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Synthesis and Antimicrobial and antifungal activities of novel Bis-imine Derivatives

Sonnekar VS[1], Jadhav WN[2], Dake SA[3] and Pawar RP[3]*

[1]Department of Chemistry, Sant Tukaram College, Parbhani-431401, (MS), India
[2]Department of Chemistry, Dnyanopasak College Parbhani-431 401, (MS), India
[3]Department of Chemistry, Deogiri College, Aurangabad-431005, (MS), India

ABSTRACT

Synthesis of bis-imines by the condensation of urea and thiourea with various substituted aromatic aldehydes in ethanol and acetic acid has been reported. All the bis-imines were further confirmed from melting point and spectral data analysis; IR, UV and elemental analysis. The compounds were screened for antimicrobial activity against bacteria *Escherichia coli*, *Staphylococcus aureus, S. typhi,* and *B.* using agar cup method and antifungal activity against *aspergillus Niger*, *P. Chrysogenum, F. Moneliforme and A. Flavus* using poison plate method.

Keywords: Antifungal, **a**ntibacterial activity; ligands, synthesis, thiourea, urea.

Corresponding author

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INTRODUCTION

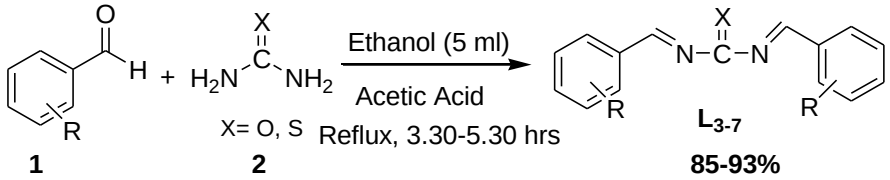
Development of environment friendly synthetic methodologies for organic synthesis is one of the recent challenges to organic chemists. Imines and bis-imines is an important class of organic compounds. These Bis-imines are biologically important due to the presence of >C=N moiety. Compound containing an azomethine group (-CH=N-) are known as schiff bases [1]. They are usually formed by the condensation of primary amine with carbonyl compound [2].

In virus replication process, the role of metal ions is extremely important. A virus penetrates into the hosts cell when mediated by some suitable metal ions. Using a suitable metal complex like $Cd(CN)_3$ which can preferably bind the sulphar site as, virus – (S)-, The penetration of the viral DNA into the host cell is arrested. As a matter of fact, for virus replication, copper and Zinc are essentially required.

Anti-biotics properties are used in metal transport across membrane or to attach the antibiotics to specific site from which it can interface the growth of bacteria.

One of the most prevalent types of catalytic mechanisms in biochemical process involves condensation of lysine residue, with a carbonyl substrate to form an imine or schiff base.

Several Schiff bases have been reported for their significant biological activities like antitumor [3], anti-inflammatory agents [4], insecticidal [5], antibacterial [6], antituberculosis [7], antimicrobial [8], anticonvulsant [9] activity. The Schiff bases are also used as versatile components in nucleophilic addition with organometallic reagents [10] and in cycloaddition reactions [11, 12].The synthesis of bis-imines by condensation of thiourea and urea with various substituted aromatic aldehydes in ethanol and acetic acid has been described. **(Scheme-1)**



1 **2**

Scheme 1

RESULT AND DISCUSSION

Preparation of Bis-imines Derivatives:

Bis-imines were prepared, isolated and characterized by melting point are given in **Table-1.** All ligands were found to be stable at room temperature.

Substituted aromatic aldehydes (1) (4 mmol) dissolved in ethanol (5 ml) and mixed with thiourea and urea (2) (2 mmol) dissolved in ethanol (5 ml). Add 2-3 drops of glycial acetic acid

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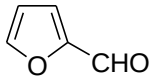
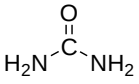
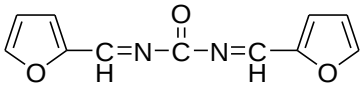
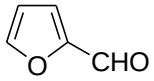
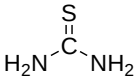
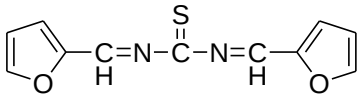
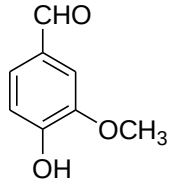
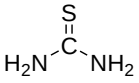
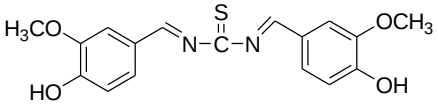
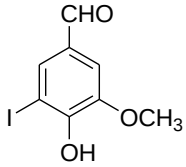
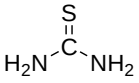
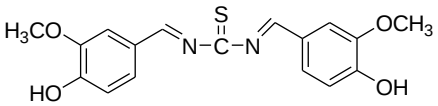
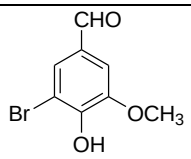
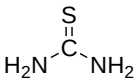
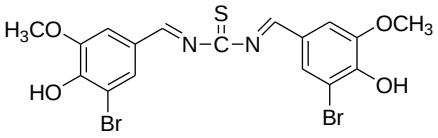
and the mixture was refluxed for appropriate time **(Table-1)** periods. On cooling the reaction mixture was poured on to 250 ml cold water. The separated solid was filtered washed with water and recrystallised from alcohol.

Table-1: Synthesis of bis-imine derivatives using Urea and Thiourea:

Comp. Codes	Reactants (1)	Reactants (2)	Products (L)	Time (hrs)	Yields (%)	M. P. (^{0}C)
L3				**4.00**	**93**	**150-153**
L4				**4.30**	**85**	**138-140**
L5				**3.30**	**87**	**85-87**
L6				**5.30**	**92**	**140-142**
L7				**5.00**	**90**	**160-162**

Infrared Spectra

The infrared spectra of the free ligands were obtained over a spectral range of 4000-400 cm^{-1}. In the infrared spectra of the ligands are found the absence of absorption bands associated with the –CHO aldehydic group and $–NH_2$ amino groups stretching of the indicating the loss of the aldehydic 1740-1720 and amine 3500-3100 cm^{-1} stretching respectively in synthesized ligands. The bands at 1690-1640 cm^{-1} and 1100-1270 respectively were assigned to the stretching vibration of the imine group of the ligands **L3- L6**.

Biological studies:

A] Antimicrobial Activity:

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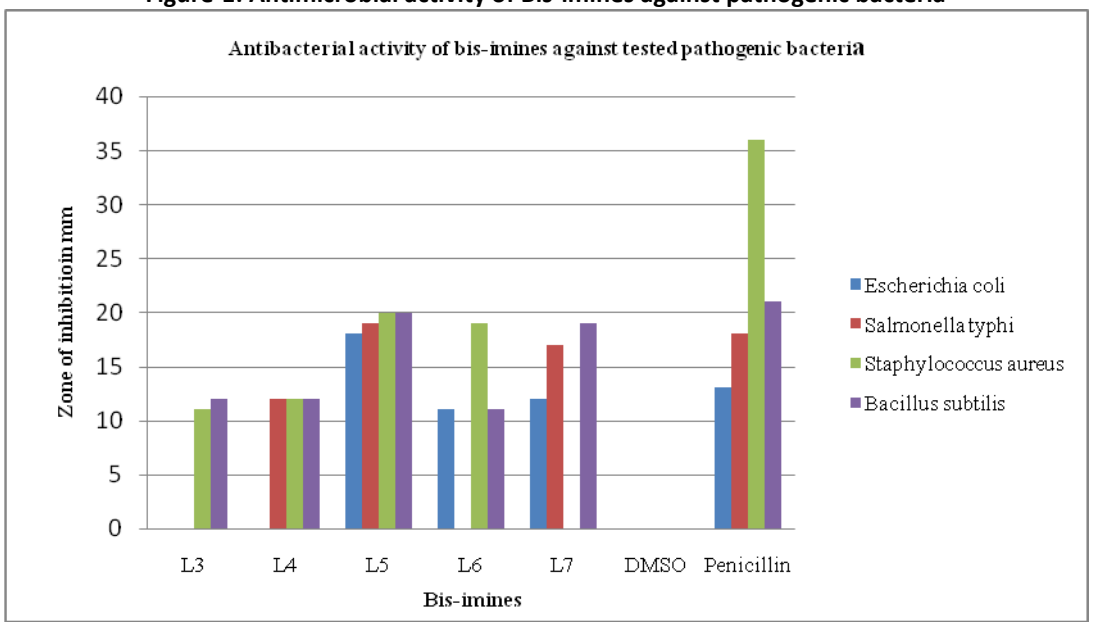
The cultures of *Escherichia coli, Salmonella typhi, Staphylococcus aureus*, and *Bacillus subtilis* grown overnight at 37°C were used for testing the antibacterial activity. Nutrient agar medium (Himedia, India) was dissolved in water (2.8% w/v) and pH was adjusted to 7.0. The solution was sterilized in autoclave at 121 °C (15 lbs/sq.in) for 20 minutes. The bacterial culture was added to the agar medium maintained at 45°C, mixed well and poured immediately in sterilized Petri plates. After hardening, four cups of 8 mm diameter each were cut into agar. 50 µL of test solutions of 1% concentrations of various chemical ligands (L3, L4, L5, L6, L7) and standard benzyl penicillin (100 µg/ml) were placed in these cups. Solvent alone in the fourth cup was kept in control. The plates were incubated at 37°C and observations recorded after 24-72 hrs. Each experiment was carried out in triplicate and the mean diameter of inhibition zone was recorded. The inhibition zones produced by the test solutions were compared with the inhibition zone produced by pure benzyl penicillin used as the standard.

Table-2: Antimicrobial screening results of the synthesized Bis-imine derivatives

Comp. Codes	Antibacterial Activity			
	E.coli	*S. typhi*	*S.aureus*	*B. Subtilis*
L3	-ve	--	11	12
L4	-ve	12	12	12
L5	18	19	20	20
L6	11	-ve	19	11
L7	12	17	-ve	19
DMSO	-ve	-ve	-ve	-ve
Penicillin	13	18	36	21

Ligand: -ve – no antibacterial activity *Zone of inhibition in mm

Figure-1: Antimicrobial activity of Bis-imines against pathogenic bacteria



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The antibacterial activity of 5 bis-imines was tested against bacterial pathogen by agar cup method and compared with standard antibiotic penicillin. It was found that all the four bacterial pathogens *Escherichia coli, Salmonella typhi, Staphylococcus aureus*, and *Bacillus subtilis* are susceptible to bis-imines L5, Salmonella typhi was resistant to L6 while others are susceptible, *Escherichia coli* was resistant to L4 while other three organisms are susceptible, while *Staphylococcus aureus*, and *Bacillus subtilis are susceptible* and other two organisms are resistant to L3, *Staphylococcus aureus* was is resistant to L7 while other three organisms are susceptible to it. The results are presented in **Table-2.**

B] Antifungal Activity: Under asceptic conditions, the test bis-imines (L3, L4, L5, L6, L7) were seeded into molten PDA medium and poured into Petri plates. The plates were covered and allowed to cool. As soon as the agar was solidified, a 9 cm sterile cork borer was used to make a disc on the pathogen plate (Mother plate). A pathogen disc was taken from pathogen plate (mother plate) and kept at the centre of test compound seeded plate with help of a sterile inoculum needle and was incubated for 3 to 4 days. The inoculum needle was sterilized with alcohol and flame before each application. The zone of inhibition was measured for pathogenecity of test compound. The experiment was repeated thrice. Triplicates were maintained for each test compound at every 24 hours interval. The diameter of mycelium growth was measured and the average value taken. The results are presented in **Table-3.**

Table-3: Antifungal screening results of the synthesized bis-imine derivatives

Comp. Codes	Antifungal Activity			
	A. Niger	***P. Chrysogenum***	***F. Moneliforme***	***A. Flavus***
L3	+ve	-ve	-ve	RG
L4	RG	-ve	-ve	RG
L5	-ve	-ve	-ve	-ve
L6	RG	-ve	-ve	RG
L7	RG	-ve	-ve	-ve
Grysofulvin	-ve	-ve	-ve	-ve
Blank	+ve	+ve	+ve	+ve

Ligand: +ve – Growth (No antifungal activity)
-ve – No Growth (Antifungal activity observed)
RG – Reduced Growth

The antifungal activity of 5 bis-imines was tested for antifungal activity of four test fungal cultures by using poison plate technique compared with the standard Grysofulvin. It was found that bis-imines L5 and inhibits the growth of all the 4 fungal cultures i.e. Penicillium chrysogenum and *Fusarium moneliforme, Aspergillus niger* and *Aspergillus flavus.* Penicillium chrysogenum and *Fusarium moneliforme* are susceptible to bis-imine L4 and L6 while in case of *Aspergillus niger* and *Aspergillus flavus* there is reduction in growth but not inhibition. *Aspergillus niger* was resistant to bis-imine L3 while others are susceptible to it. *Penicillium*

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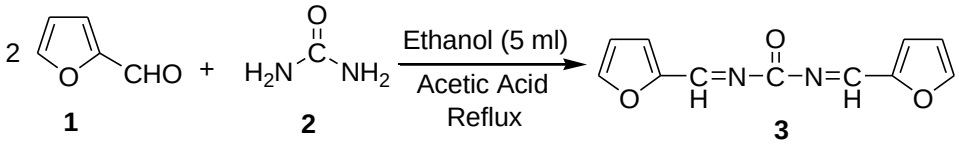
chrysogenum and Fusarium moneliforme, and Aspergillus flavus are susceptible to L7 while *Aspergillus niger there* is reduction in growth was observed.

Experimental Section:

General Experimental Procedure: Substituted aromatic aldehydes (1) (4 mmol) dissolved in ethanol (5 ml) was mixed with thiourea and urea (2) (2 mmol) dissolved in ethanol (5 ml). Add 2-3 drops of glycial acetic acid and the mixture was refluxed for appropriate time **(Table-1)** hours. On cooling the reaction mixture was poured on to 250 ml cold water. The separated solid product was filtered washed with water and recrystallised from alcohol.

Preparation of Schiff bases-Ligand using urea (L3):

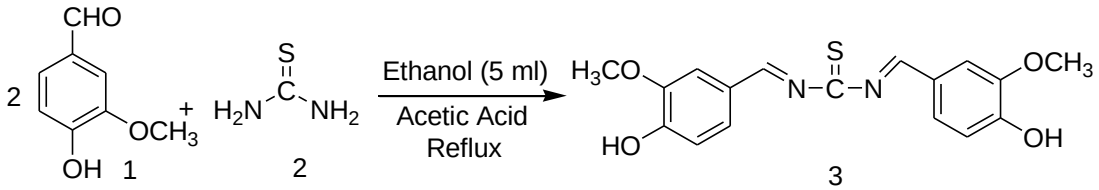
Furan-2-carbaldehyde (1) (384 mg, 4 mmol) dissolved in ethanol (5 ml) was mixed with urea (2) (120mg, 2 mmol) dissolved in ethanol (5 ml). Add 2-3 drops of glycial acetic acid and the mixture was refluxed for 4.00 hours. On cooling the reaction mixture was poured on to 250 ml cold water. The separated solid product 1,3-bis((furan-2-yl)methylene)urea was filtered washed with water and recrystallised from alcohol.



1 **2** **3**

Preparation of Schiff bases-Ligand using thiourea (L5):

3-methoxy-4-hydroxy-benzaldehyde (1) (608 mg, 4 mmol) dissolved in ethanol (5 ml) was mixed with thiourea (2) (152 mg, 2 mmol) dissolved in ethanol (5 ml). Add 2-3 drops of glycial acetic acid and the mixture was refluxed for 3.30 hours. On cooling the reaction mixture was poured on to 250 ml cold water. The separated solid product (1E,3E)-1,3-bis(4-hydroxy-3-methoxybenzylidene)thiourea of was filtered washed with water and recrystallised from alcohol



1 2 3

Spectra Data of Selected Compounds:

3-bis((furan-2-yl)methylene)urea (L3):

FTIR (KBr, cm^{-1}): 1652 (C=N), 1725 (C=O), 2990(C-H), 1606 (C=C), 1250 (C-O), 1014(C-N) cm^{-1}. Anal. Calc. for **$C_{11}H_8N_2O_3$**, C, 61.11, H, 3.73, N, 12.96, Found: C: 61.07, H: 3.81, N: 12.85.

1,3-bis((furan-2-yl)methylene)thiourea (L4):

FTIR (KBr, cm^{-1}): 2897 (C-H), 1640 (C=N), 1270 (C=S), 1610 (C=C), 1232 (C-O), 1014(C-N), cm^{-1}. Anal. Calc. for **$C_{11}H_8N_2O_2S$** C, 56.88; H, 3.47; N, 12.06; O, 13.78; S, 13.81 Found: C: 56.75, H: 3.52, N: 12.01. **UV-Vis: λmax** = 299 nm.

(1E,3E)-1,3-bis(4-hydroxy-3-methoxybenzylidene)thiourea (L5):

FTIR (KBr, cm^{-1}): 3399 (-OH), 2837 (C-H), 1668 (C=N), 1650 (C=C), 1269 (C=S), 1230 (C-O) 1029(C-N) cm^{-1}. Anal. Calc. for $C_{17}H_{16}N_2O_4S$ C, 59.29; H, 4.68; N, 8.13; Found: C: 59.22, H: 4.52, N:8.01. **UV-Vis: λmax** = 326 nm.

(1E,3E)-1,3-bis(4-hydroxy-3-iodo-5-methoxybenzylidene) thiourea (L6):

FTIR (KBr, cm^{-1}): 3650 (-OH), 1666 (C=N), 1038(C-N), 1258 (C=S), 2847 (C-H), 1458 (C=C), 1295 (C-O), 672(C-I) cm^{-1}. Anal. Calc. for $C_{17}H_{16}N_2O_4S$ C, 59.29; H, 4.68; N, 8.13; Found: C: 59.22, H: 4.52, N:8.01. **UV-Vis: λmax** = 325 nm.

CONCLUSION

In this article we are reported synthesis of bis-imines by using thiourea and urea with various substituted aromatic aldehydes in ethanol and acetic acid. Bis-imines were confirmed from melting point and spectral data analysis; IR, UV and Elemental analysis. It was found that all the four bacterial pathogens *Escherichia coli, Salmonella typhi, Staphylococcus aureus*, and *Bacillus subtilis* are susceptible to ligand **L5,** Salmonella typhi was resistant to **L6**. It was found that ligand **L5** inhibits the growth of all the 4 fungal cultures i.e. Penicillium chrysogenum and *Fusarium moneliforme, Aspergillus niger* and *Aspergillus flavus.* Penicillium chrysogenum and *Fusarium Moneliforme*. All ligands were found to be stable at room temperature.

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