Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2014, 6(2):283-287
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis, characterization and antimicrobial studies of novel 2-pyrazoline derivatives

Vidhya K. R. and Syed Shafi S.

Department of Chemistry, Thiruvalluvar University, Serkadu, Vellore

---

ABSTRACT

A simple, efficient method for the synthesis of some novel 2-pyrazoline derivatives is reported.Pyrazolines were prepared by condensing chalcones with hydrazine hydrate /isoniazid / tolylsulfonylhydrazide. The structures of the synthesized compounds were confirmed by FTIR, ^1H NMR, mass and elemental spectral data. The synthesized compounds have been screened for their antimicrobial activity against different micro-organisms. A significant level of activity was observed.

Keywords: Chalcones, Hydrazine hydrate,isoniazid,benzene sulfonyl chloride, Pyrazolines, Antimicrobial Activity.

INTRODUCTION

Piperonal,a naturally occurring derivative of piperine compound(the pyrrolidine amide of piperic acid) is an aromatic aldehyde. The choice of piperonal for the aldehyde moiety in chalcone, stemmed from the fact that many compounds containing the 3,4-methylenedioxy group have some biological activity[1,2]. 2-Pyrazoline derivatives have also been reported in the literature to exhibit various pharmacological activities such as antimicrobial [3-8], anti-inflammatory [9] and antihypertensive [10]. Its derivatives, possess a wide range of biological and physiological activities such as antitumor, antiarthritic, analgesic, anti diabetic, fungicidal, bactericidal, immunosuppressive activities.[6,8]. On the other hand anti tubercular activity of isoniazid is well documented[11,12]. Also sulfur containing heterocycles possess pharmacological activities widely occur in nature in the form of alkaloids, vitamins, pigments and as constituents of plant and animal cells. In view of these observations and in continuation of our earlier work [13],we are now reporting some novel 2-pyrazoline derivative containing benzodioxole , isoniazid / benzenesulfonyl moiety.

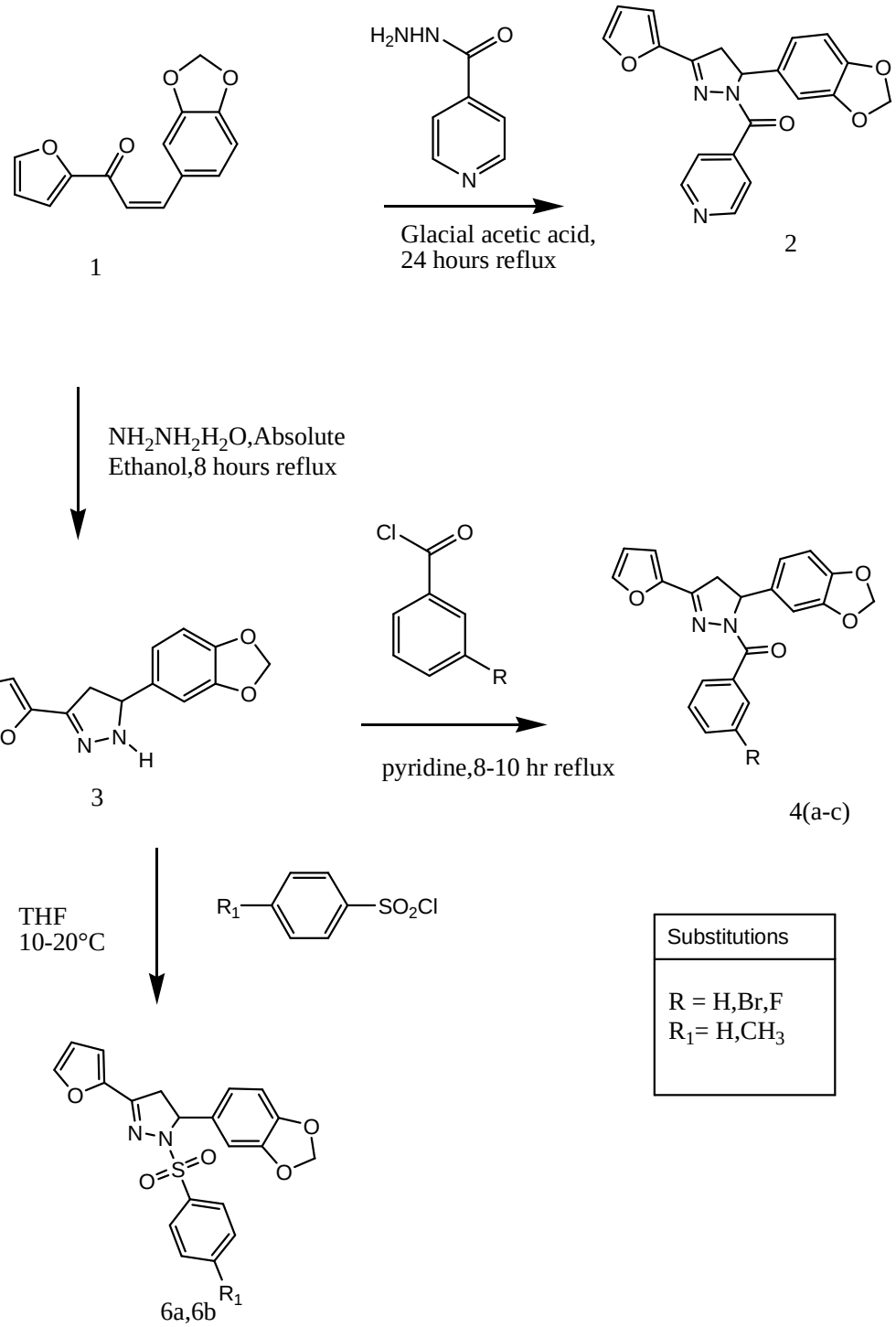
MATERIALS AND METHODS

All the reagents were purchased from Aldrich and used as received. Glacial acetic acid and dry solvents were supplied by Spectrochem, India. ^1H NMR chemical shift values were reported on the scale in ppm relative to TMS. The ^1H NMR spectra were recorded in $CDCl_3$ on BrukerAMX 400.spectrometer (400MHz). IR spectra were recorded on Perkin Elmer spectrum 100 FT-IR model. Column chromatography was performed with silica gel 60-120 mesh (Merck, Mumbai, India.). All the compounds were routinely checked for their reaction on silica gel 60 F254 TLC plates and their spots were visualized by exposing them to UV lamp or iodine vapour or $KMnO_4$ reagents. Melting points were determined by Buchi B-545 apparatus. LCMS were obtained using Agilent 1200 series LC and Micromass zQ spectrometer.Yield reported is the isolated yield after purification of the compounds.

Procedure for synthesis of 5-(benzo[d][1,3]dioxol-5-yl)-3-(furan-2-yl)-4,5-dihydropyrazol-1-yl)(pyridine-4-yl) methanone(2).

Equimolar amount of chalcone(0.01M) and Isoniazid(0.01M) in glacial acid(25 ml) was refluxed for 24 hours. Reaction was monitored by TLC, after the completion of reaction, it was diluted with water (10 mL) and extracted with ethyl acetate (50 mL) and dried over

SCHEME



Scheme:Synthesis of pyrazoline derivatives

Na_2SO_4. The organic layer was concentrated under reduced pressure to give crude product. The pure product was isolated by using column chromatography. The column was started at 10% ethyl acetate in petroleum ether and slowly increased to 70% ethyl acetate.

5-(benzo[d][1,3]dioxol-5-yl)-3-(furan-2-yl)-4,5-dihydropyrazol-1-yl)(pyridine-4-yl)methanone(2):
Off White powder,Yield:74%, M.P 186°C .; IR(KBr):1664cm^{-1}(C=O),1567cm^{-1}(C=N), 1491 cm^{-1}(C=C),1134 cm^{-1} (C-N str); ^{1}H NMR:400MHz($CDCl_3$) : 3.22 (dd, *J* = 8Hz, 8Hz, 1H, -CH_2), 3.68(dd, *J* = 4.8Hz, 5.6Hz, 1H, -CH_2), 5.2(q, *J* = 24Hz, 1H, -CH), 5.93(q, *J* = 20Hz, 2H, -O-CH_2-O), 6.80-6.74(m, 3H, Ar), 7.50(d, *J* = 1.2Hz, 1H, Ar), 7.06(t, *J* = 16Hz, 2H, Ar),7.9-8.8(d,pyridyl,2H $_a$,2H$_b$,J=8Hz); LC-MS: m/z 362 (M+1).Elemental analysis: C,66.48; H,4.18; N,11.63;O,17.71.Found:C;66.46H,4.19;N,11.64;O,17.71

Procedure for synthesis of 5-(benzo[d][1,3]dioxol-5-yl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazole(3): A mixture of chalcone(0.01M) and hydrazine hydrate 99% (0.01M) was refluxed in absolute ethanol for 8 hours. Reaction was monitored by TLC, with eluent 8:2 petether:ethylacetate. Reaction was completed. Then the resulting solid obtained was dried and washed with water. . The pure product was isolated by using column chromatography. The column was started at 10% ethyl acetate in petroleum ether and slowly increased to 70% ethyl acetate. The solid was dried and recrystallized from abs.ethanol .

5-(benzo[d][1,3]dioxol-5-yl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazole(3):
Brown powder,Yield: 78%,M.P:145°C; IR(KBr):1564cm^{-1}(C=N), 1495 cm^{-1}(C=C),1130 cm^{-1} (C-N str); ^{1}H NMR:400MHz($CDCl_3$) : 3.09(dd, *J* = 8.5Hz, 8.5Hz, 1H, -CH_2), 3.74(dd, *J* = 5Hz, 5.6Hz, 1H, -CH_2), 5.11(q, *J* = 20Hz, 1H, -CH), 5.8(q, *J* = 20Hz, 2H, -O-CH_2-O), 6.80-6.74(m, 3H, Ar), 7.50(d, *J* = 1.2Hz, 1H, Ar), 7.06(t, *J* = 16Hz, 2H, Ar), 8.6(s,1H, NH).; LC-MS: m/z 257 (M+1).; Calculated C,65.62;H,4.72;N,10.93;O,18.73,Found C,65.64;H,4.70;N,10.95;O,18.71

General procedure for synthesis of 5-(benzo[d][1,3]dioxol-5-yl)-3-(furan-2-yl)-4,5-dihydropyrazol-1-yl) (substitutedphenyl)methanone(4a-c).
To the intermediate (3) (0.002M),substituted benzoyl chloride(0.002M) was refluxed in pyridine(8-10hours).Reaction was monitored by TLC, with eluent 7:3 petether:ethylacetate. . The pure product was isolated by using column chromatography. The column was started at 10% ethyl acetate in petroleum ether and slowly increased to 60% ethyl acetate.The solid was dried and recrystallised from ethanol.

5-(benzo[d][1,3]dioxol-5-yl)-3-(furan-2-yl)-4,5-dihydropyrazol-1-yl)phenyl methanone(4a):
Brown powder,Yield:74%,M.P:152°C ; IR(KBr):1660(C=O),1567cm^{-1}(s,C=N), 1491 cm^{-1}(m,C=C),1134 cm^{-1} (s,C-N str); ^{1}H NMR:400MHz($CDCl_3$) : 400MHz($CDCl_3$) : 7.9-8.8(m,5H,) 3.1(dd, *J* = 11Hz, 11Hz, 1H, -CH_2), 3.72(dd, *J* = 4.8Hz, 5.6Hz, 1H, -CH_2), 5.1(q, *J* = 24Hz, 1H, -CH), 5.93(q, *J* = 20Hz, 2H, -O-CH_2-O), 6.80-6.74(m, 3H, Ar), 7.50(d, *J* = 1.2Hz, 1H, Ar), 7.06(t, *J* = 16Hz, 2H, Ar) 7.06 (m, *J* = 16Hz, 2H, Ar). LC-MS: m/z 361(M+1). Calculated C,69.99;H,4.48;N,7.77;O,17.76 Found C,69.97;H,4.49;N,7.79;O,17.75

5-(benzo[d][1,3]dioxol-5-yl)-3-(furan-2-yl)-4,5-dihydropyrazol-1-yl)(3-bromophenyl) methanone(4b)
Brown powder, Yield: 68%,M.P:161°C; IR(KBr):1665(C=O),1565cm^{-1}(s,C=N),1492cm^{-1}(m,C=C),1134cm^{-1}(s,C-Nstr) ; ^{1}H NMR:400MHz($CDCl_3$) : 400MHz($CDCl_3$) : 7.23-7.00 (m, 5H, Ar),), 6.80-6.74(m, 3H, Ar), 5.1(q, *J* = 24Hz, 1H, -CH),6.02 (t, *J* = 20Hz, 3H, -CH, -O-CH_2-O)3.98 (dd, *J* = 12Hz, 12Hz, 1H, -CH_2),3.3 (dd, *J* = 8Hz, 8Hz, 1H, -CH_2) LC-MS: m/z 440(M+1). Calculated: C,57.42; H,3.44;Br,18.19; N,6.38; O,14.57. Found:C,57.38; H,3.46; Br,18.21;N,6.36;O,14.59

5-(benzo[d][1,3]dioxol-5-yl)-3-(furan-2-yl)-4,5-dihydropyrazol-1-yl)(3-fluorophenyl) methanone(4c):
Brown powder,Yield:72%,M.P:178°C; IR(KBr):1665(C=O),1565cm^{-1}(s,C=N),1492cm^{-1}(m,C=C),1134cm^{-1}(s,C-Nstr) ^{1}HMR:400MHz($CDCl_3$) : 400MHz($CDCl_3$) : 7.4-7.00 (m, 5H, Ar),), 6.9-6.74(m, 3H, Ar), 5.3(q, *J* = 24Hz, 1H, -CH),6.6 (t, *J* = 20Hz, 3H, -CH, -O-CH_2-O)3.9 (dd, *J* = 12.3Hz, 12Hz, 1H, -CH_2),3.5 (dd, *J* = 9Hz, 8.8Hz, 1H, -CH_2) LC-MS: m/z 379 (M+1).; Calculated: C,66.66; H, 4.00; F,5.02; N,7.40; O,16.91.; Found: C,66.64; H,4.02; F,5.01; N,7.39; O,16.91

Procedure for synthesis of 5-(benzo[d][1,3]dioxol-5-yl)-3-(furan-2-yl)-4,5-dihydro-1-(phenyl sulfonyl) -1H-pyrazole(6a):
Benzene sulfonyl chloride (0.002M) was dissolved in tetrahydrofuran(5ml) with stirring.The stirred mixture was cooled in an ice bath to 5-10°C;followed by gradual addition of a solution of compound[3](0.002M) in tetrahydrofuran so that the temperature was maintained between 10-20°C.Stirring was continued for half an hour after the addition was complete. Reaction mixture was directly concentrated to remove organic volatiles. The residue was dissolved in water (10 mL) and extracted with ethyl acetate (30 mL) and the combined organic layer was

washed with water (30mL), brine solution (20 mL), dried over Na_2SO_4 and evaporated to dryness to get the crude product . The pure product was isolated by using column chromatography. The column was started at 10% ethyl acetate in petroleum ether and slowly ethanol.

5-(benzo[d][1,3]dioxol-5-yl)-3-(furan-2-yl)-4,5-dihydro-1-(phenylsulfonyl)-1H-pyrazole(6a): Brown powder, Yield:74%,M.P:180°C; IR(KBr):1570m^{-1}(s,C=N),1486cm^{-1}(m,C=C),1134cm^{-1}(s,C-Nstr),1172,1384(s,SO_2) ^{1}HMR: 400MHz($CDCl_3$) : 7.23-7.00 (m, 5H, Ar), 6.80-6.74(m, 3H, Ar), 5.1(q, *J* = 24Hz, 1H, -CH),6.02 (t, *J* = 20Hz, 3H, -CH, -O-CH_2-O)3.98 (dd, *J* = 12Hz, 12Hz, 1H, -CH_2),3.3 (dd, *J* = 8Hz, 8Hz, 1H, -CH_2) LC MS:m/z,397(M+1). Calculated:C,60.60;H,4.07;N,07.07;O,20.18;S,8.09. Found:C,60.58;H, 4.09;N,07.09;O,20.16;S,8.09

Procedure for synthesis of 5-(benzo[d][1,3]dioxol-5-yl)-3-(furan-2-yl)-4,5-dihydro-1-tosyl-1H-pyrazole(6b) :
P-Toluene sulfonyl chloride (0.002M) was dissolved in tetrahydrofuran(2ml) with stirring.The stirred mixture was cooled in an ice bath to 5-10°C and followed by gradual addition of a solution of compound[3](0.002M) in tetrahydrofuran so that the temperature was maintained between 10-20°C.Stirring was continued for half an hour after the addition was complete. Reaction mixture was directly concentrated to remove organic volatiles. The residue was dissolved in water (10 mL), and extracted with ethyl acetate (30 mL) and the combined organic layer was washed with water (30 mL), brine solution (20 mL), dried over Na_2SO_4 and evaporated to dryness to get the crude product. The crude product was recrytallized with diethyl ether and dried under vacuum.The solid separated was filtered,dried and recrystallised from ethanol.

5-(benzo[d][1,3]dioxol-5-yl)-3-(furan-2-yl)-4,5-dihydro-1-tosyl-1H-pyrazole(6b) :
Brown powder, Yield:73%, M.P: 190°C IR(KBr): 1565cm^{-1}(s, C=N), 1488cm^{-1}(m, C=C),1134cm^{-1}(s, C-Nstr)1178, 1389(s, SO_2); ^{1}H NMR: 400MHz($CDCl_3$) : 7.23-7.00 (m, 5H, Ar),), 6.80-6.74(m, 3H, Ar), 5.1(q, *J* = 24Hz, 1H, -CH), 6.02 (t, *J* = 20Hz, 3H, -CH, -O-CH_2-O)3.98 (dd, *J* = 12Hz, 12Hz, 1H, -CH_2),3.3 (dd, *J* = 8Hz, 8Hz, 1H, -CH_2). LC-MS: m/z 411(M+1). Calculated:C,61.45; H,4.42; N,6.83; O,19.49; S 7.81. Found: C,61.44; H,4.44; N,6.83; O,19.46;S,7.83

ANTIMICROBIAL ACTIVITY

We have investigated newly synthesised pyrazolines for their antibacterial activity against *Escherichia coli* , *Staphylococcus aureus* , *Pseudomonas aeruginosa* and *Klebsiella pneumonia* bacterial strains by the disc diffusion method. Solvent and growth controls were kept, the zones of inhibition and minimum inhibitory concentrations (MIC) noted. Results of these studies are given in Table-1. We have investigated newly synthesised pyrazolines were screened for their antifungal activity against *Aspergillus niger* , *Candida albicans* obtained. Antifungal activity was determined by measuring the inhibition zone and (MIC) was noted.

Table-1: Minimum inhibitory concentration in µg/mL given in parenthesis

Compounds (10 µg/ml)	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsilla pneumoniae*
2	23(6.25)	21.5 (6.25)	23.5(6.25)	20(6.25)
4a	20(6.25)	<10 (50)	13.5(12.5)	22(6.25)
4b	17.5(12.5)	<10(50)	<10(50)	14(12.5)
4c	12.5(12.5)	<10(50)	<10(50)	<10(50)
6a	20(6.25)	21.5 (6.25)	23(6.25)	19(12.5)
6b	23(6.25)	20.5 (6.25)	22.5(6.25)	18(12.5)
Cipro	24.5(6.25)	25(6.25)	24(6.25)	25.5(6.25)

Table-2 Antifungal activities of the newly synthesised compounds (Zone of Inhibition in mm, MIC in µg/mL given in parenthesis)

Compounds (10 µg/ml)	*Aspergillus niger*	*Candida albicans*
2	32(6.25)	35.5(6.25)
4a	<10(50)	<10(50)
4b	<10(50)	<10(50)
4c	<10(50)	<10(50)
6a	36.5(6.25)	37.5(6.25)
6b	36.5(6.25)	37.5(6.25)
Ketaconazole	38.5(6.25)	34.5(6.25)

RESULTS AND DISCUSSION

A series of novel 2-pyrazoline derivatives were synthesised and evaluated for their antibacterial and antifungal activity. All the derivatives were efficiently synthesised by two or three step process.The structure of the newly synthesised compounds was elucidated by their ^{1}H NMR,LC-MS/MS, IR spectral data and melting point analysis.

We have investigated newly synthesised pyrazoline bearing piperonal for their antibacterial activity against *Escherichia coli* , *Staphylococcus aureus* , *Pseudomonas aeruginosa* and *Klebsiella pneumonia* bacterial strains by the disc diffusion method. Solvent and growth controls were kept, the zones of inhibition and minimum inhibitory concentrations (MIC) noted. Results of these studies are given in Table-1 and compared with the standard ciprofloxacin. Most of them showed the moderate to low antibacterial activity.Among the compound 2 was showed good inhibition towards all the four bacteria tested. Compounds 6a,6b were showed good activity in *Staphylococcus aureus*,*Escherichia coli* and Pseudomonas aeruginosa. Compounds 4a,4b and 5c shows moderate to low active against all the strains tested. Synthesised pyrazolines were screened for their antifungal activity against *Aspergillus niger* ,*Candida albicans.* Antifungal activity was determined by measuring the inhibition zone and MIC. The results of these studies were given in Table-2 and compared with the standard Keta conazole. Most of the compounds synthesised showed the moderate to low activity against all the fungi tested. Particularly compounds 2,6a,6b were active against all the above fungi tested.

CONCLUSION

we have successfully synthesised a new series of 2-pyrazoline derivatives and moreover, some of compounds contains bioactive heterocyclic moiety. The antimicrobial screening suggests that all the newly synthesised compounds showed moderate to good activity against the tested organisms.

REFERENCES

[1] Jithan Aukunuru,keerthana Eedula,Venkanna Pasham,Venumadhav Katla and Srinivas Reddy.*International Journal of Pharmaceutical Sciences and Nanotechnology*,Volume 2,Issue 1,April-**June2009**
[2] Zhen-yu Shi, Yong-qiang Li, Yu-hua Kang, Guo-qiang Hu, Chao-shen Huang-Fu, Jin-bo Deng and Bin Liu.*Acta Pharmacologica Sinica* 33, 271-278 (February **2012**) | doi:10.1038/aps.2011.158.
[3] Hareesh M, Srinivas Mahanti, Sailu B, Subramanyam D, Saidu Reddy Sakam,Tara B, Balram B, Vasudha B, Ram B, *Der Pharma Chemica*, **2012**, 4(4):1637-1643.
[4] Safak C.; Tayhan A.; Sarac S.. *J. Indian Chem. Soc*. **1990**, 67, 571–574.
[5] Sandip Y. Patil,Rajesh J. Oswal, Atul S. Sayare, Sudhir S. Landge, Rishikesh V. Antre., *Der Pharma Chemica*, **2012**, 4 (1):33-38.
[6] Anjani Solankee, Rajanikant Patel and Kirti Patel *Der Pharma Chemica*, **2011**, 3 (6):317-324
[7] B.Sivarama Holla,M.K.Shivananda,P.M.Akberaliand shalini shenoy.*Indian Journal of Chemistry*.Vol, 39B,June **2000**,pp 440-447
[8] Tandrima Majumder, Biplab D, Binoy Behari Goswami and Subrat Kar. *Der Pharma Chemica*, **2011**, 3 (6):268-281
[9] Nasar, M.N.A.; Said, S.A. *Arch. Pharm. Pharm. Med. Chem*. **2003**, 336, 551
[10] Turan-Zitouni G; Chevallet P; Kiliç F.S; Erol K. *Eur. J. Med. Chem*. **2000**, 35, 635–641.
[11] Clemmenson J & Hjalgrim-Jenson S,*Ecotex Environ safety*,3,**1979**,439
[12] Jansen J.D,Clemmenson J,Sundaram K,*Mutat Res*,76,**1980**,85
[13] Balapragalathan Thappali Jothikrishnan,;Sriram Narasimhan,; Suban Syed Shafi;.*Molbank*. **2010**, M668;