IOSR Journal of Pharmacy
ISSN: 2250-3013, www.iosrphr.org
Ⅲ Volume 2 Issue 5 Ⅲ Sep-Oct 2012 Ⅲ PP.18-22

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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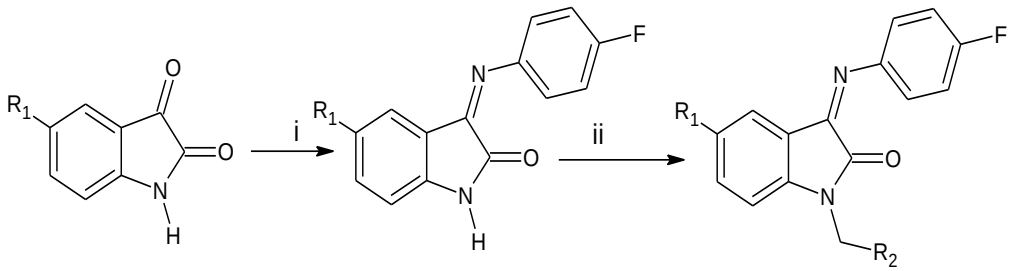
Abstract—Docking of the few fluroaniline isatin derivatives were done. Various substituted 3-(4-fluorophenylimino) indolin-2-one and 5-chloro-3-(4-fluorophenylimino) indolin-2-one derivatives [II a-h] were synthesized. All of these compounds were screen for antimicrobial as well as beta lactamase activity. 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.

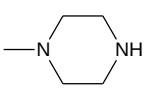
I. INTRODUCTION

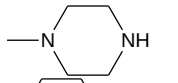
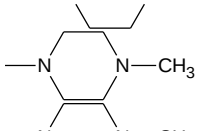
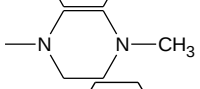
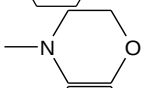
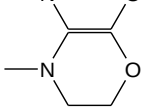
The synthetic versatility of the molecule has stemmed from the interest in the biological and pharmacological properties of the molecule and its derivatives. Schiff base & Mannich base of isatin were reported to possess antimicrobial activity and various other pharmacological activities. Extensive literature 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 fluroaniline 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].



Ia-b II a-b II c-h

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

Comp. Code	R_1	R_2
IIa	H	--
IIb	Cl	--
IIc	H	

IId	Cl	
IIe	H	
IIf	Cl	
IIg	H	
IIh	Cl	

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 spectra : Shimadzu Pharmspec 1700, UV-VIS spectrophotometer, IR spectra: Shimadzu 8400 S, FT-IR, ^{1}H 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 fluroaniline 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); ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.4-7.5 (m, 8H, Ar-H), 10.9 (s, 1H, NH); calc. for $C_{14}H_9FN_2O$: 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),; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.3-7.6 (m, 7H, Ar-H), 11.10 (s, 1H, NH); calc. for $C_{14}H_8ClFN_2O$: 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 (CH_2),1612 (C=N), 1728 (C=O), 3074 (Ar-CH), 3355 (NH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.0 (s, 1H, NH), 2.4 (t, 4H, CH_2), 2.6 (q, 4H, CH_2), 4.03 (s 2H, CH_2), 7.0-7.6 (m, 8H, Ar-H); calc. for $C_{19}H_{19}FN_4O$: 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-(piperazin-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 (CH_2), 1604 (C=N), 1735 (C=O), 3047 (Ar-CH), 3359 (NH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.67 (m, 8H, CH_2), 4.03 (s 2H, CH_2), 6.7-7.7 (m, 7H, Ar-H), 11.08 (s, 1H, NH); calc. for $C_{19}H_{18}ClFN_4O$: 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 (CH_2), 1608 (C=N), 1728 (C=O), 2958 (CH_3), 3074 (Ar-CH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.4 (s, 3H, CH_3), 2.4 (t, 8H, CH_2), 4.4 (s 2H, CH_2), 6.4-7.4 (m, 8H, Ar-H); calc. $C_{20}H_{21}FN_4O$: C-68.16%, H-6.01% and N-15.90%, found C-49.52%, H-5.12% and N-10.36%.

2.2.2.6. 5-chloro-3-(4-fluorophenylimino)-1-((4-methylpiperazin-1-yl) methyl) indolin-2-one IIf

% Yield: 71, m.p. 247-251 °C; UV λ_{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); ^{1}H-NMR ($CDCl_3$) δ (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. $C_{20}H_{20}ClFN_4O$: 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 λ_{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 ($CDCl_3$) δ (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. $C_{19}H_{18}FN_3O_2$: 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 λ_{max} 243.5nm; IR (KBr) (cm^{-1}): 771 (C-Cl), 1049 (C-F), 1442 (CH_2), 1604 (C=N), 1728 (C=O); ^{1}H-NMR ($CDCl_3$) δ (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. $C_{19}H_{17}ClFN_3O_2$: 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 micro organisms [inoculums] were prepared in different tubes in sterile distilled water. When the temperature of the sterile molten media reached to 40-45 ^{0}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^{0}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^{0}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 0.1*M* phosphate buffer, P^H 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, P^H 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 [0.01*N* iodine in 0.1*M* 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 P^H 7.0 at 30^{0}C [12; 13; 14].

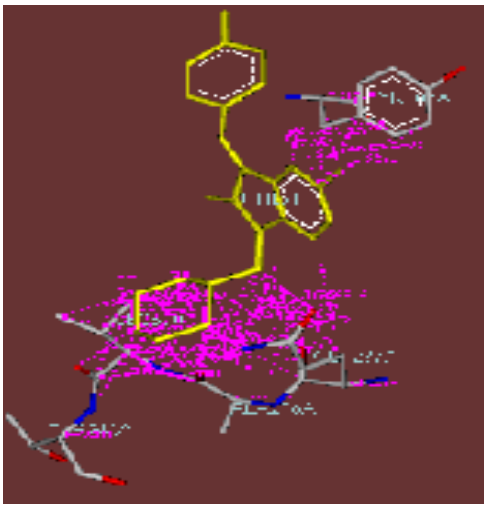


Fig. 1 Compound IIh showing Vander Waal interaction

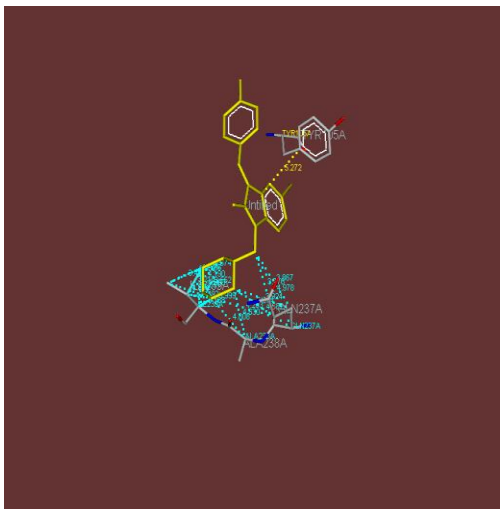


Fig. 2 Compound IIh showing Hydrophobic & Pi staking interaction

Table 1: Antimicrobial screening result [zone of inhibition in mm] of synthesized Isatin Fluroaniline derivatives

M.O.*	ZONE OF INHIBITION(mm) n=3									
	Std	**Control**	**IIa**	**IIb**	**IIc**	**IId**	**IIe**	**IIf**	**IIg**	**IIh**
E.coli	++	+	+++	+	++	+	++	++	++	+++
p.aeruginosa	++	+	+	+	+	++	+	++	++	++
S. typhi	++	+	++	++	++	++	++	++	++	++
S.aureus	++	+	++	++	+	++	+++	+	+	+++

- < [6], + [6-12], ++ [12-16], +++ > [16]
* M.O. – Microorganisms
E. coli – Escherichia coli, P aeruginosa– Psudomonas aeruginosa, S. typhi–Salmonella typhi, S. aureus – Staphylococcus aureus.

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

Sr. No.	Comp. Code	Time for decolorization of I_2 in Sec.	Activity u/ml
1	Control	79.5	75.47
2	IIa	125.5	47.9
6	IIb	125.3	47.85
5	IIc	122.9	48.8
8	IId	83.1	37.8
4	IIe	230.5	37.4
7	IIf	160.4	25.9
3	IIg	158.8	72
9	IIh	182.9	32.8

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 > IId 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 bata lactamase inhibitory activity and to some extent antibacterial activity of compound II a-h is comparable with doking result. This would serve as economic advantage to the country.

V. ACKNOWLEDGEMENT

Authors are thankful for providing funds to carryout this research, to university of Pune. Authors also thankful to Instrumentation department of Pune University, Pune for providing NMR Spectra.

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