Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(1):272-278

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis and biological screening of some Pyridine and Pyrrole derivatives of Pyrazolo [3, 4-*c*] pyrazoles

R. MallikarjunaRao[1]*, J. Sreeramulu[1], L. K. Ravindranath[1], G. NagarajaReddy[1], K. Hanumanthurayudu[1], G. Nageswara Reddy[1], A. Jayaraju[1], P. Madhusudhan[2]

[1]Department of Chemistry, Sri Krishnadevaraya University, Anantapur
[2]Department of Bio-technology, Acharya Nagarjuna University, Guntur

---

ABSTRACT

The synthesis of two series of pyrazolo [3, 4-c]pyrazoles linked to pyridine (3a-d) and pyrrole (5a-d) is achieved by reaction of Chalcones of pyrazolone (3) and (5) with hydrazine hydrate, Thiosemicarbazide, semicarbazide and phenyl hydrazine in the presence of NaOH /EtOH or AcONa/AcOH. Chalcones (3) and (5) required for the synthesis are prepared by the condensation reaction of 5-methyl-2,4-dihydro-3H-pyrazol-3-one (1) with hetero Aromatic aldehydes (2) and (4). Compound 1 intern is prepared by the cyclization reaction between ethylacetoacetate and hydrazine hydrate in absolute alcohol. Structures of the synthesized compounds have been elucidated by means of IR, ^{1}H NMR and mass spectral data. Biological screening of all compounds is reported.

Keywords: Ethylacetoacetate, Chalcones, Pyrazolone, Pyrazolo[3,4-*c*]pyrazoles, Antimicrobial activity.

INTRODUCTION

Heterocycles and medicines are both interrelated because humans are totally dependent on the drugs derived from heterocyclic rings. Heterocycles and their derivatives have attracted the attention of chemists, mainly because of broad spectrum biological and pharmacological activities associated with this class of compounds specially having nitrogen, Sulphur and oxygen or three hetero atoms. The pyrazole ring is a prominent structural motif found in numerous pharmaceutically active compounds. Pyrazole and pyrazolone ring systems represent an important class of compounds not only for their theoretical interest but also for their anti-inflammatory, postmenopausal, osteoporosis, angiotension, antagonists, and anticoagulant activities [1-3]. This is mainly due to the ease preparation and the important biological activity. Pyrazole framework plays an essential role in biologically active compounds and therefore represents an interesting template for combinatorial as well as medicinal chemistry [4-5]. Such as antibacterial [6], antifungal [7], antiviral [8], antitubercular [9], antioxidant [10], antiandrogenic [11] etc. Some of these compounds have also exhibited antidiabetic [12], herbicidal activity [13], analgesic [14] and antiparasitic [15] properties. Many pyrazoles have been found to be luminescent and fluorescent [16, 17] agents. In addition pyrazoles have played a crucial role in the development of theory in heterocyclic chemistry and also used extensively as useful synthon in organic synthesis [18-22]. It is interesting to note that fused bis-pyrazoles are reported as well known pharmacophores [23, 24].The activities of pyrazole derivatives include main topics like remarkable bactericidal, bacteriostatic, sedative, antipyretic, antiamoebic, anti-inflammatory, muscle relaxant, [25-33] hypoglycemic and sex stimulating agents This has prompted us to synthesize some of the pyrazolopyrazole derivatives by using hydrazine

hydrate, phenyl hydrazine Thiosemicarbazide and semicarbazide. It has been considered worthwhile to incorporate a suitable functionality into these derivatives to potentate their pharmacological activity [34, 35]. Encouraged by these results, it was planned to synthesize new chemical entities incorporating the two active pharmacophores namely, pyrazoline and Heteronucles in a single molecular framework using chalcones of pyrazolone and heterocyclic aldehydes as basic building blocks.

EXPERIMENTAL SECTION

Melting points were taken in open capillary tubes and are therefore uncorrected. Purity of the compounds was checked on silica gel G TLC plates of 2 mm thickness using n-hexane and ethyl acetate as solvent system. The visualization of spot was carried out in an iodine chamber. The IR spectra were recorded on Perkin-Elmer spectrometer. The ^{1}H NMR spectra were scanned on a Bruker DRX-300 MHz. spectrometer (300 MHz) in CDCl3 using TMS as internal standard and chemical shifts are expressed in δ ppm. The mass spectra were recorded on a Jeol SX-102 (FAB) spectrometer.

Synthesis of 3-methylpyrazol-5-one 1 [38]:
Ethyl acetoacetate (0.5 mole) was taken in conical flask and hydrazine hydrate (1 mole) in ethanol (40 ml) was added dropwise to it with stirring. The temperature raised during this addition and it was maintained at 60°C when a crystalline solid separated. The reaction-mixture was further stirred for 80 min at room temperature then cooled in an ice bath to complete the crystallization. Separated solid was washed with ice cold ethanol.

Synthesis of 3-methyl-4-((pyridin-4-yl)methylene)-1H-pyrazol-5(4H)-one 3:
5-Methyl-2,4-dihydro-3*H*-pyrazol-3-one (1) (0.01 mole), Pyridine aldehyde (2) (0.01 mole) and anhydrous sodium acetate (0.02 mole) were dissolved in acetic acid refluxed for 10 hr. The reaction-mixture was filtered and the filtrate was poured on crushed ice. The solid obtained was recrystallized from ethanol.

Yield: 78% Yellow coloured compound, m.p: 275-276 ^{o}C IR (KBr, cm^{-1}): 3440 (N-H str.), 3053 (C-H str., Ar-H), 2949 (C-H str., CH_3), 1709(C=O str.), 1616 (C=N str.), ^{1}H NMR ($CDCl_3$) δ 8.7(s 1H, NH), 7.5-7.8 (m, 4H, Py-H), 6.9 (s, 1H, =CH-Py), 2.51 (s, 3H, CH_3).

Similarly, compound **5** was also prepared with some change in reflux time and reaction work up. Their characteristic spectral and analytical data are given below:

1,3a,4,5-tetrahydro-3-methyl-4-(pyridin-4-yl)pyrazolo[3,4-c]pyrazole 3a:
To a mixture of compound 3 (0.01mole) and hydrazene hydrate (o.o1ml) in 30ml of ethanol a solution of NaOH (0.02 mole) in 5 ml of water was added and refluxed for 10 hr. The product was poured into crushed ice, which was filtered, dried and recrystalised from DMF.

Yield: 70% Light yellow coloured compound, m.p: 285-286 ^{o}C. IR (KBr) cm^{-1}: 3422, (N-H str.), 3022(C-H str.Py-H) , 2922 (C-H str., CH_3), 1632 (C=N str.), 1680 (C=Ostr.),1422(C=Cstr).^{1}H NMR ($CDCl_3$): δ8.01 (s, 1H, N-H),10.9(s 1H NH) δ6.9-7.8 (m, 4H, H-Pyridine),), 2.65(s, 3H, CH_3); MS : *m/z* 200 $[M]^{+\cdot}$,196,168,145,98..

Compounds **3b-d** were also prepared in similar manner with change in solvent (EtOH/AcOH or AcONa/NaOH) reflux time. Their characteristic spectral and physical data are given below:

3,3a-dihydro-4-methyl-3-(pyridin-4-yl)pyrazolo[3,4-c]pyrazole-2(6H)-carboxamide 3b
Yield: 57% Yellow coloured crystalline compound m.p: 301-305 ^{o}C IR (KBr,cm^{-1}): 3391,3330 (N-H str., NH_2), 3025 (C-H str., Py-H), 2950 (C-H str., CH_3), 1670, (C=O str.), 1615 (C=N str.), 1571(C=C), ^{1}H NMR ($CDCl_3$): δ 8.48 (s, 1H, N-$H_{(Pyrazole)}$), 7.63-7.65(m, 4H, Py) 6.55 (s, 2H, NH_2), 2..51 (s, 3H, CH_3); MS : *m/z* 245 $[M+1]^{+\cdot}$, 227,209.191,177,143.

3,3a-dihydro-4-methyl-3-(pyridin-4-yl)pyrazolo[3,4-c]pyrazole-2(6H)-carbothioamide 3c
Yield: 66% Gray coloured compound, m.p: 330-331 ^{o}C IR (KBr, cm^{-1}): 3448(N-H str., NH_2), 3033 (C-H str., Ar-H), 2899 (C-H str., CH_3), 1615 (C=N str.), 1440 (C=C str.), 1280 (C=S str.), ^{1}H NMR ($CDCl_3$): δ 8.7 (s, 1H, N-H), 6.62 (s, 2H, NH_2), 2.56 (s, 3H, CH_3);

1,3a,4,5-tetrahydro-3-methyl-5-phenyl-4-(pyridin-4-yl)pyrazolo[3,4-c]pyrazole 3d
Yield: 69% Yellow coloured compound, m.p: 288-289 ^{o}C IR (KBr, cm^{-1}): 3435 (N-H str., NH_2), 2947(C-H str., Py-H), 2922 (C-H str., CH_3), 1599 (C=N str.), 1493 (C=C str.), ^{1}H NMR ($CDCl_3$): δ 8.63 (s, 1H, N-$H_{(Pyrazole)}$), 7.5-7.3 (m,4H,Py-H), 7.1-7.19 (m, 5H, Ar-H), 2.50 (s, 3H, CH_3); MS : *m/z* 278 $[M+1]^{+\cdot}$, 280 $[M+2]^{+\cdot}$, 260,189, 173,156,144.

1,3a,4,5-tetrahydro-3-methyl-4-(1H-pyrrol-2-yl)pyrazolo[3,4-c]pyrazole 5a
To a mixture of compound **5** (0.01mole) and hydrazine hydrate (0.01mole) and anhydrous sodium acetate (0.02 mole) were dissolved in acetic acid refluxed for 10 hr. The reaction-mixture was filtered and the filtrate was poured on crushed ice. The solid obtained was recrystallized from ethanol.

Yield: 66% Buff coloured compound, m.p: 301-302 ^{o}C IR (KBr) cm^{-1}: 3435, (N-H str.,) 3050 (C-H str., Pyr-H) , 2990 (C-H str., CH_3), 1660, (C=O str.), 1578 (C=N str.), 1474 (C=Cstr.), 1090 (C-O str.),; ^{1}H NMR ($CDCl_3$): δ 8.12-9.2 (s, 2H, N-H/$NH_{(Pyrazole)}$), 11.5 (s, 1H, NH), 8.3-7.8 (m, 4H, Pyr-H), 2.8 (s, 3H, CH_3).

Compounds **5b-d** were also prepared in similar manner with change in reflux time. Their characteristic spectral and physical data are given below:

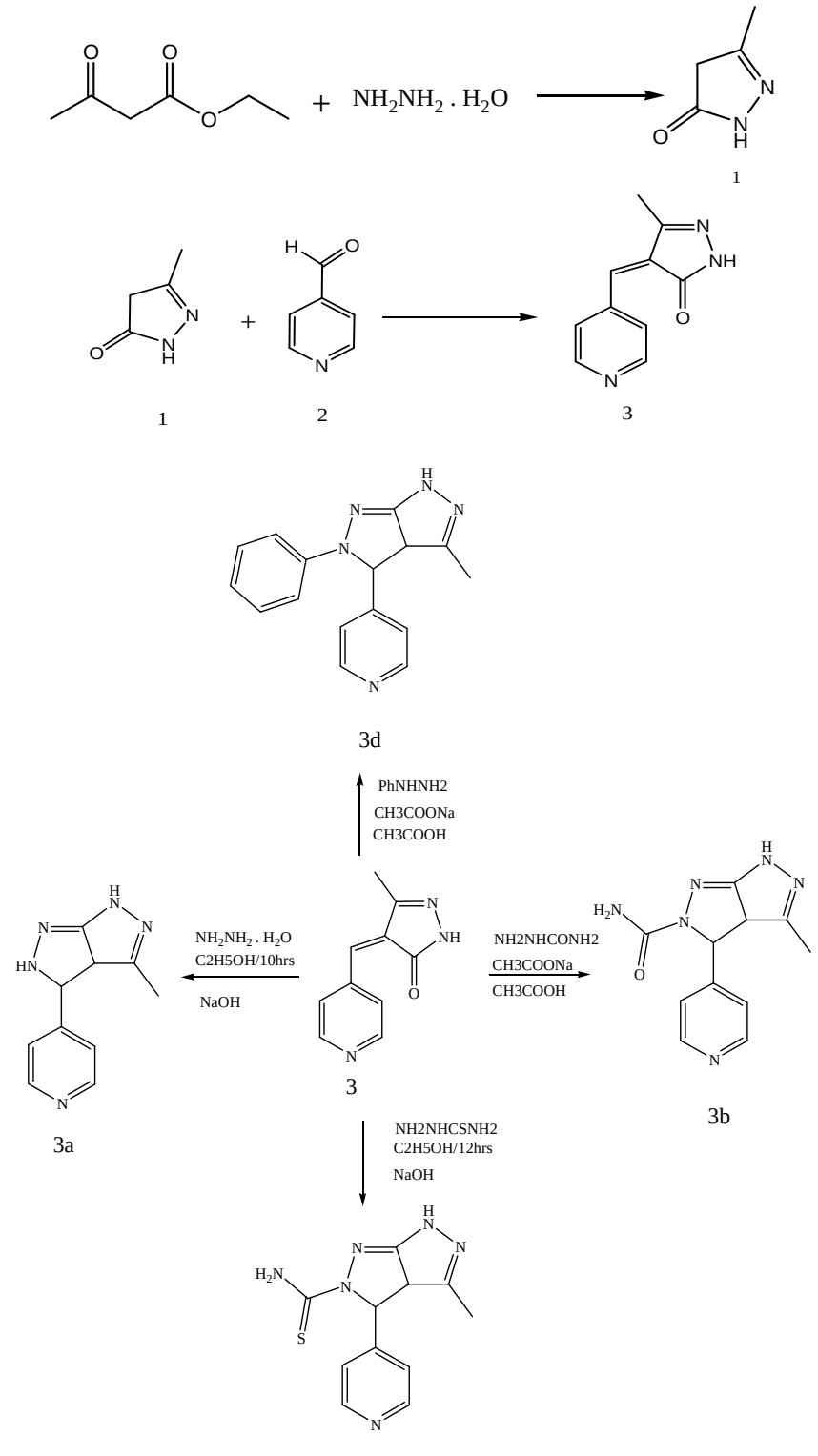
3,3a-dihydro-4-methyl-3-(1H-pyrrol-2-yl)pyrazolo[3,4-c]pyrazole-2(6H)-carboxamide 5b
Yield: 65% Light coloured compound, m.p: 330-331 ^{o}C IR (KBr) cm^{-1}: 3370, (N-H/NH_2 str.,) 3050 (C-H str., Pyrole-H) , 2990 (C-H str., CH_3), 1690, (C=O str.), 1619(C=N str.), 1480 (C=Cstr.), ^{1}H NMR ($CDCl_3$): δ 8.1-9.2 (s 2H, N-H/$NH_{(Pyrazole)}$), 6.9-7.1 (m, 3H, Pyr-H), 7.1(S, 2H, NH_2), 2.51 (s, 3H, CH_3). MS : *m/z* 233 $[M+1]^+$

3,3a-dihydro-4-methyl-3-(1H-pyrrol-2-yl)pyrazolo[3,4-c]pyrazole-2(6H)-carbothioamide 5c
Yield: 58% Yellow coloured compound, m.p.355-356 ^{o}C IR (KBr) cm^{-1}: 3370-3420 (N-H/NH_2 str.,) 3050 (C-H str., Pyr-H) , 2990 (C-H str., CH_3), 1620(C=N str.), 1532 (C=Cstr.), 1284(C=S) ^{1}H NMR ($CDCl_3$): δ 8.1-9.2 (s 2H, N-H/$NH_{(Pyrazole)}$), 6.9-7.1(m, 3H, Pyr-H), 7.1(S, 2H, NH_2.), 2.51(s, 3H, CH_3). MS : *m/z* 249 $[M+1]^{+\cdot}$, 237,211,197,183,163.

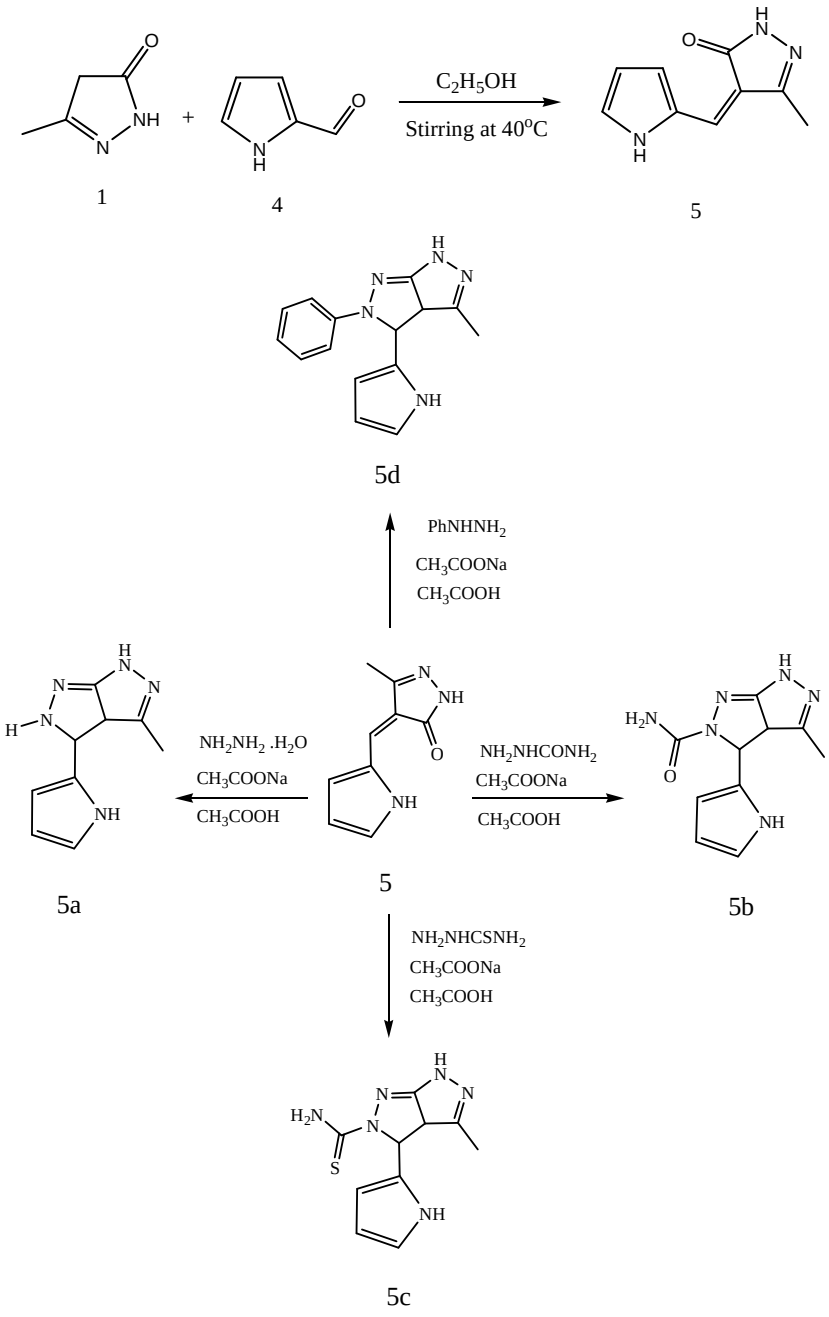
1,3a,4,5-tetrahydro-3-methyl-5-phenyl-4-(1H-pyrrol-2-yl)pyrazolo[3,4-c]pyrazole 5d
Yield: 70% Black coloured compound, m.p: 320-321 ^{o}C IR (KBr) cm^{-1}: 3401, (N-H/NH_2 str.,) 3050 (C-H str., Pyr-H) , 2860 (C-H str., CH_3),2990(=C-H, Ar-H) 1630(C=N str.), 1489 (C=Cstr.), ^{1}H NMR ($CDCl_3$): δ-8.3 (s 1H, $NH_{(Pyrazole)}$), 9.3(s,1H,$NH_{Pyrrole}$) 6.5-6.9(m, 5H, Ar-H), 2.51(s,3H,CH_3), 6.22-6.4 (m,3H,Py-H). MS : *m/z* 266 $[M+1]^{+\cdot}$, 251,204,194,161,141.

RESULTS AND DISCUSSION

In the present work the synthesis of pyridine and pyrrole derivatives of some pyrazolo pyrazoles 3a-d (Scheme 1) and 5a-d (Scheme 2) from a series of reactions was carried out. In order to achieve this aim, 5-methyl-2, 4-dihydro-3*H*-pyrazol-3-one 1 was used as starting material, which was prepared by the reaction between ethylacetoacetate and hydrazine hydrate in absolute alcohol. Compound 1 on condensation with heterocyclic aldehyde 2 and 4 in the presence of sodium acetate as a base furnished 3-methyl-4-((pyridin-4-yl)methylene)-1H-pyrazol-5(4H)-one and 4-((1H-pyrrol-2-yl)methylene)-3-methyl-1H-pyrazol-5(4H)-one 3 and 5. Their structures were confirmed by means of IR and ^{1}H NMR spectral analysis. An intense band between 3444-3492 cm^{-1} for NHstr, 3493 (N-$H_{PYRROLE}$) stretching appeared in IR and ^{1}H NMR signal for CH of C=CH-Ar at 6.5-6.7 and for NH at 8.8-8.85 confirmed the formation of 3 and 5. Compounds 3 and 5 were used as common precursors for the target pyrazolopyrazole 3a-d and 5a-d. The 1,3a,4,5-tetrahydro-3-methyl-4-(pyridin-4-yl)pyrazolo[3,4-c]pyrazole 3,3a-dihydro-4-methyl-3-(pyridin-4-yl)pyrazolo[3,4-c]pyrazole-2(6H)-carboxamide 3,3a-dihydro-4-methyl-3-(pyridin-4-yl)pyrazolo[3,4-c]pyrazole-2(6H)-carbothioamide 1,3a,4,5-tetrahydro-3-methyl-5-phenyl-4-(pyridin-4-yl)pyrazolo[3,4-c]pyrazole 3a-d were obtained by the treatment of 3a-d with hydrazene hydrate, semicorbozide ,Thiosemicarbazide and phenyl hydrazene in the presence of Acetic acid and sodium acetate. The spectral data are in good agreement with the proposed structure. IR showed intense bands at 3430 and 3380 cm^{-1}, corresponding to the NH_2 group,2985cm^{-1},1680cm^{-1} indicate aromatic =C-H,C=O groups and a weak absorption band at 1250 cm^{-1}, corresponding to C=S group, confirming the occurrence of ring closure in the form of thioaminopyrazoline ring. The ^{1}H NMR spectra substantiated the results of the IR analysis and exhibited a singlet at & 9.5-8.5 for NH_2 /NH protons, disappearance of singlet at 6.5-6.7 for C=CH-Py, The structure of compound 3a-d was also confirmed by mass spectral data.


NH2NH2 . H2O
1
2
3
3d
PhNHNH2
CH3COONa
CH3COOH
NH2NH2 . H2O
C2H5OH/10hrs
NaOH
NH2NHCONH2
CH3COONa
CH3COOH
3a
3b
NH2NHCSNH2
C2H5OH/12hrs
NaOH
3c

Scheme 1

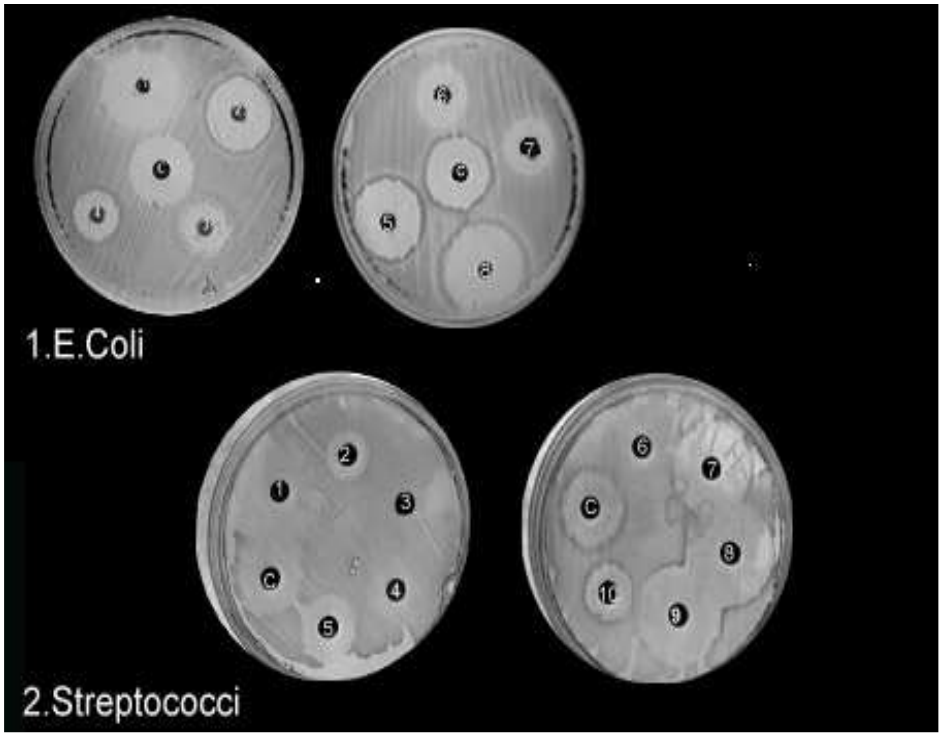


Scheme 2

In an alternative reaction pathway, 4 were converted to 1,3a,4,5-tetrahydro-3-methyl -4-(1H-pyrrol-2-yl)pyrazolo[3,4-c]pyrazole 3,3a-dihydro-4-methyl-3-(1H-pyrrol-2-yl)pyrazolo[3,4-c]pyrazole-2(6H)-carboxamide, 3,3a-dihydro -4-methyl - 3- (1H-pyrrol-2-yl) pyrazolo [3,4-c] pyrazole-2(6H)-carbothioamide, 1,3a,4,5-tetrahydro-3-methyl-5-phenyl-4-(1H-pyrrol-2-yl) pyrazolo [3,4-c]pyrazole 5a-d by the cyclization with hydrazine hydrate, semicarbazide, thiosemicarbazide and phenyl hydrazine in the presence of acetic acid and sodium acetate. Their ^{1}H NMR spectra were conclusive is assigning the structures. Rings and disappearance of singlet for C=CH-Ar in ^{1}H NMR spectra confirmed the cyclization. Compounds **5a-d** were also verified by their NMR analysis and MS fragmentation pattern data**.**

Antimicrobial Activity

Newly synthesized compounds were screened for their antibacterial activity against the Gram –ve bacteria Escherchia *coli* and the other Gram +ve bacteria Streptococcus pneumoniae in addition to their antifungal activity against *Aspergillus niger* and *Candida albicans* using the agar diffusion method [36-37] at a concentration 1 mg/mL using DMSO as a solvent. The results are recorded as average diameter of inhibition zone in mm and are given in Table 1.



C is standard, 1-8 are samples

Fig-1

Table-1

Compound No.	Zone of inhibition in mm			
	Antibacterial activity		Antifungal activity	
	Escherichia coli	*Streptococcus pneumoniae*	*Aspergillus niger*	*Candida albicans*
3a	13.5	8	12	15
3b	8	3	11	12
3c	8	14	10	15
3d	12.8	14	13.5	15
5a	8	12	16	10
5b	8	N	16	14
5c	11	7	13	15
5d	14.2	2	16	17
Standard (Gentamycin)	10	8	-----	-----
Standard (Fluconazole)	-----	-----	22	21

N = No inhibition

Compound **3a, 3d, 5c and 5d** showed highest activity against E. Coli and compound **3b,3c,5a and 5b** showed moderate activity against ***E.Coli*** species. Compounds **3c**, **3d and 5a** showed very good activity against **streptococcus pneumoniae** and compound **5c** showed moderate activity against **streptococcus** species. Amongst

the tested compounds **5a**, **5b and 5d,** showed good activity against ***A.niger*** and **3d, 5c** have shows moderate activity against *A.niger*. Compounds **3a, 4d, 5c** and **5d** have shown very good activity and compound **3c**and **5b** have shown moderate activity against candida albicans due to the above synthesized compounds exibit very good activity of against antibacterial and antifungal activity.

Acknowledgements
We are grateful to University Grants Commission, New Delhi for providing Meritorious Research Fellowship under Special Assistance Programme.

REFERENCES

[1] P Francisco; AMO deRetana; PA Jaiore, *Tetrahedron.*, **1999**, 55, 14451.
[2] KY Lee; JM Kim; JN Kim, *Tetrahedron Lett*.**, 2003**, 44, 6737.
[3] J Jiaz; Y Wu; W. Hung; P. Zhang; Y. Song; J. Woolfrey; U.Sinha; AE Arfsten; ST Edwards; A Hutchaleelaha; SJ Hollennbach; JL Lambing; RM Scarborough; BY Zhu, *Bioorg med chem Lett.*, **2004,** 14, 1229.
[4] DP Thomas; K Albert; BC Barbara; AR Mark; LB Mark; W Yaping; DV Tiffany; E Wayne; BF Mary; KF Sandra, *Bioorg Med Chem Lett*.**, 2006**, 16, 3156.
[5] V Manuela; P Valeria; V Paola; C Alexander; C Marina; M Ciro, *Bioorg Med Chem Lett*., **2006**, 16, 1084.
[6] SG Roelfvan; C Arnold; K Wellnga, *J Agric Food Chem*.**, 1979**, 84, 406.
[7] Rajendra; Krushanji Wanare, *J. Chem.Pharm.Res*., **2011**, 3(5): 136-144.
[8] Meghasham; Narayanrao Narule, *J. Chem. Pharm. Res.*, **2011**, 3(3):38-47
[9] HZ Katri; SA Vunii, *J Indian Chem Soc*.**, 1981,** 58, 168.
[10] Singarave; Mohan; Sarkkarai ;Ananthan, *J. Chem. Pharm. Res.*, 2011, 3(1):402-413
[11] G Amr Ael; NA Abdel-Lalif; MM. Abdalla, *Bioorg Med Chem.*, **2006**, 14(2), 373.
[12] HG Garge; Chandraprakash, *J Pharm Sc.*, 1971, 14, 649.
[13] HA Regaila; AK El-Bayonk; M Hammad. Egypt *J Chem.*, **1979**, 197,20.
[14] Bharat Parashar; Sudhir; Bhardwaj; Sharda Sharma; G. D. Gupta; V. K. Sharma; P. B. Punjabi, *J. Chem. Pharm. Res*., **2010**, 2(3):33-42
[15] B Chai; X Qian; S Cao; H Liu; G Song, *ARKIVOC.*, **2003**, (ii), 141.
[16] A Singh; S Rathod; BN Berad; SD Patil; AG Dosh. *Orient J Chem.*, **2000**, 16,315.
[17] VY Vernon; D William; EI Richard. *International Minerals and Chemical Corpo, US* 4221791 (C1-424-248).
[18] Y Ura; G Sakata; KO Makmo. [Nessam Chemicals Industries Ltd], *Eur Pat Appl EP* 46467 (C1 C07 D241/14).
[19] YV Tomilovi; GP Okonnishnikova; EV Shulishov; OM. Nefedov, *Russ Chem Bt.*, **1995**, 44, 211.
[20] EI Klimova; M Marcos; TB Klimova; AT Cecilio; AT Ruben; RR Lena, *J Organometallic Chem.*, **1999**, 106, 585.
[21] D Bhaskarreddy; A Padmaja; PV Ramanareddy; B Seenaiah. *Sulfur Lett*., **1993**, 16, 227.
[22] V Padmavathi; RP Sumathi; BN Chandrasekhar; D Bhaskarreddy. *J Chem Research.*, **1999**, 610.
[23] D Bhaskarreddy; BN Chandrasekhar; V Padmavathi; RD Sumathi. *Synthesis*, **1998**, 491.
[24] U Hartfiel; G Dorfmeister; H Franke; J Geisler; G Johann; R Rees. *US Patent*, **1995**, 5, 405.
[25] Kosuge; Okeda. *J. Biochem*., **1954**, 41, 183.
[26] E. Hernab; JJ Gabhks. *Cancer Chemotherapy Rept*.**, 1961**, 14, 85.
[27] KJ Potts. Comprehensive Heterocyclic Chemistry., Pergaman press, Oxford**, 1986**, 5(4A).
[28] S Richi; *J Horsfall. Phytopathology*., **1952**, 42, 457.
[29] GN Pershin; NA Novitskola; AN Kost; II Grandberg. Dokl. Acad. Nank. **1959**, 123, 200.
[30] LG Polevoi. *Chem. Abstr*., **1966**, 65, 19147d.
[31] M Nakanishi; Y Naka; R.Kobayashi. *J. Chem. Abstr*., **1975**, 82, 14092.
[32] J Clayden; N Greeves; S Warren; P Wother, Organic Chemistry, Oxford University Press., **2001**, 1196-1197.
[33] MN Narule. *J. Chem. Pharm. Res*., **2011**, 3(3), 38-47.
[34] A Kreutzberger; A Tantawy, *Chem Zeitg*, **1983**, 107, 205.
[35] M El-Kerdawy; A Tamtawy; LM Gad. *Chin Pharm J*., **1991**, 5, 43.
[36] RJ Grayer; JB Harborne, A Survey of Antifungal Compounds from Higher Plants. *Phytochemistry.*, **1994**, *37*, 19-42.
[37] ON Irob; M Moo-Young; W Anderson, Antimicrobial Activity of Annatto Extract. *Int. J. Pharm.*, **1996**, 34, 87-90.
[38] BS Furniss; AJ Hannaford; PWG Smith; AR Tatchell, Vogel's Textook of Practical Organic Chemistry, Fifth Edition, Longman Scientific & Technial., **1989**, 807.