Synthesis of Imine Derivatives Bearing Pyrazole Moiety and Evaluation of Their Biological Activities

S. M. THAKUR[1*], Dr.V.J.MEDHANE[2], Dr.G.R.JADHAV[3], Dr.K.A.MAHALE[3], Dr.P.B.AWARE[3]
[1*]Department of Chemistry, K.S.K.W. College (Affiliated to Savitribai Phule Pune University, Pune), CIDCO, Nashik-422008, India
2Department of Chemistry, S.V.K.T. College (Affiliated to Savitribai Phule Pune University, Pune), Deolali Camp, Nashik-422401, India
3Chemistry Research Centre, P.G. Department of Chemistry, K.T.H.M. College (Affiliated to Savitribai Phule Pune University, Pune), Gangapur Road, Nashik-422002, India
Tel : 9850599650, email : kskwsonali@gmail.com

Abstract
Literature survey disclose that the imine derivatives show pharmacokinetics or pharmacological importance. Thus, by targeting to design new biological activity, a new series of imine derivative (1-A-L) having pyrazole moiety has been synthesized. Chemical structure of the newly synthesized imine derivatives has been confirmed by IR, NMR, and Mass spectroscopy. All newly synthesized compounds were subjected to biological activity by estimating zone of inhibition towards Gram- positive bacteria like *staphylococcus aureus* ATCC 25923, Gram-negative bacteria like *E-Coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853 and *Candida Species*.

Keywords: Imine, Pyrazole, Anti-microbial activity, Antibacterial activity.

Introduction As defined by Schiff, “Bases are called imines that contain an aryl group bound to the N or to the C atom”

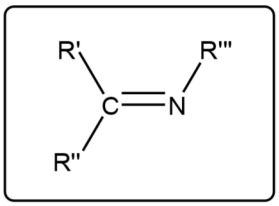

Fig- 1

R',R",R'"=Aryl or alkyl
General structure of imine

To define imine structurally, it is a similar with nitrogen of aldehyde and ketone functional group from that the carbonyl group are exchanged by an imine or azomethines group. Imines are most extensively utilized organic compounds. They have more utilizations in finding aldehydes and ketones functional group, regeneration of carbonyl and amino compounds, while defensing carbonyl and ketone during their reactions. Aromatic aldehydes of imines are most strong with an active conjugation system, while aliphatic aldehydes of imines are weak and undergoes polymerization. In the development of chemistry science the carbon nitrogen double bond chemistry plays an essential role. The mechanism of imine reaction involved in two steps. In first step, the primary amine acts as nucleophile and that attack on carbonyl carbon gives hydroxyl compound after dehydration gives imine. The second step there is a removal of water molecule from reaction mixture. It is rate dependent step. In azeotropic distillation there is a condensation between carbonyl compound and amine this classical synthesis reported by Schiff. For completely dehydration from the system molecular sieves are used. In 1990s for water elimination in situ method was developed, in this method using different dehydrating solvent like tetramethyl orthosilicate or trimethy orthoformate. By refluxing the reaction mixture of aldehydes or ketone with amine in organic medium and also carried out with acid catalyzed gives the formation of imine. The capability of these method is based on carbonyl compound (strongly electrophilic) and amines (strongly nucleophilic) in 2004 demonstrated by chakraborti et.al. They suggested the different substances which work as Bronsted- lowery or lewis acids to initiate the

activity of the carbonyl group of aldehydes, initiate nucleophile attack by amines, apart from that dehydration too eliminate the H_2O molecule from the last stage. In imines synthetization examples of Bronsted- lowery or lewis acids used [9-20]. In last twelve years the techniques like [21-29] are reported using novel ideas. Out of these novel ideas microwave irradiation technique is used because of its simple operation, which increased reaction rates and selectivity. For the non-dependent studies of Rousell and majetich class the use of microwave irradiation begins. From the other different methods microwave irradiation is more eco-friendly as the technique stopped the immensurable utility of aromatic compounds and the Dean –Strak apparatus for azeotropic dehydration. When the compound has R = H it is called as Primary imine on the other hand if R is hrdrocarbyl, it is called as Secondary imine. Imines show variety of reactivity. Chemistry is encompast with imine which are very commonly studied[1]. In the structure no.1 when R_3 is replaced with OH then it is called as oxime. Where as if it is replaced with NH_2 it is named as hydrazone. When carbon in primary imine gets attached to hydrocarbyl and hydrogen is known as primary aldimine, while if it is secondary imine in these groups it is called as secondary aldimine [2]. When carbon in primary imine gets attached to two hydrocarbyls is known as primary ketamine, while if it is attached with secondary imine the group is known as a secondary ketamine [3]. Aldimines are named by the removal of final "e" from the radical and later addition of "imine" to it. Example- Methanimine. Apart from this the derivative of carbonyl is called as imine. As imine, when the word imine is added to the carbonyl compound which falls under oxo group and has been changed by an imino group, like Sydnone as well as acetone imine. When a class of imines that has sulfinyl group which is combined with N it turns to N-sulfinyl imines.

Experimental

Material and Methods: All chemicals and solvents, reagents used in the present study were of analytical grade and solvents were used after distillation. All the melting points of the synthesized compounds were determined by open capillary and are uncorrected. The purity of the compounds was checked using precoated TLC plates (MERCK) using n-hexane: ethyl acetate (8:2) solvent system. The developed chromatographic plates were visualized under UV cabinet. IR spectra were recorded using KBr.[1]HNMR spectra in DMSO on a BRUKER FTNMR Instrument using TMS as internal standard and chemical shift values were expressed in ppm.

General procedure for the synthesis of imines:

A solution of substituted aromatic aldehydes (0.01M in 5 ml ethanol) was taken in a flask and amine derivative (0.01M: 5 ml ethanol) was slowly added with continuous stirring, the content of the flask were refluxed for four hours (TLC checked) and left over night in ice bath. The imine separated out, collected and further purified by recrystallization from ethanol.

(E)-N-(4-chlorobenzylidine) 3-4-chlorophenyl) 1H-pyrazol 5 amine (1A)

A solution of substituted aromatic aldehydes (0.01M in 5 ml ethanol) was taken in a flask and amine derivative (0.01M: 5 ml ethanol) was slowly added with continuous stirring, The content of the flask were refluxed for four hours (TLC checked) and left over night in ice bath. The imine separated out, collected and further purified by recrystallization from ethanol.

Yield (2.19g,77%), m.p.: 189 °C; IR(KBr): cm-1= 3195,1675,1657,1092;1HNMR(DMSO): δ=7.33(dd; 2H, J=7-8Hz, Ar-H), δ=7.42(dd; 2H, J=7Hz, Ar-H), δ=7.58(dd; 2H, J=8Hz, Ar-H) δ=7.89(dd; 2H, J=8Hz, Ar-H) 8.44(s; 1H, Ar-H), 8.72(s; 1H, CH) 11.99(s; 1H, NH) ppm.

(E)-N-(4-chlorobenzylidine) 3-4-bromophenyl) 1H-pyrazol 5 amine (1B)

Yield (2.98g,87%), m.p.:200°C; IR(KBr): cm-1=3184,1645,1640,1072.;1HNMR(DMSO): δ=7.72(dd; 2H, J=7Hz, Ar-H), δ=7.76(dd; 2H, J=7Hz, Ar-H), δ=7.58(dd; 2H, J=8Hz, Ar-H) δ=7.89(dd; 2H, J=8Hz, Ar-H), 8.44(s; 1H,Ar-H), 8.72(s; 1H, CH) 11.99(s; 1H, NH) ppm.

(E)-N-(4-chlorobenzylidine) 3-P-tolyl-1H-pyrazol 5 amine (1C)

Yield (2.03g,70%), m.p.:245°C; IR(KBr): cm-1=3100, 1615, 1600, 1055, ;1HNMR(DMSO): δ= 7.12(dd; 2H, J=7Hz, Ar-H), δ=7.36(dd; 2H, J=7Hz, Ar-H), δ=7.58(dd; 2H, J=8Hz, Ar-H) δ=7.89(dd; 2H, J=8Hz, Ar-H), 8.44(s; 1H, Ar-H), 8.72(s; 1H, CH) 11.99(s; 1H, NH),2.35(dd;3H,CH_3)ppm.

4-((E)-(3-(4-chlorophenyl)1H-pyrazol-5-ylimino)methyl)phenol (1D)

Yield (2.05g,74%), m.p.: 216 °C; IR(KBr): cm-1=3500, 3300, 1665, 1620,1014.;1HNMR(DMSO): 7.33(dd; 2H, J=7Hz, Ar-H), δ=7.42(dd; 2H, J=7Hz, Ar-H), δ=7.56(dd; 2H, J=8.1Hz, Ar-H) δ=6.84(dd; 2H, J=8.1Hz, Ar-H), 8.28(s; 1H, Ar-H), 9.95(s; 1H, CH),11.71(s; 1H, NH) ,7.77(s ;OH)ppm.

4-((E)-(3-(4-bromophenyl) 1H-pyrazol-5-ylimino) methyl) phenol (1E)
Yield (2.32g,75%), m.p.:236°C; IR(KBr): cm-1=3298, 3184, 1662, 1602, 1051.;1HNMR(DMSO): 7.72(dd; 2H, J=8Hz, Ar-H), δ=7.86(dd; 2H, J=8Hz, Ar-H), δ=7.56(dd; 2H, J=8.1Hz, Ar-H) δ=6.84(dd; 2H, J=8.1Hz, Ar-H), 8.28(s; 1H, Ar-H), 9.95(s; 1H, CH) 11.71(s; 1H, NH),7.77(s;OH) ppm.

4-((E)-3-P-tolyl-1H-pyrazol-5-ylimino) methyl) phenol (1F)
Yield (2.03g,70%), m.p.:260°C; IR(KBr): cm-1=3200, 3140, 1605, 1600, 1020.;1HNMR(DMSO): 7.12(dd; 2H, J=8Hz, Ar-H), δ=7.36(dd; 2H, J=8Hz, Ar-H), δ=7.56(dd; 2H, J=8.1Hz, Ar-H) δ=6.84(dd; 2H, J=8.1Hz, Ar-H), 8.28(s; 1H, Ar-H), 9.95(s; 1H, CH) 11.71(s; 1H, NH),7.77(s;OH),2.35(dd;3H,CH_3) ppm.

(E)-3-(4-chlorophenyl)-N-(furan-2-yl) methylene)-1H-pyrazol 5 amine (1G)
Yield (2.76g,72%), m.p.: 200 °C; IR(KBr): cm-1=3505, 2980, 1660, 1645,890.;1HNMR(DMSO): 7.33(dd; 2H, J=8.5Hz, Ar-H), δ=7.42(dd; 2H, J=8.5Hz, Ar-H), δ=6.65(dd; 1H, J=1.5 & 1.5Hz, Ar-H) δ=6.95(dd; 1H, J=1.5Hz, Ar-H), 7.84(d; 1H, J=1.5Hz), 7.86(s; 1H, Ar-H), 8.33(s; 1H, CH), 11.86 (s; 1H, NH)ppm.

(E)-3-(4-bromophenyl)-N-(furan-2-yl) methylene)-1H-pyrazol 5 amine (1H)
Yield (2.19g,77%), m.p.:212°C; IR(KBr): cm-1=3485, 2900, 1640, 1620, 875.;1HNMR(DMSO): 7.70(dd; 2H, J=8.5Hz, Ar-H), δ=7.80(dd; 2H, J=8.5Hz, Ar-H), δ=6.65(dd; 1H, J=1.5 & 1.5Hz, Ar-H) δ=6.95(dd; 1H, J=1.5Hz, Ar-H), 7.84(d; 1H, J=1.5Hz), 7.86(s; 1H, Ar-H), 8.33(s; 1H, CH), 11.86 (s; 1H, NH)ppm.

(E)-N-(furan-2-yl) methylene)-3-P-tolyl-1H-pyrazol 5 amine (1I)
Yield (2.36g,69%), m.p.:235°C; IR(KBr): cm-1=3400, 2890, 1620, 1600, 860.;1HNMR(DMSO): 7.12(dd; 2H, J=8.5Hz, Ar-H), δ=7.36(dd; 2H, J=8.5Hz, Ar-H), δ=6.65(dd; 1H, J=1.5 & 1.5Hz, Ar-H) δ=6.95(dd; 1H, J=1.5Hz, Ar-H), 7.84(d; 1H, J=1.5Hz), 7.86(s; 1H, Ar-H), 8.33(s; 1H, CH), 11.86 (s; 1H, NH)ppm.

2-((E)-3(4-chlorophenyl)-1H-pyrazol-5-ylimino) methyl) phenol (1J)
Yield (2.42g,69%), m.p.: 221 °C; IR(KBr): cm-1=3600, 3540, 3150, 1680,1645.;1HNMR(DMSO): 7.64(dd; 2H, J=8.5Hz, Ar-H), δ=7.97(dd; 2H, J=8.5Hz, Ar-H), δ=6.92(dd; 1H, J=8 & 1.5Hz, Ar-H), δ=6.98(dd; 1H, J=8 & 1.5Hz, Ar-H) δ=7.56(dd; 1H, J=1.5Hz & 1.5 Hz Ar-H), 7.31(m; 1H), 6.94(s;OH,),8.64(s; 1H, CH), 11.21(s;1H,Ar-H),12.16(s; 1H, NH) ppm.

2-((E)-3(4-bromophenyl)-1H-pyrazol-5-ylimino) methyl) phenol (1K)
Yield (2.03g,70%), m.p.:238°C; IR(KBr): cm-1=3580, 3500, 3100, 1640, 1620.;1HNMR(DMSO): 7.49(dd; 2H, J=8.5Hz, Ar-H), δ=7.37(dd; 2H, J=8.5Hz, Ar-H), δ=6.92(dd; 1H, J=8 & 1.5Hz, Ar-H), δ=6.98(dd; 1H, J=8 & 1.5Hz, Ar-H) δ=7.56(dd; 1H, J=1.5Hz & 1.5 Hz Ar-H), 7.31(m; 1H), 6.94(s;OH,),8.64(s; 1H, CH), 11.21(s;1H,Ar-H),12.16(s; 1H, NH) ppm.

2-((E)-(3-P-tolyl-1H-pyrazol-5-ylimino) methyl) phenol (1L)
Yield (2.42g,69%), m.p.:255°C; IR(KBr): cm-1=3490, 3500, 3100, 1615, 1600.;1HNMR(DMSO): 7.12(dd; 2H, J=8.5Hz, Ar-H), δ=7.36(dd; 2H, J=8.5Hz, Ar-H), δ=6.92(dd; 1H, J=8 & 1.5Hz, Ar-H), δ=6.98(dd; 1H, J=8 & 1.5Hz, Ar-H) δ=7.56(dd; 1H, J=1.5Hz & 1.5 Hz Ar-H), 7.31(m; 1H), 6.94(s;OH,),8.64(s; 1H, CH), 11.21(s;1H,Ar-H),12.16(s; 1H, NH) ppm.

Biological Activity- The newly synthesized imines derivatives (1A-L) has been exhibit for their biological activity against Gram positive bacteria like *staphylococcus aureus* ATCC 25923, Gram-negative bacteria like *E-Coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853 and *Candida Species*. The bacterial suspensions were spread
over nutrient agar plates. The compounds were tested at concentration 25 µg/mL in DMSO.Nystatin was used as standard to evaluate the potency of the tested compounds in DMSO under the same conditions. The zone of inhibition in mm were compared after 24 h of incubation at 37 °C and measured as per Chemical

Laboratory Standards. Nystatin was used as a reference drug and the obtained results were expressed in terms of zone of inhibition (mm) values.

Results and Discussion The following table show biological activities of imine and its derivatives. The antimicrobial screening results presented on above table no 1 reveals that compounds 1A,1C,1D,1F,1I,1L exhibited poor activity against E-Coli and they have shown moderate activity against Pseudomonas aeruginosa and Staphylococcus and the compound 1B exhibited good activity against E-Coli and moderate activity against Pseudomonas aeruginosa and Staphylococcus and compound 1H exhibited good activity against E-Coli and Pseudomonas aeruginosa and moderate activity against Staphylococcus and compound 1E,1G,1J and 1K exhibited moderate activity against E-Coli , Pseudomonas aeruginosa and Staphylococcus. The same compound also screened for the anti-fungal activity against Candida the compound 1A,1D,1G exhibited good activity and the compound 1B,1C,1E,1F,1J,1K,1L showed moderate activity and the compound 6h,6i showed poor activity.

Table 1: Biological activities of imines and its derivatives

Compound	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 25923	*Candida sp*
1A	**08mm**	**25mm**	**20mm**	**22mm**
1B	**15 mm**	**22mm**	**22mm**	**18 mm**
1C	**07 mm**	**20mm**	**18 mm**	**17mm**
1D	**10 mm**	**22mm**	**17 mm**	**22 mm**
1E	**13mm**	**27mm**	**18 mm**	**17 mm**
1F	**08mm**	**21mm**	**17 mm**	**17 mm**
1G	**14mm**	**22mm**	**20mm**	**22mm**
1H	**15mm**	**25mm**	**22 mm**	**14 mm**
1I	**08mm**	**20 mm**	**17mm**	**13mm**
1J	**13mm**	**22mm**	**22mm**	**20mm**
1K	**10mm**	**24mm**	**20mm**	**18mm**
1L	**08mm**	**20mm**	**18mm**	**17mm**
Gentamicin	18 mm	33 mm	33mm	-----
Nystatin	-----	-----	-----	24 mm

Conclusions In conclusion, a new series of (E)-N-(4-chlorobenzylidine) 3-4-chlorophenyl)1H-pyrazol5amine(**1A-1C and 1G-1I**) and 4-((E)-(3-(4-chlorophenyl)1H-pyrazol-5-ylimino)methyl)phenol (**1D-1F and (1J-1L**)

Derivatives were synthesized. The synthesized derivatives has been further evaluated for their antimicrobial and antifungal activity for the first time. As a result, some of the derivatives exhibited good antimicrobial and antifungal activity in comparison with used reference drug. Among the synthesized compounds 1A,1C,1D,1F,1I,1L exhibited comparable antimicrobial activity against *Pseudomonas aeruginosa* ATCC27853 and *staphylococcus aureus* ATCC 25923. 1B exhibited comparable antimicrobial activity against *Pseudomonas aeruginosa* ATCC27853 and staphylococcus *aureus* ATCC 25923.

1H exhibited comparable antimicrobial activity against staphylococcus *aureus* ATCC 25923. 1E,1G,1J and 1K exhibited comparable antimicrobial activity against *E-Coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC27853 and the same compound screened for the antifungal activity against *candida* ,the compound 1B,1C,1E,1F,1J,1K,1L show comparable activity.

ACKNOWLEDGEMENTS

The author is thankful to Pune and BCUD Savitribai Phule Pune University, Maratha Vidya Prasarak Samaj Nashik for providing infrastructure, Principal Dr V. B. Gaikwad and Prof. (Dr) J. S Aher, Vice Principal and Head, Department of Chemistry, K. R. T. Arts B. H. Commerce and A. M. Science College, Gangapur

Road, Nashik-422 002, (MS), India for providing the research facilities, Principal Dr.J.D.Sonkhaskar, K.S.K.W. Art's, Science and commerce college, CIDCO, Nashik-08.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. Schiff H., *Ann. Chim.Paris*, **1864**,131,118.
2. Patai S., The Chemistry of the carbon-nitrogen double bond, John Wiley & Sons Ltd., London, **1970**.
3. Zheng Y., Ma K., Li H., Li J., He J., Sun X., *Catal Lett* , **2009**,128,465.
4. Moffett R B., InRabjohn N., *John Wiley & Sons, Inc*,**1963**, 605.
5. Taguchi K., Westheimer F.H., *J Org Chem*, **1971**,36,1570.
6. Love B E., Ren J., *J Org Chem*, **1993**,58,5556.
7. Look G.C., Murphy M.M., Campbell D.A., Gallop M.A., *Tetrahedron Lett*, **1995**,36,2937
8. Chakraborti A.K., Bhagat S., Rudrawar S., *Tetrahedron Lett*, **2004**,45,7641.
9. Billman J.H., Tai K.M., *J Org Chem*, **1958**,23,535.
10. White W.A., Weingarten H., *J Org Chem*, **1967**,32,213–4.
11. Branchaud B.P., *J Org Chem*, **1983**,48,3531.
12. Armstrong III J.D., Wolfe C.N., Keller J.L., Lynch J., Bhupathy M., Volante R.P., *Tetrahedron Lett*, **1997**,38,1531.
13. Liu G., Cogan D.A., Owens T.D., Tang T.P., Ellman J.A., *J Org Chem*, **1999**,64,1278.
14. Roman G., Andrei M., *Bull Chem Technol Macedonia*, **2001**,20,131.
15. Samec J.S.M., Backvall J.E., *Chem Eur J*, **2002**,8,2955.
16. Baricordi N., Benetti S., Biondini G., de Risi C., Pollini G.P., *Tetrahedron Lett* **2004**,45,1373.
17. Panneerselvam P., Nair R.R., Vijayalakshmi G., Subramanian E.H., Sridhar S.K., *Eur J Med Chem*, **2005**,40,225.
18. Dalpozzo R., de Nino A., Nardi M., Russo B., Procopio A., *Synthesis*, **2006**,7,1127.
19. Naeimi H., Salimi F., Rabiei K., *J Mol Catal A Chem*, **2006**,260,100.
20. Kulkarni A., Patil S.A., Badami P.S., *Eur J Med Chem*, **2009**,44,2904.
21. Varma R.S., Dahiya R., Kumar S., *Tetrahedron Lett*, **1997**,38, 2039.
22. Schmeyers J., Toda F., Boy J., Kaupp G., *J Chem Soc Perkin Trans*, **1998**,2,989.
23. Vass A., Duda´ s J., Varma R.S., *Tetrahedron Lett*, **1999**,40,4951.
24. Tanaka K., Shiraishi R., *Green Chem*, **2000**,2,272.
25. Andrade C.K.Z., Takada S.C.S., Alves L.M., Rodrigues J.P, Suarez P.A.Z., Branda R.F., *Synlett*, **2004**,12,2135.
26. Vazquez M.A., Landa M, Reyes L., Miranda R., Tamariz J., Delgado F., *Synth Commun*, **2004**,34,2705.
27. Gopalakrishnan M., Sureshkumar P., Kanagarajan V., Thanusu J., Govindaraju R, *J Chem Res* , **2005**,5,299.
28. Gopalakrishnan M., Sureshkumar P., Kanagarajan V., Thanusu J., *Res Chem Intermed*, **2007**,33,541.