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Synthesis and Antimicrobial Activity of Some Pyrazolo[3,4-c]pyrazoles

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ABSTRACT
Nitrogen containing heterocyclic compounds and derivatives thereof are invaluable as a source of therapeutic agents. Pyrazole, with two nitrogen atoms and aromatic character, provides diverse functionality and stereochemical complexity in a five-member ring structure. In Knorr pyrazole synthesis, diimine compound gets deprotonated to regenerate the acid catalyst and provides the final pyrazole product. Formation of pyrazole derivatives from hydrazines, hydrazides, semicarbazides, thiosemicarbazide and aminoguanidines by condensation with 1, 3-dicarbonyl compounds is possible. As fused pyrazoles are reported to be well known pharmacophores, this has motivated to synthesize some of the pyrazolopyrazole derivatives by using hydrazine hydrate, thiosemicarbazide and semicarbazide. A series of 3-(aryl)-4-methyl-3a,6-dihydropyrazolo[3,4-c]pyrazole-2(3H)-carboxamides (IVa3-e3), 3-(aryl)-4-methyl-3a,6-dihydropyrazolo[3,4-c]pyrazole-2(3H)-carbothioamide (IVa2-e2) and 4-(aryl)-3-methyl-1,3a,4,5-tetrahydropyrazolo[3,4-c]pyrazoles (IVa1-e1) were synthesized by conventional method where fused pyrazopyrazoles were prepared. All the compounds were synthesized with good yield (56-81 %) and characterized by IR, ^{1}H NMR spectral data and C, H, N elemental analysis. All the synthesized compounds exhibited antimicrobial activity.

KEYWORDS: -Heterocyclic compounds, pyrazole, pyrazolopyrazole, antimicrobial, IR, ^{1}H NMR, C, H, N elemental analysis

INTRODUCTION:-

Pyrazole refers to the class of simple aromatic ring organic compounds of the heterocyclic series characterized by a 5-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions. Being so composed and having pharmacological effects on humans, they are classified as alkaloids, although they are rare in nature. In 1959, the first natural pyrazole, 1-pyrazolyl- alanine, was isolated from seeds of watermelons [1]. The term Pyrazole was given by Ludwig Knorr in 1883[2]. The Knorr pyrazole synthesis is an organic reaction used to convert a hydrazine or its derivatives and a 1,3-dicarbonyl compound to a pyrazole using an acid catalyst. The mechanism begins with an acid catalyzed imine formation, where in the case of hydrazine derivatives the attack can happen on either carbonyl carbon and result in two possible products. The other nitrogen of the hydrazine derivative then attacks the other carbonyl group which has also been protonated by the acid and forms a second imine group. This diimine compound gets deprotonated to regenerate the acid catalyst and provide the final pyrazole product[3]. Formation of pyrazole derivatives from hydrazines, hydrazides, semicarbazides and aminoguanidines by condensation with 1,3-dicarbonyl compounds is possible.Pyrazole derivatives have a long history of application in agrochemicals and pharmaceutical industry as herbicides and active pharmaceuticals[2]. Pyrazoles have been the recent target of numerous methodologies, mostly due to their prevalence as scaffolds in drug discovery programs as antimicrobials[4]as HMG-CoA reductase inhibitors[5], as inhibitors of HIV-1 reverse transcriptase [6] and synthesis in particular of bioactive compounds and reactions in different media[7]. The pyrazole ring is present as the core in a variety of leading drugs such aCelebrex[8], Viagra[9] or Rimonabant.Pyrazoles are found to possess anti-inflammatory[10, 11], analgesic[10, 12]and antimicrobial[11], activities. Many attempts were made by researchers to find out able potent pyrazole derivatives and also combining two pyrazole rings to enhance the biological activity.

MATERIALS AND METHODS:-

Well dried apparatus was used to conduct the reactions requiring anhydrous conditions. Laboratory reagent grade solvents and reagents used and purified by distillation and crystallization wherever necessary. Open capillary method was used for determining melting points of newly synthesized compounds. The final products were purified by recrystalization and purity was checked by micro TLC. The IR spectra of the compounds were recorded on JASCO FT/IR-5300 spectrometer using KBr pressed pellet. ^{1}H NMR spectra were recorded in a BRUKER DPX-200MHz spectrometer using TMS as internal standard. Perkin Elmer 2400 elemental analyzer was used for analysis of C, H and N which were found within ± 0.4 % of the theoretical values.

SYNTHETIC SCHEME:-

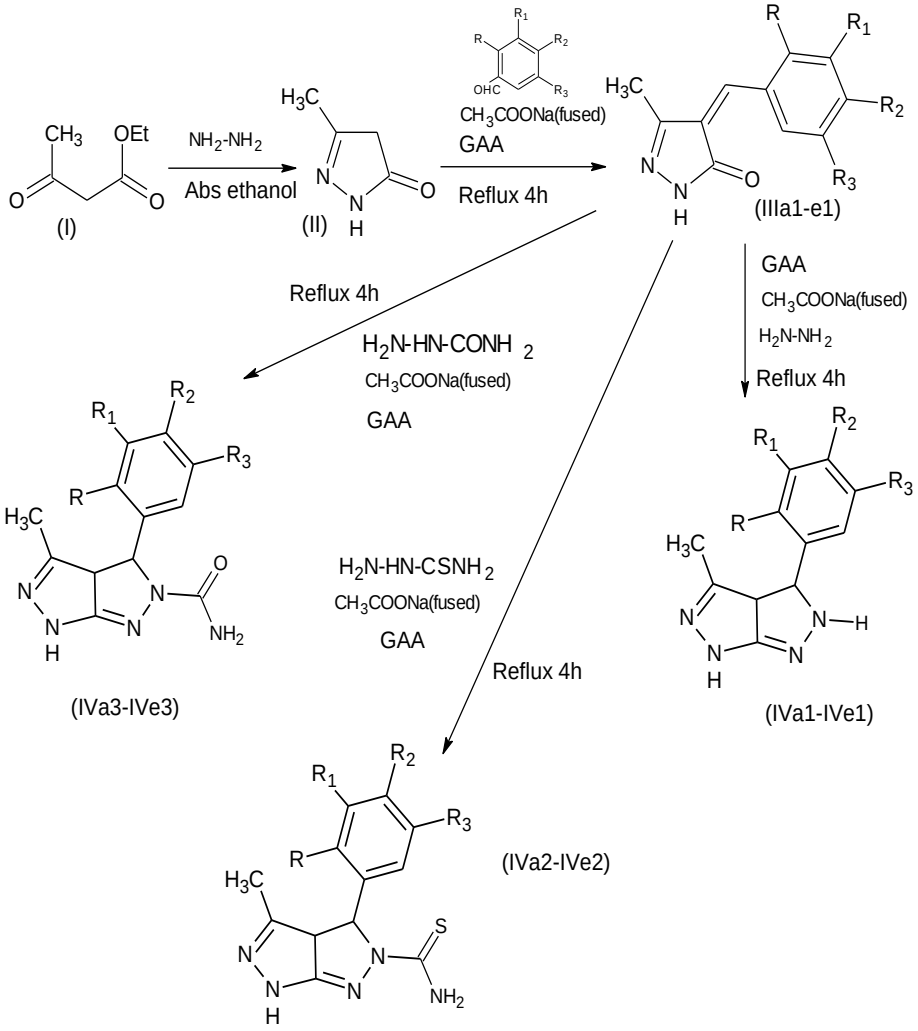

Figure1: Scheme of synthesis

As shown in figure1, A series of 3-(aryl)-4-methyl-3a,6-dihydropyrazolo[3,4-c]pyrazole-2(3H)-carboxamides (IVa3-e3) was prepared by the reaction between (4E)-4-arylidene-5-methyl-2,4–dihydro-3H-pyrazol-3-ones (IIIa1-e1) and semicarbazide refluxed in acetic acid in presence of anhydrous sodium acetate. A series of 3-(aryl)-4-methyl-3a,6-dihydropyrazolo[3,4-c]pyrazole-2(3H)-carbothioamide (IVa2-e2) was prepared by the reaction between compounds (IIIa1-e1) and thiosemicarbazide refluxed in

ethanol in presence of anhydrous sodium acetate. A series of 4-(aryl)-3-methyl-1,3a,4,5-tetrahydropyrazolo[3,4-c]pyrazoles (IVa1-e1) was prepared by the reaction between compounds (IIIa1-e1) and hydrazine hydrate, refluxed in ethanol in presence of anhydrous sodium acetate.

Conventional method for the synthesis of 5-methyl-2,4 dihydro-3*H*-pyrazol-3-one (II)

Ethylacetoacetate (1.3g, 0.01mol) was placed in a conical flask and stirred magnetically during the slow dropwise addition of solution of hydrazine hydrate (98%,0.5 ml, 0.01 mol) in absolute ethanol (1ml) and temperature of about 60^0 C was maintained, a crystalline deposit was separated. After stirring for 1 h at room temp, the reaction mixture was cooled in an ice bath to complete recrystalisation, filtered, washed with ice-cold ethanol, dried, m.p.222^0 C. Yield 0.88g,90%. [13]

General procedure for the synthesis of (4E)-4-arylidene-5-methyl-2,4–dihydro-3*H*-pyrazol-3-ones (IIIa1-e1)

A mixture of 5-methyl-2,4 dihydro-3*H*-pyrazol-3-one (II) (0.98g,0.01mol), appropriate aldehyde (0.01 mol), anhydrous sodium acetate (0.82g,0.01mol) and glacial acetic acid (40ml), was heated under reflux on heating mantle for 4 hours, cooled to room temperature and poured in an ice cold water, filtered, washed with water and recrystalised from methanol/ glacial acetic acid. The yield and m. p. were reported [14, 15].

General procedure for the Synthesis of compounds 4-(aryl)-3-methyl-1,3a,4,5-tetrahydropyrazolo[3,4-c]pyrazoles (IVa1-e1)

To a mixture of compounds (IIIa1-e1) (0.01 mol) and hydrazine hydrate (0.01 mol) in 50 ml of ethanol, anhydrous sodium acetate (0.01 mol) was added and refluxed for 4 hours. The product was poured in a mixture of crushed ice and water, filtered, dried and recrystallized from ethanol/ glacial acetic acid [15, 16].

General procedure for the Synthesis of compounds 3-(aryl)-4-methyl-3a,6-dihydropyrazolo[3,4-c]pyrazole-2(3H)-carbothioamide (IVa2-e2)

To a mixture of compounds (IIIa1-e1) (0.01 mol) and thiosemicarbazide (0.01 mol) in 40 ml of ethanol, anhydrous sodium acetate (0.01 mol) was added and refluxed for 6 hours. Reaction mixture was cooled and poured in a mixture of crushed ice and water, filtered, dried and recrystallized from ethanol/ glacial acetic acid[15, 16].

General procedure for the Synthesis of 3-(aryl)-4-methyl-3a,6-dihydropyrazolo[3,4-c]pyrazole-2(3H)-carboxamides (IVa3-e3)

A mixture of compounds (IIIa2-b2) (0.01 mol) and semicarbazide (0.01 mol) was refluxed in glacial acetic acid (40 ml) in presence of anhydrous sodium acetate (0.01 mol) for 6 hours. Reaction mixture was cooled to room temperature and poured in a mixture of crushed ice and water, filtered, dried and recrystallized from ethanol/ glacial acetic acid[15, 16].

ANTIMICROBIAL ACTIVITY:-

The antimicrobial activities of synthesized compounds were evaluated by the disc diffusion method[17]. The test microorganisms- bacteria and fungi were maintained on nutrient agar and Sabouraud's Dextrose Agar (SDA) media respectively. One loop of each strain of microorganism was transferred into a suitable

agar slant by using a sterile loop. These slants were incubated for 24h at 37^0C for bacteria and for 48-72h at 25^0C for fungi. These slants were further preserved at 4^0Cand used for antimicrobial activity studies. Antimicrobial assay: - The paper disc (No- 2 Whatmann) was cut down into a small disc (6 mm in diameter) and sterilized autoclave and then impregnated with the test solutions and standard solution. The dried discs were placed on the surface of the medium. After placing discs, Petri plates were left standing for 30 minutes at room temperature, as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of different solutions. All the Petri plates were incubated. After incubation, the diameters of the circular inhibition zones were measured. Antibacterial activity was carried out on four bacterial strains, namely *Streptococcus mutans* (gram positive), *Staphylococcus aureus* (gram positive), *Escherichia coli* (gram negative), *Shigella dysenteriae* (gram negative) and antifungal activity was carried out on two fungal strains, namely *Candida albicans* and *Rhizopus oryzae*.

Characterization of 3-methyl-4-phenyl-1,3a,4,5-tetrahydropyrazolo[3,4-*c*]pyrazole (IVa1)

The compound **IVa1** with melting point 209-211 ^{0}C was analyzed for $C_{11}H_{12}N_4$. The IR spectrum of the compound by KBr method is given in figure 5.29. It exhibits intense bands at 3352 cm^{-1}(aromatic N-H str), 3102 cm^{-1} (aromatic C-H str), 2908 cm^{-1} (C-H str in CH_3), 1585 cm^{-1} and 1617cm^{-1} (C=C and C=N), 1328 cm^{-1} (C-N str), 1049 cm^{-1} , 753 cm^{-1} (monosubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.30. It shows peaks at δ: 2.148 (d, 1H, C3a-H), 3.91 (d, 1H, C4-H), 7.27- 7.40 (m, 5H, Ar-H), 7.022 (s, 1H, pyrazoline N-H) and 1.949 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(65.98 %) H(6.04 %) N(27.98 %) found: C(65.95 %) H(6.01 %) N(27.96 %).The data confirms the structure of the compound.

Characterization of 4-(2-chlorophenyl)-3-methyl-1,3a,4,5 tetrahydropyrazolo [3,4*c*] pyrazole (IVb1)

The compound **IVb1** with melting point 198-201^0 C was analyzed for $C_{11}H_{11}ClN_4$. The IR spectrum of the compound by KBr method is given in figure 5.31. It exhibits intense bands at 3274 cm^{-1}(aromatic N-H str), 3102 cm^{-1} (aromatic C-H str), 2992 cm^{-1} (C-H str in CH_3), 1536cm^{-1} and 1656 cm^{-1} (C=C and C=N), 1344 cm^{-1} (C-N str), 1000 cm^{-1}, 750 cm^{-1} (1,2-disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.32. It shows peaks at δ: 2.412 (d, 1H, C3a-H), 3.91 (d, 1H, C4-H), 7.45-7.72 (m, 4H, Ar-H), 7.074 (s, 1H, pyrazoline N-H) and 1.940 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(56.30 %) H(4.72%) N(23.87%) found: C(56.27%) H(4.73 %) N(23.85%).The data confirms the structure of the compound.

Characterization of 2-(4-methyl-2,3,3a,6-tetrahydropyrazolo[3,4-*c*]pyrazol-3-yl)phenol (IVc1)

The compound **IVc1** with melting point 212-215^0 C was analyzed for $C_{11}H_{12}N_4O$. The IR spectrum of the compound by KBr method is given in figure 5.33. It exhibits intense bands at 3412 cm^{-1} (O-H str), 3286 cm^{-1}(aromatic N-H str), 3034 cm^{-1} (aromatic C-H str), 2921cm^{-1} (C-H str in CH_3),1519 cm^{-1} and 1620 cm^{-1} (C=C and C=N), 1286 cm^{-1} (C-N str),1037 cm^{-1}, 788 cm^{-1} (1,2-disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.34. It shows peaks at δ: 9.68 (s, 1H, OH), 2.54 (d, 1H, C3a-H), 4.97 (d, 1H, C4-H), 7.22- 7.47 (m, 4H, Ar-H), 6.832 (s, 1H, pyrazoline N-H) and 2.035 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(61.10%) H(5.59%) N(25.91%) found: C(61.07%) H(5.61%) N(25.94%). The data confirms the structure of the compound.

Characterization of 4-(2,4-dichlorophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo[3,4-*c*]pyrazole (IVd1)

The compound **IVd1** with melting point 226-228^0 C was analyzed for $C_{11}H_{10}Cl_2N_4$. The IR spectrum of the compound by KBr method is given in figure 5.35. It exhibits intense bands at 3282 cm^{-1}(aromatic N-H str), 3073 cm^{-1} (aromatic C-H str), 2921cm^{-1} (C-H str in CH_3),1581 cm^{-1} and 1615 cm^{-1} (C=C and C=N),1328 cm^{-1} (C-N str), 1123 cm^{-1}, 817 cm^{-1} (1,2,4-trisubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.36. It shows peaks at δ: 2.432 (d, 1H, C3a-H), 3.991 (d, 1H, C4-H), 7.04-7.75 (m, 3H, Ar-H), 6.9 (s, 1H, pyrazoline N-H) and 2.090 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(49.09%) H(3.75%) N(20.82%) found: C(49.04%) H(3.72%) N(20.81%).The data confirms the structure of the compound.

Characterization of *N,N*-dimethyl-4-(4-methyl-2,3,3a,6-tetrahydropyrazolo[3,4-*c*] pyrazol-3-yl) aniline (IVe1)

The compound **IVe1** with melting point 241-243^0 C was analyzed for $C_{13}H_{17}N_5$. The IR spectrum of the compound by KBr method is given in figure 5.37 It exhibits intense bands at 3365 cm^{-1}(aromatic N-H str), 3167 cm^{-1} (aromatic C-H str), 2924 cm^{-1} (C-H str in CH_3),1507 cm^{-1} and 1611 cm^{-1} (C=C and C=N), 1251 cm^{-1} (C-N str),1089 cm^{-1}, 828 cm^{-1} (1,4-disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.38. It shows peaks at δ: 2.409 (d, 1H, C3a-H), 3.933 (d, 1H, C4-H), 6.71- 7.10 (m, 4H, Ar-H), 7.02 (s, 1H, pyrazoline N-H), 3.028 (s, 6H,-N (CH_3)$_2$) and 2.091 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(64.17 %) H(7.04%) N(28.78%) found: C(64.16%) H (7.00%) N(28.81%).The data confirms the structure of the compound.

Characterization of 4-methyl-3-phenyl-3a,6-dihydropyrazolo[3,4-*c*]pyrazole-2(3*H*) carbothioamide (IVa2)

The compound**IVa2** with melting point 201-204^0 C was analyzed for $C_{12}H_{13}N_5S$. The IR spectrum of the compound by KBr method is given in figure 5.39. It exhibits intense bands at 3289 cm^{-1}(aromatic N-H str), 3090 cm^{-1} (aromatic C-H str), 2863 cm^{-1} (C-H str in CH_3),1510 cm^{-1} and 1623 cm^{-1} (C=C and C=N), 1326 cm^{-1} (C-N str), 1207 cm^{-1} (C=S str), 1051 cm^{-1} , 825 cm^{-1} (monosubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.40. It shows peaks at δ: 3.91(d, 1H, C3-H), 2.10 (d, 1H, C4-H), 7.27- 7.40 (m, 5H, Ar-H), 7.02 (s, 1H, pyrazoline N-H), 9.503 (bs, 2H,-CSNH_2) and 1.946 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(55.58%) H(5.05%) N(27.01%) found: (55.61%) H(5.07%) N(27.05%).The data confirms the structure of the compound.

Characterization of 3-(2-chlorophenyl)-4-methyl-3a,6-dihydropyrazolo[3,4-*c*]pyrazole-2(3*H*)-carbothioamide (IVb2)

The compound**IVb2** with melting point 198-201^0 C was analyzed for $C_{12}H_{12}ClN_5S$. The IR spectrum of the compound by KBr method is given in figure 5.41. It exhibits intense bands at 3330 cm^{-1}(aromatic N-H str), 3147 cm^{-1} (aromatic C-H str), 2912 cm^{-1} (C-H str in CH_3),1520 cm^{-1} and 1622 cm^{-1} (C=C and C=N), 1328 cm^{-1} (C-N str), 1133 cm^{-1} (C=S str), 1051 cm^{-1}, 825 cm^{-1} (1,2-disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.42. It shows peaks at δ: 3.91(d, 1H, C3-H), 2.31 (d, 1H, C4-H), 7.21- 7.72 (m, 5H, Ar-H), 6.92 (s, 1H, pyrazoline N-H), 9.502 (bs, 2H,-CSNH_2) and 2.029 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(49.06%) H(4.12%) N(23.84%) found: C(49.08%) H(4.15%) N(23.79%). The data confirms the structure of the compound.

Characterization of 3-(2-hydroxyphenyl)-4-methyl-3a,6-dihydropyrazolo[3,4-*c*]pyrazole-2(3*H*)-carbothioamide (IVc2)

The compound**IVc2** with melting point 212-215^0 C was analyzed for $C_{12}H_{13}N_5OS$. The IR spectrum of the compound by KBr method is given in figure 5.43. It exhibits intense bands at 3499 cm^{-1} (O-H str), 3216 cm^{-1}(aromatic N-H str), 3114 cm^{-1} (aromatic C-H str), 2922 cm^{-1} (C-H str in CH_3),1509 cm^{-1} and 1614 cm^{-1} (C=C and C=N), 1268 cm^{-1} (C-N str), 1268 cm^{-1} (C=S str), 1037 cm^{-1}, 748 cm^{-1} (1,2-disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.44. It shows peaks at δ: 9.70 (s, 1H, OH), 4.04 (d, 1H, C3-H), 2.31 (d, 1H, C4-H), 6.87- 7.44 (m, 4H, Ar-H), 7.09 (s, 1H, pyrazoline N-H), 6.49 (s, 2H,-CSNH_2) and 2.029 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(52.35%) H (4.76%) N(25.44%) found: C(52.34%) H(4.76%) N(25.43%).The data confirms the structure of the compound.

Characterization of 3-(2,4-dichlorophenyl)-4-methyl-3a,6-dihydropyrazolo[3,4-*c*]pyrazole-2(3*H*)-carbothioamide (IVd2)

The compound**IVd2** with melting point 226-228^0 C was analyzed for $C_{12}H_{11}Cl_2N_5S$. The IR spectrum of the compound by KBr method is given in figure 5.45. It exhibits intense bands at 3328 cm^{-1}(aromatic N-H str), 3082 cm^{-1} (aromatic C-H str), 2913 cm^{-1} (C-H str in CH_3),1482 cm^{-1} and 1600 cm^{-1} (C=C and C=N), 1323 cm^{-1} (C-N str), 1272 cm^{-1} (C=S str),1108 cm^{-1}, 781 cm^{-1} (1,2,4-trisubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.46. It shows peaks at δ: 3.91(d, 1H, C3-H), 2.40 (d, 1H, C4-H), 7.04- 7.75 (m, 3H, Ar-H), 76.90 (s, 1H, pyrazoline N-H), 9.720 (bs, 2H,-CSNH_2) and 2.028 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(43.91%) H(3.38%) N(21.34%) found: C(43.90%) H(3.37%) N(21.35 %).The data confirms the structure of the compound.

Characterization of 3-[4-(dimethylamino)phenyl]-4-methyl-3a,6-dihydropyrazolo[3,4-*c*]pyrazole-2(3*H*)-carbothioamide (IVe2)

The compound **IVe2** with melting point 241-243^0 C was analyzed for $C_{14}H_{18}N_6S$. The IR spectrum of the compound by KBr method is given in figure 5.47 It exhibits intense bands at 3305 cm^{-1}(aromatic N-H str), 3110 cm^{-1} (aromatic C-H str), 3007 cm^{-1} (C-H str in CH_3),1507 cm^{-1} and 1611 cm^{-1} (C=C and C=N), 1326 cm^{-1} (C-N str), 1251 cm^{-1} (C=S str), 1015 cm^{-1}, 828 cm^{-1} (1,4-disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.48. It shows peaks at δ: 3.91(d, 1H, C3-H), 2.41 (d, 1H, C4-H), 6.71- 7.10 (m, 4H, Ar-H), 7.02 (s, 1H, pyrazoline N-H), 9.72 (bs, 2H,-CSNH_2), 3.062 (s, 6H,-N(CH_3)$_2$) and 2.028 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(55.61%) H(6.00%) N(27.79%) found: C(55.60%) H(6.10%) N(27.80%).The data confirms the structure of the compound.

Characterization of 4-methyl-3-phenyl-3a,6-dihydropyrazolo[3,4-*c*]pyrazole-2(3*H*)-carboxamide (IVa3)

The compound**IVa3** with melting point 210-213^0 C was analyzed for $C_{12}H_{13}N_5O$. The IR spectrum of the compound by KBr method is given in figure 5.49. It exhibits intense bands at 3317 cm^{-1}(aromatic N-H str), 3122 cm^{-1}(aromatic C-H str), 2993 cm^{-1} (C-H str in CH_3),1707 cm^{-1} (C=O), 1557 cm^{-1} and 1610 cm^{-1} (C=C and C=N), 1320 cm^{-1} (C-N str), 1092 cm^{-1} , 820 cm^{-1} (monosubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.50. It shows peaks at δ: 2.10 (d, 1H, C3a-H), 3.916 (d, 1H, C3-H),

7.27- 7.40 (m, 5H, Ar-H), 7.02 (s, 1H, pyrazoline N-H), 6.21 (bs, 2H,-CONH$_2$) and 1.948 (s, 3H,-CH$_3$). Elemental analysis for composition of C, H and N is given as calculated: C(59.25%) H(5.39%) N(28.79%) found: C(59.23 %) H(5.40%) N(28.79%).The data confirms the structure of the compound.

Characterization of 3-(2-chlorophenyl)-4-methyl-3a,6-dihydropyrazolo[3,4-*c*]pyrazole-2(3*H*)-carboxamide (IVb3)

The compound**IVb3** with melting point 198-201^0 C was analyzed for $C_{12}H_{12}ClN_5O$. The IR spectrum of the compound by KBr method is given in figure 5.51. It exhibits intense bands at 3428 cm^{-1}(aromatic N-H str), 3118 cm^{-1} (aromatic C-H str), 2992 cm^{-1} (C-H str in CH$_3$),1706 cm^{-1} (C=O), 1482 cm^{-1} and 1682 cm^{-1} (C=C and C=N), 1278 cm^{-1} (C-N str), 1108 cm^{-1}, 733 cm^{-1} (1,2-disubstituted benzene ring). The ^{1}H NMR spectrum in CDCl$_3$ is given in figure 5.52. It shows peaks at δ: 2.49 (d, 1H, C3a-H), 4.91 (d, 1H, C3-H), 7.21- 7.72 (m, 4H, Ar-H), 7.07 (s, 1H, pyrazoline N-H), 6.21 (bs, 2H,-CONH$_2$) and 1.940 (s, 3H,-CH$_3$). Elemental analysis for composition of C, H and N is given as calculated: C(51.90%) H(4.36%) N(25.22%) found: C(51.92%) H (4.37%) N(25.23%).The data confirms the structure of the compound.

Characterization of 3-(2-hydroxyphenyl)-4-methyl-3a,6-dihydropyrazolo[3,4-*c*] pyrazole-2(3*H*)-carboxamide (IVc3)

The compound**IVc3** with melting point 212-215 ^{0}C was analyzed for $C_{12}H_{13}N_5O_2$. The IR spectrum of the compound by KBr method is given in figure 5.53. It exhibits intense bands at 3511 cm^{-1} (O-H str), 3246 cm^{-1}(aromatic N-H str), 3114 cm^{-1}(aromatic C-H str), 2930 cm^{-1} (C-H str in CH$_3$), 1701 cm^{-1} (C=O),1539 cm^{-1} and 1638 cm^{-1} (C=C and C=N), 1261 cm^{-1} (C-N str), 1107 cm^{-1}, 748cm^{-1} (1,2-disubstituted benzene ring). The ^{1}H NMR spectrum in CDCl$_3$ is given in figure 5.54. It shows peaks at δ: 9.68 (s, 1H, OH), 2.53 (d, 1H, C3a-H), 4.93 (d, 1H, C3-H), 7.22- 7.47 (m, 4H, Ar-H), 6.83 (s, 1H, pyrazoline N-H), 6.42 (bs, 2H,-CONH$_2$) and 2.03 (s, 3H,-CH$_3$). Elemental analysis for composition of C, H and N is given as calculated: C(55.59%) H(5.05%) N (27.01%) found: C(55.58 %) H(5.03 %) N(27.03%).The data confirms the structure of the compound.

Characterization of 3-(2,4-dichlorophenyl)-4-methyl-3a, 6-dihydropyrazolo[3,4-*c*] pyrazole-2(3*H*)-carboxamide (IVd3)

The compound**IVd3** with melting point 226-228^0 C was analyzed for $C_{12}H_{11}Cl_2N_5O$. The IR spectrum of the compound by KBr method is given in figure 5.55. It exhibits intense bands at 3318 cm^{-1}(aromatic N-H str), 3127 cm^{-1} (aromatic C-H str), 2987 cm^{-1} (C-H str in CH$_3$),1707 cm^{-1} (C=O), 1512 cm^{-1} and 1602 cm^{-1} (C=C and C=N), 1382 cm^{-1} (C-N str), 1098 cm^{-1}, 751 cm^{-1} (1,2,4-trisubstituted benzene ring). The ^{1}H NMR spectrum in CDCl$_3$ is given in figure 5.56. It shows peaks at δ: 2.50 (d, 1H, C3a-H), 4.90 (d, 1H, C3-H), 7.04- 7.75 (m, 3H, Ar-H), 6.90 (s, 1H, pyrazoline N-H), 6.20 (bs, 2H,-CONH$_2$) and 2.29 (s, 3H,-CH$_3$). Elemental analysis for composition of C, H and N is given as calculated: C(46.17%) H(3.55%) N(22.44%) found: C(46.16%) H(3.53%) N(22.43%).The data confirms the structure of the compound.

Characterization of 3-[4-(dimethylamino)phenyl]-4-methyl-3a,6-dihydropyrazolo[3,4-*c*]pyrazole-2(3*H*)-carboxamide (IVe3)

The compound**IVe3** with melting point 241-243 ^{0}C was analyzed for $C_{14}H_{18}N_6O$. The IR spectrum of the compound by KBr method is given in figure 5.57. It exhibits intense bands at 3268 cm^{-1}(aromatic N-H

str), 3060 cm^{-1} (aromatic C-H str), 2891cm^{-1} (C-H str in CH_3),1700 cm^{-1}(C=O), 1505 cm^{-1} and 1636 cm^{-1} (C=C and C=N), 1300 cm^{-1} (C-N str), 755 cm^{-1} (1,4-disubstituted benzene ring). The ^1H NMR spectrum in $CDCl_3$ is given in figure 5.58. It shows peaks at δ: 2.41 (d, 1H, C3a-H), 3.90 (d, 1H, C3-H), 6.71- 7.10 (m, 4H, Ar-H), 7.02 (s, 1H, pyrazoline N-H), 6.15 (bs, 2H,-CONH_2), 3.028 (s, 6H,-N (CH_3)$_2$) and 2.091 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(58.73%) H(6.34%) N(29.35%) found: C(58.71%) H(6.34%) N(29.36%).The data confirms the structure of the compound.

RESULTS:-

Table 1: Physical constants of compounds IVa1-e1, IVa2-e2 and IVa3-e3

Compound	Recrystalization Solvent	% yield	m. p. (^{0}c)	Molecular formula	Molecular weight	*Rf
IVa1	Ethanol	59	209-211	$C_{11}H_{12}N_4$	200.239	0.66
IVb1	Glacial acetic acid + Ethanol(1:1)	68	198-201	$C_{11}H_{11}ClN_4$	234.684	0.44
IVc1	Glacial acetic acid	63	212-215	$C_{11}H_{12}N_4O$	216.239	0.64
IVd1	Glacial acetic acid	81	226-228	$C_{11}H_{10}Cl_2N_4$	269.129	0.59
IVe1	Ethanol	78	241-243	$C_{13}H_{17}N_5$	243.307	0.43
IVa2	Ethanol	56	201-204	$C_{12}H_{13}N_5S$	259.330	0.66
IVb2	Glacial acetic acid	73	198-201	$C_{12}H_{12}ClN_5S$	293.775	0.42
IVc2	Ethanol	61	212-215	$C_{12}H_{13}N_5OS$	275.329	0.60
IVd2	Glacial acetic acid	76	226-228	$C_{12}H_{11}Cl_2N_5S$	328.220	0.62
IVe2	Ethanol	73	241-243	$C_{14}H_{18}N_6S$	302.397	0.46
IVa3	Ethanol	64	210-213	$C_{12}H_{13}N_5O$	243.264	0.65
IVb3	Glacial acetic acid	68	198-201	$C_{12}H_{12}ClN_5O$	277.709	0.43
IVc3	Glacial acetic acid	71	212-215	$C_{12}H_{13}N_5O_2$	259.263	0.64
IVd3	Glacial acetic acid	58	226-228	$C_{12}H_{11}Cl_2N_5O$	312.154	0.58
IVe3	Ethanol	70	241-243	$C_{14}H_{18}N_6O$	286.332	0.39

R_f value was determined in benzene: acetone (1:1)

Table 2: Antimicrobial activity of compounds IVa1-e1, IVa2-e2 and IVa3-e3

Sr. No.	Design of treatment (1mg/ml)	Diameter of zone of inhibition (mm)					
		Escherichia coli	*Staphylococcus aureus*	*Shigella dysenteriae*	*Streptococcus mutans*	*Candida albicans*	*Rhizopus oryzae*

	Standard*	20	14	24	16	26	22
	Compound IVa1	14	8	16	15	10	7
	Compound IVb1	12	2	12	13	10	11
	Compound IVc1	4	NA	3	6	6	12
	Compound IVd1	10	4	NA	NA	8	2
	CompoundIVe1	4	4	12	12	22	16
	Compound IVa2	16	10	18	15	12	10
	Compound IVb2	14	4	14	12	14	14
	Compound IVc2	6	6	7	NA	10	16
	Compound IVd2	12	6	6	8	8	6
.	Compound IVe2	10	7	15	12	22	20
.	Compound IVa3	12	10	16	15	9	6
.	Compound IVb3	12	2	11	15	12	12
.	Compound IVc3	4	NA	2	NA	8	10
.	Compound IVd3	11	4	NA	6	2	6
.	Compound IVe3	6	4	12	14	20	14

DISCUSSION:-

Synthesis of new chemical entity incorporating the same active pharmacophore to another namely pyrazole in a single molecular framework was successfully carried out. Conventional synthesis of new series of pyrazolo-pyrazoles, characterization of synthesized compounds by Infra Red, Nuclear Magnetic Resonance spectroscopy, elemental analysis and screening for the antimicrobial activity are the major highlights.pyrazolo-pyrazoles, pyrazolo-pyrazole carbothiomides and carboxamides were synthesized by conventional method. The yield was quantitative. The structures of synthesized compounds were in agreement with elemental analysis and IR and NMR spectral data. The synthesized compounds exhibited antimicrobial activity, shown in table 2.Compound IVa2 and IVa3 showed significant activity against *Streptococcus mutans*and *Shigella dysenteriae*. IVe1, IVe3 and IVa2 showed significant activity against *Candida albicans*. The synthesized compounds are believed to exert various other activities such as anti-inflammatory, analgesic, anticonvulsant, CNS depressant, ulcerogenic and anthelmintic.

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REFERENCES:-

1. Eicher T., Hauptmann S., 'The Chemistry of Heterocycles: Structure, Reactions, Syntheses, and Applications', IIndEdition, Wiley-VCH, 2003.
2. Chauhan A., Sharma P. K., Kaushik N.; Pyrazole: A Versatile Moiety, International Journal of Chem Tech Research,2011, 11-17.
3. Jian-An Jiang, Cai-Yan Du, Chun-Hui Gu, Ya-Fei Ji , One-Pot Synthesis of 4-Substituted 1,5-Diaryl-1H-pyrazole-3-carboxylic Acids via a MeONa/LiCl-Mediated Sterically Hindered Claisen Condensation-Knorr Reaction-Hydrolysis Sequence, Synlett, 2012, 23:2965-2968.DOI: 10.1055/s-0032-1317668
4. Haque T. S., Tadesse S., Marcinkeviciene J., Rogers M. J., Sizemore C., Kopcho L.M., Parallel Synthesis of Potent, Pyrazole-Based Inhibitors of Helicobacter pylori Dihydroorotate Dehydrogenase, J. Med. Chem., 2002, 45:4669–4678.
5. Pfifferkorn J. A., Harris S.F., Arora S.F., Javanbakht H., Li Y., Fretland J., Davidson J.P., Substituted pyrazoles as hepatoselective HMG-CoA reductase inhibitors: discovery of (3R,5R)-7-[2-(4-fluoro-phenyl)-4-isopropyl-5-(4-methyl-benzylcarbamoyl)-2H-pyrazol-3-yl]-3,5 dihydroxyheptanoic acid (PF-3052334) as a candidate for the treatment of hypercholesterolemia, J. Med. Chem., 2008, 51:31-45.
6. Sweeney Z. K., Harris S.F., Arora S.F., Javanbakht H., Li Y., Fretland J., Design of annulated pyrazoles as inhibitors of HIV-1 reverse transcriptase, J. Med. Chem., 2008, 51:7449-7458.
7. Katritzky Alan R. and Singh Sandeep K., Microwave-assisted heterocyclic synthesis, Arkivoc, Archive for organic chemistry, 2003, 13:68-86.
8. Thomas D. Penning, John J. Talley, Stephen R. Bertenshaw, Jeffery S. Carter, Paul W. Collins, Stephen Docter., Synthesis and Biological Evaluation of the 1,5-Diarylpyrazole Class of Cyclooxygenase-2 Inhibitors: Identification of 4-[5-(4-Methylphenyl)-3- (trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide (SC-58635, Celecoxib), J. Med. Chem., 1997, 40:1347–1365.
9. Nicholas K. Terrett, Andrew S. Bell, David Brown, Peter Ellis, Sildenafil (VIAGRATM), A potent and selective inhibitor of type 5 cGMP phosphodiesterase with utility for the treatment of male erectile dysfunction, Bioorganic & Medicinal Chemistry Letters, 1996, 6:1819-1824.
10. Waheed A., Khan S. A., Venugopala K. N., Synthesis of carboxamide of 2 amino-4'-(6-bromo-3 coumarinyl) thiazole as analgesic and anti-inflammatory agent, Indian J. Heterocyclic Chem., 2001, 11:59-62.
11. Udupi R. H., Rao S. N. and Bhat A. R., Synthesis of some pyrazoline as antimicrobial, antiinflammatory and analgesic agents, Indian J. Heterocyclic Chem., 1998, 7:217-220.
12. Ravi T. K., Sam Solomon W.D.; Annadurai K., Synthesis of Quinazoline Derivatives and their possible Biological activities, Indian drugs, 1999, 36(7):466-469.
13. Vogel A.I., Text book of practical org. chem., 5th Edition, ELBS Publication, London, 1989
14. Mariappan G., Saha B. P., Sutharson L. and Haldar A., Synthesis and bioactivity evaluation of pyrazolone derivatives, Indian journal of Chemistry- B, 2010, 49B:1671-1674.
15. Rao R. Mallikarjuna, G. Nagaraja Reddy, J. Sreeramulu, Synthesis of some new pyrazolo-pyrazole derivatives containing indoles with antimicrobial activity, Der Pharma Chemica, 2011, 3:301-309.
16. Rao R. Mallikarjunna, M. MusthakAhamad and J. Sreeramulu, Synthesis and antimicrobial activity of linked heterocycles containing pyrazolyle-indole derivatives, Journal of Pharmacy Research, 2012, 5:1518-1521.
17. Robert A. Turner., Peter Hebborn, Screening methods in Pharmacology, Academic press, New York and London, 1965.