SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SOME IMINE ANALOGS

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ABSTRACT

A series of imine analogs were synthesized by the condensation of 3-methoxy 4-hydroxy benzaldehyde with substituted amines in ethanol. The structures of newly synthesized compounds were characterized by IR and $^1$HNMR spectroscopy. The purity of the synthesized imines analogs has been ascertained by thin layer chromatography using an acetone-n-hexane mixture (1:3). Antibacterial activity of imines was tested against a panel of sensitive and resistant Gram-positive and Gram-negative strains using disc diffusion assay.

KEYWORDS: Vanilllin, substituted amines, antibacterial activity.

1. INTRODUCTION

The chemistry of >C=N is studied extensively because of its high synthesis, flexibility, varied coordinating ability and medicinal utility. The compounds containing >C=N include mainly the products of the reaction between an aldehyde or ketonic components and primary aliphatic or aromatic amines, ammonia, hydrazine, N-phenylhydrazine, hydroxylamine, hydrochloride, semicarbazide, thiosemicarbazide and their substituted derivatives.

The condensation of >C=O group compound with a varied primary amine or amino compound on the elimination of water molecule forms a weakly basic compound known as an imine or an anil or an azomethine which is commonly known as Imine, named to Honor Schiff[1] who synthesized such compounds.

Imines and their derivatives has been a research subject[2-5] owing to their pharmacological characteristics and striking complexometric behavior. These properties allow them to play a pivotal role in various biological activities[6-8] viz., antibacterial[9] antifungal[10], anti-
carcinogenic\textsuperscript{[11]}, antitubercular\textsuperscript{[12]}, anticonvulsant\textsuperscript{[13]}, anti-HIV\textsuperscript{[14]}, antiamoebic\textsuperscript{[15]}, anti-inflammatory\textsuperscript{[16]}, antinociceptive\textsuperscript{[17]}, antimouse hepatitis virus (MHV)\textsuperscript{[18]} inhibition of herpes simplex virus type-1 (HSV-1) and adenovirus type 5 (AD-5)\textsuperscript{[19]}, antimalarial\textsuperscript{[20]} pesticide thymidine phosphorylase inhibitors\textsuperscript{[21]} antitumor\textsuperscript{[22]} and herbicidal.\textsuperscript{[23]}

Vanillin (4-hydroxy-3-methoxy benzaldehyde) is one of the most important widely used flavoring materials worldwide.\textsuperscript{[24-26]} Synthetic vanillin is used in both food and non-food applications, in fragrances and as a flavoring in pharmaceutical preparations. Currently, approximately 50\% of the worldwide production of synthetic vanillin is used as an intermediate in the chemical and pharmaceutical industries for the production of herbicides, antifoaming agents or drugs such as papaverine, l–dopa, methyldopa and the antimicrobial agent, trimethoprim.\textsuperscript{[27]} Konstantinovic \textit{et al}\textsuperscript{[28]} synthesized vanillin Imines.

2. MATERIAL AND METHODS

2.1. Chemicals
All reagents and solvents were commercially available and used as supplied. All the chemicals used were of AR grade. The melting points of the compounds were determined in open capillaries on an electrothermal apparatus and are uncorrected. Analytical TLC was performed on silica gel plates; visualization was done by exposing to iodine vapor. Infrared spectra were measured using KBr pellets with Perkin Elmer BX series FT-IR spectrometer. \textsuperscript{1}H-NMR spectra were recorded on Varian 500 MHz NMR spectrophotometer using CDCl\textsubscript{3}/DMSO-d\textsubscript{6} as solvent and TMS as an internal standard (chemical shift in ppm). C, H, N, estimation was recorded on Carlo Erba 1108 (CHN) Elemental analyzer.

2.2. General procedure for the synthesis of imine analogs (2a-j)
3-methoxy 4-hydroxy benzaldehyde (0.005M) was dissolved in absolute ethanol and the contents were refluxed for 5 hrs. The reaction mixture was cooled in ice water and acidified with a drop of sulphuric acid. It was filtered under suction washed with ethanol and recrystallized from aq, ethyl alcohol when yellow crystals of the imines was obtained.
R= H, CH₃ (p-, m), OCH₃ (p-, m-), F (p-, m-), NO₂ (p-, m-), p- Cl

Scheme-1: Synthetic scheme of newly synthesized imines

Table I: Physical data of all the synthesized imine analogs

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compounds</th>
<th>R</th>
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<th>Molecular weight</th>
<th>Melting point</th>
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<td>128</td>
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<td>C₁₄H₁₄NO₂</td>
<td>227</td>
<td>102</td>
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3. SPECTRAL ANALYSIS

4-[(4-chlorophenyl)imino]methyl]-2-methoxyphenol (2a)

FT-IR (cm⁻¹): ν (O-H) 3000, ν (C-H aromatic) 3019, ν (C-H aliphatic) 2979, ν (C=N) 1623, ν (C=C phenyl) 1599, 1584, 1514, 1486 & 1463, ν (C-O) 1255, 1212 & 1182 ν (C-Cl) 721. ¹H-NMR (DMSO): δ, ppm: 9.8 (s, 1H, -OH), 8.44 (s, 1H, -CH=N-), 7.33 (d, 2H, Ar-H), 7.28 (d, 2H, Ar-H), 7.13 (d, 1H, Ar-H), 6.91 (t, 1H, Ar-H), 7.23 (d, 1H, Ar-H), 3.84 (s, 3H, -OCH₃).

2-methoxy-4-[(4-methylphenyl)imino]methyl]phenol (2b)

FT-IR (cm⁻¹): ν (O-H) 3261, ν (C-H aromatic) 3029, ν (C-H aliphatic) 2920, ν (C=N) 1616, ν (OCH₃) 1456, ν (C=C phenyl) 1595, 1575, 1509, ν (C-O) 1255. ¹H-NMR (DMSO): δ, ppm: 13.35 (s, 1H, -OH), 8.93 (s, 1H, -CH=N-), 7.33 (d, 2H, Ar-H), 7.28 (d, 2H, Ar-H), 7.13 (d, 1H, Ar-H), 6.91 (t, 1H, Ar-H), 7.23 (d, 1H, Ar-H), 3.84 (s, 3H, -OCH₃), 2.35 (s, 3H, -CH₃).
2-methoxy-4-[(4-methoxyphenyl)imino]methyl]phenol (2d)
FT-IR (cm⁻¹): ν (O-H) 3261, ν (C-H aromatic) 3029, ν (C-H aliphatic) 2920, ν (C=O) 1616, ν (OCH3) 1456, ν (C=C phenyl) 1595, 1575, 1509, ν (C-O) 1255. ¹H-NMR (DMSO): δ, ppm: 13.35 (s, 1H, -OH), 8.93 (s, 1H, -CH=N-), 7.33 (d, 2H, Ar-H), 7.28 (d, 2H, Ar-H), 7.13 (d, 1H, Ar-H), 6.91 (t, 1H, Ar-H), 7.23 (d, 1H, Ar-H), 3.84 (s, 3H, -OCH3), 2.35 (s, 3H, -CH3).

4-[(4-fluorophenyl)imino]methyl]-2-methoxyphenol (2f)
FT-IR (cm⁻¹): ν (C=N) 1588, ν (C=C) 1514, ν (C–H) 2964–2941, ν (C–O) 1283–1160. ¹H-NMR (DMSO, δ ppm): 3.85 (3H, s, OCH3), 6.93 (1H, d, Ar-H); 7.28–7.18 (4H, m, Ar-H), 7.37 (1H, dd, Ar-H), 7.55 (H9, s, 1H, s, Ar-H), 8.46 (1H, s, HC=N), 9.86 (1H, s, OH).

2-methoxy-4-[(4-nitrophenyl)imino]methyl]phenol (2h)
FT-IR (cm⁻¹): ν (O-H) 3323, ν (C-H aromatic) 3219, ν (C-H aliphatic) 1550, ν (C=O) 1628, ν (OCH3) 1456, ν (C=C phenyl) 1589, ν (C-O) 1367, ν (N=O) 1504. ¹H-NMR (DMSO): δ, ppm: 13.65 (s, 1H, -OH), 8.68 (s, 1H, -CH=N-), 7.52 (d, 2H, Ar-H), 7.36 (d, 2H, Ar-H), 7.06 (d, 1H, Ar-H), 6.93 (t, 1H, Ar-H), 3.98 (s, 3H, -OCH3).

4. ANTIBACTERIAL ACTIVITY
Antibacterial activity of imines was tested against a panel of sensitive and resistant Gram-positive and Gram-negative strains using disc diffusion assay.[29] Tested microorganisms included Gram-positive (Staphylococcus aureus and Bacillus subtilis) and Gram-negative (Escherichia coli and Pneumonia aeruginosa). For this, sterile filter paper disks (6 mm) impregnated with fixed doses viz; 800µg/ml of compounds was placed on pre-inoculated surface. The disc bearing plates were incubated within 30 min at 37° for 24 h. After incubation, the inhibition zone diameters were measured. The standard compound chosen was a known, highly active, broad–spectrum antibiotic, chloramphenicol in order to compare the relative activity of synthesized compounds with a standard. The antibacterial data of tested compounds are presented in Table II.
Table II: Antibacterial activity of Imine analogs.

(Zone of inhibition in mm.)

<table>
<thead>
<tr>
<th>MICROBIAL SPECIES</th>
<th>Compound no.</th>
<th>E. coli</th>
<th>B.subtilis</th>
<th>P.aeruginosa</th>
<th>S.Aureus</th>
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*Control DMF- No activity.

CHART-1: Activity Profile of Imines against 4 bacterial strains @ 800 µg/mL

5. RESULT AND DISCUSSION

3-methoxy 4-hydroxy benzaldehyde was condensed with substituted anilines to yield new imines. The imines obtained were colored, soluble in dimethylformamide and the have been recrystallized by using ethanol; water (1:1) ratio mixture. The purity of azomethines was ascertained by thin layer & column chromatography using acetone; n-hexane (1:3) solvent mixture.
The synthesized compounds were screened for their in vitro antibacterial activity by disc diffusion method against Gram +ve \textit{(Staphylococcus aureus, Bacillus subtilis)} & Gram –ve \textit{(Escherichia coli, Pseudomonas aeruginosa)} microorganisms by preparing 800 μg/ml of test solution of each compound taking chloramphenicol as a standard drug. Zone of inhibitions in mm were noted. The zone of inhibition for imines varied from 0 to 20 mm. The results have been shown in Table II, the activity of control (dimethyl formamide) was also checked for its toxicity.

All the tested compounds exhibited promising antibacterial activity comparable with standard drugs. The inhibition depends on the type of bacterial strain, a solvent used as well as the structure of the compound. All the imine compounds contain the same central moiety (-CH=N) with different side chains. So in a particular solvent, for a particular effect side chains play important role in inhibition. All the compounds exhibited promising antibacterial activity.

6. CONCLUSION

All the compounds have shown mild to moderate antibacterial activities. Among these imine analogs having methoxy and methyl moieties have shown good activity in all the species. Two compounds (2d, 2b) were slightly more potent. It is observed that \textit{S. aureus} show good to maximum inhibition nearly in all compounds while \textit{P. aeruginosa} show moderate inhibition antibacterial activity. \textit{E. coli} and \textit{B. subtilis} show less to good inhibition activity. All compounds show better activity in comparison to the reference drug.

7. ACKNOWLEDGEMENT

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8. REFERENCES


