Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 4, 2013

Research Article

SYNTHESIS OF NOVEL PYRAZOLE DERIVATIVES AND THEIR EFFICACY AS ANTIMICROBIAL AGENTS

M. GOVINDARAJU[1], B.N. MYLARAPPA[2], K. AJAY KUMAR[1]*

[1]Post Graduate Department of Chemistry, Yuvaraja's College, University of Mysore, India, [2]Transplant Surgery, Rangos Research Center, University of Pittsburgh, PA 15201, USA.

Received: 27 July 2013, Revised and Accepted: 29 Aug 2013

ABSTRACT

The aim of the present study was to synthesize series of new tetrasubstituted pyrazoles as potential antimicrobial agents.

The tetrasubstituted pyrazoles were synthesized by 1,3-dipolar cycloaddition of nitrile imines generated *in situ* by the oxidative dehydrogenation of aldehyde hydrazones (**4**) using chloramine-T as mild oxidant with 1, 3-diphenylprop-2-yn-1-one (**3**) in good yields. The synthesized compounds were tested for their antimicrobial susceptibility activity against different fungi and bacteria species by paper disc diffusion method.

The structures of the new pyrazoles were confirmed by spectral studies and elemental analysis.

Results of the antimicrobial activity reveal that some of the compounds particularly with chloro substituents act as potential antimicrobial agents against different fungal and bacterial organisms.

Keywords: Antibacterial, Antifungal, Cycloaddition, Dipolar, Inhibition.

INTRODUCTION

Five membered nitrogen heterocycles, particularly pyrazoles and their derivatives are regarded as important molecules in organic synthesis; they serve as building blocks for the construction of biologically potent molecules. Numerous methods have been developed for synthesis of substituted pyrazoles viz. by (i) the reaction of 1, 3-diketones with hydrazines, (ii) the reaction of α, β-unsaturated aldehyde and ketones with hydrazines. However the classical method employed for the synthesis of pyrazolines and pyrazoles involves 1, 3-dipolar cycloaddition reactions of nitrile imines to alkenes and alkynes [1,2]. An efficient regioselective synthetic route to multisubstituted pyrazoles by cyclocondensation of β-thioalkyl-α, β-unsaturated ketones with hydrazines was developed by Jin et al [3]. A convenient and efficient synthesis of a series of 1, 3-diaryl-4-halo-1*H*-pyrazoles in moderate to excellent yields by 1, 3-dipolar cycloaddition of 3-arylsydnones and 2-aryl-1, 1-dihalo-1-alkenes was reported [4].

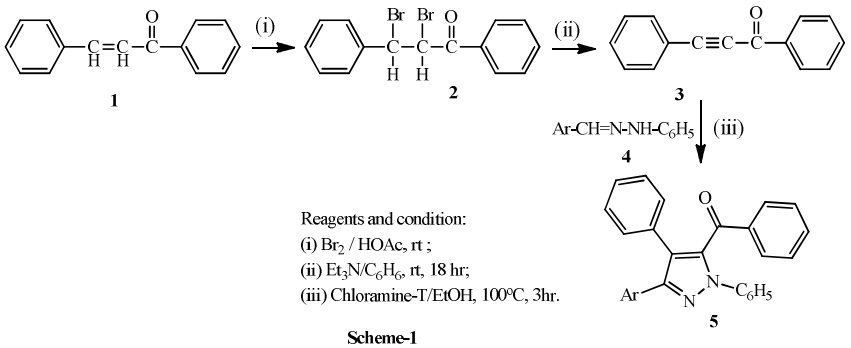
Pyrazole derivatives have been used as important pharmacores and synthons in the field of organic chemistry and drug designing. For instance, a series of 1-acetyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazoles synthesised were investigated for their ability to inhibit selectively monoamine oxidases, swine kidney diamine oxidase (SKDAO) and bovine serum amine oxidase (BSAO) [5]. Pyrazoles have known to exhibit antifungal, antibacterial, antioxidant [6,7], anti-tubercular [8], anticancer [9] activities. A series of structurally related 1*H*-pyrazolyl derivatives synthesized compounds were tested for their anti-inflammatory and antimicrobial activities.

The enormous pharmacological applications associated with pyrazoles prompted us to work in this area. In continuation of our work on pyrazoles and in search of new potential antifungal and antibacterial agents, we herein report the synthesis of a series of new novel pyrazoles by 1,3-dipolar cycloaddition reaction and in vitro evaluation of their antibacterial and antifungal activities against different organisms.

MATERIALS AND METHODS

The chemicals/reagents were obtained from Aldrich and Merck Chemicals (India). The NMR spectra were recorded on a Bruker supercon 400 MHz spectrophotometer in $CDCl_3$ using TMS as an internal standard. The Chemical shifts are expressed in δ ppm. Mass spectra were obtained on Shimadzu LCMS-2010A spectrophotometer. Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyser. Chromatographic separations were carried out on silica gel (70-230 mesh, Merck) column using hexane: ethyl acetate (8:2) as eluent.

In a typical 1, 3-dipolar cycloaddition reaction, a mixture of 1, 3-diphenylprop-2-yn-1-one **3**, aldehyde phenylhydrazones **4,** and Chloramine-T in ethyl alcohol was refluxed on a water bath conditions for 2-3 hours. After the completion, the reaction yielded 3-aryl-1, 4-diphenyl-1*H*-pyrazol-5-yl-(phenyl)methanone **5** in 70-84% yield. The 1, 3-diphenylprop-2-yn-1-one **3** was obtained by the treatment of 2, 3-dibromo-1, 3-diphenylpropan-1-one **2** with excess of triethylamine in benzene (scheme-1).



Scheme-1

Preparation of 2, 3-dibromo-1, 3-diphenylpropan-1-one (**2**): 2,3-Dibromo-1,3-diphenylpropan-1-one **2** was obtained by the reaction of chalcone **1** (1 mmol) and bromine (1.5 mmol) in acetic acid at room temperature in 1 hr as an yellow crystalline solid, m.p. 112-114ºC. ^{1}H NMR ($CDCl_3$): δ 5.64 (d, 2H, CH), 5.72 (d, 2H, CH), 7.32-7.68 (m, 10H, Ph-H). ^{1}C NMR ($CDCl_3$): δ 45.4, 62.3, 128.0, 128.2, 128.6, 128.9, 129.2, 129.4, 133.0 135.8, 138.2, 178.1.

Preparation of 1, 3-diphenylprop-2-yn-1-one (3): To a stirred solution of 2, 3-Dibromo-1, 3-diphenylpropan-1-one **2** (1 mmol) in dry benzene (100 mL), a solution of triethylamine (4 mmol) in dry benzene (30 mL) was added. The reaction mixture was stirred at room temperature for 24 hrs. The triethylamine hydrobromide formed was removed by filtration and the filtrate was concentrated by distilling the benzene under reduced pressure. The resulting mixture was extracted in to ether (50 mL); the solvent was evaporated to dryness to get the light brown yellow oily product **3**.

General procedure for the synthesis of 3-aryl-1, 4-diphenyl-1*H*-pyrazol-5-yl-(phenyl)methanone (5): A mixture of 1, 3-diphenylprop-2-yn-1-one **3** (1.0 mmol), aldehyde phenylhydrazone **4** (1.0 mmol), and chloramine-T trihydrate (2.0 mmol) in ethyl alcohol (30 mL) was refluxed on water bath for 3 hours. The progress of the reaction was monitored by TLC. After the completion of the reaction, the sodium chloride formed was filtered off, and the filtrate was evaporated in vacuo. The residual mass was extracted into ether (30 mL), washed successively with water (2 X 20 mL), 10% sodium hydroxide (2 X 20 mL) and saturated brine solution (1 X 15 mL). The organic layer was dried over anhydrous sodium sulphate. Evaporation of the solvent produced crude products and was purified by column chromatography using hexane: ethyl acetate (8:2 v/v) as eluent.

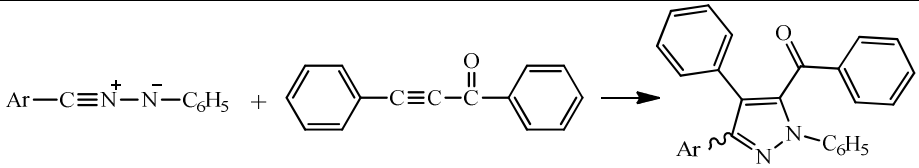
Antimicrobial activity: Antimicrobial activity of the synthesized compounds was done by paper disc diffusion method [10-12]. The test compounds **5** at the concentration of 50 µg/mL in methanol in the nutrient agar media were screened for their antibacterial activity against bacteria species *Escherichia coli, Salmonella typhimurium, Bacillus subtilis*, *Staphylococus aureus* and antifungal activity against fungal species *Aspergillus niger, Aspergillus flavus, Candida albicans, Fusarium oxysporium*. The antibiotics ciprofloxacin and nystatin were used as standard drugs against bacteria and fungi species respectively. The screening tests were carried out in triplicate and the results were expressed as a mean of three determinations.

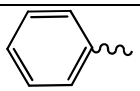
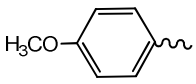
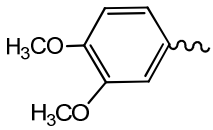
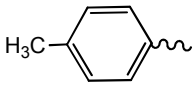
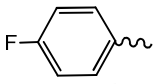
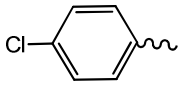
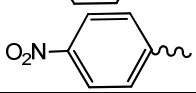
RESULTS AND DISCUSSION

The general synthetic pathway employed is depicted in the scheme-1. The structures of the synthesised compounds were confirmed by spectral studies and elemental analysis. For instance, in ^{1}H NMR spectra, the compound **2** showed the signals due to -CHBr-CHBr-CO- protons as doublet at δ 5.64 ppm and 5.72 ppm., the shift in the absorption from the expected signals due to –CH=CH-CO- protons of chalcone **1** at δ 7.70 ppm. and 7.50 ppm. confirm the structure of **2**. In ^{13}C NMR spectra, **2** showed the signals due to -CHBr-CHBr-CO- carbons at δ 45.4 ppm. and 62.3 ppm., the shift in the absorption from the expected signals due to –CH=CH-CO- carbons of chalcone at δ 145.0 ppm. and 121.6 ppm. further supports the structure of 2, 3-dibromo-1,3-diphenylpropan-1-one. The absence of absorption signals at δ 5.64 ppm and 5.72 ppm. observed for **2**, in ^{1}H NMR spectra of **3** indicated its formation by the loss of 2HBr.

The physical and analytical data of the synthesised pyrazoles were summarised in table-1. Elemental analysis data observed were in good agreement with that of theoretically calculated values; a deviation of ±0.02% strongly favors the formation of the pyrazoles.

Table 1: Physical and analytical data of substituted pyrazoles obtained by 1,3-dipolar cycloaddition of *in situ* generated nitrile imines to alkynes



Entry	Ar =	Nature of the Product	Yield (%)	Molecular formula	Elemental analysis data (Observed)*		
					C (%)	H (%)	N (%)
1		Brown oil	74	$C_{28}H_{20}N_2O$	83.92	5.01	7.08
2		Brown oil	79	$C_{29}H_{22}N_2O_2$	80.81	5.23	6.62
3		Brown oil	71	$C_{30}H_{24}N_2O_3$	78.14	5.22	6.01
4		Brown oil	86	$C_{29}H_{22}N_2O$	84.10	5.28	6.71
5		Brown oil	72	$C_{28}H_{19}FN_2O$	80.25	4.51	6.75
6		Brown oil	78	$C_{28}H_{19}ClN_2O$	77.44	4.42	6.40
7		Brown oil	70	$C_{28}H_{19}N_3O_3$	75.41	4.40	9.40

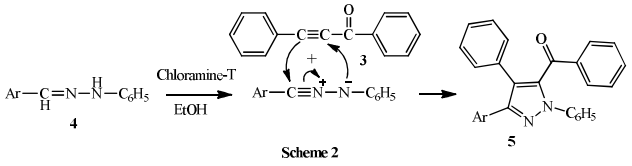
*The values are in good agreement with the theoritical values within a deviation of ±0.02%.

In ^{1}H NMR spectra, all the substituted pyrazoles **5** gave the signals due to aromatic and substituent protons in the expected region. In ^{13}C NMR spectra, all showed the signals due to aromatic and substituent carbons in the expected region. The consistent pattern signals observed for C_3-, C_4- and C_5-carbons of newly formed five membered pyrazole rings in the region δ 139-141 ppm, δ 128-129 ppm and δ 125-26 ppm respectively. All the substituted pyrazoles **5** gave MH^+ ion as base peak and significantly stable molecular ion peaks with a relative abundance ranging from 10-40%. The results of the spectral studies confirm the formation of the products **5**. The spectral data of the synthesised compounds were depicted in table-2.

Table 2: Characterization data of synthesised pyrazoles 5

Entry	^{1}H NMR (δ ppm)	^{13}C NMR (δ ppm)	MS (m/z)
1	7.15-7.80 (m, 20H, Aromatic-H).	124.9, 125.3, 126.0, 126.5, 127.6, 128.0, 128.5, 129.2, 129.6, 131.8, 134.0, 136.7, 138.7, 140.1, 169.8.	401 (MH^+, 100), 316, 301, 286, 271, 235.
2	3.86 (s, 3H, OCH_3), 7.06 (dd, 2H, Ar-H), 7.24 (dd, 2H, Ar-H), 7.32-7.78 (m, 15H, Aromatic-H).	55.3, 114.2, 124.6, 125.1, 126.0, 126.3, 127.0, 127.8, 128.0, 128.2, 128.6, 129.4, 131.0, 135.1, 136.4, 138.1, 140.2, 158.0, 170.0.	----
3	3.85 (s, 6H, OCH_3), 6.97-7.52 (m, 18H, Aromatic-H).	----	461 (MH^+, 100), 430, 400, 376, 361, 346, 331, 295.
4	2.22 (s, 3H, CH_3), 7.08 (dd, 2H, Ar-H), 7.20 (dd, 2H, Ar-H), 7.30-7.72 (m, 15H, Aromatic-H).	21.3, 125.3, 126.8, 127.3, 128.2, 128.2, 129.4, 130.1, 131.2, 132.6, 135.7, 136.2, 136.8, 138.9, 140.1, 170.6.	415 (MH^+, 100), 330, 315, 300, 285, 249.
5	7.28 (dd, 2H, Ar-H), 7.35-7.82 (m, 15H, Aromatic-H), 8.12 (dd, 2H, Ar-H).	----	419 (MH^+, 100), 334, 319, 304, 289, 253.
6	7.30 (dd, 2H, Ar-H), 7.35-7.82 (m, 15H, Aromatic-H), 7.96 (dd, 2H, Ar-H).	125.6, 126.0, 126.3, 127.2, 128.0, 128.6, 128.9, 129.4, 129.8, 131.8, 134.3, 136.4, 137.1, 138.9, 140.7, 170.1.	437 (MH^+, ^{37}Cl, 33%), 435 (MH^+, ^{35}Cl, 100%), 434, 350, 335, 320, 305, 269.
7	7.30-7.74 (m, 15H, Aromatic-H), 7.98 (dd, 2H, Ar-H), 8.24 (dd, 2H, Ar-H).	124.2, 125.6, 126.2), 126.6, 127.2, 128.1, 128.5, 128.8, 129.3, 129.7, 131.8, 136.8, 137.1, 138.9, 140.0, 146.8, 169.8.	-----

The mechanism for the formation of substituted pyrazoles via 1, 3-dipolar cycloaddition is depicted in scheme-2. In a reaction, the chloramine-T oxidizes aldehyde phenylhydrazones **4** to generate versatile intermediate nitrile imines which acts as dipole. The nitrile imines generated *in situ* undergo cycloaddition with alkyne function of 1, 3-diphenylprop-2-yn-1-one **3** to form substituted pyrazoles **5**.



Scheme 2

The results of antibacterial activity of the synthesized compounds **5** against different bacterial species were tabulated in table-3. The study revealed that all compounds exerted moderate to good antibacterial activity against the tested organisms, except 3-(4-nitrophenyl)-1,4-diphenyl-1*H*-pyrazol-5-yl)(phenyl)methanone the that contain a strong electron withdrawing nitro substituent failed to exhibit inhibition against all the organisms. 3-(4-Methoxyphenyl)-1, 4-diphenyl-1*H*-pyrazol-5-yl) (phenyl)methanone and 3-(4-chlorophenyl)-1, 4-diphenyl-1*H*-pyrazol-5-yl) (phenyl)methanone have exhibited excellent activity against all the tested organisms. 3-(4-methylphenyl)-1, 4-diphenyl-1*H*-pyrazol-5-yl) (phenyl) methanone found highly active against *E. coli* and *S. typhimurium* organisms but moderately active against *B. subtilis* and *S. aureus* species. Phenyl(1,3,4-triphenyl-1*H*-pyrazol-5-yl)methanone, 3-(4-fluorophenyl)-1,4-diphenyl-1*H*-pyrazol-5-yl)(phenyl)methanone and 3-(3,4-dimethoxyphenyl)-1,4-diphenyl-1*H*-pyrazol-5-yl)(phenyl)methanone exhibited moderate to good activity against the organisms tested.

The results of antifungal activity of the pyrazoles **5** against different fungal species were summarised in table-4. The study revealed that the compounds 3-(4-nitrophenyl)-1,4-diphenyl-1*H*-pyrazol-5-yl)(phenyl)methanone and 3-(3,4-dimethoxyphenyl)-1,4-diphenyl-1*H*-pyrazol-5-yl)(phenyl)methanone found inactive; 3-(4-methoxyphenyl)-1,4-diphenyl-1*H*-pyrazol-5-yl)(phenyl)methanone and 3-(4-chlorophenyl)-1,4-diphenyl-1*H*-pyrazol-5-yl)(phenyl)methanone are highly active against the tested organisms. 3-(4-Methylphenyl)-1, 4-diphenyl-1*H*-pyrazol-5-yl) (phenyl)methanone showed promising activity against *C. albicans* moderate against *A. niger, A. flavus* and *F. oxysporium* species. Phenyl(1, 3,4-triphenyl-1*H*-pyrazol-5-yl)methanone and 3-(4-fluorophenyl)-1,4-diphenyl-1*H*-pyrazol-5-yl) (phenyl) methanone have moderately active against the tested organisms.

Table 3: Zone of Inhibition of synthesised pyrazoles tested against bacteria species. (measured in mm)*

Entry	*E. coli*	*S. typhimurium*	*B. subtilis*	*S. aureus*
1	38	38	37	42
2	36	35	32	38
3	40	38	36	44
4	36	35	38	46
5	39	44	42	48
6	34	30	30	36
7	***	***	***	***
Std**	36	35	32	40

*results are expressed as a mean of the three determinations (n=3); **ciprofloxacin (25 µg/disc) was used as positive reference standard drug; ***no inhibition observed

Table 4: Zone of Inhibition of synthesised pyrazoles tested against fungal species. (measured in mm)*

Entry	*A. niger*	*A. flavus*	*C. albicans*	*F. oxysporium*
1	36	38	28	33
2	28	31	25	26
3	***	***	***	***
4	28	34	30	32
5	36	40	30	31
6	24	24	20	23
7	***	***	***	***
Std**	28	32	24	27

*the results are expressed as a mean of three determinations (n=3); **nystatin (25 µg/disc) was used as positive reference standard drug; *** no inhibition observed.

CONCLUSIONS

The easy and accessible procedure for the synthesis of tetrasubstituted pyrazoles and efficacy of some of the molecules as antimicrobial agents validates the significance of this study. Among the series of the compounds reported, 3-(4-methoxyphenyl)-1, 4-

diphenyl-1*H*-pyrazol-5-yl) (phenyl)methanone and 3-(4-chlorophenyl)-1, 4-diphenyl-1*H*-pyrazol-5-yl) (phenyl)methanone acts as potential antifungal and antibacterial agents.

ACKNOWLEDGEMENTS

The authors are grateful to NMR research center, SAIF, Madras for recording NMR spectra of the compounds reported.

REFERENCES

1. Umesha KB, Lokanatha Rai KM, Ajay Kumar K. A new approach to the synthesis of pyrazoles via 1,3-dipolar cycloaddition of nitrile imines with acetyl acetone. Indian J Chem 2002; 41B:1450-1453.
2. Dalloul HM. Heterocyclic synthesis using nitrile imines: part 14. Synthesis of new pyrazole derivatives. Turk J Chem 2010; 34:529-535.
3. Jin W, Yu H, Yu Z. Regioselective synthesis of multisubstituted pyrazoles via cyclo condensation of β-thioalkyl-α,β-unsaturated ketones with hydrazines. Tetrahedron Lett 2011; 52:5884-5887.
4. Y. Yang, C. Kuang, H. Jin, Q. Yang, Z. Zhang. Beilstein J Org Chem 2011; 7:1656-1658.
5. Fedele M, Franco C, Adriana B, Daniela S, Bruna B, Olivia B, Paola T, Bruno M, Stefano A, Andrea T. Inhibition of amine oxidases activity by 1-acetyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives. Bioorg Med Chem Lett 2002; 12:3629-3633.
6. Jayaroopa P, Vasanth Kumar G, Renuka N, Harish Nayaka MA, Ajay Kumar K. Evaluation of new pyrazole derivatives for their biological activity: Structure-activity relationship. Int J PharmTech Res 2013; 5(1):264-270.
7. Govindaraju M, Vasanth Kumar G, Pavithra G, Harish Nayaka MA, Mylarappa BN, Ajay Kumar K. Evaluation of new tetra substituted pyrazolines for their antimicrobial and antioxidant activity; Structure-activity relationship. IOSR J Pharm Biolog Sci 2012; 2(6): 30-34.
8. Shih S-R, Chu T-Y, Reddy GR, Tseng S-N, Chen H-L, Tang W-F, Wu M-S, Yeh J-Y, Chao Y-S, Hsu JTA, Hsieh H-P, Horng J-T. Pyrazole compound BPR1P0034 with potent and selective anti-influenza virus activity. J Biomed Sci 2010; 17(13):1-9.
9. Mathew A, Mary STL, Arun KT, Radha K. Design, synthesis and biological evaluation of pyrazole analogues of natural piperine. Hygeia J D Med 2011; 3(2):48-56.
10. Ajay Kumar K, Lokanatha Rai KM, Umesha KB. Synthesis and evaluation of antifungal and antibacterial activity of ethyl 3,5-diarylisoxazole-4-carboxylates. J Chem Res (S) 2001: 436-438.
11. Vasanth Kumar G, Govindaraju M, Renuka N, Bi Bi Ahmadi Khatoon, Mylarappa BN, Ajay Kumar K. Synthesis of 1,3,5-triaryl-4,6-dioxo-pyrrolo[3,4-*d*]-7,8-dihydropyrzoles and their antimicrobial and antioxidant activity. Rasayan J Chem 2012; 5(3): 338-342.
12. Ajay Kumar K, Lokanatha Rai KM, Vasanth Kumar G, Mylarappa BN. A facile route for the synthesis of ethyl *N*-aryl-2,6-dioxo-piperid-3-ene-4-carboxylates and their biological activity. Int J Pharm Pharm Sci 2012; 4 Suppl 4:564-568.