Docking, synthesis, antimicrobial screening and beta lactamase inhibitory activity of 3-(4-fluorophenylimino) indolin-2-one and 5-chloro-3-(4-fluorophenylimino) indolin-2-one derivatives

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**Abstract**—Docking of the few fluoroaniline isatin derivatives were done. Various substituted 3-(4-fluorophenylimino) indolin-2-one and 5-chloro-3-(4-fluorophenylimino) indolin-2-one derivatives [II a-h] were synthesized. All of these compounds were screen for antimicrobial as well as beta lactamase activity. And it was observe that docking result as well as antimicrobial and beta lactamase shown good activity for 5-chloro-3-(4-fluorophenylimino)-1-(morpholinomethyl)indolin-2-one[II h].

**Keywords**—3- (4-fluorophenylimino) indolin-2-one, beta lactamase inhibitors.

**I. INTRODUCTION**

The synthetic versatility of the molecule has stemmed from the interest in the biological and pharmacological properties of the molecule and its derivatives. Schiff base & Mannich base of isatin were reported to possess antimicrobial activity and various other pharmacological activities. Extensive literature review has been made regarding the activities of the isatin, especially for antimicrobial activity. The main focus is given to beta lactamase inhibitor activity and it is done by iodometric assay. The docking of the same molecule was done using VLife Molecular Design Suite [VLife MDS] [1;2]. Few fluoroaniline derivatives of isatin and chloro isatin were chosen as ligands for docking in beta lactamase protein [from Staphylococcus aureus PDB code: 3blm] in Cavity 1. A systematic conformational search was performed to obtain the low energy conformations of the ligands. Docking of the low energy conformer of each molecule, into the 3blm was done by using Genetic algorithm [GA] method. The complexes were then minimized using the MMFF method and ligand–receptor interactions were studied. Binding energy of all molecules was determined in order to give ranking [see fig.1, fig. 2] [3;4;5;6;7;8].

![Chemical Structures](image)

\(i\) = Ethanol, GAA; \(ii\) = THF, Formaline solution, secondary amines [Morpholine, Piperazine, n-Methyl piperazine].

<table>
<thead>
<tr>
<th>Comp. Code</th>
<th>(R_1)</th>
<th>(R_2)</th>
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<tbody>
<tr>
<td>IIa</td>
<td>H</td>
<td>--</td>
</tr>
<tr>
<td>IIb</td>
<td>Cl</td>
<td>--</td>
</tr>
<tr>
<td>IIc</td>
<td>H</td>
<td>––N(\bigcirc)NH</td>
</tr>
</tbody>
</table>
II. EXPERIMENTAL

2.1. General
All reagents were obtained from Sigma Aldrich and Loba Chem Ltd. [India]. All the solvents used in these studies were dried and distilled before use. Melting points [m.p.]: Veego VMP-PM digital melting point apparatus, TLC: solvent Benzene: Ethanol [8:2], UV: Shimadzu Pharmspec 1700, UV-VIS spectrophotometer, IR spectra: Shimadzu 8400 S, FT-IR, ¹H NMR spectra: 300 MHz JEOL NMR Spectrophotometer.

2.2. Synthesis
2.2.1. General procedure for synthesis of Schiff base of isatin and chloroisatin [II a-b]
Equimolar quantities of substituted isatin [I a-b] and fluoroaniline were dissolved in warm ethanol containing 1ml of glacial acetic acid. The reaction mixture was irradiated in a microwave oven at 80% intensity with 30s per cycle. The number of cycle in turn depended on the completion of the reaction, which was checked by TLC. After completion of the reaction the mixture was poured in crushed ice. The resulting precipitate was filtered recrystallized and dried [II a-b] [9].

2.2.2. General procedure for synthesis of Mannich Base [II c-h]
A slurry consisting of the Schiff base of substituted isatin [0.005 mol] synthesized by using a literature methodology, THF [5 ml] & 37% formalin [2 ml] was made. To this Morpholine/ piperazine/ n-methyl piperazine [0.005mol] was added drop wise with cooling and shaking. The reaction mixture was allowed to stand at room temperature for 1 hr with occasional shaking after which it was warmed on a steam bath for 15 min. At the end of the period the contents were cooled and the product was obtained, which was further recrystallized from chloroform-petroleum ether [10].

2.2.2.1. 3-(4-fluorophenylimino) indolin-2-one IIa
% Yield: 70, m.p. 210-214 °C; UV λ max 248 nm; IR (KBr) (cm⁻¹): 995 (C-F), 1334 (C-N), 1739 (C=O), 3008 (Ar-CH), 3263 (NH); ¹H-NMR (CDCl₃) δ (ppm): 6.4-7.5 (m, 8H, Ar-H), 10.9 (s, 1H, NH); calc. for C₁₅H₁₁FN₂O: C-69.99%, H-3.78% and N-11.66%, found C-49.19%, H-1.96% and N-8.86%.

2.2.2.2. 5-chloro-3-(4-fluorophenylimino) indolin-2-one IIb
% Yield: 68, m.p. 236-240 °C; UV λ max 250 nm; IR (KBr) (cm⁻¹): 790 (C-Cl), 1114 (C-F), 1612 (C-N), 1743 (C=O), 3001 (Ar-CH); ¹H-NMR (CDCl₃) δ (ppm): 6.3-7.6 (m, 7H, Ar-H), 11.10 (s, 1H, NH); calc. for C₁₅H₁₁ClFN₂O: C-61.22%, H-2.94% and N-10.20%, found: C-45.12%, H-1.82% and N-10.11%.

2.2.2.3. 3-(4-fluorophenylimino)-1-(piperazin-1-ylmethyl) indolin-2-one IIC
% Yield: 79 , m.p. 166-170 °C; UV λ max 246 nm; IR (KBr) (cm⁻¹): 1056 (C-F), 1465 (CH₂), 1612 (C=N), 1728 (C=O), 3074 (Ar-CH), 3355 (NH); ¹H-NMR (CDCl₃) δ (ppm): 2.0 (s, 1H, NH), 2.4 (t, 4H, CH₂), 2.6 (q, 4H, CH₂), 4.03 (s 2H, CH₂), 7.0-7.6 (m, 8H, Ar-H); calc. for C₁₅H₁₁FN₂O: C-67.44%, H-5.66% and N-16.56%, found C-58.31%, H-4.19% and N-14.65%.

2.2.2.4. 5-chloro-3-(4-fluorophenylimino)-1-(piperezin-1-ylmethyl) indolin-2-one IID
% Yield: 77, m.p. 245-249 °C; UV λ max 244.5 nm; IR (KBr) (cm⁻¹): 771(C-Cl), 1053 (C-F), 1438 (CH₂), 1604 (C=N), 1735 (C=O), 3047 (Ar-CH), 3359 (NH); ¹H-NMR (CDCl₃) δ (ppm): 2.67 (m, 8H, CH₂), 4.03 (s 2H, CH₂), 6.7-7.7 (m, 7H, Ar-H), 11.08 (s, 1H, NH); calc. for C₁₉H₁₃FN₄O: C-61.21%, H-4.87% and N-15.03%, found C-45.691%, H-3.98% and N-12.64%.

2.2.2.5. 3-(4-fluorophenylimino)-1-((4-methylpiperazin-1-yl)methyl)indolin-2-one IIE
% Yield: 66, m.p. 169-172°C; UV λ max 248nm;IR (KBr) (cm⁻¹): 1060 (C-F), 1469 (CH₂), 1608 (C=N), 1728 (C=O), 2958 (CH₃), 3074 (Ar-CH); ¹H-NMR (CDCl₃) δ (ppm): 2.4 (s, 3H, CH₃), 2.4 (t, 8H, CH₂), 4.4 (s 2H, CH₂), 6.4-7.4 (m, 8H, Ar-H); calc. C₁₉H₁₅FN₄O: C-68.16%, H-6.01% and N-15.90%, found C-49.52%, H-5.12% and N-10.36%.

IId  Cl  N  H
Ile  H  N  -NCH₃
IIf  Cl  N  -NCH₃
Ilg  H  N  O
IIh  Cl  N  O
2.2.2.6. 5-chloro-3-(4-fluorophenylimino)-1-[(4-methylpiperazin-1-yl) methyl] indolin-2-one II

% Yield: 71, m.p. 247-251 °C; UV $\lambda_{max}$ 247 nm; IR (KBr) (cm$^{-1}$): 771 (C-Cl), 1053 (C-F), 1438 (CH$_2$), 1604 (C=N), 1735 (C=O), 3047 (Ar-CH$_3$); $^1$H-NMR (CDCl$_3$) $\delta$ (ppm): 2.2 (s, 3H, CH$_3$), 2.3, 2.71 (t, 8H, CH$_2$), 4.3 (s 2H, CH$_2$), 6.4-7.3 (m, 7H, Ar-H); calc. C$_{29}$H$_{30}$ClF$_3$N$_2$O: C-62.09%, H-5.21% and N-14.48%, found C-55.21%, H-4.10% and N-13.78%.

2.2.2.7. 3-(4-fluorophenylimino)-1-(morpholinomethyl)indolin-2-one IIg

% Yield: 72, m.p. 153-155 °C; UV $\lambda_{max}$ 240 nm; IR (KBr) (cm$^{-1}$): 1060 (C-F), 1469 (CH$_2$), 1608 (C=N), 1728 (C=O), 3078 (Ar-C-H); $^1$H-NMR (CDCl$_3$) $\delta$ (ppm): 2.4 (t, 4H, CH$_2$), 3.5 (t, 4H, CH$_2$), 4.3 (s 2H, CH$_2$), 6.3-7.4 (m, 8H, Ar-H); calc. C$_{19}$H$_{18}$FN$_2$O: C-67.24%, H-5.35% and N-12.38%, found C-60.39%, H-2.65% and N-11.51%.

2.2.2.8. 5-chloro-3-(4-fluorophenylimino)-1-(morpholinomethyl)indolin-2-one IIh

% Yield: 75, m.p. 170-174 °C; UV $\lambda_{max}$ 243.5 nm; IR (KBr) (cm$^{-1}$): 771 (C-Cl), 1049 (C-F), 1442 (CH$_2$), 1604 (C=N), 1728 (C=O); $^1$H-NMR (CDCl$_3$) $\delta$ (ppm): 2.3 (t, 4H, CH$_2$), 3.6 (t, 4H, CH$_2$), 4.03 (s 2H, CH$_2$), 7.0-7.67 (m, 7H, Ar-H); calc. C$_{19}$H$_{17}$ClF$_3$N$_2$O: C-61.05%, H-4.58% and N-11.24%, found C-49.32%, H-3.52% and N-10.25%.

2.2.3. Antimicrobial Activity

The nutrient agar media [for antibacterial activity] were prepared in conical flasks and sterilized in autoclave. Suspensions of different microorganisms [inoculums] were prepared in different tubes in sterile distilled water. When the temperature of the sterile molten media reached to 40-45 °C, 0.5 ml of the inoculum was added, mixed and poured immediately into the sterile petri plates [10 cm diameter] and labeled accordingly. The agar was then allowed to solidify. The wells were prepared in plates using sterilized borer of 6 mm in diameter [3 wells/plate]. About 50 µL of the control [Ethanol], sample solutions [II a-h 300µg/ml] and the standard drugs [ampicillin and griseofulvin 300µg/ml] were transferred into the wells in each plate using micropipettes. The plates were then refrigerated to allow for 1 hr of prediffusion to occur and the plates were then transferred to the incubator (temperature maintained at 37°C). And was kept in incubator for 24 hr. The zones of inhibition were measured as average of 3 readings [see table 1] [11].

2.2.4. Beta lactamase Assay

All reagents are equilibrated to 30°C in a water bath before adding them to the reaction tubes [20 x 150 mm. Pyrex test tubes] in the following order: first 1 ml of gelatin Solution [1 per cent c. p. grade, E. Merck in 0.1M phosphate buffer, pH 7.0], 50 µl of enzyme, 1 drop of Starch Solution [1 per cent soluble starch], 1 ml of Penicillin Solution [Crystalline Sodium Penicillin G [Hindustan Antibiotics Ltd.] 1660 µ /mg, dissolved in 0.1M phosphate buffer, pH 7.0, to contain not less than 5,000 µ/ml], 3 ml of sample solution II a-h and finally add 2 ml of iodine [0.01N iodine in 0.1M potassium iodide]. Then the time of decolorization of iodine was recorded with a stop-watch, after addition of substrate blank should always be determined using water in place of sample solution [see table 2].

Unit: Penicillinase activity is expressed in Pollock and Torriani unit. One unit is that amount of enzyme which will hydrolyse 1 µM Sodium Penicillin G in one hour at pH 7.0 at 30°C [12; 13; 14].
Table 1: Antimicrobial screening result [zone of inhibition in mm] of synthesized Isatin Fluoroaniline derivatives

<table>
<thead>
<tr>
<th>M.O.*</th>
<th>Std</th>
<th>Control</th>
<th>IIa</th>
<th>IIb</th>
<th>IIc</th>
<th>IIId</th>
<th>IIe</th>
<th>IIIf</th>
<th>IIg</th>
<th>IIh</th>
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<tbody>
<tr>
<td>E. coli</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+</td>
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<td>p. aeruginosa</td>
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<td>++</td>
<td>++</td>
<td>++</td>
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<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

- < [6], + [6-12], ++ [12-16], +++ > [16]
* M.O. – Microorganisms

Table 2: Penicillinase Corresponding to Time of Decolorization of 2 ml of 0.01 N Iodine

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Comp. Code</th>
<th>Time for decolorization of I₂ in Sec.</th>
<th>Activity u/ml</th>
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<td>1</td>
<td>Control</td>
<td>79.5</td>
<td>75.47</td>
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<td>6</td>
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<td>125.3</td>
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<tr>
<td>5</td>
<td>IIc</td>
<td>122.9</td>
<td>48.8</td>
</tr>
<tr>
<td>8</td>
<td>IIId</td>
<td>83.1</td>
<td>37.8</td>
</tr>
<tr>
<td>4</td>
<td>IIe</td>
<td>230.5</td>
<td>37.4</td>
</tr>
<tr>
<td>7</td>
<td>IIf</td>
<td>160.4</td>
<td>25.9</td>
</tr>
<tr>
<td>3</td>
<td>IIg</td>
<td>158.8</td>
<td>72</td>
</tr>
<tr>
<td>9</td>
<td>IIh</td>
<td>182.9</td>
<td>32.8</td>
</tr>
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III. RESULT AND DISCUSSION
All the synthesized compounds [II a-h] were screened for antibacterial activity against E. coli, P aeruginosa, S. typhi, S. aureus strains, by agar diffusion method and the average diameter of zone of inhibition was recorded. The screened results were compared with the standard Ampicillin at a concentration of 300 μg/ml. Result was shown in table 1. The beta lactamase inhibitor activity was shown in the table 2 and both the result were compare with dock score. According to docking prediction the compound IIh > IIf > IIId and according to both activity compound IIh was having highest activity. The most important interaction was found to be Van der Waal, H-bond & Hydrophobic. It was found that the predicted docking results using VLife MDS Software were quite accurate after comparing it with the actual antimicrobial and beta lactamase inhibitor activity.

IV. CONCLUSION
In conclusion, results from this study has demonstrated the beta lactamase inhibitory activity and to some extent antibacterial activity of compound II a-h is comparable with docking result. This would serve as economic advantage to the country.

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REFERENCES

[2]. WWW.vlifesciences.com