Journal of the Korean Chemical Society
2012, *Vol. 56, No. 2*
Printed in the Republic of Korea
http://dx.doi.org/10.5012/jkcs.2012.56.2.246

A Facile Greener Assisted Protocol for the Synthesis of Some New 4-aryl-(5-chloro-3-Methyl-1-phenyl-1H-Pyrazol-4-yl)-4,5-dihydroisoxazol-3-yl) Derivatives and their *in vitro* Antimicrobial Activity

Baseer M. Shaikh, Shankaraiah G. Konda†, Omprakash S Yemul, and Bhaskar S. Dawane*
School of Chemical Sciences, Swami Ramanand Teerth Marathwada University Nanded-431606 (M.S) India.
**E-mail: bhaskardawane@rediffmail.com*
†*Department of Chemistry, K. J. Somaiya College of Arts, Commerce and Science, Kopargaon-423601 (M.S) India*
(Received September 26, 2011; Accepted February 21, 2012)

ABSTRACT. An efficient access, single step and environmentally benign synthesis of a new series of pyrazole containing isoxazolines derivatives were prepared by the condensation of chalcones bearing pyrazole moiety with hydroxyl amine hydrochloride in basic condition by using polyethylene glycol-400 (PEG) as a greener reaction solvent. The advantages of the present methodology are mild reaction condition and avoidance of volatile organic solvent. Furthermore, these newly synthesized compounds were screened for their antimicrobial activity against various pathogens like *Escherichia coli* (MTCC 2939), Salmonella typhi (MTCC 98), *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441), *Aspergillus niger* (MTCC 281), *Aspergillus flavus* (MTCC 2501), *Penicillium chrsogenum* (MTCC 160) and *Fusarium moniliformae* (MTCC 156). Especially compound containing the hydroxyl group in C_2-position and presence of halo (I, Br and Cl) groups as substituents at C_3 and C_5 position on the benzene nucleus showed the higher activity. Furthermore, compounds bearing methyl groups in combination with I and Br which enhanced the activity.

Key words: PEG-400, Chalcones, Hydroxyl amine hydrochloride, Isoxazolines, Antimicrobial activity

INTRODUCTION

Emerging infection diseases and the increasing number of multi-drug resistant microbial pathogen still make the treatment of infection disease an important pressing global problem therefore a substantial research for the discovery and new class of antimicrobial agents is needed. Keeping in mind isoxazoline derivatives are useful as intermediates in the organic synthesis, are found to possess a wide important pharmacophore and privileged structure in medicinal chemistry. They possess fungicidal, antimicrobial, bactericidal and mutagenic activities. Isoxazoline possess various biological and pharmacological activities. In addition, they find application as dyestuffs, auxiliaries in fiber finishes, dropping dye in the electroluminescence device and in liquid crystalline mixture. Synthesis of novel isoxazoline derivative remains a main focus of medicinal chemist, due to their diverse pharmacological activity. Isoxazoline derivatives have been reported to possess antifungal[1,2] antibacterial,[3] anticonvulsant,[4] anti-inflammatory,[5] antiviral[6] and analgesic[7]activity. Much research has been carried out with the aim to finding therapeutic values of isoxazoline moiety since their discovery. A large number of substituted isoxazoline derivatives are prepared and tested for variety of biological activities. Such as antimicrobial activity[8,9] and hypolipemics.[10] J. T. Desai[11] *et al* has been reported the synthesis and antimicrobial activity of some new isoxazoline moiety. All the compounds have been screened for antibacterial activity against bacteria Gram +ve & Gram -ve antifungal activity. Y. Rajendra Prasad[12] *et al* has been reported the 3-(2"-hydroxy napthalen-1"-yl)-5-phenyl-2-isoxazolines and tested their antidepressant activity. Hae Suk Youn *et al.*[13] has been reported the synthesis and biological evaluation of isoxazoline. Amber L. Norman *et al.*[14] reported that 3,5-disubstitued D^2-isoxazolines can be prepared from the corresponding α,β-unsaturated ketone by treatment with hydroxyl amine hydrochloride and sodium hydroxide.

These finding prompted us to synthesize the substituted isoxazoline derivatives containing antimicrobial active profile like pyrazole nucleus enhances the activity of parent compound by using greener reaction solvent i.e. polyethylene glycol-400 (PEG). One of the key areas of green chemistry is the replacement of the hazardous solvent as with environmentally benign solvents like polyethylene glycol-400 (PEG). Recently, liquid polymers or low melting polymers like PEG's have emerged as alternative green reaction media with unique properties such as thermal sta-

bility, commercial availability, non-volatility, immiscibility with a number of organic solvents and recyclability. PEGs are preferred over other polymers because they are inexpensive, completely non-halogenated, easily degradable and of low toxicity.[15] Many organic reactions have been carried out using PEGs as solvent or co-solvent such as Heck reaction,[16] asymmetric dihydroxylation,[17] Suzuki cross-coupling reaction[18] oxy-dehydrogenation of alcohols and cyclic dienes, oxidation of sulfides and the Wacker reaction[19] and partial reduction reaction of alkynes.[20]

MATERIAL AND METHOD

Melting points were uncorrected and determined in an open capillary tube. IR spectra were recorded on FTIR Shimadzu spectrometer. ^{1}H NMR spectra were recorded in DMSO-d_6 on Avance 300 MHz spectrometer using TMS as an internal standard. The mass spectra were recorded on EI-Shimadzu-GC-MS spectrometer. Elemental analyses were performed on a Carlo Erba 106 Perkin-Elmer model 240 analyzer.

Synthesis of 4-Chloro-3-(5-(5-Chloro-3-Methyl-1-Phenyl-1H-Pyrazol-4-yl)-4,5-Dihydroisoxazol-3-yl)-5-Iodophenol (12b)

A mixture (5-chloro-2-hydroxy-3-iodophenyl)-3-(5-chloro-3-Methyl-1-phenyl-1H-Pyrazol-4-yl)-prop-2-en-1-one (0.001 mmol) and hydroxyl amine hydrochloride (0.0015 mmol) in PEG-400 (15 mL) was heated at 80 ºC for 2-3 hours as shown in *Scheme* 1. After completion of reaction (monitored by TLC), the reaction mixture was extracted with ethyl acetate (2×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The obtained product was recrystallized by aqueous acetic acid to give pure product.

Spectral Data of Selected Compounds

4-chloro-2-(5-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydroisoxazol-3-yl)phenol (2a):

IR (KBr): 3374, 2956, 1595, 1456, 820,710, 719 cm^{-1}. ^{1}H NMR (DMSO-d_6), δ 1.4 (s, 3H, CH_3), 3.52 (d, 2H, CH_2), 4.10 (m, 1H, CH), 7.4-8.00 (m, 8H, Ar-H), 12.3 (s, 1H, OH) ppm. Mass (m/z), 388 (M^+ ion), Anal. Calcd for $C_{19}H_{15}Cl_2N_3O_2$: C, 58.28; H, 3.89; N, 10.8; Found: C, 50.15; H, 3.70; N, 10.0%.

4-chloro-2-(5-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydroisoxazol-3-yl)-6-iodophenol (2b):

IR (KBr): 3374, 2956, 1595, 1456, 820, 710, 719 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.4 (s, 3H, CH_3), 3.52 (d, 2H, CH_2),4.10 (m, 1H, CH), 7.4-8.00 (m, 7H, Ar-H), 12.3 (s, 1H, OH) ppm. Mass (m/z), 514 (M^+ ion), Anal. Calcd for $C_{19}H_{14}Cl_2IN_3O_2$: C, 44.39; H, 2.74; N, 8.17; Found: C, 44.29; H, 2.70; N, 8.11%.

2,4-dichloro-6-(5-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydroisoxazol-3-yl)phenol (2d):

IR (KBr): 3374, 2956, 1595, 1456, 820,710, 719 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.4 (s, 3H, CH_3), 3.52 (d, 2H, CH_2), 4.10 (m, 1H, CH), 7.4-8.00 (m, 7H, Ar-H), 12.3 (s, 1H, OH) ppm. Mass (m/z), 423 (M^+ ion), Anal. Calcd for $C_{19}H_{14}Cl_3N_3O_2$: C, 55.99; H, 3.34; N, 9.94; Found: C, 55.89; H, 3.30; N, 9.88%.

4-chloro-2-(5-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydroisoxazol-3-yl)-5-methylphenol(2e):

IR (KBr): 3374, 2956, 1595, 1456, 820, 710, 719 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.4 (s, 3H, CH_3), 1.4 (s, 3H, CH_3), 3.52 (d, 2H, CH_2), 4.10 (m, 1H, CH), 7.4-8.00 (m, 7H, Ar-H), 12.3 (s, 1H, OH) ppm. Mass (m/z), 402 (M^+ ion), Anal. Calcd for $C_{20}H_{17}Cl_2N_3O_2$: C, 59.71; H, 3.34; N, 10.45; Found: C, 59.61; H, 3.30; N, 10.23%.

4-chloro-6-(5-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydroisoxazol-3-yl)-2-iodo-3-methylphenol (2f):

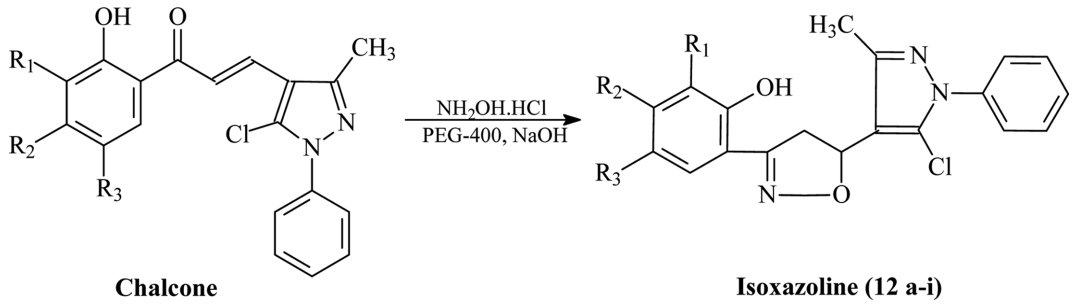
IR (KBr): 3354, 2948, 1595, 1456, 820,710, 719 cm^{-1}. ^{1}H NMR (DMSO-d_6), δ 1.4 (s, 3H, CH_3), 1.4 (s, 3H, CH_3), 3.52 (d, 2H, CH_2), 4.10 (m, 1H, CH), 7.4-8.00 (m, 6H, Ar-H), 12.3 (s, 1H, OH) ppm. Mass (m/z), 528 (M^+ ion), Anal. Calcd for $C_{20}H_{16}Cl_2IN_3O_2$: C, 45.48; H, 3.05; N, 7.96; Found: C, 45.33; H, 3.00; N, 7.75%.

2-(5-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydroisoxazol-3-yl)-4-methylphenol (2h):

IR (KBr): 3370, 2946, 1575, 1455, 820, 710, 729 cm^{-1}. ^{1}H NMR (DMSO-d_6), δ 1.4 (s, 3H, CH_3), 1.4 (s, 3H, CH_3), 3.52 (d, 2H, CH_2), 4.10 (m, 1H, CH), 7.4-8.00 (m, 6H, Ar-H),12.3 (s, 1H, OH) ppm. Mass (m/z), 368 (M^+ ion), Anal. Calcd for $C_{20}H_{18}ClN_3O_2$: C, 65.31; H, 4.93; N, 11.42; Found: C, 65.22; H, 4.88; N, 11.40%.

RESULT AND DISCUSSION

In continuation of our work on the synthesis of some new bioactive heterocyclic compounds,[21-23] herein we report new series of pyrazole containing isoxazolines derivatives was described here by the condensation of chalcones with



Chalcone **Isoxazoline (12 a-i)**

***Scheme* 1.** Synthesis of pyrazole containing isoxazoline derivatives.

hydroxyl amine hydrochloride in basic condition by using polyethylene glycol-400 (PEG) as a green reaction solvent. The starting chalcones are prepared by the reported method.[24] Initially, we attempted the condensation of (5-chloro-2-hydroxy-3-iodophenyl)-3-(5-chloro-3-methyl-1-phenyl-1H-Pyrazol-4-yl)-prop-2-en-1-one (0.001 mmol) with hydroxyl amine hydrochloride (0.0015 mmol) in PEG-400 (15 mL) as reaction solvent. The reaction went to completion and corresponding product (**2a**) was obtained in 90% yield and rest of the product obtained is summarized in *Table* 1.

The antimicrobial activities of the synthesized compounds were determined by agar well diffusion method.[25] The compounds were evaluated for antibacterial activity against *Escherichia coli* (MTCC 2939), *Salmonella typhi* (MTCC 98), *Staphylococcus aureus* (MTCC 96), and *Bacillus subtilis* (MTCC 441). The antifungal activity was evaluated against *Aspergillus niger* (MTCC 281), *Aspergillus flavus* (MTCC 2501), *Penicillium chrsogenum* (MTCC 160) and *Fusarium moniliformae* (MTCC 156), and were procured from Institute of Microbial technology (IMTech), Chandigarh, India. The antibiotic penicillin (25 μg/mL) was used as reference drug for both antibacterial and Nystatin was used as reference drug for antifungal activity. Dimethyl sulphoxide (1%, DMSO) was used a control with out compound.

The culture strains of bacteria were maintained on nutrient agar slant at 37±0.5 °C for 24 h. The antibacterial activity was evaluated using nutrient agar plate seeded with 0.1 mL of respective bacterial culture strain suspension prepared in sterile saline (0.85%) of 10^5 CFU/mL dilutions. The wells of 6 mm diameter were filled with 0.1 mL of compound solution at fixed concentration 25 μg/mL separately for each bacterial strain. All the plates were incubated at 37±0.5 °C for 24 h. Zone of inhibition of compounds in mm were noted.

For antifungal activity, all the culture strains of fungi maintained on potato dextrose agar (PDA) slant at 27±0.2 °C for 24-48 hr till sporulation. Spore of strains were transferred in to 5 mL of sterile distilled water containing 1% Tween-80 (to suspend the spore properly). The spores were counted by haemocytometer (10^6 CFU/mL). Sterile PDA plate was prepared containing 2% agar; 0.1 mL of each fungal spore suspension was spread on each plate and incubated at 27±0.2 °C for 12 hrs. After incubation well prepared using sterile cork borer and each agar well was

***Table* 1.** Physico-chemical data synthesized isoxazoline derivatives

Entry	Product	R_1	R_2	R_3	Yield %	M.P. (°C)
1	2a	H	H	Cl	85	111
2	2b	I	H	Cl	84	118
3	2c	Br	H	Cl	90	149
4	2d	Cl	H	Cl	92	138
5	2e	H	CH_3	Cl	88	128
6	2f	I	CH_3	Cl	84	150
7	2g	Br	CH_3	Cl	90	170
8	2h	H	H	CH_3	82	194
9	2 i	H	H	H	86	135
10	2j	Br	H	CH_3	92	138
11	2k	I	H	I	88	128
12	2l	Br	H	Br	84	150

***Table* 2.** Antimicrobial activity of synthesized isoxazolines (**2a-l**)

Product	Bacteria				Fungi			
	Ec	St	Sa	Bs	An	Af	Pc	Fm
2a	16	10	09	11	18	16	14	19
2b	10	16	18	17	09	12	15	11
2c	11	17	15	17	12	10	14	20
2d	14	16	17	9	18	16	12	18
2e	08	10	11	13	17	15	14	17
2f	16	18	14	16	10	-	13	13
2g	16	20	18	14	11	10	12	14
2h	12	-	13	10	09	-	10	11
2i	10	08	09	10	17	15	15	18
2j	16	11	08	14	18	11	13	08
2k	12	14	16	12	17	17	16	11
2l	10	18	16	14	13	17	14	14
Penicillin	22	22	24	24	NA	NA	NA	NA
Nystatin	NA	NA	NA	NA	20	22	24	24

Zone of inhibition is expressed in mm. Ec-Escherichia coli, An-Aspergillus niger, St-Salmonella typhi, Af-Aspergillus flavus, Sa-Staphylococcus aureus, Fm-Fusarium moniliformae, Bs-Bacillus subtillis, Pc-Penicillium chrysogenum, -No activity, NA-Not Applicable

filled with 0.1 mL of compound solution at fixed concentration 25 μg/mL. The plates were kept in refrigerator for 20 minutes for diffusion and then incubated at 27±0.2 ºC for 24-28 hrs. After incubation, zone of inhibition of compounds were measured in mm along with standard.

The results of in vitro antibacterial activities of compounds (**2a-l**) against various bacterial strains are summarized in *Table* 2. It has been observed that some of compounds exhibited interesting antibacterial activities. Compounds **2a**, **2d**, **2f**, **2g**, and **2j** showed effective activity against *E. coli*, and compounds **2c**, **2f**, **2g**, and **2l** were displayed a good zone of inhibition against *S. typhi*. Compounds **2b**, **2d**, **2g** and **2k** showed good activity for *S. aureus* and compounds **2c** and **2f** effective activity against *B. subtilis*. Compounds **2e**, **2h**, **2i** were displayed less active against all tested bacteria. The results of antifungal activities of synthesized compounds **(2a-l)** were summarized in *Table* 2. Most of the compounds were showed a significant level of activity in comparison with standard antifungal. Compounds **2a**, **2d**, **2e**, **2i**, were showed a good inhibitory activity against *A. niger* and Compounds **2a**, **2j** and **2k** were showed good activity against *A. flavus* and also compounds **2d**, **2e** shows moderate to good activity. Also the compounds **2a**, **2b**, **2c**, **2e**, and **2i** show good to moderate activity against P. chrysogenum. Compounds **2a**, **2c**, **2d** and **2e** were active against *F. moniliformae.*

CONCLUSION

In summary, we have designed and synthesized some new pyrazole containing isoxazolines derivatives by the condensation of chalcones with hydroxyl amine hydrochloride in basic condition by using polyethylene glycol-400 (PEG) as a green reaction solvent. The preliminary *in vitro* antimicrobial screening of this series revealed that, compounds showed potent activity. Therefore, the present study is useful for finding the new drugs in medicinal investigation against bacterial and fungal diseases.

Acknowledgments. One of the author BSD gratefully acknowledge to UGC-New Delhi, for Post Doctoral research award. (F. 30-1/2009, SA-II). The authors are grateful to Principal Yeshwant Mahavidyalaya for providing laboratory facility and the Director, IICT, Hyderabad for providing necessary instrumental facilities.

REFERENCES

1. Mizabuchis, Satoy. *Agri. Bio. Chem*. **1984**, *48*, 2771.
2. Bhakunin, D. S.; Chaturvedi, R. *J. Nat. Prod*. **1984**, *47*, 585.
3. Vittorio, F.; Ronsisvalle, G.; Pappalardo, M. S.; Blandino, G. *Chem. Abstr*. **1985**, *103*, 19721.
4. Lapage, Hublot, B. *Chem. Abstr*. **1996**, *113*, 211964.
5. Shivkumar, B.; Nargund, L. V. G. *Indian J. Heterocyclic Chem*. **1998**, *8*, 27.
6. Simmonds, M. S.; Blaney, W. M.; Monuche F. D.; Bettollo, M. *J. Chem. Ecol*. **1996**, *16*, 365.
7. Nagano, M.; Sakai, J.; Mizukai, M.; Nakamura, N.; Misaka, E.; Kobayashi S.; Tomita, K.; Kokai, I. *Chem. Abstr*. **1979**, *92*, 41922.

8. Varma, B. L. *Indian. J. Heterocyclic Chem.* **2003**, *13*, 111.
9. Nagar, D. N.; Shah, V. H. *Indian. J. Heterocyclic Chem.* **2003**, *13*, 173.
10. Natale, N. R.; Rogers, M. E.; Stoples, R. T. *J. Med. Chem*. **1999**, *42*, 3087.
11. Desai, J. T.; Desai, C. K.; Desai, K. R. *J. Iran. Chem. Soc.* **2008**, *5*(1), 67.
12. Rajendra Prasad, Y.; Ravi Kumar, P.; Ramesh B. *Int. J. Chem. Sci*. **2007**, *5*(2), 542.
13. Youn, H. S.; Lee, E. J.; Lee, J. E.; Park, W. K. *Bull. Korean Chem. Soc.* **2009**, *30*(8), 1873.
14. Norman, A. L.; Shurrush, K. A.; Calleroz, A. T.; Mosher, M. D. *Tetrahedron Lett.* **2007**, *48*(39), 6849.
15. Chandrasekhar, S.; Narsihmulu, Ch.; Sultana, S. S.; Reddy, N. R. K. *Org. Lett.* **2002**, *4*, 4399.
16. (a) Chandrasekhar, S.; Narsihmulu, Ch.; Sultana, S. S.; Reddy, N. R. K. *Chem. Commun.* **2003**, *1*, 716. (b) Jiang, R. Y.; Kuang, Q.; Sun, X.-L.; Zhang, S. Y. *Tetrahedron: Asymmetry* **2004**, *15*, 743.
17. Namboodiri, V. V.; Verma, R. S. *Green Chemistry* **2001**, *3*, 146.
18. Haimov, A.; Neumann, R. *Chem. Commun,* **2002**, *14*, 876.
19. Chandrasekhar, S.; Narsihmulu, Ch.; Chandrashekar, G.; Shyamsunder, T. *Tetrahedron Lett.* **2004**, *45*, 2421.
20. Perrier, S.; Gemini, H.; Li, S. *Chem. Commu*. **2004**, *45*, 604.
21. Dawane, B. S.; Konda, S. G.; Shaikh, B. M.; Bhosale, R. B. *Acta Pharm*. **2009**, *59*, 473.
22. Dawane, B. S.; Konda, S. G.; Shaikh, B. M.; Mandawad, G. G. *Eur. J. Med. Chem.* **2010**, *45*, 387.
23. Dawane, B. S.; Shaikh, B. M.; Khandare, N. T. *Green Chemistry Letters and Review* **2010**, *3*(3), 205.
24. Dawane, B. S.; Konda, S. G.; Shaikh, B. M.; Mandawad, G. G. *Asian J. Res. Chem.* **2010**, *13*(1), 90.
25. Shrinivasan, D.; Sangeetha, N.; Suresh, T.; Lakshman, P. *J. Ethnophrmacol* **2001**, *74*, 217.