial, antituberculosis antineoplastic, antidiabetic anti fertility and anti hydthyroid activity. anthelmintic, antifungal, anti-inflammatory, antiviral and analgesic properties. The potency of these clinically useful drugs encouraged the synthetic chemists for the synthesis of some more potent and significant compounds..

Keywords: pyrazole- pyrazolones, Mannich Bases, Antimicrobial, antituberculosis, antidiabetic anti fertility

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INTRODUCTION

The chemistry of pyrazolo pyrazolones has generated intensive scientific interest due to their biological and Industrial applications. Pyrazolones are natural biocides and are well known intermediates in the synthesis of heterocyclic compounds exhibiting various biological activities. pyrazoles and their derivatives possess some interesting biological properties such as antibacterial, antifungal, insecticidal, anesthetic, anti-inflammatory, analgesic etc. Pyrazole is a class of compounds, which has many applications in different field. One of the methods for the synthesis of such compound is from unsaturated carbonyls (chalcone) by the cyclization with hydrazine and substituted hydrazine. Pyrazole and their derivatives are to be important for drugs and agricultural chemicals. Some substituted pyrazoles and theirderivatives have been reported to possess several interesting biological activities such as hypnotic properties, antimicrobial, antitumor and antifungal. Many pyrazoles are used for the treatment of thyroid Antimicrobial drugs or chemicals are the substances used to kill or slow down the growth of microorganisms. They include antibiotics, antiviral, antifungal and anti-parasitic agents.[1] Antimicrobial chemotherapy has been used from last six decades against infectious diseases caused by a variety of pathogens. Since then, many antimicrobial drugs were discovered, hundreds of drugs using now a days. Anti-microbial drugs are most commonly available today.[2] Since the introduction of penicillin as antibiotics in the control of infectious diseases, frequent use of antimicrobial drugs cause a variety of problems, such as drug resistance, allergic reactions, nutritional loss, toxicity and much more. Almost all of the major categories of antibiotics in the clinical application showed resistance to microorganism specially β- lactam, macrolides, vancomycin and quinolones derived bacterial drug's resistance is a source of concern for healthcare officials. The effective treatment against microbial agents is limiting day by

day.[3,4] Many other antimicrobial drugs are toxic too. So, there is a real need to discover new compounds with high efficiency towards pathogens and less toxicity, which may be different from available resistant drugs. This provides a great opportunity to synthetic chemists for the synthesis of such new compounds having lower cytotoxicity and better antimicrobial properties. The biological activity of the compounds depends on structure of molecule.[5] It has been shown that heterocyclic compounds are more biological active as compared to others.[6] Heterocyclic compounds particularly five and six member heterocycles have attracted the attention of pharmaceutical community over the years due to their therapeutic value.[7] Polyfunctionalized heterocyclic compounds containing Nitrogen, sulphur, oxygen as heteroatoms play important roles in the drug discovery process.This compound has various applications in a number of fields. Pyrazolone nucleus contain plays an important role in various medicines.[7]. This stimulated great interest in the structural study of pyrazole and related compounds and much success was made in pharmaceutical industry. Some commercially used pyrazole pyrazolone; In the view of the varied biological and pharmacological application, we synthesized some new Heterocyclic derivatives of prazole-pyrazolones (I(a-f), 2(a-f), 3(a-f),4(a-f),5(a-f),6(a-f,)7(a-f)) This review is summarized to know about Mannich Base derivatives of pyrazole-pyrazolones along with their anti-microbial properties

MATERIALS AND METHODS

Apparatus

Melting points were determined in open capillary tubes and were found uncorrected. IR spectra were recorded on FT-IR spectrometer (Perkin Elmer) using KBr disc method. 1HNMR spectra were recorded on 1H FT-NMR (Brucker AMX 400\ MHz) spectrometer in DMSO. The comp ounds were analyzed for elemental analysis and the percentage of elements were found to be very near that of the calculated values.

Experimental section

The required primary amine is diazotized with sodium nitrite and HCl mixture at 0–5^{o}C and it is coupled with pentane 2,4-dione (B) to afford 3-(2-phenylhydrazono)pentane-2,4-dione (C).

Synthesis of (E)-1-(3,5-dimethyl-1H-pyrazol-4-yl)-2-phenyldiazene:

Condensation of 3-(2-phenylhydrazono) pentane-2,4-dione (C), hydrazine in the presence of catalytic amount of dimethyl formamide under microwave irradiation afforded (E)-1-(3,5-dimethyl-1H-pyrazol-4-yl)-2-phenyldiazene (1). In a typical experimental procedure, a mixture of 3-(2-phenylhydrazono) pentane-2,4-dione (C), hydrazine and dimethyl formamide (10 drops) 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 treated with cold water. The precipitate (E)-1-(3,5-dimethyl-1H-pyrazol-4-yl)-2-phenyldiazene (1) was filtered recrystalized from ethanol. The yield is 88 %.

Synthesis of (2R)-2-(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl)acetic acid (2).

A mixture of (1) and ethyl 2-chloroacetate, anhydrous K_2 CO_3 and DMF was stirred at room temperature for 8 hours. The reaction mixture was diluted with ice cold water. The separated solid was identified as methyl (2R)-2-(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl) acetate (2).The compound 2 was dissolved in THF and treated with Aq. 5N NaOH solution stirred at room temperature for 4 hours. The completion of reaction monitor by TLC, the THF distilled under reduced pressure and acidified with con HCl separated solid was filtered, washed with water to afforded (2R)-2-(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl)acetic acid 3a. Similar procedure was adapted for the synthesis of (3) (b-f) from 2 (b-f).

The compound synthesized (2)a has been characterized by means of their elemental analysis, I.R, and ^{1}HNMR data.

IR spectra:

The IR (KBr) spectra of (2R)-2-(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl) acetic acid(2). Fig. 2.1 showed absorptions around 2210,1690,1460 and 1455 cm^{-1} due to N=N, C=O of COOH, cyclic carbonyl and five member hetero cyclic pyrazole ring respectively;

^{1}H-NMR Spectra:

^{1}H-NMR (200 MHz, CDCl3+DMSO-d_6); δ 11.0 (br, s, COOH), 7.3-7.40 (m, 5H, C_6H_5) , 4.67 (S, 2H, N-CH_2-COOH), 2.8 (S, 6H, $(CH_3)_2$, pyrazole) ppm

Synthesis of methyl (2R)-2-(2-aminoacetamido) propionate Hydrochloride (3).

The compound of Alanine methyl ester Hydrochloride was dissolved in Chloroform and cooled to 0^{o}C then add N-Methyl morpholine (NMM) and stirred for 15min. then add N-Boc- Glycine was dissolved in $CHCl_3$ and dicyclohexyl carbodiimide (DCC) and stirred for 24 h, the reaction was monitored by TLC. After completion of the reaction the unwanted solid was filtered and separated the filtrate was washed with 5% sodium bicarbonate and saturated sodium chloride solution. Further the solvent was distilled under reduced pressure to give N-Boc-Glycyl-Alanine methyl ester as a crude product it was recrystallization with chloroform and hexane to give semi solid. The yield is 80 %. Then the deprotection of ^{t}Butyloxycoronyl-Glycyl-alanine methyl ester was dissolved in Dichloromethane and treated with trifluoroaceticacid and stirred for 2h. The reaction was monitor by TLC. After completion of the reaction it was basified with saturated sodium bicarbonate solution and extracted with dichloromethane and the solvent was distilled off and treated with isopropyl alcohol hydrochloride. The precipitate was filtered to give methyl (2R)-2-(2-aminoacetamido) propionate hydrochloride 4. The yield was 60%.

The compound synthesized 4 have been characterized by means of their elemental analysis, I.R, and ^{1}HNMR.

IR spectra:
The IR (KBr) spectra of methyl (2R)-2-(2-aminoacetamido)propionate 4. showed absorptions around 3445,3425, (m, -NH_2 str, Gly) 3122 (m,-NH str, amide), 2954,2925(m, -CH str, asym, CH_3 and CH_2), 2852(m, -CH str, sym, CH_2), 1748 (s, -C=O str, ester), 1645, 1636 (s, -C=O str, 2^{o} amide), 1534 (m, -NH bend, 2^{o} amide), 1272 (s, C-O str, ester)

^{1}H-NMR Spectra:
^{1}H-NMR (200 MHz, CDCl3); δ 6.50 (1H, br, s, -NH), 6.22 (1H, br, s, -NH), 4.74-4.69 (1H, m, α-H, Ala), 3.59 (3H, s, OCH_3), 3.49-3.47 (2H, d, CH_2, Gly), 2.0 (br, s, -NH_2), 1.29-1.27 (3H, d, β-H's, Ala) ppm;

Synthesis of Methyl (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl) acetamido ethanamido] propanoate 5.
The compound of (2R)-2-(2-aminoacetamido) propionate Hydrochloride 4 was dissolved in Chloroform and cooled to 0^{o}C then add N-Methylmorpholine(NMM) and stirred for 15min. then add (2R)-2-(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl)acetic acid 3a in $CHCl_3$ and dicyclohexylcarbodiimide(DCC) and stirred for 24 h, the reaction was monitored by TLC. After completion of the reaction the unwanted solid was filtered and separated filtrate was washed with 5% sodium bicarbonate and saturated sodium chloride solution. . Further the solvent was distilled under reduced pressure to give crude product, it was stirred in hexane to give solid. It was filtered to give Methyl (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl)acetamido ethanamido] propanoate 5a. The yield was 60%. The similar procedure was adopted for the synthesis of 5(b-f) from 3(b-f)

The compound synthesized Methyl (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl) acetamido ethanamido] propanoate 5a have been characterized by means of their elemental analysis, I.R and ^{1}HNMR.

IR spectra:
IR spectral data of Methyl (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl) acetamido ethanamido] propanoate 5a Showed around absorptions around 3122 (m,-NH str, amide), 2954-2925(m, -CH str, asym, CH_3 and CH_2), 2852(m, -CH str, sym, CH_2), 2210, (s, -N=N str, Azo) 1748 (s, -C=O str, ester), 1645 & 1636 (s, -C=O str, 2^{o} amide), 1534 (m, -NH bend, 2^{o} amide), 1272 (s, C-O str, ester), 1460 and 1455 cm^{-1} due to five member hetero cyclic pyrazole ring respectively;

^{1}H-NMR Spetra:
^{1}H-NMR (200 MHz,$CDCl_3$); δ 7.3-7.40 (m, 5H, C_6H_5), 6.50 (1H, br, s, -NH), 6.22 (1H, br, s, -NH , amide), 4.74-4.69 (1H, m, α-H, Ala), 4.67 (S, 2H, CH_2, amide), 3.67(3H, s, OCH_3), 3.49-3.47 (2H, d, CH_2, Gly), 2.8 (S, 6H, $(CH_3)_2$, pyrazol), 1.29-1.27 (3H, d, β-H's, Ala) ppm;

Synthesis of (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl)acetamido ethanamido] propyl hydrazide 48.
A solution of Methyl (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl)acetamido ethanamido] propanoate 5a and hydrazine hydrate in ethanol was refluxed for 5 hours. The reaction mixture was cooled and poured on to ice cold water with stirring. The separated solid was filtered, washed with water and recrystalized from ethanol to afford (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl)acetamido ethanamido]propyl hydrazide 6a. The similar procedure was adopted for the synthesis of 6(b-f) from 5(b-f).

The compound synthesized (2R)-2-[(4-(4'-substitutedphenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl)acetamido ethanamido] propyl hydrazide 6(a-f) have been characterized by means of their elemental analysis, I.R, ^{1}HNMR and MS data

IR spectra:
IR spectral data of (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl)acetamido ethanamido]propylhydrazide 6a. Showed absorptions around 3445 & 3425 (m, -NH_2, str, hydrazine), 3305 (m, -NH, str, hydrazine), 2954-2925(m, -CH str, asym, CH_3 and CH_2), 2852(m, -CH str, sym, CH_2), 2210, (s, -N=N str, Azo), 1710, 1645 & 1636 (s, -C=O str, 2^o amides), 1540 (m, -NH bend, 2^o amide), 1460 and 1455 cm^{-1} due to five member hetero cyclic pyrazole ring respectively;

^{1}H NMR Data:
The ^{1}HNMR spectra of (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl) acetamido ethanamido]propyl hydrazide 6a was recorded in^{1}HNMR (200MHz) ($CDCl_3$+DMSO-d_6) (δppm): δ 7.3-7.40(m, 5H, C_6H_5), 6.22(br, s, -NH, amide), 4.86-4.8(1H, m, α-H, Ala), 4.67(S, 2H, CH_2, amide), 3.49-3.47(2H, d, CH_2, Gly), 2.8(S, 6H, $(CH_3)_2$, pyrazol), 2.0(br, s -NH_2), 1.29-1.27(3H, d, -CH_3, β-H's of Ala).

Mass spectra
The mass spectra (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl)acetamido ethanamido]propyl hydrazide (R=H) displayed molecular ion (M^+) peaks at m/z 372.4

The significant fragmentation process occurring in the mass spectrum of [3,5-Dimethyl-4-(4'penyl hydrazono)-4,5-dihydro pyrazol-1-yl]-acetic acid hydrazide 6a The spectrum showed the molecular ion (M^+) peak at m/z 274 with an intensity of 14.7%. Decomposition of molecular ion A at path a resulted in the formation of the fragment "B" at m/z 243 (8.2%) by the loss of H radical atom from B yielded cation C at m/z 243 (46.2%) elimination of $C_2N_2H_5CO$ molecule from "C" yielded cation D at m/z 216 (77.5%). Loss of methylene radical (CH_2) from D yield cation E at m/z 202 (25.2%).Elimination of $C_7H_9N_{20}$ molecule from E yield radical cation F at m/z 148 (100%). Loss of C_6H_5 radical from molecular ion yelid cation G at m/z 197 (65.7%).

Synthesisof(2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl)acetamido ethan]-N'-(2-oxoindolin-3-ylidene) propyl hydrazide 50

The synthon, isatin 7 was prepared by the procedure described by Marvel and Heins[41].

Condensation of (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl)acetamido ethanamido] propyl hydrazide 6a with Isatin 7 in DMF furnished the (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl) acetamido ethanamido]-N'-(2-oxoindolin-3-ylidene) propyl hydrazide 8a in excellent yields.

In a typical example, a mixture of 6a (R = H) and 7 in 1 : 1 molar preparation when heated in DMF and water bath for 45 minutes yielded a compound M.P. 230^{0}C. Based on spectral data, the compound was assigned structure as (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl) acetamido ethanamido]-N'-(2-oxoindolin-3-ylidene) propyl hydrazide 8a (R=H).

The similar procedure was adopted for the synthesis of 8(b-f) from 6(b-f)

The compound synthesized (2R)-2-[(4-(4'-substitutedphenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl) acetamido ethanamido]-N'-(2-oxoindolin-3-ylidene) propyl hydrazide8(a-f) have been characterized by means of their elemental analysis, I.R, ^{1}HNMR and MS data

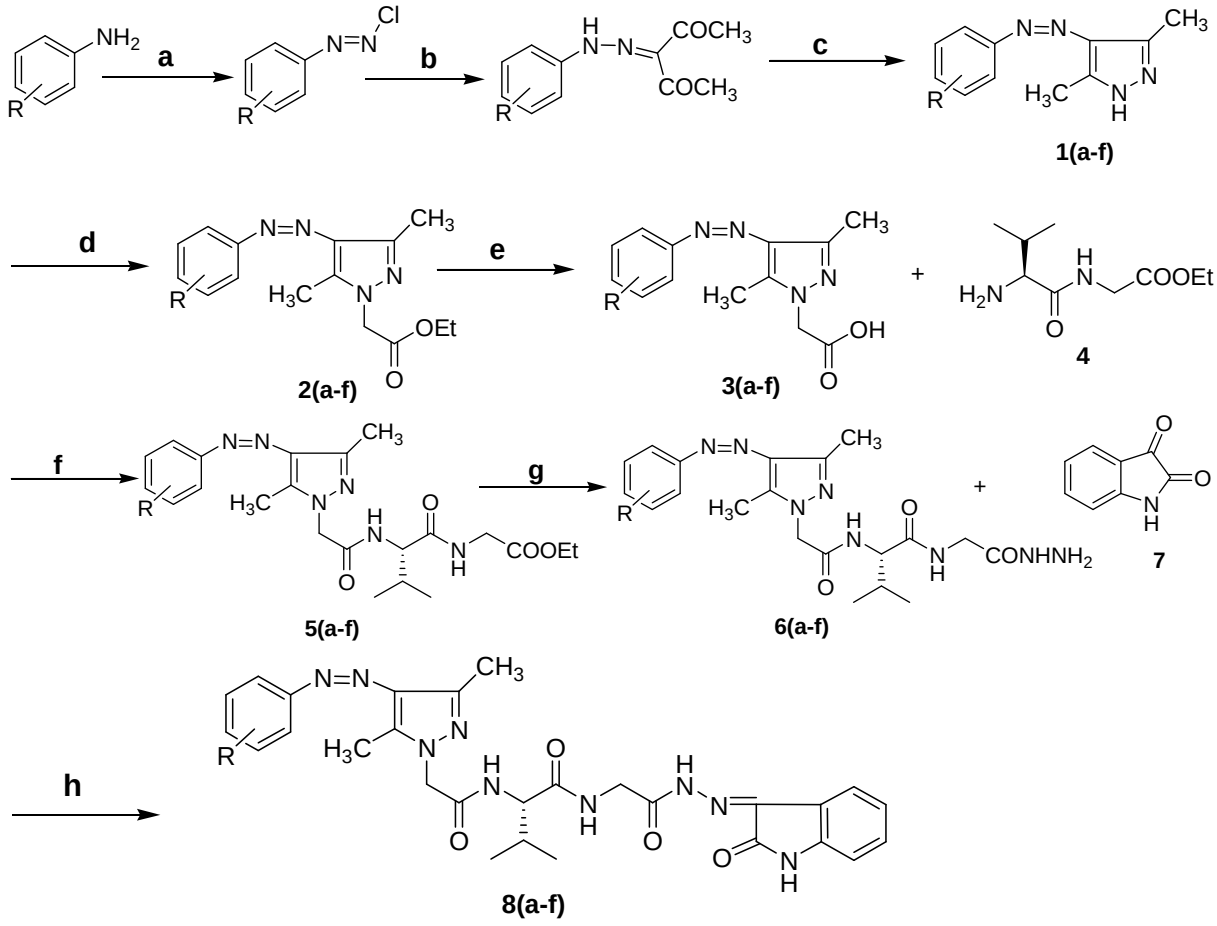
IR Spectra: IR spectral data of (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl)acetamido ethanamido]-N'-(2-oxoindolin-3-ylidene)propyl hydrazide 8a(R = H). Showed absorptions around, 3305 (m, -NH, str, hydrazide),3170 (m, str, -NH indole), 2954-2925(m, -CH str, asym, CH_3 and CH_2), 2852(m, -CH str, sym, CH_2), 2210, (s, -N=N str, Azo), 1710, 1645 & 1636 (s, -C=O str, 2^o amides), 1618 (m, -C=O str, indole), 1602 (m, -C=N str, indole), 1540 (m, -NH bend, 2^o amide), 1460 and 1455 cm^{-1} due to five member hetero cyclic pyrazole ring respectively

^{1}H NMR Data: The ^{1}H NMR spectra of (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl)acetamido ethanamido]-N'-(2-oxoindolin-3-ylidene)propyl hydrazide 8a was recorded in^{1}HNMR (200MHz) ($CDCl_3$+DMSO-d_6) (δppm): δ 8.98(S, 1H, indole-NH), 7.2-7.70(m, 9H, Ar-H), 6.22(br, S, -NH, amide), 4.86-4.8(1H, m, α-H, Ala), 4.67(S, 2H, CH_2, amide), 4.1(2H, S, CH_2, Gly), 2.8(S, 6H, $(CH_3)_2$, pyrazol), 1.29-1.32 (3H, m, β-H's, Ala). .

Mass spectra:The mass spectra of (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl)acetamido ethanamido]-N'-(2-oxoindolin-3-ylidene)propyl hydrazide 8a (R=H) showed molecular ion (M^+) peaks at m/z 403.

The mass spectral fragmentation patterns of (2R)-2-[(4-phenyldiazenyl-3,5-dimethyl-1H-pyrazol-1-yl)acetamidoethanamido]-N'-(2-oxoindolin-3-ylidene)propyl hydrazide 8a. The molecular ion observed at m/z 403 (100%), other important peaks appeared at m/z 387 (37.3%), 362 (78.4%), 299 (7.5%), 244 (38.1%), 243 (65.7%).

Structures of synthesized compounds:



R = H, 4' -CH3, 4' -OCH3, 4'-OC_2H_5, 4' -Cl, 4' -Br

Antibacterial activity:

The synthesized compounds were subjected nto antimicrobial screening by cup plate method for zone of inhibition. The antibacterial activity was tested against various Gram positive and Gram negative bacteria

Antimicrobial activity of synthesized compounds
Zone of inhibition (mm)

Compound (100 µg/mL)	Gram positive		Gram negative	
	B.Subtilis	*S.aureus*	*K.Penimonia*	*E.Coli*
1a	22	--	18	19
2a	---	----	----	20
3a	21	17	19	24
5a	18	18	21	19
6a	24	21	18	23
8a	25	22	17	22

RESULTS AND DISCUSSION

All the data of compounds confirms the synthesis of the above mentioned derivatives like melting point of all the pyrazole derivatives ranges between 180-215□C, the thin layer chromatography by using silica gel-G as adsorbent and benzene: methanol (6:4) as a solvent system, solubility data represents that the synthesized compounds were polar in nature like it is soluble in water, methanol, ethanol and insoluble in benzene, acetone, hexane. The UV spectroscopic data represents the λmax(nm) values of the compounds. IR spectroscopy helps to identify the chemical structure of the compounds, like in the given data all the compounds show the peak values of the representing group which is present in the compounds so here all these pyrazole derivatives show C-H stretch, N-H stretch (amides) C-C stretch (aromatic), N-H (amine)= stretch, C-N stretch, C-H bend, C=C stretch (aromatics), and in compound C=O stretch are present due to furfurylamine , peaks that confirms the structures of the above derivatives. NMR spectroscopical data also helps to confirm the presence of hydrogen bonds in the compound so here the NMR spectroscopy was done by using CDCl3 as the solvent and tetra methyl silane as the internal standard and the chemical shift values (ppm) gave the much more conformation about the structural determination of the compound that it contains. After structural determination the in-vivo testing of the synthesized compounds. The compounds were evaluated for their antimicrobial activity by cup-plate method against various Gram positive, Gram negative bacteria All the compounds have highly significant activity.

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