Synthesis and antibacterial profile of novel azomethine derivatives of β-phenylacrolein moiety

Sridevi Chigurupati1*, Neeraj Kumar Fuloria1, Shivkanya Fuloria1, Sundram Karupiah1, Ravichandran Veerasamy1, Appala Raju Nemala2, Lim Jun Yi1, Ang xiang Ilan1 and Syed Adnan Ali Shah3

1AIMST University, Faculty of Pharmacy, Semeling, 08100 Bedong, Kedah, Malaysia, 2Department of Pharmaceutical chemistry, Sultan–Uj–Uloom College of Pharmacy, Road No. 3, Banjara hills, Hyderabad, India, 3Universiti Teknologi MARA (UiTM), Faculty of Pharmacy, Puncak Alam Campus, Selangor D.E, Malaysia

*For correspondence: Email: sridevi.phd@gmail.com; Tel: +6-014-9449846

Received: 16 December 2015 Revised accepted: 24 March 2016

Abstract

Purpose: To develop some novel molecules effective against antibiotic-resistant bacterial infections.

Methods: A series of azomethines (SB-1 to SB-6) were synthesized from β-phenyl acrolein moiety. The structures of the synthesized compounds were confirmed on the basis of their UV ultra-violet (UV) spectroscopy (λmax 200 - 400 nm), Fourier transform infra-red (FTIR, vibrational frequency: 500-4000 cm-1), 1H nuclear magnetic resonance (NMR, chemical shift: 0 - 10 ppm), 13C NMR (chemical shift: 0 - 200 ppm), mass spectrometry (m/z values: 0 - 500) and carbon hydrogen nitrogen (CHN) elemental analysis. The new compounds were screened for antibacterial activity by test-tube dilution and disc diffusion methods using gentamicin as reference standard.

Results: The structures of azomethine were in full agreement with their spectral data. Among all the synthesized compounds, compounds SB-5 and SB-6 exhibited the highest minimum inhibitory concentration (MIC) of 62.5 µg/mL. At MIC of 250 µg/mL, all compounds SB-1 to SB-6 displayed significant antibacterial activity, compared to gentamycin (p < 0.05). SB-5 and SB-6 were active against S. aureus, P. aeruginosa and K. pneumoniae; SB-3 was active against B. subtilis and S. aureus. SB-4 was active against P. aeruginosa and S. aureus while SB-1 and SB-2 were active against S. aureus.

Conclusion: The synthesized compounds possess antibacterial activities compared to those of gentamycin.

Keywords: Acrolein, Imines, Azomethine, Antibacterial, Gentamycin, Minimum inhibitory concentration

INTRODUCTION

Antimicrobial drugs occupy a unique niche in the history of medicine. The increased incidence of severe opportunistic bacterial infections in immunological deficient patients together with the development of resistance among pathogenic Gram-positive and Gram-negative bacteria have revealed great need to search for new compounds that are effective against antibiotic-resistant bacteria. Literature highlights the potentials of β-phenyl acrolein as an antimicrobial [1], anticancer [2] and flavoring agent for chewing gums [3]. Many investigators have observed the importance of azomethines for their antibacterial [4,5], antifungal [6], anti-proliferative [7,8] and antipyretic properties. It is evident that azomethines with aryl substituents are more stable and readily synthesized, whereas those containing alkyl substituents are...
RELATIVELY UNSTABLE. AZOMETHINES OF ALIPHATIC ALDEHYDES ARE USUALLY UNSTABLE AND READILY POLYMERIC, WHILE THOSE WITH AROMATIC ALDEHYDES HAVING EFFECTIVE CONJUGATION ARE MORE STABLE [9].

IN THE PRESENT RESEARCH WORK, A NEW SERIES OF AZOMETHINE DERIVATIVES OF β-PHENYL ACRYLEIN WAS SYNTHESIZED (SB-1 TO SB-6) AND SCREENED FOR ANTIBACTERIAL ACTIVITY AGAINST GRAM-POSITIVE (BACILLUS SUBTILIS AND STAPHYLOCOCCUS AUREUS) AND GRAM-NEGATIVE (KLEBSIELLA PNEUMONIAE AND PSEUDOMONAS AERUGINOSA) BACTERIA IN ORDER TO GENERATE POTENT AND SAFER ANTIBACTERIAL AGENTS.

EXPERIMENTAL

MATERIALS

ALL THE SOLVENTS AND CHEMICALS USED WERE OF ANALYTICAL GRADE AND OBTAINED FROM SIGMA-ALDRICH AND MERCK PVT LTD, INDIA AND WERE USED WITHOUT FURTHER PURIFICATION.

GENERAL PROCEDURE FOR SYNTHESIS OF AZOMETHINE DERIVATIVES OF β-PHENYL ACRYLEIN

EQUIMOLAR CONCENTRATION OF β-PHENYL ACRYLEIN (0.01 M) AND SUBSTITUTED AROMATIC AMINES (0.01 M) WERE DISSOLVED IN 50 ML OF ANHYDROUS ETHANOL SEPARATELY. SOLUTION OF SUBSTITUTED AROMATIC AMINE WAS THEN ADDED DROP-WISE INTO β-PHENYL ACRYLEIN SOLUTION IN A CONICAL FLASK. THE MIXTURE WAS MADE UP TO 150 ML WITH 95 % ANHYDROUS ETHANOL, AND 2 TO 3 DROPS OF TRIETHYLAMINE (BASIC CATALYST) WAS ADDED [10]. THE MIXTURE WAS THEN STIRRED USING MAGNETIC STIRRER AT 60 TO 70 °C FOR 6 HOURS OVER A WATER BATH. THE REACTION WAS MONITORED BY TLC. THE SAMPLE MIXTURE WAS EVAPORATED UNDER PRESSURE AT 65 °C USING ROTARY evaporator [11,12]. THE SOLID OBTAINED ON CONCENTRATION OF FILTRATE WAS RECRYSTALLIZED FROM AQUEOUS ETHANOL TO YIELD THE PURE COMPOUNDS SB-1 TO SB-6 (PHYSICAL DATA ARE GIVEN IN TABLE 1). THE MELTING POINTS OF THE COMPOUNDS WERE DETERMINED ON A THOSHNIWAL ELECTRIC MELTING POINT APPARATUS AND THE VALUES WERE UNCORRECTED. THE REACTION WAS MENTIONED BY TLC ON SILICA gel-GF 254 (MERCK) COATED PLATES. SPOTS OF TLC WERE IDENTIFIED IN IODINE CHAMBER.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

TEST TUBE DILUTION METHOD WAS USED TO DETERMINE MINIMUM INHIBITORY CONCENTRATION. ONE ML OF STERILIZED MEDIA (NUTRIENT AGAR) WAS Poured INTO STERILE TEST TUBES. ONE ML OF 2000 µG/ML TEST SOLUTION WAS TRANSFERRED IN ONE TUBE AND SERIALLY DILUTED TO GIVE CONCENTRATIONS OF 1000, 500, 250 AND 125 µG/ML. TO ALL THE TEST TUBES, 0.1 ML OF SUSPENSION OF BACTERIA IN SALINE WAS ADDED AND THE TUBES WERE INCUBATED AT 37 °C FOR 24 H. THE GROWTH IN THE TUBES WAS OBSERVED VISUALLY FOR TURBIDITY. MIC WAS DETERMINED WITH THE LOWEST CONCENTRATION OF THE SAMPLE THAT RETARDED THE DEVELOPMENT OF TURBIDITY [13].

DISC-DIFFUSION METHOD


STATISTICAL ANALYSIS

EACH EXPERIMENTAL VALUE IS EXPRESSED AS THE MEAN ± STANDARD ERROR MEAN (N = 3). STATISTICAL ANALYSIS PERFORMED USING GRAPHPAD PRISM 5.0 AND DATA ANALYZED USING ONE-WAY ANALYSIS OF VARIANCE (ANOVA) FOR COMPARISON BETWEEN GROUPS FOLLOWED BY DUNNETT’S MULTIPLE COMPARISON TEST AT A SIGNIFICANT LEVEL OF p < 0.05.

RESULTS

CHEMISTRY

UV-VISIBLE, IR, NMR AND MASS SPECTRAL DATA
N-(3-phenylallylidene)benzamine (SB-1): Yield: 70%; mp: 98-100 °C; Anal. Calcld. for C_{16}H_{15}N: C, 86.92; H, 6.32; N, 6.76 %. Found C, 86.89; H, 6.28; N, 6.66 %. IR (KBr, cm⁻¹): 3085 (≈C-H stretching of aromatic ring), 3039 (≈C-H stretching of alkenyl group), 1600 (C=O stretching from azomethine group), 1540-1600 (C=C stretching of aromatic ring), 1320 (C-N, stretching of azomethine group); ¹³C-NMR (500.1 MHz, CDCl₃, δ/ppm): 5.76 (1H, t, J = 9.5 Hz, H-2'), 6.52 (1H, d, J = 12 Hz, H-3'), 7.23 (10H, m, phenyl), 8.24 (1H, d, J = 7.8 Hz, H-1'); ¹³C-NMR (100 MHz, CDCl₃, δ/ppm): 120.3 (C₂), 128.6 (C₆), 127.5 (C₁), 128.4 (C₆'), 129.2 (C₆''), 131.1 (C₂''), 135.8 (C₉), 139.2 (C₆), 150.4 (C₂), 164.3 (C₁); MS (m/z, relative abundance, %): 207 (M⁺, 10), 107, 77, 53, 51, 130 (BP, 100); UV-Vis (MeOH) (λ_{max}/nm): 353.

4-(3-Phenylallylideneamino)benzaldehyde (SB-2): Yield: 79%; mp: 130-134 °C; Anal. Calcld. for C_{16}H_{15}NO: C, 81.68; H, 5.57; N, 5.95 %. Found C, 81.56; H, 5.48; N, 5.87 %. IR (KBr, cm⁻¹): 3050 (≈C-H stretching of aromatic ring), 3038 (≈C-H stretching of alkenyl group), 2720, 2820 (C-H stretching of aldehyde group), 1725 (C=O stretching of aldehyde group), 1540-1620 (C=C stretching of aromatic ring), 1320 (C-N, stretching of azomethine group); ¹³C-NMR (500.1 MHz, CDCl₃, δ/ppm): 5.81 (1H, t, J = 9.5 Hz, H-2'), 6.63 (1H, d, J = 12 Hz, H-3'), 7.5 - 7.9 (10H, m, phenyl), 8.32 (1H, d, J = 7.8 Hz, H-1'), 9.94 (s, 1H, aldehyde); ¹³C-NMR (100 MHz, CDCl₃, δ/ppm): 120.5 (C₂), 123.8 (C₆), 126.2 (C₆''), 128.3 (C‘), 129.4 (C₆'), 131.8 (C₂''), 135.4 (C₉), 136.3 (C₁), 139.6 (C₆), 155.2 (C₂), 164.6 (C₁'); 192.4 (−CHO); MS (m/z, relative abundance, %): 235 (M⁺, 20.4), 158, 105, 102, 77, 53, 51 (BP, 100); UV-Vis (MeOH) (λ_{max}/nm): 307.

4-(3-Phenylallylideneamino)phenol (SB-3): Yield: 80%; mp: 120-130 °C; Anal. Calcld. for C_{16}H_{15}NO: C, 80.69; H, 5.87; N, 6.27 %. Found C, 80.56; H, 5.78; N, 5.82 %. IR (KBr, cm⁻¹): 3640 (Broad, O-H Str), 3055 (≈C-H stretching of aromatic ring), 3038 (≈C-H stretching of alkenyl group), 1668 (C≡N stretching azomethine group), 1540-1600 (C=C stretching of aromatic ring), 1320 (C-N, stretching of azomethine group); ¹³C-NMR (500.1 MHz, CDCl₃, δ/ppm): 5.4 (1H, s, −OH, D₂O exchangeable), 5.72 (1H, t, J = 9.5 Hz, H-2'), 6.59 (1H, d, J = 12 Hz, H-3'), 7.4 - 7.8 (10H, m, phenyl), 8.29 (1H, d, J = 7.8 Hz, H-1'); ¹³C NMR (100 MHz, CDCl₃, δ/ppm): 118.4 (C₆), 120.3 (C₂), 123.9 (C₆''), 126.5 (C₆'), 128.4 (C‘), 129.2 (C₆'), 135.8 (C₉), 139.2 (C₂), 141.6 (C₆), 157.2 (C₁); MS (m/z, relative abundance, %): 223 (M⁺, 18.5), 146, 130, 102, 93, 77, 53, 51 (BP, 100); UV-Vis (MeOH) (λ_{max}/nm): 302.

4-Methoxy-N-(3-phenylallylidene)benzamine (SB-4): Yield: 87%; mp: 140-143 °C; Anal. Calcld. for C_{18}H_{16}NO: C, 80.98; H, 6.37; N, 5.90 %. Found C, 80.96; H, 6.33; N, 4.89 %. IR (KBr, cm⁻¹): 3052 (≈C-H stretching of aromatic ring), 3037 (≈C-H stretching of alkenyl group), 2934, 2876 (C-H, Str), 1658 (C≡N stretching azomethine group), 1540-1620 (C=C stretching of aromatic ring), 1320 (C-N, stretching of azomethine group), 1255 (C-O-C, Str); ¹³C-NMR (500.1 MHz, CDCl₃, δ/ppm): 3.73 (3H, s, CH₃), 5.74 (1H, t, J = 9.5 Hz, H-2'), 6.56 (1H, d, J = 12 Hz, H-3'), 7.3 - 7.75 (10H, m, phenyl), 8.36 (1H, d, J = 7.8 Hz, H-1'); ¹³C-NMR (100 MHz, CDCl₃, δ/ppm): 56.3 (−CH₃), 116.2 (C₆), 120.4 (C₂), 123.7 (C₆ & C₆'), 126.2 (C₆''), 126.9 (C₁), 128.1 (C₆') 135.8 (C₉), 139.3 (C₆), 141.2 (C₂), 160.4 (C₁); 164.3; MS (m/z, relative abundance, %): 237 (M⁺, 19.0), 160, 130, 107, 102, 77, 53, 51 (BP, 100); UV-Vis (MeOH) (λ_{max}/nm): 306.

4-Methoxy-2-(3-phenylallylideneamino)benzoic acid (SB-5): Yield: 80%; mp: 150-154 °C; Anal. Calcld. for C_{17}H_{15}NO₃: C, 72.58; H, 5.37; N, 4.98 %. Found C, 72.52; H, 5.39; N, 4.92 %. IR (KBr, cm⁻¹): 3400-2500 (OH Str of COOH), 3057 (≈C-H stretching of aromatic ring), 3038 (≈C-H stretching of alkenyl group), 2500-2900 (O-H of COOH, Str), 1720 (C=O of COOH Str), 1658 (C≡N stretching azomethine group), 1540-1620 (C=C stretching of aromatic ring), 1280 (C-O stretching of COOH group), 1233 (C-N, stretching of azomethine group), 1250 (C=C stretching of other group); ¹³C-NMR (500.1 MHz, CDCl₃, δ/ppm): 3.74 (3H, s, OCH₃), 5.76 (1H, t, J = 9.5 Hz, H-2'), 6.8 (1H, d, J = 12 Hz, H-3'), 7.64 - 7.92 (10H, m, phenyl), 8.38 (1H, d, J = 7.8 Hz, H-1'), 11.00 (1H, s, COOH); ¹³C-NMR (100 MHz, CDCl₃, δ/ppm): 56.8 (−CH₃), 108.4 (C₆), 109.1 (C₉), 112.6 (C₁), 119.8 (C₆'), 126.1 (C₆''), 127.2 (C₇), 128.6 (C₆''), 132.4 (C₂), 135.7 (C₉), 138.3 (C₆'), 153.5 (C₂), 167.8 (C₆), 169.6 (−COOH); MS (m/z, relative abundance, %): 281 (M⁺, 20.7), 204, 151, 130, 102, 77, 53, 51 (BP, 100); UV-Vis (MeOH) (λ_{max}/nm): 338.


Chigurupati et al.
Table 2: Minimum inhibitory concentration (MIC) of β-phenyl acrolein derivatives, SB-1 to SB-6

<table>
<thead>
<tr>
<th>Compound</th>
<th>Gram positive bacteria (µg/mL)</th>
<th>Gram negative bacteria (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. subtilis</td>
<td>S. aureus</td>
</tr>
<tr>
<td>SB-1</td>
<td>250</td>
<td>62.5</td>
</tr>
<tr>
<td>SB-2</td>
<td>125</td>
<td>62.5</td>
</tr>
<tr>
<td>SB-3</td>
<td>62.5</td>
<td>62.5</td>
</tr>
<tr>
<td>SB-4</td>
<td>125</td>
<td>62.5</td>
</tr>
<tr>
<td>SB-5</td>
<td>125</td>
<td>62.5</td>
</tr>
<tr>
<td>SB-6</td>
<td>125</td>
<td>62.5</td>
</tr>
</tbody>
</table>

Table 3: Zone of inhibition of β-phenyl acrolein derivatives, SB-1 to SB-6

<table>
<thead>
<tr>
<th>Compound</th>
<th>Gram positive bacteria (mm, 250µg/mL)</th>
<th>Gram negative bacteria (mm, 250µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. subtilis</td>
<td>S. aureus</td>
</tr>
<tr>
<td>SB-1</td>
<td>8.5±0.28</td>
<td>9.9±0.57*</td>
</tr>
<tr>
<td>SB-2</td>
<td>10.1±0.72</td>
<td>10.8±0.92*</td>
</tr>
<tr>
<td>SB-3</td>
<td>12.0±0.57*</td>
<td>10.0±1.00*</td>
</tr>
<tr>
<td>SB-4</td>
<td>9.6±0.33</td>
<td>10.6±0.33*</td>
</tr>
<tr>
<td>SB-5</td>
<td>10.3±0.33</td>
<td>11.3±0.33*</td>
</tr>
<tr>
<td>SB-6</td>
<td>9.6±0.33</td>
<td>10.3±0.88*</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>12.0±0.33</td>
<td>9.3±0.57</td>
</tr>
</tbody>
</table>

*p<0.05, compared to Gentamycin (one-way ANOVA followed by Dunnett’s multiple comparison test (p < 0.05); values are mean ± SEM (n = 3)

C, 72.51; H, 5.32N; IR (KBr cm⁻¹): 3052 (C-H stretching of aromatic ring), 3036 (C-H stretching of alkenyl group), 2926, 2872 (C-H stretching of methyl group), 1668 (C=N stretching of azomethine group), 1540-1600 (C=C stretching of aromatic ring), 1320 (C-N, stretching of azomethine group); ¹H-NMR (500.1 MHz, CDCl₃-d, δ/ppm): 5.85 (1H, δ = 9.5 Hz, H-2'), 6.76 (1H, d, J = 12 Hz, H-3'), 7.64 - 7.92 (10H, m-phenyl), 8.41 (1H, d, J = 7.8 Hz, H-1'), 11.00 (1H, s, COOH); ¹³C-NMR (100 MHz, CDCl₃, δ/ppm): 120.4 (C₂), 123.5 (C₃& C₄), 126.7 (C₅& C₆), 127.4 (C₇), 128.4 (C₈& C₉), 129.2 (C₁₀), 131.2 (C₁₁& C₁₂), 135.9 (C₁₃), 139.4 (C₁₄), 155.4 (C₁₅), 164.8 (C₁₆), 169.6 (-COOH); MS (m/z, relative abundance, %): 251 (M⁺, 32.6), 174, 130, 121, 102, 77, 53, 51 (BP, 100); UV-Vis (MeOH) (λmax/nm): 307.

Antibacterial activity

The newly synthesized β-phenyl acrolein derivatives were tested for their antibacterial potential against B. subtilis and S. aureus, K. pneumoniae, and P. aeruginosa, using gentamycin as standard. The results are given in Table 2 and Table 3.

DISCUSSION

Equimolar concentration of β-phenyl acrolein and substituted aromatic amines in the presence of basic catalyst, triethylamine resulted in formation of azomethine derivatives of β-phenyl acrolein moiety shown in Figure 1.

The λmax for the newly synthesized azomethines was found to be in range from 300-440 nm. The IR stretch at around 1650-1680 cm⁻¹ showed the C=N bond formation. The formation of azomethines was identified by the presence of triplet between 5.7 to 5.8 ppm, in proton NMR spectra. All other aliphatic and aromatic protons were observed within the expected regions. The novel compounds were further confirmed by their characteristic mass fragment spectra. The mass fragment pattern of compound SB-4 given in Figure 2, displayed parent ion peak at 237, base peak at 51, and different fragment peaks at 160, 130, 107, 102, 77, and 53. Similarly, all the new compounds were characterized. This part confirmed the synthesis of a series of six new azomethines derivatives of β-phenyl acrolein.

The antibacterial potential of newly synthesized molecules was estimated by tube dilution and disc diffusion method; using Gram positive bacteria (Bacillus subtilis and Staphylococcus aureus) and Gram negative bacteria (Klebsiella pneumoniae and Pseudomonas aeruginosa). Tube dilution method depends upon the inhibition of growth of a microbial culture in a uniform solution of antibiotic in a fluid medium that is favorable to its rapid growth in the absence of the antibiotic [17]. In this method minimum inhibitory...
Among all synthesized compounds, study results given in Table 2 and Table 3, as per the drug [18].

Determined. Gentamycin was used as a standard concentration of the test compounds was determined. Gentamycin was used as a standard drug [18].

As per the minimum inhibitory concentration study results given in Table 2 and Table 3, among all synthesized compounds, SB-5 and SB-6 displayed highest MIC value of 62.5 µg/ml. The zone of inhibition experiment, revealed that at MIC of 250 µg/ml, all compounds SB-1 to SB-6 showed significant antibacterial activity (p < 0.05). SB-5 and SB-6 were active against S. aureus, P. aeruginosa and K. pneumoniae; SB-3 was active against B. subtilis and S. aureus, SB-4 was active against P. aeruginosa and S. aureus. SB-1 and SB-2 were active against S. aureus. The antimicrobial results proved that all

**Figure 1**: Scheme for synthesis of azomethine derivatives of β-phenyl acrolein moiety

**Figure 2**: Mass fragmentation pattern of SB-4

Where,

\[
\begin{align*}
R1 &= -\text{H}, & R2 &= -\text{H}, & R3 &= -\text{H}, & \text{for SB-1} \\
R2 &= -\text{CHO}, & R2 &= -\text{H}, & R3 &= -\text{H}, & \text{for SB-2} \\
R1 &= -\text{OH}, & R2 &= -\text{H}, & R3 &= -\text{H}, & \text{for SB-3} \\
R1 &= -\text{OCH}_3, & R2 &= -\text{H}, & R3 &= -\text{H}, & \text{for SB-4} \\
R1 &= -\text{H}, & R2 &= -\text{OCH}_3, & R3 &= -\text{COOH}, & \text{for SB-5} \\
R1 &= -\text{COOH}, & R2 &= -\text{H}, & R3 &= -\text{H}, & \text{for SB-6}
\end{align*}
\]
synthesized azomethines of β-phenyl acrolein moiety possess significant antibiotic potential.

CONCLUSION

β-Phenyl acrolein derivatives have been successfully synthesized and appear to be a novel and important class of antibacterial agents against Gram-positive and Gram-negative bacteria including S. aureus, P. aeruginosa, and K. pneumonia. The synthetic route and antibacterial potential of the compounds may be useful in guiding future efforts to synthesize new compounds with improved antibacterial activity.

ACKNOWLEDGEMENT

The authors are thankful to AIMST University, Malaysia, for providing funds via a grant, and also for the facilities to carry out the research. The authors are also thankful to UiTM, Malaysia for providing technical support in generating the analytical data.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

AUTHORS’ CONTRIBUTION

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Sridevi Chigurupati, Neeraj kumar Fuloria and Ravichandran Veerasamy carried out the synthetic work, Syed adnan ali Shah, Shivkanya Fuloria and Sundram Karupia performed the analytical work while Appala raju Nemala, Lim jun Yi and Ang xiang llan carried out the antibacterial activities. All authors approved the manuscript for publication.

REFERENCES